Event-related Potential Additivity as an Index of Neural Independence

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Abstract

Background Scalp-recorded event-related potentials (ERPs) are poorly suited for certain types of source analysis. For example, it is often difficult to precisely assess whether two ERP waveforms were produced by similar neural sources, especially when the waveforms share the same polarity and a similar scalp topography and temporal dynamics. We report here an alternative method to establishing independence of neural sources grounded in the principle of superposition, which stipulates that electrical fields summate where they intersect in time and space. We assessed the independence of two frequently reported positive waves in the ERP literature, the P300 (elicited by unexpected stimuli) and P600 (elicited by syntactic anomalies). Subjects read sentences that contained a word that was either non-anomalous, unexpected in one feature (capitalized, different font, different font color, or ungrammatical), or unexpected in two features (capitalized and different font style, capitalized and different font color, or capitalized and ungrammatical). Thus, in the double anomaly condition, the similarity between a shared feature (i.e., capitalization) and a second feature was systematically manipulated across conditions from larger degree (i.e., font style) to lesser degree (i.e., ungrammatical) of feature similarity. Results We quantified the degree of source independence for the features of interest by applying a novel Additivity Index, which compares ERPs elicited by the doubly anomalous words to composite waveforms formed by mathematically summing the ERP response to singly anomalous words. The degree of source independence is reflected by the degree of summation, with Additivity scores ranging from 0 (completely non-independent) to 1 (completely independent). The computed Additivity Index values varied with feature similarity in the predicted direction: similar features demonstrated lower Additivity Index values, or lower degrees of independence. On the other hand, dissimilar features manifested robust additivity, resulting in larger AI values. Conclusion We quantified the degree to which the P600 and P300 effects are neurally distinct across stimulus features with varying degrees of similarity by computing a continuous measure of independence via the Additivity Index. These findings indicate that the Additivity Index provides a valid and general method for quantifying the neural independence of scalp-recorded brain potentials.

Background

The study of the electrophysiology of cognitive processes fundamentally relies on the additive nature of electrical fields, which summate across both time and space[1]. This property of electrical signals is what enables the recording of event-related potentials (ERPs), which reflect the summed, simultaneously occurring post-synaptic potentials of large numbers of cortical pyramidal neurons, as recorded on the scalp[2]. As such, ERPs provide an online measurement of the neural response to a particular stimulus feature, including the electrical polarity, timing, and scalp topography of that neural response.

Furthermore, the physics-based *principle of superposition* states that every charge in space creates an electric field that is independent of the presence of other charges in a conductive medium. Thus, if two electrical sources are neurally independent, then the combined, simultaneously occurring electrical fields produced by these two sources will be equivalent to the sum of the fields produced by each of the sources individually. The degree of additivity will reflect (at least to a useful approximation) the degree to which the two brain responses are neurobiologically distinct[3-6]. Previously reported ERP studies have used this approach to assess the neural independence of the brain response to a pair of stimulus features[5]; these studies have typically adopted an “anomaly” paradigm in which subjects are presented with a first stimulus that contains a specific feature of interest, a second stimulus with a different feature, and a third stimulus containing both features. Following the principle of superposition, if the two features are independently processed in the brain, then the neural responses associated with each individual stimulus feature should summate when the two features are presented simultaneously.

This logic has been successfully used to assess the neural independence of certain types of linguistic contrasts. For example, prior work has shown that sentence-embedded semantic (e.g., “The cat will *bake* the food.”) and syntactic (e.g., “The cat will *eating* the food.”) anomalies elicit distinct ERP responses (the N400 and P600 effects, respectively)[7-11]. Osterhout and Nicol[6] assessed the independence of these responses by adding a third condition that was anomalous both syntactically and semantically (e.g., “The cat will *baking* the food.”). The doubly anomalous stimuli elicited a response that approximated the summation of the two effects when they were elicited in isolation, indicating that the effects are (to a significant degree) generated independently[9,12].

A similar result was reported by Osterhout et al[5], who studied the independence of two positive waves that overlap in time and space: the P600 elicited by syntactic anomalies, and the P300 elicited by a wide range of unexpected or unlikely but task-relevant events. Because the ERP effects are superficially similar—both are late positivities broadly distributed with maximal amplitudes over central posterior sites—there has been a debate over whether the P600 is a member of the P300 ‘family’[13-16]. Osterhout et al[5] applied an additivity paradigm to assess the neural independence of the P300 and P600 components. Participants read sentences that contained a syntactic anomaly, a word that was in an unexpected physical form (all uppercase letters), or a word that was both in uppercase and syntactically anomalous.

Osterhout et al[5] reported that the ERP response to the doubly anomalous words closely approximated the sum of the ERP response to each type of anomaly when they were presented separately. This result was taken as evidence of a considerable degree of independence with respect to the neural systems that respond to each type of anomaly.

However, in these and related studies, the strength of the conclusions is limited to a binary logic, in which the two processes of interest are determined to be either independent or non-independent. It seems likely that meaningful and measurable gradations of neural independence may exist. If so, then the *relative* neural independence of two brain responses could be quantified on a continuous scale of additivity, with the endpoints representing no independence (little or no additivity) and complete independence (close to perfect additivity, assuming low quantities of random noise). Here, we explore this possibility and also attempt to validate a simple scale of independence, the Additivity Index (AI). AI values range positively from 1 (reflecting complete separation of the neural mechanisms underlying processing of the two stimuli) to 0 (reflecting the complete overlap of the neural responses). The AI represents a straightforward application of the principle of superposition[1].

