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RUNNING HEAD: Synesthesia, a Learned Association?

On the Nature of Synesthesia: A Learned Association or Something Different?

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Abstract

Synesthesia is a phenomenon that has captivated the interest of many researchers, as it is a unique experience of the blending of the senses. The following study was conducted in an effort to understand whether synesthetic experiences can be learned, as Bor, Rothen, Schwartzman, Clayton, & Seth (2014) claimed. While there has been much research demonstrating that synesthesia is more common in the population than previously thought, and likely to develop in young children as a learning mechanism (Watson et al., 2017a), there have not been as many event-related brain potential (ERP) studies conducted on synesthesia. ERP studies are important for synesthesia, since neural phenomena are often best measured through brain monitoring technologies, such as magneto/electroencephalography (MEG/EEG), ERP, functional Magnetic Resonance Imaging (fMRI). The current study utilizes the measurement of continuous neural data through a pre- and post-test ERP study monitoring changes occurring at the Pz electrode site for the visual N100, auditory N100, and the P300 ERP components in 15 neurotypical, non-synesthetic college-age adults. The goal of this study was to understand whether non-synesthetes can have a synesthetic experience or are merely forming a learned association after 4 weeks of grapheme-color task, and chromesthesia task training. The results show that while participants can be trained to form learned associations between letters and colors, and sounds and color as shown by a significantly reduced P300 amplitude, they do not seem to have synesthetic experiences as was previously indicated—as demonstrated by the nonsignificant change in both N100 components.

Keywords: Synesthesia, ERP, N100, P300, Learning

On the Nature of Synesthesia: A Learned Association or Something Different?

Understanding the nature of psychological phenomena can be a challenging task. Often, there are a large confluence of factors which combine together to produce the effects we observe as a singular phenomenon. One such multifaceted phenomenon is synesthesia. Synesthesia can be defined (rather simplistically) as a blending of the senses. In particular, it is the involuntary, automatic activation of one sense as a result of the direct activation of another. For example, some people with synesthesia can listen to particular sounds and will, involuntarily, visualize that sound to have a particular color. There are many different types of synesthesia, the most common of which is known as grapheme-color synesthesia, or the association of a particular color with a particular grapheme, i.e. a letter or number (Bor et al. 2014). Other types include time-space synesthesia—the association of discrete chunks of time (calendar year, specific dates, months) with a particular location in space (see Fig. 1) (Watson et al. 2017a)—and chromesthesia—the association of a particular sound with a particular color (Brang, Teuscher, Ramachandran, & Coulson, 2010). Synesthesia is an important phenomenon to comprehend, because it alters the way in which people experience and interact with the world around them. It has also caught the attention of researchers who seek to determine the nature of the synesthetic experience (Bor et al., 2014). In order to understand why these questions about synesthesia's origins, nature, and teachability, it is important to delve a little more into synesthesia's background.

Synesthesia Prevalence and Origins

Synesthesia was once believed to be a rare condition in the population (Baron-Cohen, Burt, Smit-Laittan, Harrison, & Bolton, 1996), and estimates on its prevalence varied widely (Sagiv & Ward, 2006). Current estimates still have a large range, putting the prevalence of

synesthesia anywhere between 0.05% of the population and 4% of the population (Simner et al., 2006). Much of this variation is a result of the self-referral of synesthetes to psychological studies that was present in previous studies (Baron-Cohen et al., 1996). Additionally, prior to the work of Simner et al. (2006), evidence suggested that synesthesia is more prevalent among females (Baron-Cohen et al., 1996 estimated the ratio to be approximately 6:1). Objective evidence by Simner et al. (2006) demonstrated that synesthesia had a female to male ratio of 1.1:1. There are also some researchers who have argued that synesthesia is partly related to one's inherited genetics, because of the high rates of synesthesia within families who have at least one other synesthete (Baron-Cohen et al., 1996). Beyond family links, and disproven female bias for synesthesia, however, lies the complex ideas behind synesthesia's origins. Synesthesia's origins are valuable to understand, because they provide important information on understanding the roles that developmental processes and learning styles/methods in infants' and childrens' brains play in the behaviors seen in adults.

Competing hypotheses. While there are currently five competing hypotheses about synesthesia's origins, only three of them are relevant to the current study, the neonatal synesthesia hypothesis (Deroy & Spence, 2013; Maurer & Mondloch, 2005), the neural pruning hypothesis (Hubbard & Ramachandran, 2005), and the local cross-activation model.

The neonatal hypothesis. In a review of the current standing and validity of the neonatal hypothesis, Maurer and Mondloch mention that it (the neonatal hypothesis) was born out of the seemingly paradoxical evidence on the development of cross-modal perception (p. 193 in Robertson & Sagiv, 2005). This evidence showed that babies successfully linked information across sensory modalities early after birth, failed at those same tasks later, only to relearn those cross-modal connections between six and twelve months of age (Maurer & Mondloch, 2005, p.

193 in Robertson & Sagiv, 2005). Maurer and Mondloch (2005, in Roberson & Sagiv, 2005) argued the cross-modal interactions seen in infants resembled that of adult synesthesia because neonates may have some transient connections in their developing cortex that resemble and/or behave similarly to the cross-modal interactions seen in adults, as a result of learned behavior during the ages of 6-12 months (Maurer & Mondloch, 2005, in Robertson & Sagiv, 2005). Eventually, a competing hypothesis to synesthesia's origins arose—the neural pruning hypothesis.

The neural pruning hypothesis. The neural pruning hypothesis states that synesthesia arises and persists through adulthood, because the synaptic pruning process fails to properly occur during early development (Hubbard & Ramachandran, 2005; Petanjek et al, 2011). During normal development, synaptic density increases from birth to a maximum between ages 1-2, declines from ages 2-16, and remains steady from ages 16-72 (Huttenlocher, 1979). Between 2 and 6 years of age, the neurotypical brain will start trimming synapses that are not often used, e.g., cross-modal connections between different sensory modalities. The failure of the normal pruning process leaves extra synaptic connections in the brain, which, as Hubbard & Ramachandran (2005) mention, is hypothesized to allow the enhanced cross-modal connections seen in synesthesia. Like the neonatal hypothesis, the neural pruning hypothesis also fell a little short in explaining the origins of synesthesia because it focused mainly upon the physiological, biological, and genetic origin of synesthesia. However, there is another debate about synesthesia's origins that is taking place—one that occurs at the architectural level (Hubbard & Ramachandran, 2005).

The local cross-activation model. Unlike the neural pruning and neonatal hypotheses, the local cross-activation model (Hubbard & Ramachandran, 2005) is based on the idea that areas of

the brain near to one another, e.g., the fusiform gyrus—identified as having roles in color detection and recognition as well as grapheme and number recognition (Hubbard & Ramachandran, 2005)—are activated together because of a high level of connections between those two regions. However, both the neonatal and neural pruning hypotheses do have a few problems.

Some issues with the above hypotheses. While the neonatal synesthesia and neural pruning hypotheses explains how synesthesia can arise for people who have it for the vast majority of their lives, it fails to take into account synesthesia which arises from incidents of neural trauma, drug usage (such as LSD), or the acquisition of synesthesia later in life (Afra, Funke, & Matsuo 2009; Schweizer et al., 2013). On the other hand, while the local cross-activation model of synesthesia provides a solid foundation for the role of learning in synesthesia (Hubbard, 2007)—since learning a task repeatedly can lead to the stronger synaptic connections, (i.e. the basis of the local cross-activation model of synesthesia)—it isn't always able to explain the acquired, drug induced, or trauma induced forms of synesthesia. In order to tease out what hypothesis or model (neonatal, neural pruning, local-cross activation) plays a greater role in the development of synesthesia, researchers focused on grapheme-color synesthesia, because it has the ability to provide answers to questions asked about any of the above hypotheses or model described. This is because people must learn what graphemes are (and they vary by language), and the regions of the brain that are associated with color recognition and grapheme recognition are near one another (as described by Hubbard, 2007). Additionally, grapheme-color has a high prevalence among people with synesthesia—another factor which makes it such a highly studied form of synesthesia (Grossenbacher & Lovelace, 2001; Hubbard, 2007; Hubbard et al., 2011; Hubbard & Ramachandran, 2005; Ramachandran and Hubbard, 2001a, 2001b; Zamm, Schlaug,

Eagleman, & Loui, 2013). Because grapheme-color synesthesia is so highly studied, researchers appear to have a better handle on some of the possible mechanisms that may cause or be highly correlated with grapheme-color synesthesia. For this reason, the current study utilizes grapheme-color synesthesia in testing. Chromesthesia is also utilized because of the rapid registration of auditory stimuli that is easily observable using event-related brain potential (ERP) technology.

A possible mechanism of grapheme-color synesthesia and chromesthesia. Since the current study focuses on grapheme-color and chromesthesia, it is valuable to try to comprehend the mechanism(s) that drive these forms of synesthesia. One possible mechanism is the hyperconnectivity (also called hyperbinding) hypothesis—mainly put forward by Hubbard and Ramachandran (as mentioned in Zamm et al., 2013). The hyperconnectivity hypothesis is the biological basis for the neural pruning hypothesis and the local cross-activation model. It (hyperconnectivity) arises from the idea that grapheme-color, chromesthesia, and other forms of synesthesia are caused because of greater than normal neural, and synaptic, connections present in the regions of the brain that connect different recognition and processing areas for the different senses. Zamm et al. (2013) provided evidence to support the hyperconnectivity hypothesis.

In their study, Zamm et al. (2013) state that the synesthetes with chromesthesia that they tested had both more neurons and more synaptic connections, in one hemisphere versus another along a white matter pathway known as the “inferior front-occipital fasciculus (IFOF)” (p. 359). The IFOF is responsible for connecting the auditory and visual association areas with the frontal lobe (Zamm et al., 2013). This increased integrity, found mainly in the left hemisphere (Zamm et al., 2013), seemed to suggest that increased white matter connectivity in the IFOF was responsible for increased cross-modal associations (Zamm et al., 2013). Essentially, this means

that synesthetes not only have more neurons (and therefore more connections) in their left versus right hemispheres, but also that these neurons increase the connections between different sensory modalities, as compared to non-synesthetes. Since this data was found in synesthetes, Zamm et al. (2013) believe that this increased white matter in the IFOF is responsible (at least in part) for synesthesia.

Zamm et al.'s 2013 study provides a potential connection to learning. It is known that neuronal connections can be strengthened over time the more they are used (Trafton, 2015), and the process of learning hinges upon being able to create, strengthen, and maintain synaptic connections between different regions of the brain (Fields, 2013). In fact, multiple neuroimaging studies have demonstrated over the years that learning alters the structure of the brain itself, as Fields (2012) describes in his review. Therefore, if, as some synesthesia researchers hypothesize (Hubbard & Ramachandran, 2005; Maruer & Mondloch, 2005, in Robertson & Sagiv, 2005; Petanjek et al., 2011), it is not difficult to consider that synesthesia, a process where two senses (and therefore regions of the brain) are linked together likely stems from some learning process early in development Watson et al. (2017). This understanding of that learning may play a role in the development of synesthesia, leads to a natural question—how, if at all, is learning involved in the phenomenon of synesthesia?

Synesthesia and Learning. While it takes time to learn to associate colors with specific words and meanings, neurotypical people possess the instinctual ability to see, hear, taste, smell, and touch. For example, the cross-modal connections that are created between, say smell and memory (Willander & Larsson, 2006), is not something that one has to actively try to create, as the underlying neuronal connections between smell and memory are already present at birth.

Synesthesia, on the other hand, is not necessarily something that one is born with. Time-space synesthesia is one such example. In this form of synesthesia, certain events in time (such as calendar days, months, years, etc.) are associated with a particular shape or area of space in a region around the synesthete (Fig. 1). However, as with other forms of synesthesia, while there are many variations of those spatial representations among synesthetes, an individual synesthete's representation is consistent over a long stretch of time (Teuscher et al, 2010). While time-space synesthesia is likely related to one's sense of proprioception, it is very unlikely to be related to how humans conceptualize and understand time at birth.

There are, in fact, two ways of comprehending time. One method is the understanding the *passage* of time, which infants as young as six weeks are capable of doing (Berg, 1974). Indeed, numerous studies (Addyman, Rocha, & Mareschal, 2014; Brannon, Roussel, Meck, & Waldorff, 2004; Colombo & Richman, 2002) show that infants have a concept of the passage of time, even if they do not organize into discrete units as adult humans do (Addyman et al, 2014). The second is the *cultural context* of time—that is what is a Gregorian calendar year versus a fiscal year versus a lunar year. This second concept varies depending on the culture one learns while growing up, and it is the cultural context of time, which is the basis of temporo-spatial synesthesia (Fig. 1). Given that an understanding of time in its cultural context is developed through learning, the implication remains that there must be some connection between learning and synesthesia. After all, if, as Maurer and Mondloch (2005) state, the cross-modal linkages present in babies are lost only to be re-learned several months later, then it makes sense that synesthesia is—to some degree—an alteration in the learning process that leads to increased cross-modal connections, that can develop into synesthesia.

Indeed, it is important to note that during the opening salvo of scientific research into synesthesia (during the 1890s as Watson et al., 2017a indicates); many researchers (Calkins, Flournoy, and Galton, as mentioned in Watson et al., 2017a) asserted that there is a strong connection between synesthesia and learning. Calkins in particular (Watson et al., 2017a) argued that most types of number-form synesthesia—where numbers are in a complex spatial arrangement around the synesthete (Watson et al., 2017a)—likely originates from the way in which children learn numbers and words early in childhood. Calkins further states that while unusual neurophysiology in the child’s brain could be a factor (Watson et al., 2017a), the preservation of that synesthesia into adulthood is only possible if that synesthesia form is cultivated for learning (Watson et al., 2017a).

As the genetics argument gained steam, however, the learning argument for synesthesia faded into the background. While there have been some studies by Asher et al. and Tomson et al. (as cited in Watson et al., 2017a) which provide potential genetic loci that may be related to synesthesia, these studies don’t explain the cases of synesthesia wherein people develop the phenomenon after some type of brain trauma (Schweizer et al., 2013). As a result, scientists are beginning to take a more balanced view between the learning and genetics arguments. This change means that “the appropriate question is [no longer] ‘does learning influence synesthetic development?’, but rather ‘to what extent and in what manner does this influence occur’” (p. 213, Watson et al., 2017a).

In pursuing this question, several researchers have tried to develop training regimes to train non-synesthetic participants to acquire synesthesia (Bor et al., 2014). As Bor and his colleagues (2014) point out, however, few synesthesia training studies have gone longer than a week. Bor et al. (2014) attempted to do just that, by extending the training regime to 9 weeks,

and providing increased task difficulty with improved performance (something Bor and his colleagues mention has not been done by most training paradigms). Bor et al. (2014) choose to utilize behavioral methods and self-reporting measures to determine whether participants developed grapheme-color synesthesia by the end of the study. Bor and colleagues (2014) do point out that it is unlikely that their participants actually developed synesthesia after 9 weeks training as their participants lost their acquired “synesthesia” three months post training. While behavioral methods can be useful, as can self-reporting measures, in a multi-faceted phenomenon such as synesthesia, electroencephalographic (EEG) and event-related potential (ERP) methods are better suited to help pull apart some of the nuances. In particular, EEG methods can help determine whether the training regime actually results in the participants acquiring synesthesia, or merely forming associations between letters and colors. The answer to that question is best found through EEG and ERP technologies, which allow researchers to monitor changes in a participant’s behavior and/or understanding without relying on behavioral data alone.

Event-Related Potential Technique

Often, when looking at EEG data, it is important to narrow the information down to event-related brain potentials (ERPs) which allow for a more focused view of the data. This is because event-related brain potentials are voltage potentials measured at the surface of the scalp, which are time-locked to specific events. They also provide a high temporal resolution—millisecond-to-millisecond resolution as compared to the hundreds of milliseconds or second to second resolution of behavioral measures such as reaction time. This study, in particular, looks at the N100 (N1) and the P300b (P3b) components (see Fig. 2 for examples of typical waveforms for these ERP components). These two components have been indicated to be important to measure in most ERP studies of synesthesia—of any kind (Yamashita, Hikosaka, Nanba, &

Tabuchi, 2010; Sinka, Neufeld, Wiswede, Emrich, Bleich, Münte, & Szycik, 2014; Beeli, Esslen, & Jänke, 2007). The descriptions from these ERP components (provided below) can be found in Stephen Luck's book *An Introduction to Event-Related Potential Technique* (2005).

There are, in fact, two types of N1 components, the visual, and the auditory N1, and like most ERP components, each N1 wave is composed of several sub-components. One peaking anteriorly around 150-200 ms, the second subcomponent peaking parietally around 150-200 ms post stimulus (Hopf et al., 2002, Ritter et al., 1979, and Vogel & Luck, 2000 in Luck, 2005), while the third subcomponent originates from the lateral occipital cortex (also between 150-200 ms). In the case of the auditory N1, it has several distinct subcomponents, a frontocentral subcomponent generated in the auditory cortex peaking around 75 ms (Luck, 2005), a second subcomponent around 100 ms whose origin is unknown (Luck, 2005), and a third, laterally distributed subcomponent which peaks around 150 ms and is generated in the superior temporal gyrus (Luck, 2005). The N1 wave is also sensitive to attention (Luck 2005).

Since the lateral occipital N1 subcomponent of the visual N1 wave is larger when involved in discrimination tasks over detection tasks (Luck, 2005), leads to the following question: What happens to the visual N1 wave when participants are given direct information from two different modalities at the same time, and asked to associate/recognize both? In the case of the auditory N1 component, we are led to another related question: What happens to the auditory N1 wave when attention is split between the auditory and visual sensory modalities? This study seeks to understand try to understand both of these questions by determining what happens when non-synesthetes are trained in an attempt to develop chromesthesia.

In the most likely scenario, we expected that participants will demonstrate a more enhanced visual N1 over parietal and occipital regions and a more enhanced auditory N1 (longer

latency, lower amplitude) over the parietal sites after training (as the effects of split attention have hopefully been reduced due to training). This would be consistent with previous research that indicates that synesthetes have greater N1 enhancement over occipital regions as compared to non-synesthetes (Sinke et al., 2014). However, we also expected that the enhancement would not be as greatly pronounced as we see in chromesthetic synesthetes, as it is more likely that the participant generates learned associations between color and sound, as opposed to true synesthesia.

For the P3 component, Luck (2005) writes that there are two components, both of which are elicited by unpredictable, infrequent shifts in tone pitch or intensity. The P3a however, is elicited mainly by novel stimuli; unlike the P3b, which is elicited when the shifts in tone pitch or intensity are, task relevant (Luck 2005). Sutton, Braren, Zubin & John (1965) performed a series of experiments on uncertainty resolution, which demonstrated that when participants are given information about the nature of a stimulus prior to its presentation, versus when they are kept uncertain about that stimulus, they found that a large positivity which peaked around 300 ms post stimulus. Sutton and colleagues (1965) would also show that the more improbable a stimulus the greater the amplitude of the P3, and greater for incorrect answers than correct ones (Luck, 2005). The latency of the P3 varies depending on when the uncertainty is resolved. After training, for example, an improbable stimulus would become less improbable in nature to the participant, so their P3 amplitude would be reduced. To that end, this study constructed the visual synesthesia tasks to present information on the nature of the stimulus in some trials, without providing the same information in other trials (for example, whether a letter or number will be presented will be indicated by a particular symbol, e.g., an arrow pointing up for letter or down for a number). If the participants have synesthetic experiences, we would expect that the P3 would have a

significantly larger amplitude, but if the participants are merely forming a learned association it is far more likely that the P3 amplitude will be much lower after-training, since the stimuli are no longer improbable or novel to the participant.

While the current study is limited in scope, if we were to find a significantly larger N1 and P3 amplitudes post-training, it would be possible to say that the participants' brains have rewired themselves to change their comprehension between graphemes and color, and/or sound and color. Therefore, learning has occurred, potentially lending credence to Zamm et al.'s idea that what matters in synesthesia is the number and strength of the synaptic connections, rather than simply the proximity or genetics of a synesthete.

Methods

Sample and Participant Selection

A total of 23 subjects were recruited from the Illinois Wesleyan University (IWU) student population. During an initial recruitment briefing, participants were asked whether they had either grapheme-color synesthesia or chromesthesia. As no participants were synesthetes, all 23 were allowed to continue. Participants were also asked whether they suffered from photosensitive epilepsy, or colorblindness. None answered yes to any of the conditions, and so all were allowed to continue. If they answered yes to either condition, then those participants were not included in the study. Additionally, as in Bor et al. (2014), participants were given several color consistency tests through www.synesthete.org, which also provided three questionnaires to participants. The color consistency scores help determine whether any participants might possibly have either grapheme-color synesthesia or chromesthesia. The three participants who did have color consistency scores comparable to synesthetes were monitored, but not removed from the study, due to a lack of available participants. It is possible that these

participants (who remained in the final sample size) could have skewed the results in favor of synesthetic experiences—however the results did not indicate *any* results similar to synesthetic experiences, even with these participants present, and so the three participants were left in the sample size. In regards to the questionnaires, the first two questionnaires asked questions relating to age, gender, and the participants’ medical and family history relating to synesthesia. These two questionnaires were voluntary, and not required for the study. The third questionnaire was also untraceable back to individual participants; however, they were asked to fill this final questionnaire out—if they could—as it provides some base information for potential types of synesthesia that the participants may experience. Of the 23 participants, only 15 returned to complete post-test (an attrition rate of 34.8%). Therefore, the final sample size was 15. This study was approved by the IWU Institutional Review Board. Informed consent was obtained from all subjects prior to the start of testing.

Color Consistency Test

Prior to the pre-test EEG, all participants were required to complete the internet-based standardized synesthesia battery test found at www.synesthete.org (Eagleman, Kagan, Nelson, Sagaram, & Sarma, 2007) for “Letters→Color” and “Musical Pitch→Color,” as these were the types of synesthesia that the study is designed to train participants in. In the “Letters→Color” test, participants were presented with 78 trials with the following instructions (www.synesthete.org): “Click and drag the triangles or circle to choose a color [from the color gradient] which most closely resembles the synesthetic color associated with the letter or word presented. You may also use the arrow keys on your keyboard to adjust the color. The test will end automatically when all trials are complete.” Figure 3 provides a screenshot of the task, as participants would see it. If the color consistency of the participant for letters and color is

comparable with that of a synesthete, that participant was asked whether the color associations are consistent with their previous experiences. All participants mentioned that they only formed these associations in the moment, not because of long-time synesthetic experiences.

In the “Musical Pitch→Color” test, participants were presented with 39 trials with the following instructions (www.synesthete.org): “Click on the color bar and/or color square to choose a color which most closely resembles the synesthetic color associated with the presented sound. The test will end after all trials are complete.” Participants were instructed that “synesthetic color” meant the color they believed the letter or sound to represent. Figure 4 provides a screenshot of the task, as participants would see it. None of the participants had any sound color associations comparable with that of a synesthete for the “Musical Pitch→Color” test. Data were collected via the synesthete.org website which transferred participant results directly to the researcher’s account on the www.synesthete.org site. It is important to note, that www.synesthete.org’s synesthesia battery tests were chosen because they allowed participants to pick out any color (including along all gradients) with the presentation of any letter or pitch, there are no predetermined colors chosen by the synesthesia battery test designed by Eagleman et al. (2007).

EEG Tasks

Synesthetic Stroop Task. In the Synesthetic Stroop Task, participants were placed approximately 60 cm from a computer screen and presented with black letters on a white screen with small six squares of color, as depicted in Figure 5, and asked to select a specified color for each letter (see Table 1), with each 2 of the 4 response pad buttons correlating to two colors (Button 2 = Yellow and Blue, Button 3 = Purple and Orange). Participants were provided with Table 1 prior to the start of the task. Additionally, on six small sheets of paper, with the color

(Red, Yellow, Blue, Purple, Orange, and Green) written at the top of each sheet, with the letters that correspond to each of those colors written below. For example, on the “Red” sheet, the letters written were A, G, M, S, Y. Each of these small sheets was then taped to a corner of the screen corresponding to the color’s location on the stimulus image (see Fig. 5). Each participant was given three blocks each with 52 images each with the letters presented in a randomized order to prevent order/memorization effects (see Fig. 5). Prior to the presentation of each letter stimulus, participants were either given a white number on a black background between 1 and 4 (with each number corresponding to a specific color, as Fig. 5 shows), or a left arrow colored white, on a black background (as a non-indicator stimulus). Participants were instructed that if a number was presented prior to stimulus presentation, that they should be ready to press that particular button (e.g., a “1” before stimulus onset meant that the color of letter will be red, and the participant should get ready to press button one). The images were created via Microsoft® PowerPoint®, and presented through the stimulus presentation software Stim2 created by Compumedics® Neuroscan™. Each image was presented to the participant for 500 ms with a 700 ms response window. This task was conducted twice, once as a pre-test prior to synesthesia training onset, and once post-synesthesia training onset (approximately one month after the pre-test).

Chromesthesia Task. In the Chromesthesia Task, participants were placed approximately 60 cm from a computer screen and presented with sounds and colors in the following order. They were given the following instructions for Block 1: A sound and two grids of color (Fig. 6, 7) would be presented simultaneously for one second. Participants were instructed to press button one if the sound heard matched one of the colors on the left-hand side of the screen (Fig. 6), and button four if the sound heard matched one of the colors on the right-

hand side of the screen (Fig. 7). All participants were asked to select the correct color (as defined by Table 2) as fast as possible after stimulus presentation. Each participant was given the correct colors for each sound prior to the start of Block 1 and again prior to the start of Block 2. There were a total of 96 trials presented to the participant (with the sounds presented twelve times, in a randomized order to prevent participants from memorizing the sequence).

In Block 2, participants were instructed that a sound would be presented for approximately a second, and would be immediately followed by eight images, each with a single color (in the order Red, Yellow, Blue, Green, Orange, Purple, Brown). They were instructed to Button 4 when the correct color flashed on screen. Due to time constraints on participants, there were only 40 trials within this block, with the sounds presented in orders different from those in Block 1. This task was conducted twice, once as a pre-test prior to synesthesia training onset, and once post-synesthesia training onset (approximately one month after pre-test).

Electrophysiological Recording. The Electroencephalogram (EEG) recording for this study was taken utilizing 64 Ag/AgCl electrodes (size 10 mm) arranged on an elastic cap (Neuro, Inc., El Paso, Texas). All impedance levels were kept below 10 k Ω for each electrode. The electrode position at AFz was utilized as ground, while the mastoid electrodes were utilized as references to remove non-brain signals/artifacts. Additionally, another reference electrode was placed between Cz and CPz.

The EEG data was then digitized, amplified and filtered in DC mode using a NeuroscanTM Synamps2 bioamplifier. In order to digitize the signal, an AC to DC converter was utilized at a sampling rate of 500 Hz in accordance with the cardinal theorem of interpolation (aka the Nyquist-Shannon Sampling Theorem). NeuroscanTM SCAN software (v. 4.5.1) recorded the EEG activity, and Stim2 was utilized for both stimulation and recording participant responses

during the Synesthetic Stroop and Chromesthesia tasks. EEG epochs began 100 ms prior to stimulus presentation, and ended 700 ms post response. In order to account for blinks, patients had an electrooculogram recorded via four electrodes: two were placed above each eye (lateral movement detection), and two were placed below each eye (detect vertical movement and blinks). EEG data were analyzed for blinks and other eye movements (such as saccades) and utilizing artifact analysis and removal tools, these blinks and saccades were removed from the data without affecting the EEG data significantly. Any trials in which there were other artifacts (e.g., the participant sneezed) had to be removed, as the results were no longer possible to use. These trials were analyzed using artifact rejection tools, and removed when necessary.

The amplitudes for the N1, and P3 were analyzed, as previous research has indicated that these signals are best to analyze for synesthesia research (Yamashita et al., 2010; Sinka et al., 2014; Beeli et al., 2007). The N1 and P3 amplitudes were determined for each participant by finding the average waveform of each participant in response to all stimuli in a given block (if present) in a given task, the auditory N1 was observed at Pz, since Pz is directly between both temporal lobes, and the sound is played from both sides of the participant. The visual N1 is also analyzed at Pz since the visual N1 component of interest originates from both the parietal cortex. Finally, the P3 was observed at Pz, as the P3 is parietally maximal. The visual N1 was analyzed between 80 ms and 170 ms at the occipital sites (as it was unclear what the visual N1's specific latency would be for the tasks in this study), while the auditory N1 was analyzed between 10 ms and 150 ms at temporal sites (both post stimulus presentation). The P3 was analyzed at from 250 ms to 600 ms at the parietal sites. Once the average waveform for each participant was found in the both the Synesthetic Stroop task condition, and the Chromesthesia task the results for the participants' post-test were compared to the pre-test results at Pz. The post-training EEG results

were compared with that of the known synesthetes to further determine whether synesthesia has truly been acquired, or whether an association has been formed between the grapheme and color, or between the sound and color.

Training Tasks

Grapheme-Color Synesthesia Training. Participants were provided with a PDF shared via Google Drive containing a modified version of the Grimm’s Fairy Tales, where the color of the lettering has been changed to match the colors described in Table 1. Participants were asked to read at least one story a day, for a minimum of three days a week (and a maximum of five days a week). Participation was monitored through email check-ins with participants once every two weeks. This training continued for 4 weeks after the pre-test EEG.

Chromesthesia Training. Participants were provided with two website links (provided prior to the references section), which took each participant to flat.io, a website which allows the creation of color-coded music sheets and plays a piano when the play button is pressed. The two music sheets transcribed were “High Hopes” by Panic! At the Disco and “Hedwig’s Theme” by John Williams (files are available as QR codes in the Supplemental Materials, see Fig. 8 and 9). Each participant was provided with the following instructions:

Press the play button on the screen with your volume turned up, and make sure to listen to the song. Once you are done listening to the song at least once (preferably twice), listen to it a third time with your eyes closed and do your best to recall the color for as many of the notes as you can. Do this a minimum of one time, but try and repeat the process for a second time to get yourself more comfortable with this training task. Feel free to listen to the song as many times as you want, but at a minimum try to listen to the song (or songs) for 20 minutes, identifying the sounds and colors (and notes) as best you

can. Please repeat the above process a minimum of three times a week and a maximum of five times a week.

Participation was monitored through email check-ins with participants once every two weeks. In total, between the Chromesthesia and Grapheme-Color synesthesia training, most participants completed each task an average of 10 times. Training continued for participants until they returned for their post-test, approximately four weeks from their pre-test EEG.

Statistical Analysis

Once ERP data were collected for all participants at the end of training, the average waveforms were found using the Neuroscan software. Once the average latency and amplitude was determined at site Pz for all three components (visual and auditory N1, and P3b) for each participant. These data were compared using a paired samples t-test analysis, conducted through the software IBM SPSS Statistics 25, between the pre- and post-test results at Pz sites for both tasks. The visual N1 and auditory N1 are separated by tasks (synesthetic Stroop task and chromesthesia, respectively). Grand average waveforms were found for the pre- and post-tests group of participants in order provide a simpler, more visual look into the data.

Results

Synesthetic Stroop Task Results

A paired samples t-test revealed that there was not a significant difference between the pre-test results ($M=1.53$, $SD=2.57$) as compared with the post-test results ($M=1.01$, $SD=2.29$) ($t(14)=1.20$, $p=.251$) at the Pz site for the visual N100 component. These results indicate that there is not a significant difference in how the participants registered (i.e., identified) the visual stimuli presented to them. A second paired samples t-test revealed that there was a significant difference between the pre-test results ($M=2.01$, $SD=3.52$) as compared with the post-test results

($M=-0.27$, $SD=2.07$) ($t(14)=2.26$, $p=.040$) for the P3 at Pz. These results indicate that participants required less cognitive effort to comprehend the stimuli presented to them in the Synesthetic Stroop Task post-training. Figure 10 provides a look at the grand average waveform among all participants for both the pre- and post-training ERP results. Figure 11 provides a bar graph visualization of Figure 10 and the above statistics, to help compare the mean microvoltage changes pre- and post-training for both the N1 and P3 components during the Synesthetic Stroop Task.

Chromesthesia Task Results

A paired samples t-test revealed that there was not a significant difference between the pre-test results ($M=0.55$, $SD=2.57$) as compared with the post-test results ($M=-0.55$, $SD=1.73$) ($t(14)=1.542$, $p=.145$) at the Pz site for the auditory N100 component. These results indicate that there is not a significant difference in how the participants registered (i.e., identified) the auditory stimuli presented to them. A second paired samples t-test revealed that there was a significant difference ($p<0.05$) between the pre-test results ($M=2.68$, $SD=3.10$) as compared with the post-test results ($M=0.98$, $SD=1.92$) ($t(14)=2.69$, $p=.018$) for the P3 at Pz. These results indicate that participants required less cognitive effort to comprehend the stimuli presented to them in the Chromesthesia Task post-training. Figure 12 provides a look at the grand average waveform among all participants for both the pre- and post-training ERP results. Figure 13 provides a bar graph visualization of Figure 12 and the above statistics, to help compare the mean microvoltage changes pre- and post-training for both the N1 and P3 components during the Chromesthesia Task.

Discussion

The current study examined whether it is possible for non-synesthetic adults to obtain a synesthetic experience through training as indicated by Bor et al. (2014), as the very neurological nature of synesthesia seems to indicate that non-synesthetes should not be able to “learn” synesthetic experiences. A synesthetic experience, by its very nature is an experience, which once obtained or learned should not be readily lost—based on the current understanding of synesthesia (Jänke, 2007). Instead, it is far more likely that participants were forming learned associations between the graphemes and colors, and sounds and colors upon which they were trained. