THESIS

THE TEST-RETEST RELIABILITY OF THE CONTINGENT NEGATIVE VARIATION (CNV) IN CHILDREN AND ADULTS BEFORE AND AFTER REMOVING ABERRANT CNV SEGMENTS

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ABSTRACT

THE TEST-RETEST RELIABILITY OF THE CONTINGENT NEGATIVE VARIATION (CNV) IN CHILDREN AND ADULTS BEFORE AND AFTER REMOVING ABERRANT CNV SEGMENTS

The contingent negative variation (CNV) is a slow, negative drift in electroencephalographic potential that occurs between two stimuli. Researchers have examined the CNV and its embedded components, the O-wave and E-wave, in the study of development and dysfunction in attentional processing. However, few studies have tested the reliability of these components, and never in a paradigm with two visual stimuli or in children. The present study investigated the test-retest reliability of the visually-evoked CNV components in 58 children and 32 adults. The efficacy of a newly-developed procedure for reducing trial-to-trial variability in ERPs was also tested. Participants performed a visual Go-NoGo task while EEG data were recorded during two sessions scheduled one-to-two weeks apart. Developmental data agreed with previous literature such that children had significantly less negative CNV amplitudes than adults, though each component presented with a significant Group by Session interaction. Adult amplitudes became less negative from one session to the next, and children’s data shifted in the opposite direction. Correlational analyses also indicated that developmental trends were present among children; amplitudes became more negative with increasing age. Reliability analyses revealed significantly lower indices than previous findings using auditory paradigms. Although children seemed to have higher reliability \( r = .34 \text{ - .53} \) than adults \( r = .05 \text{ - .58} \), analyses revealed no significant differences between these groups. The newly developed
procedure for reducing variability did not significantly improve reliability, but it did significantly change the amplitude of the total CNV. Future investigations should further examine the efficacy of this new procedure in producing averaged ERPs. The data from the present study suggest that researchers and clinicians should be careful in interpreting visually-evoked CNV components. Changes noted over time through the course of development or intervention may largely be the result of normal fluctuations in brain processing. Further research is required to better understand what underlying mechanisms may be affecting the reliability of the CNV components, and how to improve the reliability through adjustments to data collection procedures and measurement techniques.
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CHAPTER ONE

Sustained attention is a cognitive process that is important to many realms of day-to-day life. Sustained attention goes by many names, including vigilance (Fruhstorfer & Bergstrom, 1969; Haider, Spong, & Lindsley, 1964), and the alerting system (Petersen & Posner, 2012; Posner & Petersen, 1990), and is defined as the ability to maintain focus on a particular task for an extended period of time (Hilti et al., 2013; Manly, Anderson, Nimmo-Smith, et al., 2001; Matchock & Mordkoff, 2009). Some investigators have defined sustained attention as only one subtype of attention. Other attentional constructs may include selective attention, and shifting or switching attention (Manly, Anderson, Nimmo-Smith, et al., 2001; Posner & Petersen, 1990; Robertson, Ward, Ridgeway, & Nimmo-Smith, 1996). From adults driving to work, to the child sitting in the classroom, and even to the air traffic controller watching flight patterns, sustained attention ensures that people can effectively monitor their environment and maintain attention to a task for extended periods of time. From car and plane crashes to limited success in the classroom, lapses in sustained attention may have detrimental, sometimes life changing consequences (Cubillo, Halari, Smith, Taylor, & Rubia, 2012; Petrilli, Roach, Dawson, & Lamond, 2006).

From Behavior to Brain

Certain behavioral measures have been commonly utilized in the study of sustained attention. For instance, the Test of Everyday Attention for Children (TEA-Ch) has particular subtests that specifically tap into the construct of sustained attention (Manly, Anderson, Nimmo-Smith, et al., 2001; Sutcliffe, Bishop, & Houghton, 2006; Verstraeten, Vasey, Claes, & Bijttebier, 2010). Using this assessment, therapists and researchers alike have been able to detect
significant differences in sustained attentional abilities among different subgroups of children. Heaton and colleagues (2001) examined children with attention deficit hyperactivity disorder (ADHD) and typically developing children’s performance on the subtests of the TEA-Ch. The researchers found that children with ADHD performed significantly worse on three out of five subtests directly related to sustained attention when compared to their typically developing peers (Heaton et al., 2001). Similar findings have been reported in other studies (Manly, Anderson, Nimmo-Smith, et al., 2001; Sutcliffe et al., 2006). Developmental effects have also been noted such that with increasing age, children tend to perform better on all subtests (Verstraeten et al., 2010).

In the past, behavioral measures have been invaluable in understanding group differences and changes throughout development, and in turn have provided information for developing intervention and educational programs. However, there is a piece of the story that cannot be answered from the perspective of behavioral assessments alone: what underlying mechanisms may be responsible for the behavioral differences measured with tools like the TEA-Ch? Only recently have researchers begun to bridge the gap between behavioral research and neuroscience (Filoteo, 2004; Horton, 2008; Rothenberger, 2006; Tansey, 2001).

Segalowitz and Davies (2004) highlighted the need for a better understanding of the brain-behavior relationship as more and more research linking brain structure with behavioral indices of development, both typical and atypical, surfaces in the literature. The importance of combining brain and behavioral measures lies in understanding the why behind the what. Specifically, neuroscience-based tools can elucidate the brain mechanisms underlying particular observable behaviors. Specific studies have been able to connect behavior and brain in the realms of specific disorders like depression (Theus, 2003) and ADHD (Semrud-Clikeman et al.,
The literature connecting brain and behavior throughout development is accumulating (Rothenberger, 2006; Rypma & D'Esposito, 2001; Schmajuk, 2005; Tansey, 2001), and such findings have recently been able to inform intervention efficacy on a whole new level (Dawson et al., 2012).

Although a number of neuroscience tools are available to researchers, not every neuroimaging technology is appropriate for use in any given study. Different neuroimaging techniques will provide different measures of brain processes and brain structures, thus investigators must carefully select which technique will best address the issues of interest. For example, tools such as functional magnetic resonance imaging (fMRI), diffusor tensor imaging (DTI), positron emission tomography (PET), and single-photon emission computer tomography (SPECT) each vary in the methods used to produce a structural image of the brain and provide unique information for better understanding the brain-behavior relationship. However, all of these tools tend to be fairly expensive and potentially challenging for use with certain populations, particularly children.

Challenges are presented by the nature of the tool itself; for instance, PET and SPECT, can be used to create three-dimensional images of the brain and measure localized metabolic activity such as glucose uptake. These procedures require that the participant receive a radioactive tracer injection and then be exposed to gamma radiation. Although the practices are deemed safe for adults, it is challenging to justify exposing children to such toxins. Likewise, DTI and fMRI have been useful in imaging white- and gray-matter structure, and in examining functional water flow and oxygenation during neural processing. These techniques have been used with children, however they can induce unintentional stress or fear without prior participant training (Skouras, Gray, Critchley, & Koelsch, 2013). These techniques require that the
participant be physically constrained to immobilize the head in order for clear, precise images of the brain to be obtained. The participant must lie still in the MRI machine tunnel, which can further increase stress levels in individuals who fear small spaces. The large magnet within the tunnel makes loud noises that can also be unsettling for participants. Overall, the experience can be frightening or uncomfortable thus inducing anxiety that may alter the brain’s responses to stimuli.

A final consideration in choosing the appropriate neuroimaging tool for a given study is whether spatial or temporal resolution is more important for addressing a specific hypothesis. All of the aforementioned techniques have high spatial resolution, meaning they are valuable for the localization of neural activity. However, these methods tend to have poor temporal resolution, meaning that it is challenging to determine exactly when each of the different structures of the brain start to process information after a stimulus event. One specific classification of neuroimaging tools tends to have excellent temporal resolution: electrophysiological measures. Electrophysiology tends to be less expensive than the previously mentioned tools, provides excellent temporal resolution on the order of milliseconds, and has been commonly used with specific populations like children.

Electrophysiological Measures

Electroencephalography (EEG) is a neuroimaging technique in which the electrical activity produced by the brain is recorded by metal sensors placed on the scalp of an individual (Stern, Ray, & Quigley, 2001). A major advantage of EEG over other neuroimaging technologies is its temporal precision, which allows researchers to examine the timing of neural processing and reactivity with accuracy at the millisecond level. Because EEG is non-invasive, relatively risk-free way of studying neural activity, it is an ideal method for use with populations such as
children (Schmidt & Segalowitz, 2008). Although there are many ways to analyze the data collected by EEG, one main method has been used in the study of sustained attention, the study of event-related potentials (ERPs).

An ERP represents the brain’s electrical response time-locked to a particular event (e.g., the onset of a stimulus presentation). Typically, researchers will create an averaged ERP by averaging the brain’s response to an event over the course of multiple presentations. The result is a waveform with distinctive positive and negative voltage deflections (i.e., components), each of which is associated with specific cognitive and motor functions in response to the event (Stern et al., 2001). Two main features of ERP components are measured: the amplitude and the latency of the component. Amplitude, which is measure in microvolts, has been associated with the intensity of neural processing as well as the amount of neural resources dedicated to processing the event within a particular cognitive domain (Walhovd, Rosquist, & Fjell, 2008). In turn, latency, measured in milliseconds, has been associated with the efficiency of processing of the event within a particular cognitive domain. Using ERPs, it is possible to study a specific component related to sustained attention known as the contingent negative variation (CNV).

The Contingent Negative Variation

The CNV is a slow negative drift first discovered nearly 50 years ago (Walter, Winter, Cooper, McCallum, & Aldridge, 1964). One way in which this component is elicited using a Go-NoGo task. In a Go-NoGo task the participant is presented a conditional stimulus followed several seconds later by an imperative stimulus. The conditional stimulus indicates whether the participant should respond to the following imperative stimulus. An averaged ERP is calculated to examine the brain’s response between the conditional and imperative stimulus. For both Go and NoGo trials, the brain’s response to the conditional stimulus (i.e., early sensory and
attentional processing) can be examined (Segalowitz & Davies, 2004). In NoGo trials, following the initial processing (i.e., the P300 complex) of the conditional stimulus, brain activity returns to baseline with no apparent further processing preceding the presentation of the imperative stimulus. However, in Go trials, after the initial processing of the conditional stimulus is complete, a slow, negative drift builds until the presentation of the imperative stimulus where a response is required (Segalowitz & Davies, 2004; van Boxtel & Böcker, 2004; Walter et al., 1964). The total CNV is typically measured beginning approximately 500-600ms after the presentation of the conditional stimulus until the onset of the imperative stimulus during Go trials. Using a number of neuroimaging techniques including functional magnetic resonance imaging (fMRI) and dipole localization routines in EEG and magnetoencephalography (MEG) research, studies have shown that the CNV originates primarily from the dorsolateral prefrontal cortex (DLPFC) of the brain (Basile, Baldo, de Castro, & Gattaz, 2003; Basile, Ballester, de Castro, & Gattaz, 2002; Basile, Brunder, Tarkka, & Papanicolaou, 1997; Strack, Kaufmann, Kehrer, Brandt, & Stürmer, 2013), though other areas of the prefrontal cortex also seem to contribute.

Localization

Basile et al. (1997) performed a study using MEG to investigate the localization of the CNV in the adult brain. Five adult participants were presented with a three-stimulus task in which a warning stimulus (S1) indicated what information should be focused on in the subsequently presented images. The second and third stimuli (S2 and S3 respectively) were pictures of faces with superimposed abstract patterns overlaid on the image. Depending on the nature of S1, participants would focus on either the faces or the abstract patterns. Participants were instructed to decide whether the images presented in S2 and S3 matched and respond to
matching trials with a button press using their right index finger (Basile et al., 1997). Findings indicated that for all five participants, sources of activation localized to the DLPFC for pattern recognition, and to the medial prefrontal cortex for facial recognition.

Subsequent studies by Bareš, Rektor, Kanovsky, and Streitová (2000, 2003) examined the generators of the CNV using intracranially-implanted electrodes in patients with thalamic pain and drug-resistant epilepsy. The procedure included a Go-only paradigm in which a tone was presented binaurally through earphones, and then a flash was presented via goggles three seconds later. Participants responded to the flash by flexing their right hand. The implanted electrodes recorded neural activations across several areas in the prefrontal cortex at the time of the CNV, including orbitofrontal cortex and DLPFC (Bareš et al., 2000, 2003). Activations in supplementary motor cortex, the primary motor cortex, anterior cingulate cortex, the basal ganglia, and the thalamus were also noted. These areas are postulated, at least in part, to be reflective of the sensory and motor processing necessary to achieve a response.

**Interpretation of the CNV**

Over the years, research has related the CNV to sustained attention such that a larger negativity (i.e., a greater negative drift preceding the imperative stimulus) is indicative of greater sustained attentional abilities (Bareš et al., 2000; Segalowitz & Davies, 2004; van Boxtel & Böcker, 2004; Zappoli, 2003). Individuals known to have impaired attentional abilities, including children with ADHD and individuals with brain lesions, have been shown to exhibit smaller, or sometimes a total lack of a CNV response in Go-NoGo tasks (Spronk, Jonkman, & Kemner, 2008; Zappoli, 2003). Further investigations of this slow negative drift have revealed two distinct components, the O-wave and the E-wave, each of which is related to a unique cognitive process (Connor & Lang, 1969).
The O-wave

The first unique component of the CNV is known as the O-wave, sometimes also referred to as the initial CNV (iCNV; e.g., Bender, Resch, Weisbrod, & Oelkers-Ax, 2004; Kropp, Kiewitt, Göbel, Vetter, & Gerber, 2000), and has been described as a representation of the brain’s orienting response to the conditional stimulus (Rohrbaugh, Newlin, Varner, & Ellingson, 1984; Zimmer & Demmel, 2000). Research has predominantly studied the O-wave during a 200 millisecond window directly following the P300 to the conditional stimulus, thus many researchers have investigated the O-wave from approximately 500-700 milliseconds following the conditional stimulus. The O-wave is thought to be generated in frontal regions of the brain (Falkenstein, Hoormann, Hohnsbein, & Kleinsorge, 2003; Giard, Perrin, Pernier, & Bouchet, 1990; Rohrbaugh et al., 1984; Zimmer & Demmel, 2000).

The E-wave

The second unique component of the CNV is known as the E-wave, sometimes referred to as the late CNV (lCNV; e.g., Darabaneanu, Kropp, Niederberger, Strenge, & Gerber, 2008; Kropp et al., 2000; Zaepffel & Brochier, 2012), and has been related to cognitive expectancy and motor preparation for the upcoming imperative stimulus (Knott et al., 1991; Simons, Hoffman, & MacMillian, 1983). The E-wave is typically measured during the last 200 milliseconds preceding the imperative stimulus. Decades of research has examined the nature of the E-wave. Some studies suggest that the E-wave can occur even when no response to the imperative stimulus is required, whereas others showing no evidence of an E-wave when no response is necessary (Rohrbaugh et al., 1984). The E-wave is thought to be generated in prefrontal, precentral, and parietal areas of the brain (Basile et al., 2002; Bender et al., 2004; Falkenstein et al., 2003; Knott et al., 1991).
With the total CNV, the O-wave, and the E-wave being produced primarily by frontal and prefrontal regions of the brain (Falkenstein et al., 2003; Segalowitz & Davies, 2004), the issue of developmental progression of these components becomes quite interesting. Specifically, the CNV and its components may not be as stable or organized in children as they are in adults due to the nature of neural development.

**Development**

**Maturation of the frontal cortices**

The frontal and prefrontal cortices of the brain are known to be the latest brain regions to fully develop (Risberg & Grafman, 2006; Segalowitz & Davies, 2004), with maturation lasting until an individual’s mid-to-late twenties. The CNV and its components have been localized to frontal regions in the past, thus understanding the development of implicated regions may help explain variability in the CNV through the course of development. Research has shown that specific regions of the prefrontal cortex activated during Go-NoGo tasks, including the DLPFC and the ventromedial prefrontal cortex (VMPFC), mature in their connectivity to other brain regions through the course of development. The DLPFC has been implicated in top-down control, motor behavior selection and control, and working memory processes, and it has also been noted in the literature as modulating the activation of other cortical areas (Gilbert, Zamenopoulos, Alexiou, & Johnson, 2010; Hosseini et al., 2010; Kaller et al., 2012; Poletti, 2009). The VMPFC, including the orbitofrontal cortex, has been implicated in other processes. For instance, literature has linked activation of the VMPFC to emotional processing, representations of somatosensory information, and the reward and motivational systems of the brain (Beer, John, Scabini, & Knight, 2006; Beer, Lombardo, & Bhanji, 2010; Smith, Jones, Bullmore, Robbins, & Ersche, 2014; Wilbertz et al., 2012).
Developmental changes in the connectivity of the VMPFC and the DLPFC have been related to differences in cognitive abilities in children versus adults (Barber, Caffo, Pekar, & Mostofsky, 2013; Crone, Zanolie, van Leijenhorst, Westenberg, & Rombouts, 2008; Jaeger, 2013). For example, Barber et al. (2013) performed a study investigating the differences in cognitive control based on connectivity of frontal lobe regions in children versus adults during two Go-NoGo tasks. The study included 28 healthy adults age 18-to-47 and 63 neurotypical children age 8-to-12. Participants’ brain activity was recorded via fMRI during two separate Go-NoGo tasks: one task was deemed simple, and the other complex. For the simple task, participants were presented with either a red circle or a green circle and instructed to immediately respond to green circles with a button press, but inhibit responses to red circles. In the complex task, there was an added working memory component (Barber et al., 2013). Participants were presented with either a blue circle or a yellow circle. If the preceding circle was a different color, participants were instructed to immediately respond with a button press. When the preceding circle was the same color, participants were instructed to inhibit their response.

Examination of the resting-state functional-connectivity MRI results revealed that adults, overall, had greater activations in the DLPFC and the VMPFC during both tasks when compared to children (Barber et al., 2013). Interestingly, adults showed much more activation in these frontal regions in conjunction with other brain areas related to attentional and inhibitory processing. These areas include, for instance, the right inferior frontal cortex, the insula, and the inferior parietal lobe. Concurrent activations were more highly correlated within adults than within children, suggesting that adults had stronger integration of brain processes between
regions than children. In other words, adults exhibited better connectivity of the DLPFC and VMPFC to other brain regions during Go-NoGo tasks than children.

Such developmental research has illuminated specific differences in functional connectivity between children and adults. Given that children and adults have different structural nuances, one might question what differences are present in the actual processing of information across development. In other words, because children do exhibit significant structural differences in regions that have been shown to produce the CNV, researchers may investigate changes in the topography of the CNV and its components through the course of development. As the connectivity between frontal regions responsible for eliciting the CNV develops with age, there could be changes in the presentation of the CNV component itself.

**Maturation of the CNV**

Individuals with still-developing or disrupted frontal and prefrontal cortices exhibit less efficient performance in many cognitive tasks, such as attentional processing, when compared to individuals with fully-developed functional frontal and prefrontal cortices (McCallum & Walter, 1968; Segalowitz & Davies, 2004). With this consideration, several researchers have examined the developmental progression of ERP components that are known to be frontally- and prefrontally-generated; components like the CNV.

For instance, Segalowitz and Davies (2004) examined the maturation of the CNV in 57 children age 7-to-17 years, and 20 young adults age 19-to-27 years. Participants performed a simple Go-NoGo task in which either a green or a red circle was presented as the conditional stimulus for 250ms. After a 2000ms interstimulus interval, a picture of a car was presented for 250ms as the imperative stimulus. Participants were instructed to respond by pressing a button as quickly as possible when the car appeared if it was preceded by a green circle (i.e., a Go trial). A
car preceded by a red circle was a NoGo trial and required that the participants inhibit their response. Data were analyzed by examining the averaged amplitude in five different time windows: 400-800ms, 800-1200ms, 1200-1600ms, 1600-2000ms, and 800-2000ms following the presentation of the conditional stimulus. Amplitudes were measured from sites Fz, Cz, and Pz for Go trials and NoGo trials separately (Segalowitz & Davies, 2004).

Examination of the NoGo trials indicated no relationship between amplitude and age for any of the five time windows, suggesting that brain processing during NoGo trials was similar between children and adults. However, averaged amplitudes measured during each of the five time windows in Go trials revealed significant correlations with age (Segalowitz & Davies, 2004). Correlations were only significant when measured from site Cz; sites Fz and Pz showed little-to-no correlation between age and CNV amplitude. The data suggested that for all but the 800-1200ms time windows, amplitudes became more negative with increasing age ($r = -0.34$ to $-0.54$, $p < 0.002$). For the 800-1200ms window, the opposite was true; amplitudes tended to become more positive with increasing age ($r = 0.49$, $p < 0.002$). The opposing trend was not discussed further in the article. The authors concluded that in general, CNV amplitudes tend to become more negative through the course of maturation (Segalowitz & Davies, 2004).

Through research on the developmental trends of the CNV and its components, researchers have found that children tend to exhibit significantly smaller amplitudes than do adults (Bender et al., 2002; Segalowitz & Davies, 2004). Specifically, one research group examined the CNV across the lifespan including data from children ($M = 10.15$ years, $SD = .59$), adolescents ($M = 14.42$ years, $SD = .55$), young adults ($M = 24.27$ years, $SD = 2.07$), and older adults ($M = 71.24$ years, $SD = 2.91$; Hämmerer, Li, Müller, & Lindenberger, 2010). Participants performed a variant of a CPT-AX Go-NoGo task while EEG data were recorded. Rather than
presenting participants with letters as stimuli, participants were presented with colored squares. One of 12 different colored squares was presented at any given time. A blue square was the conditional stimulus, and a yellow square was the imperative stimulus. The remaining 10 colored squares were non-cue stimuli. In a given trial, a square was presented for 200ms with a 2150ms interstimulus interval before the next colored square was presented. Participants were instructed to respond with a button press any time that they saw a yellow square preceded by a blue square, the Go trials (Hämmerer et al., 2010).

The CNV amplitude was then measured as the amplitude directly preceding the presentation of the imperative stimulus on Go trials (i.e., the amplitude at 2150ms) at site Cz. Data were analyzed using a repeated measures MANOVA design where age group was a between-subject factor, and “cue versus non-cue” (i.e., Go vs. NoGo trial) was a within-subjects factor. The data indicated that children had significantly smaller CNV amplitudes than did all other age groups, and that none of the other age groups significantly differed in CNV measurements (Hämmerer et al., 2010). Although mean amplitudes were not reported for each age group, mean differences between age groups were reported. Between children and adolescents, the mean difference was 3.01µV; between adolescents and young adults, the mean difference was .88 µV; and between younger and older adults, the mean difference was .36 µV. The findings of this study agree with other studies such that children tend to have smaller CNVs than adults.

Jonkman, Lansbergen, and Stauder (2003) examined the development of the CNV in 12 children age 9-to-10, and in 13 young adults age 19-to-23. Participants were asked to perform a standard CPT-AX task. In this paradigm, 11 different letters of the alphabet were presented in a pseudorandom order, one-at-a-time in the center of a computer screen. Participants were
instructed to press a button when the letter X was presented on the screen, but only if it was preceded by the letter A. For the sake of analysis, trials in which the letter A was followed by the letter X were considered Go trials, and trials in which the letter A was followed by any letter other than an X were considered to be NoGo trials. Stimuli were presented for 150ms each, and there was a consistent interstimulus interval of 1500ms. Go and NoGo trials comprised 10% of the total presentations and were equiprobable being presented 12 times each in a single block. Four blocks of trials were performed for a total of 48 possible Go trials, and 48 possible NoGo trials.

The CNV was measured as the averaged amplitude at two separate time points: 850 – 1250ms, and 1250 – 1650ms. Although the authors did state that they chose two separate measurements to capture differences in amplitude at varying time points, they did not specify that the time windows chosen were indicative of O-wave or E-wave measurements, nor did they justify the later-than-typical starting point (i.e., 850ms) for their measurement of the CNV (Jonkman et al., 2003). The CNV was explored using a multivariate repeated measures ANOVA at a number of scalp sites, including Fz, Cz, Pz, Oz. Findings indicated that children had smaller CNV amplitudes than adults at sites Cz and Pz in the earlier CNV window, but not during the later CNV window. The authors attributed the difference in findings to the presence of an elongated P300 component in children that was captured in the earlier time window.

Jonkman (2006) expanded this research by examining not only 9-to-10 year old children (n = 17) and young adults age 19-to-23 (n = 17), but also children age 6-to-7 (n = 16). Using the same CPT-AX task as the previous study (Jonkman et al., 2003), Jonkman (2006) examined the CNV as the averaged amplitude from 1300-1650ms, again without explanation of the decision to start examining the CNV at a later time point than is typical in the literature. Interestingly,
because Jonkman (2006) did not find any interaction effects between electrode sites, the CNV was measured as the averaged amplitude across the combination of four midline scalp sites: Fz, Cz, Pz, and Oz. Findings from a repeated measures ANOVA indicated that younger children had significantly smaller CNV amplitudes than did older children and adults (Jonkman, 2006). Again, older children (age 9-10) did not exhibit significantly different CNV amplitudes than the adults.

Interestingly, Jonkman (2006) did not examine the earlier window of the CNV that was investigated in Jonkman et al. (2003), thus differences in the early amplitude of the CNV among children of different ages is still unknown. Additionally, the procedures used for measuring CNV amplitude differed between the two studies. Jonkman et al. (2003) inspected individual scalp sites, and Jonkman (2006) the CNV amplitude averaged across four scalp sites. The latter technique is not common in the measurement of the CNV in the literature, thus the findings from Jonkman (2006) may not be replicated when using more standard techniques. If the findings are not replicable, one may question the reliability of the findings; were the differences uncovered between younger and older children a result of study procedures, or were there actual differences between the groups? It is also possible that the CNV is simply not a stable component within children, thus yielding different results in children of different ages. Knowledge of the reliability of the CNV within children may help in understanding these discrepancies.

**Reliability of the CNV**

The test-retest reliability of the CNV and its components has been sparsely studied in the past (Griesel & Bartel, 1975; Kropp et al., 2000; Roth, Kopell, Tinklenberg, Huntsberger, & Kraemer, 1975), and studies examining the reliability of the CNV used adult samples exclusively. It is also noteworthy that to date, no known studies have examined the reliability of
the CNV in the context of a paradigm in which both the conditional and imperative stimuli are visual.

Griesel and Bartel (1975) used a paradigm in which 24 adult participants first saw a flash of light and then responded to an auditory tone with a button press. The CNV was measured as the averaged amplitude of a straight line drawn from the highest positivity of the P300 to the lowest negativity immediately preceding the imperative stimulus. Reliability was assessed by measuring the CNV produced during two sessions scheduled seven days apart. The researchers were able to obtain Pearson product-moment reliability coefficients near .80 for the total CNV, but the O-wave and E-wave were not individually measured. The authors suggested that although the CNV does appear to be relatively stable in adults, researchers must be aware of individual variability that may result in apparent instability of the initial and late aspects of the total CNV (Griesel & Bartel, 1975).

A second study by Roth et al. (1975) used a completely Go-trial paradigm (i.e., the paradigm did not contain NoGo trials) in which 20 young adult men were asked to view a star that appeared on a screen in front of them, then respond to the subsequently presented auditory tone by pressing a button. Participants were given feedback such that delayed responses (more than 250ms after the presentation of the tone) and premature responses were punished with a burst of white noise. There were 70 total trials presented during each of three separate sessions, each of which was scheduled one week apart. It is noteworthy that these data were collected as part of a study examining the effects of alcohol and marijuana consumption, and the authors do not explicitly state that the participants included in the following reliability analysis were members of the marijuana, alcohol, or placebo group. All testing did occur after the
administration of a beverage with either dissolved marijuana, alcohol, or a placebo (Roth et al., 1975).

In their study, Roth et al. (1975) measured the CNV as the amplitude at three separate time points measured by hand at 600, 800, and 1000ms after the presentation of the cue (conditional) stimulus. Measurements were performed by two different people and interrater reliability was established for each of the three time points ($r = .97$, $r = .96$, and $r = .98$ respectively). Test-retest reliability was only measured between the first and second session for each participant. Using Pearson product moment correlations, CNV test-retest reliability coefficients of $r = .64$, $r = .67$, and $r = .60$ were established for the three separate amplitude measurements (i.e., 600ms, 800ms, and 1000ms) respectively (Roth et al., 1975).

In contrast, Kropp et al. (2000) used an entirely auditory Go-NoGo task, both the conditional stimulus and the imperative stimulus were auditory, and subsequently examined the total CNV, the O-wave (i.e., iCNV) and the E-wave (i.e., lCNV). Twenty-seven young adults were presented a conditional stimulus of a single tone of variable frequency (Go trials: variable frequency 250 – 2000Hz; NoGo trials: 200Hz) indicating whether the trial was a Go or a NoGo trial. The imperative stimulus was a 2500Hz tone that would play for 1500ms, or until it was deactivated by a button-press response by the participant. There was an interstimulus interval of 3000ms between the conditional and imperative stimuli. All tones were presented at a stable intensity of 75dB from a speaker placed behind the participant. Two sessions were recorded ten days apart. During each session, participants were presented with 32 Go trials, and eight NoGo trials.

The amplitude of the total CNV was measured as the average amplitude of the signal between the conditional and imperative stimulus. The O-wave was measured by first finding the
maximum amplitude in the timeframe between 550 and 750ms after the presentation of the conditional stimulus. The latency of this maximum was used as the center-point for a 200ms window in which the averaged amplitude was measured as the O-wave. The E-wave was measured as the averaged amplitude in the last 200ms preceding the presentation of the imperative stimulus. Using Pearson product-moment coefficients, the researchers found that the O-wave was the most reliable component, $r = .86$, followed by the total CNV, $r = .68$, and then the E-wave, $r = .63$ (Kropp et al., 2000).

These studies all showed moderate-to-high reliability for the CNV and its components, however they are limited in generalizability. To date, no studies have examined the reliability of the CNV and its components when evoked during a completely visual paradigm; auditory-evoked potentials and visually-evoked potentials have been known to differ in amplitude and latency among other features (Kappenman & Luck, 2012). Thus a difference in stimulus modality may result in differences in stability of the CNV and its components. The studies also only examined the CNV and its components in adult samples. Knowing that children generally exhibit CNV components that significantly differ from those of adults (Hämmerer et al., 2010; Jonkman, 2006; Jonkman et al., 2003; Segalowitz & Davies, 2004), the findings from the previously performed reliability studies may not be generalizable to children’s brain activity in response to Go-NoGo tasks. Further research could be done to investigate the test-retest reliability of the CNV and its components in children and adults during a completely visual Go-NoGo task.

**Managing variability in CNV analyses**

Researchers have struggled to analyze CNV data, particularly when handling data from children participants. Because of the length of a trial (often 2000ms or longer), the likelihood of
having an eye blink or other artifact within the EEG trace is much higher than in a typical ERP paradigm where the components of interest occur within a 500ms duration. A common data reduction technique to create an averaged ERP involves the use of an artifact rejection procedure to remove all trials with voltages exceeding ±100µV due to eye activity in specified channels. This technique does remove artifacts such as eye blinks or muscle movements from the analysis process, but may be too stringent for a CNV paradigm when used with children. Children are known to produce more artifacts from eye blinks than adults in EEG recordings (Gavin & Davies, 2008). The typical CNV paradigm consists of fewer trials than other ERP paradigms and, when too many additional trials are lost, the resulting averaged ERP may no longer be a valid representation of individual’s brain activity. For instance, Klein and Feige (2005) performed a study in which child and adolescent participants performed a saccadic CNV paradigm. Even after careful calibration of eye movements that were and were not appropriate given the task, over 20% of all participants were excluded from the study due to the small number of remaining trials after rejecting segments with eye blinks and muscle activity.

A number of techniques have been used to remove unwanted electrical activity from EEG data while still retaining the integrity of the underlying brain signal. From artifact rejection (Jonkman, 2006; Klein & Feige, 2005), to independent components analysis decompositions (Hämmerer et al., 2010), to uniquely developed algorithms to reduce or remove eye blinks (Kononowicz & Van Rijn, 2011; Segalowitz & Davies, 2004), researchers have attempted to account for aberrant segments, often at the risk of losing a large portion of data for an individual participant. The issue of aberrant trials, and specifically outlier segments that may affect the averaging process, becomes even more apparent when examining data from children who have performed a CNV task.
One major goal of the present study is to account for aberrant CNV segments in a unique new manner. Specifically, a new procedure has been developed that performs three data reduction steps, each of which handles aberrant segments in a unique manner. First, the procedure will perform a previously developed eye regression technique designed to remove eye blinks from trials wherever possible (Segalowitz, 1996). Specifically, the procedure removes large voltage shifts in the EEG signal due to eye blinks leaving behind only the brain activity. This is accomplished using a multiple regression approach where each scalp channel serves as an independent variable. Following eye regression, an artifact rejection is performed to remove trials with voltages ±100µV. Finally, the unique aspect of the procedure examines the average voltage within a specified window (e.g., 1500-2000ms post-conditional stimulus) on an individual basis. Segments with an average window voltage that exceeds two-times the interquartile range of the distribution of averaged window voltages for an individual are rejected as outlier segments, and thus removed from the later processing step used for creating the individual’s averaged CNV. Such a procedure could have implications for the reliability of the CNV and its components.

**Purpose**

The purpose of the present study is to examine the test-retest reliability of the visually-evoked CNV amplitude in children and adults as measured during two separate recording sessions scheduled one-to-two weeks apart. This study will also examine a new method of accounting for aberrant ERP segments (i.e., outliers) on a trial-to-trial basis, and determine whether removing outlier segments from the averaging process can improve reliability measures of the CNV amplitude. The following research questions will be addressed.

1. Is there a developmental relationship between CNV amplitude and age?
2. Which components of the CNV (if any) are the most reliable measures in adults?

3. Which components of the CNV (if any) are the most reliable measures in children?

4. How does the reliability of the CNV and its components differ between children and adults?

5. Does removing outlier segments from the averaging process significantly change the reliability of the CNV and its components in children and adults?
CHAPTER TWO

The contingent negative variation (CNV) is an event-related potential (ERP) that presents as a slow negative drift between two defined stimuli, often during a Go-NoGo task. Interestingly, the CNV is the only known ERP measure that is indicative of sustained attention processing (Segalowitz & Davies, 2004; Walter et al., 1964). Because of its unique nature, researchers have increasingly used the CNV to measure sustained attention in individuals with disorders that may impair attentional processing such as ADHD (Spronk et al., 2008; Werner, Weisbrod, Resch, Roessner, & Bender, 2011) and migraines (Bender et al., 2002; Darabaneanu et al., 2008).

The CNV follows a bimodal distribution with an early component, the O-wave, and a late component, the E-wave. The O-wave has been noted as an orienting response (Giard et al., 1990; Rohrbaugh et al., 1984; Zimmer & Demmel, 2000), and the E-wave is hypothesized to represent expectancy and response preparation (Basile et al., 2002; Bender et al., 2004; Knott et al., 1991). The generators of the CNV and its components have been localized to frontal regions including the dorsolateral prefrontal cortex (Bareš et al., 2000; Bareš et al., 2003; Basile et al., 2002). Decades of neuroscience research has indicated that the frontal lobes, and particularly the prefrontal cortex, are the last to fully mature with development spanning into the mid-to-late twenties (Risberg & Grafman, 2006; Segalowitz & Davies, 2004). Thus, in addition to examining attentional implications for disordered populations, researchers have taken interest in the developmental trajectory of the CNV components as the frontal lobes mature throughout childhood and adolescence (Hämmerer et al., 2010; Jonkman, 2006; Jonkman et al., 2003; Segalowitz & Davies, 2004).
Developmental research has found that children tend to have smaller, less negative CNV components compared to adults (Hämmerer et al., 2010; Jonkman, 2006; Jonkman et al., 2003). Additionally, data indicate that the CNV component amplitudes gradually become more negative throughout development into young adulthood (Segalowitz & Davies, 2004). Significant differences in amplitude have been found even between seven-year-old children and ten-year-old children (Jonkman et al., 2003).

Surprisingly, a topic that has been little-explored in CNV research is test-retest reliability. In fact, the few known studies that have examined the stability of the CNV components have only tested adult samples, and each study measured the amplitude of the CNV at different time points within the component (Griesel & Bartel, 1975; Kropp et al., 2000; Roth et al., 1975). For example, Roth et al. (1975) had 20 adult participants perform an auditory Go-NoGo task on two separate occasions scheduled seven days apart. The CNV was measured as the peak amplitude at three time points during the ERP segment for each of the sessions. The authors reported moderate reliability for each of the three measurements ($r = .60 - .67$). The O-wave and E-wave were not measured in this study. A later study by Kropp et al. (2000) had 27 adult participants perform an auditory Go-NoGo paradigm. Unlike the previous study, Kropp et al. (2000) did measure all three CNV components for two separate recording sessions scheduled ten days apart. The O-wave was measured as the averaged amplitude in a 200ms window surrounding each individual’s maximum amplitude between 550 and 750ms. The E-wave was measured as the averaged amplitude within the last 200ms directly preceding the presentation of the imperative stimulus. Finally, the total CNV was measured as the averaged amplitude within the total timeframe between the conditional and imperative stimuli. The authors reported moderate-to-
high reliability for all three components ($r = .63 - .86$) where the O-wave was the most reliable followed by the E-wave and total CNV showing similar reliability indices.

To date, there have been no studies, to our knowledge, examining the reliability of the CNV components in a completely visually-based paradigm, which is commonly utilized with children in order to keep participants engaged in the task. It has been well-established in the literature that different sensory modalities can affect the amplitude and latency of the resulting ERP components (Kappenman & Luck, 2012). That is, the amplitude and latency of ERP components are characteristics of each sensory modality which differ. Thus, it is possible that the visually-evoked CNV components may not present with the same reliability indices as auditory-evoked components.

Additionally, no reliability studies, to our knowledge, have included children in their samples. As developmental research continues to emerge, it becomes more and more clear that children tend to have more variability in their EEG and ERP tracings compared to adults. Specifically, children’s ERPs tend to have a lower signal-to-noise ratio (SNR) compared to adults, in part due to the reduced number of segments included in the averaging process. SNR decreases in a non-linear fashion as a function of the number segments included in the averaging process decreases (Voitinskii & Pryanishnikov, 1968). Children tend to produce more movement-related artifacts during EEG recording than adults (e.g., eye blinks, saccades, muscle activity), which often leads to greater segment rejection during data reduction. Furthermore, unlike other ERP investigations that study a component of interest occurring within a 500ms period, CNV paradigms may require segments in lengths upwards of 2000ms. Because of the longer length of the segments necessary to examine CNV components, it is more likely that a participant, and particularly a child participant, will produce an artifact during a trial. The more
segments that must be rejected due to artifacts, the lower the SNR and the poorer depiction researchers have of the invoked brain activity elicited by the stimulus.

Given the possible implications of clinical and developmental research, the question of stability becomes one of necessity. For instance, if a clinical researcher decides to use the CNV components as a measure of intervention efficacy for a child with ADHD, the researcher should first have evidence that the CNV components are stable over a certain period of time. Otherwise, any noted changes in the components after completion of the intervention may not be attributable to the intervention itself, but rather day-to-day instability in the brain signal. Likewise, longitudinal studies of development may incorrectly attribute changes to development that merely reflect normal variability in the brain signal.

The purpose of the present study was to examine the test-retest reliability of the visually-evoked CNV components in children and adults. To examine the validity of the data produced, we first determined whether the data follow developmental trends described in the literature (Hämmerer et al., 2010; Jonkman, 2006; Jonkman et al., 2003; Segalowitz & Davies, 2004). The CNV components were expected to be significantly less negative in children compared to adults. Additionally, among children the CNV components were expected to become more negative with increasing age. Following validation, the test-retest reliability of the data was determined. In accordance with previous reliability studies (Kropp et al., 2000), the O-wave was expected to be the most reliable component followed by the E-wave and finally the total CNV for both children and adults. We expected that adults would have significantly better reliability indices for all components compared to children due to the generally higher SNR of adult EEG data. Finally, the efficacy of a new technique for producing ERPs was examined. The CNV outlier rejection (CNVor) procedure was developed to produce ERPs in two ways: 1) using traditional data
reduction methods, and 2) using a distribution-based outlier rejection approach to remove aberrant segments from the averaging process. By rejecting outlier segments, the purpose of the CNVor procedure was to reduce the variability in the averaged ERP for a given individual. The CNVor procedure was expected to improve reliability indices for both children and adults due to the reduced intraindividual variability in the ERP tracing.

Methods

Participants

Participants for this study were 32 adults between the ages of 19 and 28 years ($M = 23.28$, $SD = 2.31$; 22 men), and 58 children between the ages of 7 and 13 years ($M = 10.30$, $SD = 1.56$; 26 boys). All participants were screened for neurological or developmental disorders, use of psychopharmaceutical drugs (e.g., antidepressants), and history of head trauma via a self-report by adult participants and parent-report for child participants. Before data collection procedures began, all adult participants and parents of child participants signed informed consent forms. All child participants signed assent forms. After completing their first session, participants were compensated with a small thank you gift. Following the second session, participants received $15. All procedures were approved by the local university Institutional Review Board.

Procedure

Participants completed two recording sessions scheduled one or two weeks apart (i.e., the same day and time). During the first session, participants and parents of child participants were asked to review and sign all informed consent and assent forms as necessary depending on the age of the participant. Participants were then seated in a comfortable chair at a table in front of a computer screen. Two research assistants placed the EEG cap and sensors on the participant’s
head and face, respectively. A research assistant gave each participant a brief training on how to reduce production of artifacts from eye blinks and muscle movements. Then three minutes of eyes-opened resting EEG were recorded while participants stared at a fixation point on the screen. Participants then performed two other unrelated EEG tasks as part of a larger data collection effort; these tasks will not be discussed further in the current study. After these tasks were completed, participants were given instructions regarding the visual Go-NoGo task.

Participants completed approximately 10 practice trials that provided feedback that applauded correct trials and offered instruction to correct behavior on incorrect trials. Participants were also shown their reaction times for each correct Go trial and encouraged to try and beat their best time. After the participant was comfortable with the practice and appeared to understand the instructions, the complete paradigm was presented which lasted approximately 5-10 minutes. Performance feedback was not given after any trials during the test session nor at the end of the session. After completion of the Go-NoGo task, participants performed one more unrelated EEG task that will not be discussed further in this study. Aside from completing informed consent and assent forms, the same procedure was followed during the second session. Each participant performed all EEG tasks in the same exact order during both sessions. Following EEG tasks, participants completed approximately one hour of paper and pencil behavioral assessments examining cognitive and executive functioning, but these assessments will not be discussed further in this study.

**Visual Go-NoGo Paradigm**

The design of the Go-NoGo paradigm used for this study was fairly simple so that participants of all ages could easily understand the instructions and perform the task correctly. First, a conditional stimulus was presented on a computer screen positioned in front of the
participant. Participants saw either a red circle or a green circle for a duration of 250ms. Then, 1750ms after the circle disappeared, the imperative stimulus, an image of a red car, appeared in the center of the screen for a duration of 250ms (see Figure 1). If a trial began with conditional stimulus being an image of a green circle, participants were instructed to press a button on a response pad placed in front of them with their right index finger as quickly as possible after the car appeared (i.e., a Go trial). Button presses that occurred before the presentation of the car were considered incorrect responses. For trials with the conditional stimulus being an image of a red circle, participants were instructed to refrain from pressing the button when the car appeared (i.e., a NoGo trial). A random, variable intertrial interval of 2000 to 7000ms was used to avoid eliciting a CNV response between trials. There were a total of 40 Go trials and 40 NoGo trials presented in a pseudorandom order during each of the two recording sessions.

**Electrophysiological Recording**

EEG recordings were obtained using the BioSemi ActiveTwo system (BioSemi, Inc., Amsterdam, The Netherlands). Active EEG was recorded from 33 Ag-AgCl sintered electrodes based on the American Electroencephalographic Society nomenclature guidelines (1994) with a Common Mode Sense (CMS) active electrode and a Driven Right Leg (DRL) passive electrode as the ground (http://www.biosemi.com/faq/cms&drl.htm). Reference electrodes were placed on the left and right earlobes. Two bipolar electrooculograms (EOG) were used to account for vertical and horizontal eye movements. The vertical EOG was derived from electrodes placed on the supra- and infraorbital regions of the left eye. The horizontal EOG was derived from two electrodes placed on the left and right outer canthi. Data were sampled at a rate of 1024Hz.
Electrophysiological Data Reduction

Data from the continuous EEG recording were filtered with a .03 to 30Hz bandpass filter (12dB/octave) and then segmented from 200ms prior to the conditional stimulus onset to 2250ms after the conditional stimulus onset. Baseline correction was performed on each segment using the EEG data from -200 – 0ms (i.e., until conditional stimulus onset). Segments were then divided into correct Go and NoGo trials. Correct Go trials were any trials in which a green circle appeared before the car, and the button was pressed after the car appeared. Correct NoGo trials were any trials in which a red circle appeared before the car, and there was no button press. Trials in which a button was pressed before the onset of the imperative stimulus (i.e., the car) or in which a response was made during a NoGo trial were considered incorrect trials and were not included in the averaging process. Retained segments were passed through the newly developed CNV outlier rejection (CNVor) procedure executed in Matlab code.

The CNVor procedure analyzed the ERP data in three different ways: 1) examined segments retained after artifact rejection (i.e., removing trials with amplitudes that ±100µV in the vertical EOG channel, Fz, Cz, and Pz); 2) examined segments retained after eye regression (Segalowitz, 1996) and artifact rejection; and 3) examined segments retained after eye regression, artifact rejection, and removal of aberrant segments based on individual segments with average window voltages exceeding two-times the interquartile range of the average window voltages of all segments for an individual. The CNVor procedure rejected segments with outliers in the timeframe of 1500-2000ms. This window was selected because most individuals’ P300 components should be resolved by 1500ms, and thus would likely not influence or bias the analysis of outliers. Further analyses in this study only used the data obtained from methods 2 and 3 of the CNVor procedure. Method 2 is a traditional means of producing ERPs consistent
with the literature on CNV measurements, and method 3 is a newly developed, distribution-based, outliers rejected (DBOR) approach. These data were processed using a Matlab routine to calculate the averaged amplitudes of the CNV components. The O-wave was calculated as the average voltage in the 600-800ms window, the E-wave was calculated as the average voltage in the 1800-2000ms window, and the total CNV was calculated as the average voltage in the 600-2000ms window.

**Data Analysis**

**Descriptive Data.** Behavioral data were examined based on participants’ performance on the Go-NoGo task including measures of correct responses and reaction times to stimuli. The number of correct Go and NoGo segments retained before and after outlier rejection are reported. The number of outlier segments rejected by the CNVor procedure is also reported. Reaction times for correct Go trials were calculated as the time in milliseconds from onset of the imperative stimulus to the button press. A 2 (Group) x 2 (Session) ANOVA was performed to determine whether children and adults had similar reaction times across sessions.

Additionally, the number of segments included in the averaging process were examined for children and adults. A 2 (Group) x 2 (Session) repeated measures ANOVA was performed specifically to determine whether children and adults had a significantly different number of segments included in the averaging process. Analyses were performed based on the number of segments included in the traditionally-measured ERPs.

**CNV Component Data.**

**Go versus NoGo amplitudes.** To determine whether the average amplitude in the time windows of the O-wave, E-wave, and total CNV were significantly different between Go and NoGo trials, three separate 2 (Session) x 2 (Trial Condition) x 2 (Group) repeated measures
ANOVAs were performed group using traditionally-measured amplitudes as the dependent measures. A Bonferroni correction was applied to account for inflated type I error rates.

**Children versus adult average amplitudes.** To determine whether children had significantly smaller CNV amplitudes than adults, three 2 (Group) x 2 (Session) ANOVAs were performed using each traditionally-measured CNV component as a dependent measure. The second analysis, a Pearson correlation, was used to determine whether CNV amplitudes increased with age within children.

**Test-retest reliability.** To examine the test-retest reliability of the CNV and its components, three different reliability analyses were conducted. Specifically, Pearson product-moment correlations, and two forms of intraclass correlation coefficients (ICC), ICC (3,1), consistency, and ICC (3,1) absolute agreement were performed to correlate the average amplitudes of the traditionally-measured and DBOR-measured CNV components between session 1 and session 2. Using Pearson coefficients maintains consistency with the previous studies on test-retest reliability of the CNV, thus providing a proper comparison of reliability measurements. The ICC measurements offer a somewhat more accurate measurement of reliability; the consistency ICC should be fairly similar to the Pearson coefficients simply due to the nature of the statistical test. The absolute agreement ICC will likely result in lower reliability because it takes into account not only the consistency of change in amplitudes, but the disparity in values as well. These measurements were reported for adults and children separately.

In order to address whether the reliability of the CNV and its components differed between children and adults, a series of Fisher’s $r$ to $Z$ transformations were performed. Specifically, the Pearson correlations indicating the reliability of each CNV component were
compared between children and adults to determine whether adults had significantly larger reliability coefficients than children.

**CNVor results.** To address the success and utility of the CNVor procedure in improving reliability of the CNV and its components, a series of Fishers $r$ to $Z$ transformations were performed. The analyses were used to determine whether Pearson reliability coefficients were significantly larger for DBOR data than for traditional data for children and adults separately. Another analysis, a 2 (Session) x 2 (Group) x 2 (Procedure) repeated measures ANOVA was performed in order to further examine the impact of the CNVor procedure on the measurement of the total CNV amplitude.

**Results**

**Descriptive Data**

Analyses were performed in order to determine whether children and adults significantly differed in reaction times to correctly-performed Go trials across sessions. The assumption of homogeneity of variances was not met, thus a Greenhouse-Geisser correction was used as is reflected in the degrees of freedom. Results of a 2 (Group) x 2 (Session) repeated measures ANOVA showed that adults had faster reaction times compared to children, $F (1, 81) = 29.86, p < .001, \eta^2_p = .27$. However, there were no significant differences in reaction times across sessions for either group. The means and standard deviations of reactions times for Go trials are reported in Table 1. Despite applying regression techniques to remove eye-blinks from the segments, after artifact removal procedures seven children were excluded from the study because they had fewer than 12 correctly performed Go segments retained for the averaging process ($< 30\%$ of all Go trials) for either session 1 or session 2. The means and standard deviations of the
number of segments included in the averaging process for the traditional and DBOR data are reported in Table 2.

In order to examine whether children and adults had a significantly different number of retained segments, a 2 (Group) x 2 (Session) repeated measures ANOVA was performed with the number of segments retained after outlier rejection as the dependent measure. Findings revealed that adults retained significantly more segments for the averaging process when compared to children, $F(1, 81) = 25.17, p < .0005, \eta_p^2 = .24$. Additionally, individuals tended to have a slightly larger number of segments for session 1 data than for session 2 data, $F(1, 81) = 4.78, p = .03, \eta_p^2 = .06$.

In order to create a fair comparison of performance between age groups and between sessions, the ERP amplitudes were adjusted for the number of segments included in the average by dividing the averaged amplitude for each participant by the square root of the number of segments included in the average. Further examination of statistical results using both adjusted and unadjusted amplitudes yielded similar findings, thus only results based on the unadjusted amplitudes will be reported.

**CNV Component Data**

The means and standard deviations for the unadjusted averaged amplitudes of the CNV and its components are reported in Table 3. The averaged ERPs including Go and NoGo trials can be seen in Figures 2 through 9 for adults (see Figures 2-5) and children (see Figures 6-9) separately, and for traditionally measured data (see Figures 2, 3, 6, and 7) and DBOR data (see Figures 4, 5, 8, and 9) separately.

**Go versus NoGo amplitudes.** The data were examined to determine whether brain processing significantly differed during Go trials compared to NoGo trials. The analyses served
to confirm the presence of the CNV components. The averaged amplitudes in the time windows of the O-wave (600-800ms), the E-wave (1800-2000ms), and the total CNV (600-2000ms) were examined to see whether Go trial amplitudes significantly differed from NoGo trial amplitudes. The means and standard deviations of the amplitudes for each of the time windows can be seen in Table 4. Three separate 2 (Session) x 2 (Group) x 2 (Trial Condition) repeated measures ANOVAs were performed. Averaged amplitude in each of the three time windows for traditionally-measured CNV components served as the dependent measures. A Bonferroni correction was applied resulting in a family-wise error rate of $\alpha = .017$ for the interpretation of findings for each age group.

Statistics showed that for the time window of the O-wave, when the data was collapsed across groups, there was not a significant difference in amplitudes between Go and NoGo trials, though the Trial Condition by Group interaction approached statistical significance, $F(1, 81) = 5.25, p = .03, \eta^2_p = .06$. The interaction suggested that adults tended to have larger differences between Go and NoGo trial amplitudes compared to children. For the time window of the E-wave, the data indicated that across both groups the amplitudes of Go trials were significantly more negative than in NoGo trials, $F(1, 81) = 77.66, p < .0005, \eta^2_p = .49$. The same was true for amplitudes measured in the time window of the total CNV, $F(1, 81) = 33.91, p < .0005, \eta^2_p = .30$. These data suggest that for both age groups, the E-wave and total CNV are present in Go trials and not in NoGo trials. However, the presence of the O-wave merits further investigation. These data suggest that the data are valid in that the CNV components are present in Go trials and not in NoGo trials. Thus, further analyses only utilized Go trial amplitude measurements. It is the brain processing on Go trials that is of particular interest in this investigation.
**Children versus adult average amplitudes.** In order to determine if the present study supported the developmental trend of children having smaller amplitudes than adults, three separate 2 (Group) x 2 (Session) repeated measures ANOVAs were performed with the averaged amplitude of each traditionally-measured CNV component as a dependent measure. Because three separate ANOVAs were performed for this analysis, a Bonferroni correction was applied resulting in a family-wise error rate of $\alpha = .017$.

Data for the O-wave showed that during both sessions, adults had significantly more negative O-wave amplitudes than children. Interestingly, from the first session to the second, adults seemed to have a less negative O-wave whereas children trended in the opposite direction, $F(1, 81) = 6.80, p = .011, \eta^2_p = .08$ (see Table 3 for averaged amplitudes). The E-wave amplitudes for adults were significantly more negative than for children, $F(1, 81) = 10.63, p = .002, \eta^2_p = .12$. Although the data followed a similar pattern to the O-wave findings, the Session x Group interaction did not reach statistical significance given the adjusted family-wise error rate, $F(1, 81) = 5.31, p = .026, \eta^2_p = .06$. The averaged amplitude of the total CNV was also significantly more negative for adults than for children, $F(1, 81) = 14.04, p < .005, \eta^2_p = .15$.

In order to examine whether the CNV and its components are related to age within children, several Pearson product moment correlations were performed using the averaged amplitudes of the traditionally-measured CNV components and the children’s age in years rounded to two decimal points. Findings showed that children’s age was negatively correlated with all CNV components, indicating that as children’s age increased, their CNV components became more negative (see Table 4). Results for the O-wave and total CNV were statistically significant; however correlations for the E-wave for session 1 and session 2 did not reach statistical significance, $p = .05$ and $p = .10$ respectively.
Test-retest reliability. To assess the test-retest reliability of the CNV components in adults and children, three separate types of correlational analyses were performed. Specifically, Pearson product moment correlations, consistency measures of intraclass correlation coefficients (ICC), and absolute agreement measures of ICCs were performed (see Table 5).

Adults showed the highest reliability coefficients for the O-wave, then the E-wave, and the total CNV was the least reliable component. Only the O-wave reliability measurements for adults reached statistical significance, \( r = .58, p = .001 \). Children resembled adults in that the O-wave was the most reliable component followed by the E-wave, and the total CNV was the least reliable component. Unlike adults, children’s reliability statistics were significant for all three components, \( r_{O-wave} = .53, p < .0005; r_{E-wave} = .50, p < .0005; r_{CNV} = .34, p = .016 \). As expected, for both children and adults, the consistency ICC and the Pearson correlation yielded similar results for all measurements, and the absolute agreement ICC yielded slightly lower or identical measurements compared to the other two correlational procedures.

To determine whether the reliability of the CNV components significantly differed between children and adults, a series of Fisher’s \( r \) to \( Z \) transformations were performed. Calculations were first done by hand using \( r \) to \( Z \) conversions from Cohen (2008) and then cross-checked using the \( r \) to \( Z \) transformation tool developed by VassarStats (http://vassarstats.net/rdiff.html). To calculate the final \( Z \) statistic, children’s transformed \( Z \) scores were subtracted from adults’ transformed \( Z \) scores. None of the \( Z \) scores calculated from the Pearson correlations were statistically significant indicating that there were no significant differences in reliability between children and adults (see Table 6). The \( Z \) scores approached significance for the E-wave, \( p = .05 \) so it is possible that with a larger sample, we would find a
statistically significant difference in reliability in traditional E-wave measurements between children and adults.

**CNVor results.** In order to examine whether the CNVor procedure significantly changed the reliability of the CNV components, a series of Fisher's $r$ to $Z$ transformations were performed for children and adults separately. Again, the calculations were first performed by hand using $r$ to $Z$ conversions from Cohen (2008) and then cross-checked using the $r$ to $Z$ transformation tool developed by VassarStats ([http://vassarstats.net/rdiff.html](http://vassarstats.net/rdiff.html)). To calculate the final $Z$ score, the traditional data transformed $z$ scores were subtracted from the DBOR data transformed $Z$ scores. Reliability indices for both DBOR and traditional data can be seen in Table 5. The results showed that there were no statistically significant differences between DBOR data and traditional data reliability coefficients for either adults or children (see Table 7).

To further investigate the utility of the CNVor procedure, a 2 (Session) x 2(Group) x 2 (Procedure) repeated measures ANOVA was performed where Procedure included traditional and DBOR measurements of the total CNV averaged amplitude. Findings indicated that even after adding in the factor of Procedure, adults had more negative CNV amplitudes than children, $F(1, 81) = 12.81, p = .001, \eta^2_p = .14$ (see Table 8). Interestingly, the results also showed that CNV amplitudes were significantly more negative when using traditional measures compared to the DBOR approach, $F(1, 81) = 8.87, p = .004, \eta^2_p = .10$. These findings suggest that the CNVor procedure does significantly impact the measurement of CNV data, even if the reliability is not significantly improved.
Discussion

Go versus NoGo Amplitudes

Findings indicated that although there was a significantly more negative amplitude for Go trials compared to NoGo trials in the time windows of the E-wave and total CNV, this was not the case for the time window of the O-wave. Examination of the grand averaged ERPs does suggest evidence of an O-wave in adults (see Figures 1 and 2). The data present with a separation of Go and NoGo voltages following the P300 where Go trials become more negative than NoGo trials. Children’s EEG data do seem to exhibit the same pattern, though at a later time point than adults (see Figures 5 and 6); that is, children’s data seem to show more of a separation between Go and NoGo trials around 1100ms. The evidence from examination of the figures accompanied by the nearly statistically significant ANOVA results warrant further investigation.

Other investigations have used a similar time window to the one selected in the present study (i.e., 600-800ms) and have shown evidence of an O-wave (e.g., Bender, 2004; Kropp et al., 2000). However, relatively few studies have examined the O-wave in a visually-based paradigm. Given that ERPs change in morphology depending on the sensory modality of the paradigm, it is possible that the component structure is different in the visually-evoked tracings. Visual inspection of the ERPs from the current study shows that the P300 component extends for a fairly long period of time in the averaged ERP, particularly in children (see Figures 1-8). If the visually-evoked components resulted in extended latencies, it is possible that the large P300 component may have obscured the O-wave in the 600-800ms window. Thus, the lack of significant differences in amplitudes between Go and NoGo trials may have been a result of the overlapping components that researchers typically expect to be resolved by the time the O-wave measurement window begins.
Developmental Indices

The data supported the hypothesis that children would exhibit significantly smaller CNV component amplitudes when compared to adults. These data agree with previous developmental literature examining the CNV (Hämmerer et al., 2010; Jonkman, 2006; Jonkman et al., 2003; Segalowitz & Davies, 2004). Interestingly, these data did reveal a significant Group x Session interaction in which children’s amplitudes became more negative from session 1 to session 2, and adults’ amplitudes changed in the opposite direction. The opposing directionality of change between children and adults is an interesting issue that warrants further investigation. It is possible that the adults’ amplitudes became less negative because they did not attend to the task as much as the paradigm continued. Future investigations may explore within- and between-session habituation effects among adults to test the hypothesis that attention attenuates throughout the paradigm. As for children producing more negative components over time, this may be a result of more organized, more mature brain processing as a result of learning. Future studies may consider examining trial-to-trial variability in brain processing among children. Reductions in the standard deviations around the mean amplitudes could support the idea that variability in brain processing decreases with practice.

Analyses examining developmental trends within children supported the hypothesis that age would be negatively correlated with amplitude. For the O-wave and total CNV, the data revealed a statistically significant, negative correlation between age and amplitude within children. Interestingly, the correlations for the E-wave only approached statistical significance. It is possible that the lack of statistical significance is merely a result of the sample size, and increasing the number of children would resolve the issue. However, it is also possible that the E-wave does not show the same developmental trends as the O-wave and total CNV.
Recall that the O-wave and total CNV have generators localized in prefrontal areas including the dorsolateral prefrontal cortex (Bareš et al., 2000; Bareš et al., 2003; Giard et al., 1990). The E-wave, though it does have some prefrontal generators, is also heavily influenced by precentral and parietal generators (Bae & Park, 2008; Knott et al., 1991; Zaepffel & Brochier, 2012). Research has suggested that the posterior sensory and motor areas develop at a different rate when compared to prefrontal regions associated with executive and attentional processes (Brun, 1999). Thus, it is possible that the E-wave may present with developmental trends that differ from the O-wave and total CNV, which are predominantly prefrontally-generated. Major developmental changes in motor and sensory areas may have already occurred by the age of seven, so future research may consider examining the same paradigm for developmental trends in younger children. Future investigations should consider expanding their examination of the development of the E-wave in a larger age range of children.

**Test-Retest Reliability**

We hypothesized that for both children and adults, the O-wave would be the most reliable component followed by the E-wave and finally the total CNV. The data supported the hypothesis to an extent. Specifically, the data for adults did present with the O-wave as the most reliable component. However the O-wave was the *only* reliable component for adults. Among children, the O-wave and E-wave presented with similar reliability indices, and the CNV exhibited the lowest reliability statistics.

The poor reliability indices for the adult data were surprising given the previous literature on CNV reliability. For example, Kropp et al. (2000) measured the O-wave, E-wave, and total CNV in 27 adult participants over two recording sessions set ten days apart. Pearson product-moment correlations revealed reliability ranging between .63 and .86 across the three
components (Kropp et al., 2000). Likewise, previous studies that only measured the total CNV in adults found reliability indices between .60 and .80 (Griesel & Bartel, 1975; Roth et al., 1975). The discrepancy between the current findings and previous literature may be due to differences in the sensory modality of the paradigm presented to the participants. Evidence has shown that the modality of the stimuli presented can affect factors including the latency and amplitude of ERP components (Eimer & Schröger, 1998; Kappenman & Luck, 2012). It is possible that the reliability of ERP components may also change based on the sensory modality that elicited the component. The current study should be replicated in order to determine whether the reliability results can be reproduced. Another follow-up study may investigate a within-person design in which participant perform both auditory and visual Go-NoGo tasks. This study would shed light on whether sensory modality does indeed affect the reliability of the CNV components, or if another factor may be responsible for our disparate findings.

Interestingly, in contrast to the hypothesis that adults would exhibit better stability than children, children seemed to have much better reliability indices than adults. However, further inspection via Fisher’s r to Z transformations showed no statistically significant differences between children and adults’ test-retest reliability. Again, future investigations should further study the effects of sensory modality on test-retest reliability, but also explore other individual factors such as learning or practice effects, fatigue, and motivation. All of these individual differences may contribute to variability in brain responses, thus altering the stability of the grand averaged ERP over time.

**CNVor Efficacy**

The CNVor procedure was developed in Matlab code in order to reduce individual variability in ERPs by removing aberrant segments from the averaging process. Because of its
ability to reduce individual variability, we hypothesized that the DBOR-calculated ERPs would show significantly better test-retest reliability than traditionally-measured ERPs that included outlier segments. The data did not support the hypothesis; Fisher’s r to Z transformations showed no significant differences between reliability indices for the two procedures. Although the CNVor procedure did not significantly impact test-retest reliability of the CNV components, the procedure does seem to have a significant effect on the data.

Results of a three-way ANOVA indicated that the amplitude of the total CNV was significantly more negative when examining traditionally-measured ERPs compared to DBOR-measured ERPs. This finding was true for both age groups in both sessions. Additionally, developmental trends remained intact after the DBOR analysis such that adults still had significantly more negative amplitudes than children in accordance with previous literature (Hämmerer et al., 2010; Jonkman, 2006; Jonkman et al., 2003; Segalowitz & Davies, 2004). These data suggest that although stability was not significantly impacted, the DBOR approach to producing ERPs provided a new view of brain signal processing by tapping into individual differences across trials.

Researchers abound have been testing innovative ways to reduce noise and artifacts in EEG tracings while maintaining the integrity of the brain signal. Some have attempted to use specifically developed algorithms and calibration techniques for detecting artifacts (Klein & Feige, 2005; Kononowicz & Van Rijn, 2011) whereas others have implemented statistical methods like independent components analysis (ICA) decompositions (Hämmerer et al., 2010). Despite the utility of these methods, such routines do not always capture aberrant segments as the CNVor procedure did in this study. Specifically, the CNVor procedure provided a unique perspective by inspecting voltages segment-by-segment for each individual in order to determine
what was most representative of brain processing for one individual versus another. Most data reduction techniques are based on values and parameters derived from population norms that are then applied to each individual. Considering the field’s movement toward inspecting individual differences, the CNVor approach may prove to be quite useful in future ERP investigations. Studies should continue examining the utility of the CNVor procedure by examining changes in standard deviations and SNR. Additionally, further manipulations of the parameters by which a segment is deemed “aberrant” may prove beneficial to producing the best SNR for a given individual.

Conclusions

Given the findings of the current study, further investigation is required to understand the stability of the visually-evoked CNV and its components. The data suggest that only the O-wave is a stable component in adults whereas all three CNV components are moderately reliable in children. Clinicians and therapists who use visually-evoked CNV components to examine attentional processing in their clients should be cautious when interpreting changes over time. It is possible that the changes may be the result of instability in the ERP components rather than an intervention.

Additionally, the CNVor procedure merits further investigation as a means of examining individual differences in neural processing. Although the DBOR approach did not affect the stability of the CNV components, there was significant change in the resulting averaged amplitudes. Investigators may study the effects on individual variability via standard deviations about the averaged amplitude as well as SNR indices. As the field moves toward a more individualized study of brain processing, approaches like the DBOR method may provide valuable insight into producing the clearest brain signal possible with ERP studies.
CHAPTER THREE

Go versus NoGo Amplitudes

Analyses were performed to determine whether Go trials significantly differed from NoGo trials in averaged amplitude. Specifically, based on the definition of the Go-NoGo elicited CNV components, i.e., slow, negative drifts in amplitude that occur only during Go trials (Segalowitz & Davies, 2004; van Boxtel & Böcker, 2004; Walter et al., 1964), one should expect to see significant differences in neural processing between the two trial conditions. In this case, for a measured amplitude to truly represent one of the CNV components, one would expect that the amplitude on Go trials would be significantly more negative than that of NoGo trials. The data indicated that in the time windows of the E-wave and total CNV, averaged amplitudes measured from Go trials were significantly more negative than amplitudes measured from NoGo trials. Thus, it is reasonable to conclude that the brain processing represented in the Go trial ERP is indicative of E-wave and total CNV processes. However, such was not the case when examining the O-wave.

For both children and adults, analyses revealed no significant differences between Go and NoGo trial amplitudes for the O-wave. Given these data, the presence of the O-wave in both child and adult EEG data is inconclusive. Other analyses would be required to further investigate whether either age group engaged in brain processing that was different for Go trials compared to NoGo trials in the 600-to-800ms time window. For example, principal components analysis (PCA) has been used to distinguish specific CNV components from other overlapping brain processes (Bender et al., 2004). Bender et al. (2004) examined whether the O-wave and E-wave were distinct from one another using PCA in 81 children and adolescents between the ages of 6
and 18 years. In a completely-Go-trial paradigm, participants were presented with a warning tone followed three seconds later by an imperative tone while EEG was recorded. Participants were instructed to respond to the imperative tone as quickly as possible with a button press. The O-wave was then measured as the averaged amplitude from 550-to-750ms following the presentation of the warning stimulus at site Cz. The measurement of the E-wave was not specifically discussed in the article. Using PCA, the authors found that children and adolescents exhibited two distinct components during a broad frontal negativity between 400 and 800ms following the presentation of the warning stimulus. The first principal component, measured at site Fz, accounted for 42% of the variance in the measured O-wave amplitude. The second component was measured from centroparietal leads and accounted for 52% of the variance in the E-wave amplitude (Bender et al., 2004).

Given the utility of PCA in parsing distinct ERP components from other brain processes, it is possible that the same analysis may clarify whether data from the current study do indeed contain O-wave processing. It is also possible that the electrode site selected was not the most appropriate for the O-wave. It is standard in the literature to measure all three CNV components from site Cz (Knott et al., 1991; Rohrbaugh et al., 1984; Segalowitz & Davies, 2004). However, in their PCA analysis, Bender et al. (2004) found that signals recorded at site Fz accounted for the most variance in the O-wave component. Future investigations may explore which electrode sites are best for examining each individual CNV component and whether these sites are consistent between children and adults.
Developmental Indices

Child versus Adult Amplitudes

Analyses indicated that children had significantly smaller, less negative amplitudes for all three CNV components when compared to adults. The findings are in accordance with previous literature (Hämmerer et al., 2010; Jonkman, 2006; Jonkman et al., 2003; Segalowitz & Davies, 2004). However, there was an unexpected interaction uncovered by the analyses in the current study which differed from previous studies in that the developmental comparison was extended across two sessions rather than a single session. Across data collection sessions, adults’ amplitudes shifted to become less negative whereas children’s amplitudes shifted to become more negative. A number of explanations could be used to explain the finding, though further investigation via carefully controlled studies would be required.

One possible explanation for the interaction is learning or practice effects. ERP research has found that learning can significantly affect component amplitudes. For instance, Lang and Kotchoubey (2000) performed a study in which 11 adults between the ages of 21 and 46 years were presented with paired auditory stimuli in two phases. During the first phase, participants were instructed to attentively listen to 80 pairs of vowel sounds. Sixty trials were commonly presented, meaning the first vowel sound was always followed by the same second vowel sound (e.g., /a – u/). There were three different common pairs presented 20 times each. The other 20 trials were irregular pairs in which the vowel sound pairs were random and unpredictable. During the second phase, participants were presented with common and irregular trials as well as a third trial type in which a vowel pair began with the same first vowel sound as a common pair, but ended with an unexpected second vowel sound. A total of 180 trials were presented in the
second phase, 60 pairs of each trial type. Stimuli were presented at 70dB for 200ms each (Lang & Kotchoubey, 2000).

Lang and Kotchoubey (2000) examined the N200 and a slow negative wave (SNW) for differences in component latency and amplitude between the three trial types presented in phase two. Specifically, the authors inspected for differences between each of the two trial types from phase one and the third trial type introduced in phase two. The data showed no significant shifts in component latency, however there were significant differences in amplitudes for both ERP components. The authors suggested that the differences in amplitudes between trial types represented knowledge acquired during the first phase of the study despite participant reports that there was no explicit knowledge of learning (Lang & Kotchoubey, 2000).

The findings from Lang and Kotchoubey (2000) suggest that learning does in fact have an impact on the resulting ERPs, even within a single session. It is possible then, that within and between sessions, participants may experience learning effects that could impact the presentation of their ERPs. Further investigation would be necessary to determine how learning may differentially impact children’s and adults’ brain processing, though it could help explain the significant interaction found in the present study.

Other individual differences may also have an effect on recorded EEG signals from one day to the next. Such factors as anxiety (Aarts & Pourtois, 2010; Grillon & Ameli, 1994), motivation (D’Angiulli et al., 2012; Kleih, Nijboer, Halder, & Kübler, 2010), and even attention (Dale, Simpson, Foxe, Luks, & Worden, 2008; Saupe, Widmann, Bendixen, Müller, & Schröger, 2009) could impact the morphology of the averaged ERP. Relating to the present study, it is possible that adults were not as motivated to complete the Go-NoGo task as children were given the simplicity and the game-like nature of the paradigm. Additionally, participants may have
been, in general, more anxious about having EEG data recorded during the first session compared to the second session. Although the procedure is non-invasive and proven to be safe, it is still unfamiliar to the majority of participants who volunteer for EEG studies. Strange and uncommon situations can induce anxiety in participants, particularly children, thus interfering with the recorded EEG signals. When participants return for a second session scheduled relatively close in time to the first, the environment is likely not as unfamiliar or stress-inducing as before, which could explain some of the changes in ERP amplitudes noted across sessions. Future studies may choose to monitor stress levels throughout each session in order to control for varying anxiety from one session to the next, especially in children.

**Developmental Indices within Children**

The present study examined children between the ages of 7 and 13 years. Correlational analyses were performed to examine the relationship between age and component amplitude within children. In accordance with previous literature (Segalowitz & Davies, 2004), the O-wave and total CNV presented with significant negative correlations between age and averaged amplitude. Although they only approached statistical significance, correlations between age and E-wave amplitudes followed the same trend. These data indicated that with increasing age, CNV components became more negative in amplitude.

Studies have shown that all three CNV components have generators in the frontal and prefrontal cortices (Bareš et al., 2000; Bareš et al., 2003; Giard et al., 1990). Given that maturation of the frontal and prefrontal cortices extends into adulthood, it makes sense that the CNV components exhibit developmental trends and present with a more mature topography with increasing age (Segalowitz & Davies, 2004). However, unlike the O-wave and total CNV, the E-wave has generators in parietal and motor cortices (Basile et al., 2002; Bender et al., 2004).
fact, the E-wave overlaps with another ERP component known as the lateralized readiness potential (LRP), which represents brain processing associated with motor preparation for an upcoming response (Smulders, Kok, Kenemans, & Bashore, 1995; Zordan, Sarlo, & Stablum, 2008).

In a recent study, Bryce, Szűcs, Soltész, and Whitebread (2011) examined the development of the LRP in children and adults. Thirty-two children, ages five or eight years, and 16 adults between the ages of 23 and 31 years performed a simple forced-choice task in which paired images were presented on a computer screen. Each pair contained two animals, one of which was larger than the other. On congruent trials, the animal that was pictured as larger was larger in real life (e.g., a large rhinoceros and a small butterfly). On incongruent trials, the animal that was pictured as larger was actually smaller in real life (e.g., a large butterfly and a small rhinoceros). Stimuli were presented for 4000ms with 1000ms between trials. While EEG data were recorded, participants were instructed to press a button with either their left or right hand to signify which animal was larger (Bryce et al., 2011).

The LRP was measured at electrode sites C3 and C4 to capture the motor preparation happening in both left and right motor cortices. Analyses indicated that younger children showed more extreme responses (i.e., more negative amplitudes) than older children and adults (Bryce et al., 2011). This trend toward decreasingly negative amplitudes with increasing age is in contradiction to the expected developmental change of the E-wave, i.e., increasingly negative amplitudes with increasing age. It is possible that the overlapping processing of attentional expectancy and motor preparation with the E-wave and LRP obscured visibility of the E-wave’s developmental trends in the present study. Such an effect may explain why the correlation between age and E-wave amplitude among children was not statistically significant. Future
investigations exploring E-wave development may consider using techniques like PCA to isolate E-wave processing from other simultaneously produced brain signals.

**Test-Retest Reliability**

Reliability statistics indicated that for both children and adults, the O-wave was moderately reliable with Pearson product moment correlations near .50. For children, the E-wave and total CNV were also moderately reliable. Interestingly, for adults, the O-wave was the only reliable component. This finding was in contrast to previous research on CNV component reliability, though the discrepancies may be explained in part by differences in study methods.

To date, no known CNV reliability studies have utilized a completely visual paradigm to evoke the CNV components. For example, Kropp et al. (2000) had participants perform a Go-NoGo task in which the conditional stimulus was a tone of either 200Hz (i.e., a NoGo trial), or a tone between 250 and 2000Hz (i.e., a Go trial). The imperative stimulus was a tone of 2500Hz that would sound for 1500ms, or until a response was made. Likewise, Roth et al. (1975) utilized a completely auditory paradigm, and Griesel and Bartel (1975) utilized a visual-auditory paradigm with a flash of light for the conditional stimulus and a tone as the imperative stimulus.

It is known that the stimulus modality used to elicit an ERP component can affect the amplitude and latency of that component (Kappenman & Luck, 2012). In fact, stimulus modality can affect a number of aspects of EEG data (Krause et al., 2006; Mazaheri & Picton, 2005; Naumann et al., 1992) For example, Krause et al. (2006) performed an EEG study investigating the effects of stimulus modality on language processing. Seven adults were presented with images, and twelve adults were presented with auditory stimuli. Participants had to indicate whether the stimulus provided represented a real word or a pseudoword by pressing a button. A total of 200 stimuli were presented in each stimulus condition while EEG data were recorded.
Data were analyzed using time-frequency analyses in order to examine differences in processing in the 4-to-30Hz range. Findings indicated that visual stimuli evoked much greater theta, alpha, and beta event-related desynchronization compared to auditory stimuli (Krause et al., 2006). It is possible that the EEG signal produced by the completely visual paradigm in the present study was more variable than that produced by an auditory paradigm, thus decreasing the reliability of the CNV components. Future investigations should further examine the effects of stimulus modality on the CNV components using time frequency analyses.

Interestingly, although children seemed to have better reliability indices than adults, Fisher’s $r$ to $Z$ transformations revealed no significant differences in stability for any CNV component. This finding suggests that perhaps both children and adults exhibited a large amount of variability in their EEG signals from session to session. Future investigations should examine individual differences that may affect variability between data collection sessions. As discussed previously, such factors as attention (Dale et al., 2008; Saupe et al., 2009), motivation (D’Angiulli et al., 2012; Kleih et al., 2010), and anxiety (Aarts & Pourtois, 2010; Grillon & Ameli, 1994) could potentially alter the brain signals recorded via EEG within and between lab visits.

**CNVor Efficacy**

The CNVor procedure was developed in order to produce ERPs using both traditional data reduction methods as well as through a distribution-based approach that rejected aberrant segments from the averaging process on an individual basis. Specifically, the traditional approach consisted of averaging all segments together after applying a baseline correction, an eye regression procedure (Segalowitz, 1996), and a standard artifact rejection. The DBOR approach followed the same approach, but with one unique step interjected. Rather than
averaging all remaining segments together, the CNVor procedure inspected all segments on an individual basis. Any segment that contained voltages exceeding two times the interquartile range of voltages for a particular individual were rejected as outliers and excluded from the averaging process.

One major purpose of this study was to examine the efficacy of the newly developed CNVor procedure in reducing individual variability in CNV tracings. Preliminary data showed that on average, approximately one segment was excluded as an outlier for each individual. This statistic was true for both children and adults across both sessions. Thus, the CNVor procedure did appropriately reject aberrant segments. However, Fisher’s r to Z transformations showed no significant differences in test-retest reliability between traditionally-produced and DBOR-produced ERPs. Thus, with the parameters utilized for rejecting outliers, the CNVor procedure did not seem to significantly reduce the amount of variability noted across sessions.

However, further analyses were performed in order to investigate whether the average of one segment per individual that was rejected as an outlier significantly impacted the data in another manner. Results of a three-way ANOVA indicated a significant Procedure effect, which suggested that the DBOR approach produced significantly different averaged total CNV amplitudes compared to the traditional approach. Further examination showed that the CNV amplitudes were significantly less negative when produced via the DBOR technique. Thus, although the DBOR approach did not significantly affect the reliability of the CNV, statistics did indicate that the CNVor procedure significantly impacted the averaged CNV amplitude in both children and adults.

The present study utilized only one set of parameters for defining outlier segments. It is possible that with refined adjustments to the parameters (e.g., the time window examined, or the
boundaries of the interquartile range), the CNVor procedure could prove to be even more efficacious and possibly even improve reliability indices. Future investigators should carefully decide on the parameters to utilize a priori in order to avoid “data mining”. That is, researchers who choose to use procedures like the CNVor should decide on the parameters based on theory or prior studies in order to avoid conscientious manipulating of their data to produce positive findings.

**Conclusions and Implications**

The present study has illuminated a number of findings with important implications for researchers and clinicians alike. The CNV components are measures of sustained attention (Bareš et al., 2000; Zappoli, 2003), orientation (Rohrbaugh et al., 1984; Zimmer & Demmel, 2000), and anticipation (Knott et al., 1991; Simons et al., 1983), three cognitive processes which can be important in everyday functioning. The interpretation of the CNV components in research or clinical settings could have cascading implications for the way that future research is conducted, or for an individual’s well-being depending on the situation. Thus, it is important for researchers and clinicians to carefully interpret findings from CNV data.

**Implications for Research**

Research on the CNV has been conducted for nearly half of a century (Walter et al., 1964), yet the field of EEG research is still working to fully understand the ERP components involved. One crucial step in understanding ERPs is determining how reliable they are from one session to the next. Without a reliable component, it is challenging to apply the findings of a study outside of the single instance during which EEG data were recorded. Although relatively few studies have been conducted examining the stability of the CNV components (Griesel & Bartel, 1975; Kropp et al., 2000; Roth et al., 1975), the findings have suggested that the ERPs are
rather reliable across time in adults. However, data from the present study reveal a different story.

The data indicated that the visually-evoked CNV components were only moderately reliable at best. In children, all three components were moderately reliable with the total CNV exhibiting the poorest stability. In adults, the O-wave was the only reliable component. These data suggest that perhaps the visually-evoked CNV components are not as reliable as auditory-evoked CNV components measured in past studies (Kropp et al., 2000).

Such information becomes important for researchers to consider when designing study procedures, particularly with respect to developmental studies. Traditionally, developmental researchers examining the CNV components have utilized completely visual paradigms (Hämmerer et al., 2010; Jonkman, 2006; Jonkman et al., 2003; Segalowitz & Davies, 2004). Using visual stimuli tends to be more engaging for children, thus producing greater amounts of clean data from a recording session (Gavin & Davies, 2008). Assuming that the CNV components elicited by visual stimuli are equally as reliable as those produced from auditory stimuli, and given that children and adults have equally stable brain activity across time in response to CNV paradigms, developmental researchers would have little to consider when designing their study procedures. However, data from the present study suggest the opposite.

The reliability indices obtained in the present study were far lower than expected given previous findings (Griesel & Bartel, 1975; Kropp et al., 2000; Roth et al., 1975). It is possible that researchers using visually-based paradigms to evoke the CNV components in adults may not be obtaining reliable data simply because the visual systems of the brain process information differently than the auditory systems. Perhaps adults found the paradigm to be too easy and did not engage in attentional processing to the same extent from one session to the next because of
the limited attentional demands. Additionally, the current study was the first study to examine the test-retest reliability of the CNV components in children. Further examination is necessary to fully establish the reliability of the CNV components in children via visual- and auditory-based paradigms, however the preliminary findings suggest only moderate reliability of the CNV components in children.

Researchers examining changes in CNV amplitudes or topographies across time must be careful to take the stability of the components into consideration. It is possible that noted changes may be the result of maturation over time, or the result of normative instability in the components themselves because of the manner in which the brain processes the task. I suggest that researchers consider taking multiple measurements from each participant in order to establish measures of intraindividual variability. With this measurement, researchers could better control for instability in the brain processing across time and better understand the development of sustained attention, orienting, and expectancy.

**Implications for Clinicians**

Just as researchers need to carefully interpret their findings, clinicians must be mindful of intraindividual variability in measured brain activity from one day to the next. With attention-based disorders becoming more and more prevalent in today’s society, the need for reliable measures of cognitive processing and attentional abilities has never been greater. A number of disorders include deficits in attentional abilities as key symptoms and repercussions; some of these disorders include autism spectrum disorder (ASD; Chan et al., 2011; Sokhadze et al., 2012), attention deficit hyperactivity disorder (ADHD; Heaton et al., 2001; Spronk et al., 2008), and even migraines (Bender et al., 2002; Oelkers-Ax et al., 2004).
For decades, clinicians and therapists have utilized behavioral measures to assess cognitive functioning in individuals with disorders. Assessments like the Test of Everyday Attention (TEA; Robertson, Ward, Ridgeway, & Nimmo-Smith, 1994) and the Test of Everyday Attention for Children (TEA-Ch; Manly, Anderson, Nimmo-Smith, et al., 2001) are common tools for measuring attentional abilities in adults and children respectively. Although behavioral assessments have been incredibly useful in determining whether there are deficits in attentional processing, such measures cannot assess the underlying mechanisms responsible for those deficits. Using measures like EEG and ERPs can help to illuminate what neural mechanisms may be involved in the attentional dysfunctions measured via behavioral assessments.

It goes without saying that clinicians should utilize the most reliable tools possible when assessing an individual. Whether the intent is to diagnose a disorder or measure changes in attentional abilities over the course of an intervention, the reliability of the measure is an important factor for interpreting the results. Because the CNV is the only known ERP component that directly taps into sustained attention, it is the most reasonable component to examine as a supplement for behavioral assessments of sustained attention.

Given the findings of the present study, perhaps the visually-evoked CNV components are not the best measures for clinicians to use in the assessment of sustained attention in clients. For both children and adults, the reliability of the components reached only moderate reliability at best with coefficients near $r = .50$. For clinical purposes, statisticians have suggested that reliability indices should be upwards of $r = .70$ (Cohen, 2008). The use of CNV components as measures of intervention efficacy or for the establishment of diagnostic criteria must be carefully considered. It is possible that a single recording of EEG data is not enough information to establish a true picture of an individual’s attentional processing abilities. A clinician using ERPs
could wrongfully diagnose someone with an attentional deficit, or mistakenly overlook an individual who truly does have trouble with attentional processing. In the case of intervention efficacy, one might mistakenly interpret changes across time as improvements in processing as a result of the intervention when in reality, the changes are normative fluctuations in the EEG tracing across sessions.

The implications for clinicians are potentially severe and have real repercussions on the lives of a number of individuals seeking help for their attentional deficits. It is clear that research on sustained attention in the brain must further advance before the CNV components can be reliably utilized in clinical settings. Given prior research, clinicians may choose to examine the auditory-evoked CNV components in adults as reliability indices are relatively high and have been established across several studies (Griesel & Bartel, 1975; Kropp et al., 2000; Roth et al., 1975). However, caution should be used when interpreting the findings from children as no studies to date have examined the reliability of the auditory-evoked CNV components. Further investigation is necessary to establish reliable measures to be used in clinical practice.

**Conclusions**

The present study has shed light on a number of issues with important implications for future research and clinical practice. The visually-evoked CNV components, though not necessarily best for clinical use, demand the attention of researchers. Future investigations should examine how the brain differentially processes visual- versus auditory-based paradigms to elicit the CNV. The effects seemed to be fairly dramatic when comparing the findings of the present study against previous literature.

Additionally, the utility of procedures that can account for intraindividual variability, like the CNVor, seems promising. In the current study, the CNVor procedure was developed in order
to create ERPs based on individual participants’ voltage distributions. Recently, the field has begun to recognize the importance of accounting for intraindividual variability in EEG tracings. Tools that can properly account for intraindividual variability may prove to be prudent for both research and clinical interpretations. Although the CNVor procedure did not significantly impact reliability indices, it did have a meaningful impact on the data for both children and adults. With further refinement and exploration, procedures like the CNVor could help push the field in a new direction in which researchers can better understand individual differences in EEG signals. It is through this exploration that the field can move toward addressing the needs of individuals with atypical neural processing and apply findings to clinical practices and interventions.
Table 1

Means and Standard Deviations of Reaction Times (ms) for Go Trials during Session 1 and Session 2 for Children and Adults.

<table>
<thead>
<tr>
<th></th>
<th>Session 1</th>
<th>Session 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
</tr>
<tr>
<td><strong>Children</strong></td>
<td>301.87</td>
<td>99.71</td>
</tr>
<tr>
<td><strong>Adults</strong></td>
<td>209.11</td>
<td>43.83</td>
</tr>
</tbody>
</table>
Table 2

Means and Standard Deviations of the Number of Segments included in the Averaging Process for Children and Adults during Session 1 and Session 2 in the Traditional and DBOR Data Analyses, and the Number of Segments that were Excluded by the CNVor Procedure.

<table>
<thead>
<tr>
<th></th>
<th>Session 1</th>
<th></th>
<th>Session 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
<td>M</td>
<td>SD</td>
</tr>
<tr>
<td>Traditional</td>
<td>27.00</td>
<td>6.88</td>
<td>25.47</td>
<td>7.45</td>
</tr>
<tr>
<td>Children</td>
<td>26.25</td>
<td>6.94</td>
<td>24.82</td>
<td>7.48</td>
</tr>
<tr>
<td>Excluded</td>
<td>.75</td>
<td>1.20</td>
<td>.65</td>
<td>1.00</td>
</tr>
<tr>
<td>Adults</td>
<td>33.56</td>
<td>4.20</td>
<td>31.72</td>
<td>5.60</td>
</tr>
<tr>
<td>DBOR</td>
<td>32.59</td>
<td>4.14</td>
<td>30.66</td>
<td>5.20</td>
</tr>
<tr>
<td>Excluded</td>
<td>.97</td>
<td>1.36</td>
<td>1.06</td>
<td>1.22</td>
</tr>
</tbody>
</table>

Notes: DBOR refers to the distribution-based, outliers-rejected approach to creating averaged ERPs using the CNV outlier rejection (CNVor) procedure.
Table 3

The Means and Standard Deviations of the Averaged Amplitudes (in µV) in the Time Windows of the O-wave, E-wave, and Total CNV for Go and NoGo Trials in Session 1 and Session 2.

<table>
<thead>
<tr>
<th></th>
<th>Go Trials</th>
<th>NoGo Trials</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Session 1</td>
<td>Session 2</td>
</tr>
<tr>
<td>600-800ms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(O-Wave)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1800-2000ms</td>
<td>-1.47</td>
<td>4.20</td>
</tr>
<tr>
<td>(E-Wave)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>600-2000ms</td>
<td>0.03</td>
<td>4.03</td>
</tr>
<tr>
<td>(total CNV)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adults</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1800-2000ms</td>
<td>-4.94</td>
<td>3.04</td>
</tr>
<tr>
<td>(E-Wave)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>600-2000ms</td>
<td>-3.03</td>
<td>2.44</td>
</tr>
<tr>
<td>(total CNV)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4

*Pearson Product Moment Correlations between Children’s Age in Years and the Averaged Amplitude of the Traditionally-Measured CNV Components in Microvolts (µV).*

<table>
<thead>
<tr>
<th></th>
<th>Session 1</th>
<th>Session 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>O-wave</td>
<td>-.47**</td>
<td>-.45**</td>
</tr>
<tr>
<td>E-wave</td>
<td>-.28</td>
<td>-.24</td>
</tr>
<tr>
<td>Total CNV</td>
<td>-.39**</td>
<td>-.31*</td>
</tr>
</tbody>
</table>

* *p < .05
** *p < .01
*** *p < .001
Table 5

*Test-Retest Reliability Indices: Pearson Product Moment and Intraclass Correlation Coefficients for the Traditionally-Measured and DBOR-Measured CNV Components in Adults and Children during Session 1 and Session 2.*

<table>
<thead>
<tr>
<th></th>
<th>Traditional Data</th>
<th></th>
<th>DBOR Data</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>ICC (3,1) Consistency</td>
<td>ICC (3,1) Absolute Agreement</td>
<td>r</td>
</tr>
<tr>
<td><strong>Children</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O-wave</td>
<td>.53***</td>
<td>.52***</td>
<td>.49***</td>
<td>.57***</td>
</tr>
<tr>
<td>E-wave</td>
<td>.50***</td>
<td>.50***</td>
<td>.48***</td>
<td>.48***</td>
</tr>
<tr>
<td>Total CNV</td>
<td>.34*</td>
<td>.33**</td>
<td>.33**</td>
<td>.35*</td>
</tr>
<tr>
<td><strong>Adults</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O-wave</td>
<td>.58**</td>
<td>.58***</td>
<td>.58***</td>
<td>.59***</td>
</tr>
<tr>
<td>Total CNV</td>
<td>.05</td>
<td>.05</td>
<td>.05</td>
<td>.04</td>
</tr>
</tbody>
</table>

*Notes: DBOR refers to the distribution-based, outliers-rejected approach to creating averaged ERPs using the CNV outliers rejection (CNVor) procedure.*
Table 6

Fisher’s r to Z Transformations Comparing Children and Adults on Reliability Coefficients Based on Traditionally-Measured ERP Components.

<table>
<thead>
<tr>
<th>Component</th>
<th>z</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>O-Wave</td>
<td>.13</td>
<td>.45</td>
</tr>
<tr>
<td>E-Wave</td>
<td>-1.62</td>
<td>.05</td>
</tr>
<tr>
<td>Total CNV</td>
<td>-1.38</td>
<td>.08</td>
</tr>
</tbody>
</table>

Notes: When performing the Fisher’s r to Z transformations, final Z scores were calculated with the children’s Z score being subtracted from the adults’ z score (i.e., $z_{\text{final}} = \frac{z_{\text{adults}} - z_{\text{children}}}{\sigma}$).
Table 7

*Fisher’s r to Z Transformations Comparing Reliability Coefficients for DBOR- and Traditionally-Measured ERP Components for Adults and Children Separately.*

<table>
<thead>
<tr>
<th></th>
<th>Adults</th>
<th></th>
<th></th>
<th>Children</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>z</td>
<td>p</td>
<td></td>
<td>z</td>
<td>p</td>
</tr>
<tr>
<td>O-Wave</td>
<td>-.06</td>
<td>.48</td>
<td></td>
<td>-.25</td>
<td>.41</td>
</tr>
<tr>
<td>E-Wave</td>
<td>.19</td>
<td>.42</td>
<td></td>
<td>.16</td>
<td>.44</td>
</tr>
<tr>
<td>Total CNV</td>
<td>.04</td>
<td>.48</td>
<td></td>
<td>-.08</td>
<td>.43</td>
</tr>
</tbody>
</table>

Notes: 

a When performing the Fisher’s r to X transformations, final X scores were calculated with the traditional data Z score being subtracted from the DBOR data Z score (i.e., Z_{final} = [Z_{DBOR} – Z_{Traditional}]/\sigma).

b DBOR refers to the distribution-based, outliers-rejected approach to creating averaged ERPs using the CNV outliers rejection (CNVor) procedure.
Table 8

The Means and Standard Deviations of the Averaged Amplitude (in µV) of the Total CNV Based on Traditional Measurements and the DBOR Approach.

<table>
<thead>
<tr>
<th></th>
<th>Traditional</th>
<th></th>
<th>DBOR</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Session 1</td>
<td>Session 2</td>
<td>Session 1</td>
<td>Session 2</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
<td>M</td>
<td>SD</td>
</tr>
<tr>
<td>Children</td>
<td>0.03</td>
<td>4.03</td>
<td>-0.65</td>
<td>3.78</td>
</tr>
<tr>
<td>Adults</td>
<td>-3.03</td>
<td>2.44</td>
<td>-2.27</td>
<td>2.67</td>
</tr>
</tbody>
</table>

Notes: DBOR refers to the distribution-based, outliers-rejected approach to creating averaged ERPs using the CNV outliers rejection (CNVor) procedure.
Figure 1. The visual Go-NoGo task presented to participants during EEG recording. A green circle served as the conditional stimulus beginning a Go trial, and a red circle was the conditional stimulus beginning a NoGo trial. The car was the imperative stimulus.
Figure 2. The grand averaged ERP for adults’ traditional data, before outlier rejection procedures, for session 1 measured at site Cz.
Figure 3. The grand averaged ERP for adults’ traditional data, before outlier rejection procedures, for session 2 measured at site Cz.
Figure 4. The grand averaged ERP for adults’ DBOR data for session 1 measured at site Cz. Outliers were rejected from 1500-2000ms using the CNVor procedure.
Figure 5. The grand averaged ERP for adults’ DBOR data for session 2 measured at site Cz. Outliers were rejected from 1500-2000ms using the CNVor procedure.
Figure 6. The grand averaged ERP for children’s traditional data, before outlier rejection procedures, for session 1 measured at site Cz.
Figure 7. The grand averaged ERP for children’s traditional data, before outlier rejection procedures, for session 2 measured at site Cz.
Figure 8. The grand averaged ERP for children’s DBOR data for session 1 measured at site Cz. Outliers were rejected from 1500-2000ms using the CNVor procedure.
Figure 9. The grand averaged ERP for children’s DBOR data for session 2 measured at site Cz. Outliers were rejected from 1500-2000ms using the CNVor procedure.
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