For our purposes, a stimulus feature is any aspect of a stimulus that can be manipulated independently of some other feature or set of features. For example, the frequency and amplitude of an auditory stimulus can be manipulated independently, and so would constitute separate features[17]. In order to assess the degree of neural independence in processing two stimulus features, we chose pairs of features that can co-occur within a single stimulus. Neural independence, in this case, refers specifically to the degree to which the brain response elicited by manipulation of one stimulus feature (A) summates with the brain response elicited by manipulation of a second stimulus feature (B). If there is some degree of overlap between the neural sources responsible for processing each individual stimulus feature, then the net signal elicited by the processing of a stimulus containing both features (A&B) will be less than the sum of the signals produced in response to the individual features (A+B). This is due to the fact that the shared neural sources responsible for processing both stimulus features would presumably contribute only once to the net signal (A&B) but twice to the summed signal (A+B).

Within the framework described above, the degree of neural independence can be conceptualized as a parametric function that has a unidimensional range. At one end of this range, the brain processes the two features using separate (independent) systems–changes in one feature elicit a response that is neurally independent from the response elicited by changes to another feature. Following the rule of superposition, one would predict an additive (or near-additive, due to unsystematic noise) function in such instances. As one approaches the other end of the range, there is an increase in overlap of the processing of two given features; the neural response to change in one feature becomes increasingly similar to the neural response to change in another feature; consequently, one would expect the neural responses to summate less, if at all. Our approach, using the AI, represents a fundamentally different method for examining the "neural independence" of stimulus features, one that might allow us to define a "feature category similarity space" in terms of neural activity. Features that are within a category should have a much lower additivity index than features that are between categories.

While AI values might not directly reflect source configurations, they (theoretically) should indicate the relative neural independence of these sources regardless of how the sources of interest are configured. For instance, if it were possible that a single anatomical source could be sensitive to the "severity" of an error and increase its response in the presence of multiple errors in an additive manner, then this single source would, in fact, be responsible for the independent processing of two stimulus features, which would consequently by reflected by a higher additivity index.

Just as independent current sources sum across space and time, the brain responses to two simultaneously-presented stimulus features will summate to the extent that they are independently generated. Therefore, the ERP waveform observed to a stimulus that contains both features simultaneously will be (assuming no noise) equal to a composite waveform generated by mathematically summing the waveforms observed when the two features are presented in separate classes of stimuli. The degree of additivity would reflect the degree of neural independence, with respect to how the brain is processing the two features. Complete independence would be manifested as a near-perfect additive function, whereas the degree of non-independence would be quantitatively inferred by the proportional difference between a perfectly additive function and the observed function. That is, in the non-independent case, the ERP waveforms resulting from presentation of a stimulus containing both features will be smaller than a composite waveform generated by mathematically summing the waveforms resulting from individual presentations. This result would be obtained because the non-independent neural resources will contribute twice to the summed signal but only once to the processing of the two-feature stimulus.

Mathematically, the Additivity Index is a simple ratio represented by Equation (1):

(1):

$$AI = 1 - \frac{(A+B) - A\&B}{A\&B} \qquad (1)$$

where A + B represents the sum of the mean amplitudes of the difference waveforms elicited by two different stimulus features during a particular temporal window, and A&B represents the mean amplitude when both features are presented simultaneously in that same window. Assuming that the effects elicited by each stimulus feature are sufficiently robust (i.e. statistically significant), the range of the equation is from 0 to 1, wherein complete overlap in processing is reflected by the value of 0 and complete independence of processing is represented by the value of 1.

In order to empirically demonstrate the validity of the Additivity Index, we recorded ERPs to anomalies involving four distinct stimulus features: font type, font capitalization, font color, and syntax. We then constructed a set of sentences, each of which contained a critical word that was anomalous with respect to one of these features, or a pair of features (i.e., anomalous capitalization paired with anomalous font, color, or grammar). Given prior work, we expected that each anomalous feature would elicit a robust positive-going shift in the ERP[11,18-21]. Grammatical anomalies elicit a large positive-going wave[8,11], as do a wide variety of expectation violations (e.g., duration, intensity, frequency, modality, probability)[18-21]. Of particular interest were the ERP responses to pairs of feature anomalies, which systematically differed with respect to the similarity between the two features. These three pairs reflect a manipulation of the degree of similarity of the within-pair violations, such that the three pairs form a putative continuum of similarity with respect to their constituent features. Violations of font and capitalization both involve manipulating the physical shape of individual letters; that is, the changes necessary to go from a well-formed stimulus to an anomalous stimulus are entirely orthographic in nature. By contrast, the manipulation of capitalization and color involves two visual features, but the features are quite distinct (orthographic and color-related). The third pair involves manipulation of two other distinct features: a physical feature (capitalization) and a highly abstract linguistic constraint on the form that words can take when they appear in sentences (see Fig 1). Each category of stimulus was equiprobable in the stimulus set.

INSERT FIGURE 1 HERE

Given prior work, we expected that each anomalous feature would elicit a robust positive-going shift in the ERP[11,18-21]. Our prediction is that the magnitude of the ERP response to the doubly anomalous stimuli would be an inverse function of the similarity of the two anomalous features, within a given stimulus. Consistent with our prediction, we report here systematic covariation between the additivity of the ERP responses and the similarity of stimulus features, representing a first step in validating the Additivity Index as a metric for assessing processing independence at a neural level. We further demonstrate the utility of this index by quantifying the independence of two ERP waves that share a similarity in their temporal and topographical characteristics but that are elicited by functionally distinct anomalies, namely, graphemically anomalous words and syntactically anomalous words.

Results

Consistent with much prior research[5], ERP responses to the anomalies were uniformly largest over centro-posterior sites, as indicated by interactions between condition and by electrode site. Effects over medio-lateral and lateral electrodes trended in the same direction but were not as robust as those measured at midline electrodes. We therefore focused our statistical analyses on data acquired over midline sites.

Capitalization and Syntax

Grand average ERPs for the four critical words at Pz are shown in Fig 2A. The capitalization and double anomalies elicited large-amplitude positive-going deflections, relative to the control condition, beginning at approximately 200ms. ERPs to the syntactic anomalies began to diverge from the control condition at about 500ms, and also elicited a large positive deflection with a centro-posterior distribution. The positive wave persisted through the end of the epoch in all conditions. The omnibus ANOVA on mean amplitude within the 300 to 900ms window latency range (across midline sites) yielded a significant effect of violation type, $F(3,72) = 20.49$, $MSE = 23.29$, $p < 0.001$, as well as a violation type by electrode interaction, $F(6,144) = 8.02$, $MSE = 2.26$, $p < 0.001$. The Additivity Index was computed to be 0.85 at electrode Pz (where the effect magnitudes were most robust), indicating a substantial degree of independence in the neural sources contributing to the two positive waves.

INSERT FIGURE 2 HERE

Simple effects analyses for the individual violations along the midline sites reveal no significant differences between the experimental conditions and the control condition during the 0-150ms window. Because (as is usually the case) the electrophysiological differences among the treatment conditions were largest over midline sites, we focused our statistical analyses on data recorded from midline electrodes. There was no significant difference between the double violation waveform and the composite waveform, for brain activity acquired over midline sites. For the 150-300ms time window across midline sites, there was a significant difference between capitalization and control conditions, $F(1,24) = 4.28$, $MSE = 11.42$, $p < 0.05$, and between the double violation and control condition, $F(1,24) = 6.95$, $MSE = 8.85$, $p < 0.05$. There was no significant difference between the double violation and the composite condition. For the 300-500ms time window across midline sites there was a significant difference between capitalization and control conditions, *F*(1,24) = 24.53, *MSE* = 16.00, $p < 0.001$, no significant differences between the syntax and control condition, and a significant difference between the double violation and control condition, *F*(1,24) = 17.22, *MSE* = 23.51, $p < 0.05$. There was no significant difference between the double violation and the composite condition.

For the 500-800ms time window across midline sites there was a significant difference between capitalization and control conditions, *F*(1,24) = 26.30, *MSE* = 30.20, $p < 0.001$, a significant difference between the syntactic violation and the control, *F*(1,24) = 16.10, *MSE* = 31.27, $p < 0.001$ and a significant difference between the double violation and control condition, *F*(1,24) = 39.17, *MSE* = 45.46, $p < 0.001$. There was no significant difference between the double-violation waveform and the composite waveform. For the 300-900ms time window, across midline sites there was a significant difference between capitalization and control conditions, *F*(1,24) = 25.65, *MSE* = 19.90, $p < 0.001$, a significant difference between the syntactic violation and the control, *F*(1,24) = 10.79, *MSE* = 19.29, $p < 0.001$, and a significant difference between the double violation and control condition, *F*(1,24) = 35.14, *MSE* = 35.14, $p < 0.001$. There was no significant difference between the double violation and the composite condition. See Table 1 for all pairwise comparisons.

INSERT TABLE 1 HERE [from *Additional file 1.xlsx*]

Capitalization and Text Color

Grand average ERPs for the four critical words are shown in Fig 2B. The capitalization violation, the color violation, and the double violation all showed a positive-going deflection relative to the control condition, beginning at approximately 200ms. Although broadly distributed, all three deflections were again maximal at centro-posterior sites. All three experimental conditions continue to show a positive-going mean amplitude throughout the rest of the epoch, at which point the responses to the following word began to be manifested in the waveform. The omnibus ANOVA on mean amplitude in the 300 to 900ms latency range (and from the three midline sites) yielded a significant effect of violation type,

$F(3,72) = 25.61$, $MSE = 15.17$, $p < 0.001$, as well as a violation type by electrode interaction, $F(6,144) = 9.26$, $MSE = 3.07$, $p < 0.001$. The Additivity Index calculated at electrode site Pz was 0.42, indicating a moderate degree of independence in the neural sources contributing the two positivities.

Simple effects analyses for the individual violations along the midline sites reveal no significant differences between the experimental conditions and the control condition during the 0-150ms window. There was no significant difference between the double violation and the composite condition for the midline condition. For the 150-300ms time window across midline sites, there was a significant difference between capitalization and control conditions, $F(1,24) = 21.77$, $MSE = 7.21$, $p < 0.001$, a significant differences between the color and control condition, $F(1,24) = 24.00$, $MSE = 6.03$, $p < 0.001$, and a significant difference between the double violation and control condition, $F(1,24) = 15.06$, $MSE = 15.15$, $p < 0.001$. There was also a significant difference between the double violation and the composite condition, $F(1,24) = 9.52$, $MSE = 9.38$, $p < 0.01$. For the 300-500ms time window across midline sites, there was a significant difference between capitalization and control conditions, $F(1,24) = 26.19$, $MSE = 16.60$, $p < 0.001$, a significant difference between the color and control condition, $F(1,24) = 53.87$, $MSE = 19.08$, $p < 0.001$, and a significant difference between the double violation and control condition, $F(1,24) = 47.68$, $MSE = 38.97$, $p < 0.001$. There was also a significant difference between the double violation and the composite condition, $F(1,24) = 6.51$, $MSE = 14.76$, $p < 0.05$. For the 500-800ms time window across midline sites, there was a significant difference between capitalization and control conditions, $F(1,24) = 46.37$, $MSE = 23.00$, $p < 0.001$, a significant difference between the color violation and the control, $F(1,24) = 19.84$, $MSE = 16.99$, $p < 0.001$, and a significant difference between the double violation and control condition, $F(1,24) = 44.88$, $MSE = 66.88$, $p < 0.001$. There was also a significant difference between the double violation and the composite condition, $F(1,24) = 19.87$, $MSE = 32.12$, $p < 0.001$. For the 300-900ms time window across midline sites, there was a significant difference between capitalization and control conditions, $F(1,24) = 43.79$, $MSE = 14.67$, $p < 0.001$, a significant difference between the color violation and the control, $F(1,24) = 32.03$, $MSE = 13.27$, $p < 0.001$, and a significant difference between the double violation and control condition, $F(1,24) = 42.28$, $MSE = 19.49$, $p < 0.001$. There was also a significant difference between the double violation and the composite condition, $F(1,24) = 15.99$, $MSE = 18.63$, $p < 0.001$. See Table 1 for all pairwise comparisons.

Capitalization and Font

Grand average ERPs for the four critical words are shown in Fig 2C. The capitalization violation, the font violation, and the double violation all showed a positive-going deflection relative to the control condition, beginning at approximately 200ms. All three experimental conditions continue to show a positive-going mean amplitude throughout the rest of the epoch, at which point the responses to the following word begin to manifest. The omnibus ANOVA on the mean amplitude in the 300 to 900ms latency range across midline sites yielded a significant effect of violation type, $F(3,72) = 52.05$, $MSE = 14.35$, $p < 0.001$, as well as a violation type by electrode interaction, $F(6,144) = 32.99$, $MSE = 3.12$, $p < 0.001$. The Additivity Index calculated at electrode site Pz was 0.20, indicating a low degree of independence in the neural sources contributing the two positivities.

Simple effects analyses for the individual violations along the midline sites reveal no significant differences between the experimental conditions and the control condition during the 0-150ms window. There was no significant difference between the double violation and the composite condition across the midline sites. For the 150-300ms time window across midline sites, there was a significant difference between capitalization and control conditions, $F(1,24) = 34.51$, $MSE = 7.63$, $p < 0.001$, a significant differences between the font and control condition, $F(1,24) = 7.56$, $MSE = 10.56$, $p < 0.05$, and a significant difference between the double violation and control condition $F(1,24) = 37.28$, $MSE = 9.35$, $p < 0.001$. There was no significant difference between the double violation and the composite condition. For the 300-500ms time window across midline sites, there was a significant difference between capitalization and control conditions, $F(1,24) = 120.05$, $MSE = 13.51$, $p < 0.001$, a significant difference between the font and control condition, $F(1,24) = 47.30$, $MSE = 23.39$, $p < 0.001$, and a significant difference between the double violation and control condition, $F(1,24) = 96.03$, $MSE = 27.66$, $p < 0.001$. There was also a significant difference between the double violation and the composite condition, $F(1,24) = 26.27$, $MSE = 18.48$, $p < 0.001$. For the 500-800ms time window across midline sites, there was a significant difference between capitalization and control conditions, $F(1,24) = 67.84$, $MSE = 22.25$, $p < 0.001$, a significant difference between the font violation and the control, $F(1,24) = 56.34$, $MSE = 30.32$, $p < 0.001$, and a significant difference between the double violation and control condition, $F(1,24) = 98.10$, $MSE = 15.50$, $p < 0.001$. There was also a significant difference between the double violation and the composite condition, $F(1,24) = 36.59$, $MSE = 46.38$, $p < 0.001$. For the 300-900ms time window across midline sites there was a significant difference between capitalization and control conditions, $F(1,24) = 77.12$, $MSE = 14.22$, $p < 0.001$, a significant difference between the font violation and the control, $F(1,24) = 57.56$, $MSE = 17.87$, $p < 0.001$, and a significant difference between the double violation and control condition, $F(1,24) = 104.96$, $MSE = 12.53$, $p < 0.001$. There was also a significant difference between the double violation and the composite condition, $F(1,24) = 32.47$, $MSE = 25.78$, $p < 0.001$. See Table 1 for all pairwise comparisons.

Discussion

In order to test the validity of the Additivity Index (AI), we compared ERPs elicited by three distinct pairs of stimulus features: capitalization *vs*. syntax, capitalization *vs*. color, and capitalization *vs*. font. Within each pair, either the first, the second, or both of the features appeared in an anomalous (unexpected) form. As specified by the physics-based principle of superposition, electrical signals add together where they intersect in time and space. Therefore, the degree to which the two stimulus features were processed independently in the brain should be reflected in the degree to which the ERP “anomaly” responses summed, when those two stimulus features were presented simultaneously. We observed a parametric inverse function in which the degree of feature similarity predicted the degree of neural independence in the ERP response to the anomalous features. Specifically, the least-similar feature pair (contrasting capitalization and syntax, that is, contrasting the effects of orthographic change and grammatical well-formedness) produced the largest AI value, whereas the most similar pair (contrasting capitalization and font) produced the smallest AI value. The intermediate pairing, capitalization and color, elicited an intermediate AI value. Described in terms of overlap in neural processing, we can summarize this as follows: anomalies involving syntax and capitalization engage highly independent neural processes, and the brain seems to treat syntax and capitalization as highly distinct features. Anomalies involving capitalization and font, however, seem to elicit highly similar neural processes; that is, the brain treats them as similar features. Anomalies involving capitalization and color fall nearly halfway between these two points (Fig 3).

INSERT FIGURE 3 HERE

One of the advantages of the method described here is that it provides a continuous scale ranging from 0 (no additivity) to 1 (perfect additivity). Previously, ERP investigators have tended to treat the “independence” of two processes or brain responses as a binary feature (“independent” *vs.* “not independent”). A particularly relevant example is the debate concerning whether the P600 to syntactic anomalies is a member of the P300 family of positivities that are elicited by a wide range of unexpected stimuli[6,13,22]. Osterhout et al[5] presented sentences that contained words that were anomalous at a physical-feature level (all in upper case), a grammatical level (agreement violations), or both physically and grammatically. Osterhout et al[5] reported that the ERP responses to the two effects approximately summated when presented simultaneously. However, without some sort of empirical index of the range of additivity, claims made about “approximate” additivity are insufficiently specific. The findings reported here provide additional and quantitatively more specific information about the relative independence of these two categories of brain response.

It is conceivable that the AI will turn out to be a more generally useful metric of both similarity and categorization. There are, perhaps, two fundamentally different possibilities as to why a particular set of features would be treated by the brain as similar or dissimilar. The first possibility is that the independence or non-independence of the response is a reflection of neural organization. The visual system, for instance, has partially independent circuitry for processing form and color. The strong non-independence (AI = .20) of font and capitalization relative to color and capitalization (AI = .42) may therefore reflect fixed fundamental differences in the circuits necessary to process the features unrelated to the particulars of the task. If this were the case, then the Additivity Index would be a measure of natural categories that are fixed and reflect the modular organization of the brain. In other instances, however, the neural categorization of features might be fluid and context dependent. In these instances, the AI might change as a function of task demands. For instance, if subjects were given explicit instructions that an anomaly in font called for the participant to judge that trial to be “unacceptable,” whereas an anomaly in capitalization was to be judged as “acceptable,” a fixed categories explanation would predict that the Additivity Index would remain unchanged. On the other hand, a fluid categories model would predict that separate processes would be engaged if we explicitly inform subjects that font and capitalization are now members of separate categories—one a violation, one not—and we would expect the Additivity Index to increase substantially, reflecting the independence of the processing of the two features in this alternate context.

Although the current data do not allow us to differentiate between these two possibilities, the graded nature of the Additivity Index across the three different pairs, all of which were given in identical contexts, suggests that the AI is a reflection of natural categories. It is possible that both natural and contextual factors mediate functional categorization, such that part of the independence of two features is a reflection of some underlying fixed neural organization and another portion of their independence is reliant on whether task demands call for subjects to treat them the same.

An issue raised by our method for investigating neural independence of feature processing pertains to the neurobiology of the relevant functional sources. We should note explicitly that the methods employed here offer little information about the cerebral localization of the processes of interest, in the classical sense of that term (i.e. the determination of an anatomical source for a signal). Indeed, the Additivity Index should not be used to make claims about anatomical segregation; it is purely a tool to measure independence of function, as manifested in real-time processing. For instance, were a single cell to fire in response to individual stimuli separately and independently, such that the presence or absence of one stimulus did not affect the response to the presence or absence of a second stimulus, then, although the anatomical source of the response would be identical, the processing would be independent. The AI, as a measure of processing rather than source configurations, would be equal to 1. Therefore, high AI values do not necessarily imply that the measured ERP signal is generated in separate locations, but, rather, is purely a reflection of the ability of the brain to process multiple features without any interaction. As such, the AI can be used alone or in conjunction with methods for source localization to allow a better understanding of the processing dynamics underlying cognition.

Conclusion

The current study demonstrated the neural independence of the P300 and P600 ERP effects by varying the degree of feature similarity between stimuli in a graded manner. We showed that ERP effects elicited by stimuli with double anomalies varied in the extent of additivity of neural sources that reflected the degree of similarity between stimulus features. We thus demonstrated the feasibility of quantifying the degree of independence between neural sources recruited for processing different stimulus features from ERP data. The Additivity Index was shown to be sensitive to the amount of overlap between neural sources in a continuous, rather than dichotomous, measure. By applying the *principle of superposition*, we showed that it is possible inform an understanding of the nature of the neural substrates underlying ERP effects. By leveraging the additive nature of electrical sources intrinsic to ERPs, this method expands the toolbox of ERP researchers with a compelling metric that can contribute to interpretations regarding source analysis from scalp-recorded electroencephalograms (EEG).

Methods

Participants

Our participants included 42, 32 and 31 individuals for the capitalization/syntax, capitalization/color, and capitalization/font comparisons respectively. 17, 7, and 6 subjects' data were excluded due to excessive eye movement or other artifacts in the raw EEG, leaving 25 subjects per comparison included for analysis. All participants were strongly right-handed as assessed by an abridged version of the Edinburgh Handedness Inventory, and all had normal or corrected-to-normal vision. Participants provided informed consent and received a small amount of class credit for participation.

Procedure

Participants were tested in a single session lasting approximately 85 minutes (including about 30 minutes of experimental preparation). Upon arrival at the laboratory, each participant was asked to fill out an abridged version of the Edinburgh Handedness Questionnaire and a language history questionnaire.

Each participant was randomly assigned to one of the stimulus lists and was seated in a comfortable recliner in front of a CRT monitor. Participants were instructed to relax and minimize movements while reading, and to read each sentence as naturally as possible. Each trial consisted of the following events: each sentence was preceded by a blank screen for 1000ms, followed by a fixation cross, followed by a stimulus sentence that was presented one word at a time. The fixation cross and each word appeared on the screen for 475ms followed by a 250ms blank screen between words. Sentence-ending words appeared with a full stop followed by a response prompt asking participants if the sentence was “good” or “bad.” Participants were instructed to respond “good” if they felt it was a well-formed and meaningful sentence, and to respond “bad” if they felt the sentence was in any way abnormal. Subjects were not explicitly informed as to what constituted a “bad” sentence aside from abnormality and were not given feedback as to whether or not their response was correct. Participants were randomly assigned to use either their left or right hand for the “good” response. Note that although P300 amplitude is modulated by attention and task relevancy, the additivity function should be unaffected by any amplitude differences as long as all of the stimuli are equally task relevant, as is the case in this study.

Stimuli

For each of the three violation pairs, four word-lists were constructed using 120 target sentences. Each sentence was between six and 14 words long. Four versions of each sentence were created using a Latin square design (Fig 1). The four versions of the sentences were identical except for the target word that was always at least two words from the sentence initial position and two words from the sentence final position. Each subject saw only one version (condition) of each sentence; the conditions were equally distributed between the four lists such that each contained a combination of 30 sentences that contained a capitalization violation, 30 that contained a violation of a second type (font, color or syntax), 30 that contained a double violation (capitalization as well as font, color or syntax) and 30 that were well-formed. Each sentence appeared only once per list, and each list contained a different form of the sentence so that within each stimulus pair, each subject read the same 120 sentences of interest, 30 of which were in one of four different conditions. Example sentences are shown in Fig 1. Half of the subjects used their right hand to respond to the non-anomalous sentences, and half used their left hand.

Data Acquisition and Analysis

Continuous EEG was recorded from 19 tin electrodes attached to an elastic cap (Electro-cap International) in accordance with the extended 10-20 system. Vertical eye movements and blinks were monitored by two electrodes, one placed beneath the left eye and one placed to the right of the right eye. The 19 electrodes were referenced to an electrode placed over the left mastoid and were amplified with a bandpass of 0.01-100Hz (3db cutoff) by an SAI bioamplifier system. Impedances at scalp and mastoid electrodes were held below 5 μΩ and below 15 μΩ at eye electrodes.

Continuous analog-to-digital conversion of the EEG and stimulus trigger codes was performed at a sampling frequency of 200Hz. ERPs, time-locked to the onset of the critical word, were averaged off-line for each participant at each electrode site in each condition. Grand average wave forms were created by averaging over participants. Trials characterized by eye blinks, excessive muscle artifact, or amplifier blocking were not included in the averages; additionally, subjects who had rejections in over one-third of trials were not included in the analysis. The number of rejections did not differ significantly between conditions or groups.

ERP components of interest were quantified by computer as mean voltage within a window of activity, encompassing the effect of interest. Based on visual inspection of the waveforms, and in order to make meaningful comparisons between the three experiments, we compared mean amplitude across a large window, 300-900ms after stimulus presentation,and comparisons were made relative to a 100ms pre-stimulus baseline. This window was chosen because it encompasses the entire range of the divergence of any of the experimental conditions from the baseline (well-formed) condition as measured by visual inspection. As the latencies for the onset of the individual violations vary widely, it is important that a large window is used so that the comparison encompasses the entirety of both deflections. Using smaller time windows would obscure the additivity in the function if it failed to encompass the entire function. Nonetheless, for the sake of consistency with prior work we repeated the analyses using time windows that have been used historically to quantify the early components (N1, P2) and the language-sensitive components (N400 and P600). For these analyses, mean amplitude was computed within time windows between 0-150, 150-300, 300-500, and 500-800 milliseconds.

Within each time window, ANOVAs were calculated with violation type (no violation, syntax/color/font, capitalization, double violation and composite—the mathematical sum of the ERP response to two single violations) as a within-subjects factor. Data from midline (Fz, Cz, Pz), medio-lateral (right hemisphere: Fp2, F4, C4, P4, O2; left hemisphere: Fp1, F3, C3, P3, O1), and latero-lateral (right hemisphere: F8, T8, P8; left hemisphere: F7, T7, P7) sites were treated separately. ANOVAs on midline electrodes included electrode as an additional within-subjects factor (3 levels). ANOVAs on medio-lateral electrodes included hemisphere (2 levels) and electrode (5 levels) as additional within-subjects factors. ANOVAs over latero-lateral electrodes included hemisphere (2 levels) and electrode (3 levels) as additional within-subjects factors. The Greenhouse-Geisser correction for inhomogeneity of variance was applied to all repeated measures on ERP data with greater than one degree of freedom in the numerator. In such cases, the corrected p-value is reported.

Abbreviations

AI: Additivity Index

EEG: electroencephalogram

ERP: event-related potential

Declarations

Ethics approval and consent to participate

This study was approved by the Institutional Review Board at the University of Washington. Participants provided informed verbal consent and received a small amount of class credit for participation. Given that study procedures were classified as minimal risk by the IRB and that verbal consent would minimize potential risks and laboratory resources, a waiver of documentation of consent was requested from and approved by the IRB.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

Not applicable.

Authors' contributions

GV conceptualized the methodology behind the study, conducted data acquisition, performed formal analysis of all data, and wrote the original manuscript draft. MZ conducted figure preparation and the writing, reviewing, and editing of subsequent drafts. CK conducted the writing, reviewing, and editing of subsequent drafts. LO aided in the conceptualization and methodology of the study, along with writing, reviewing, and editing of the original manuscript draft.

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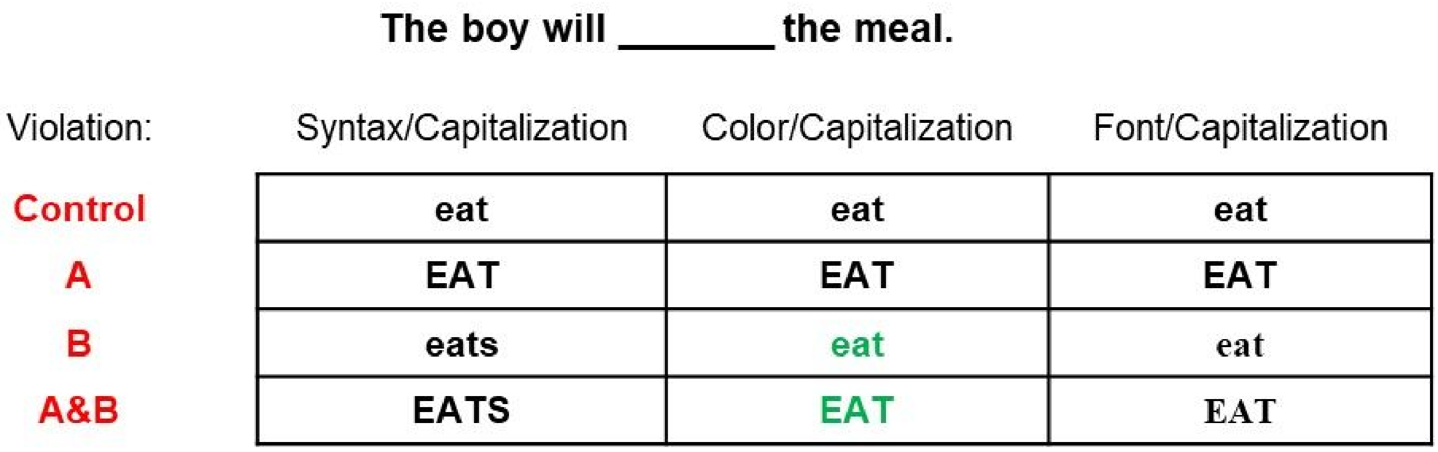
Figures



The boy will ________ the meal.

Violation:	Syntax/Capitalization	Color/Capitalization	Font/Capitalization
Control	eat	eat	eat
A	EAT	EAT	EAT
B	eats	eat	eat
A&B	EATS	EAT	EAT

Figure 1

The differing word forms for all conditions across the three additivity experiments. A = capitalization condition; B = syntax/color/font condition; A&B = double-anomaly condition.

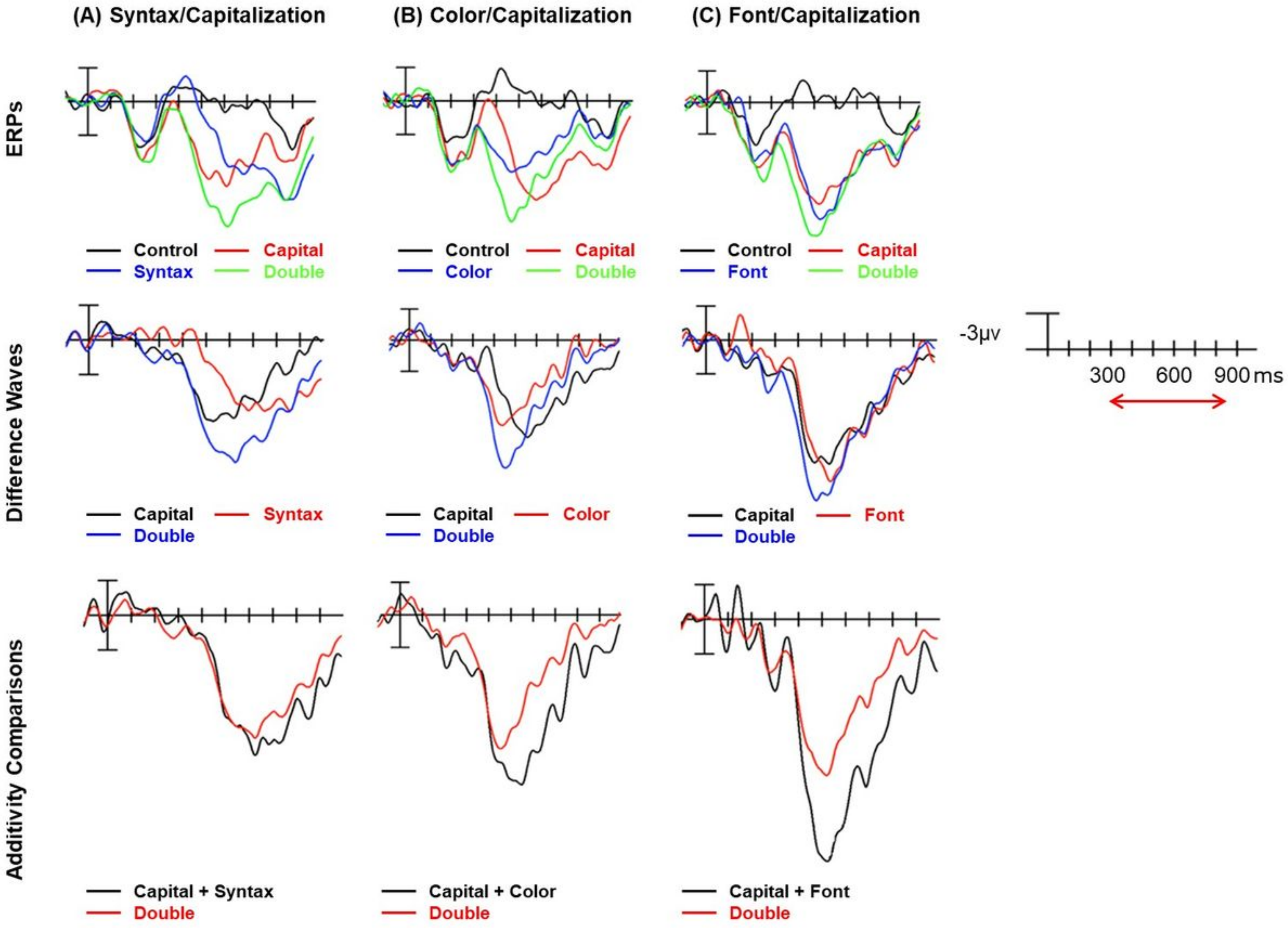


Figure 2

Grand average ERPs, difference waves, and additivity comparison waveform transformations at electrode site Pz. (A) Syntax and capitalization violations condition. (B) Color and capitalization violations condition. (C) Font and capitalization violations condition.

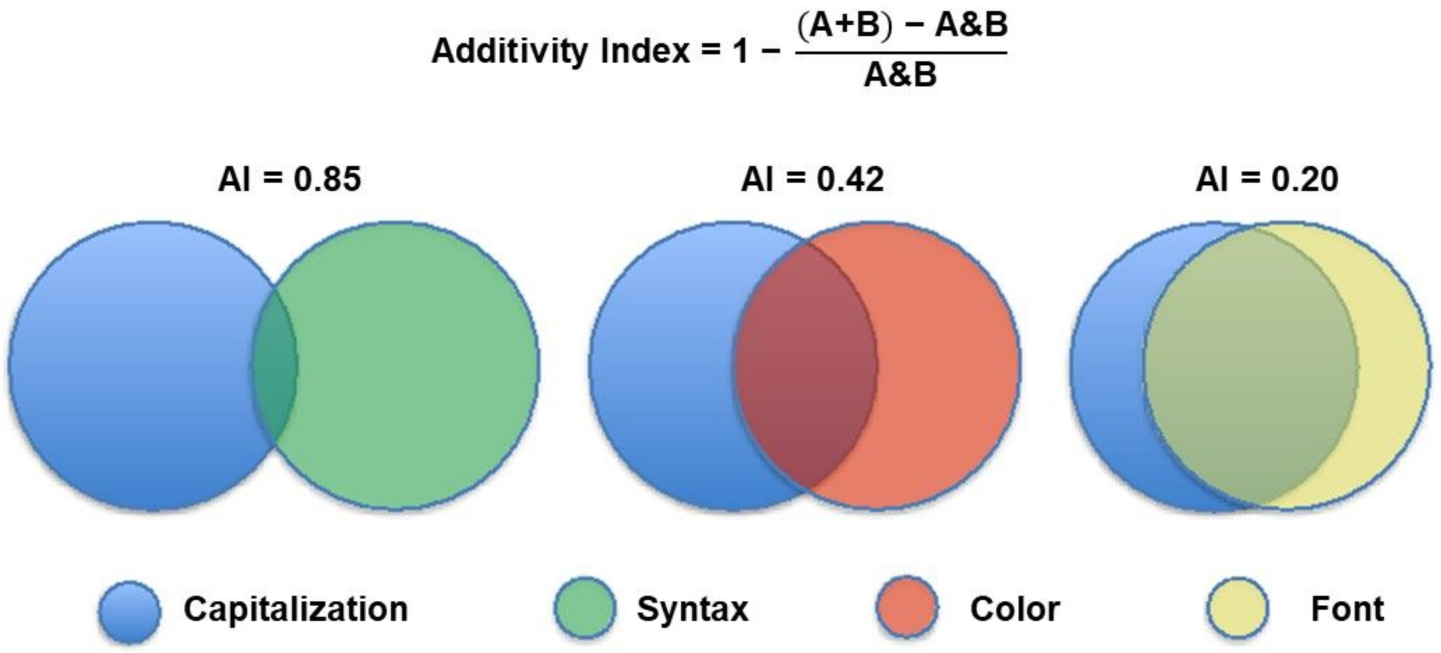


Figure 3

Visual representation of the overlap in neurocognitive resources for processing syntax, color, and font vs. capitalization.

Supplementary Files

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- Additionalfile1.xlsx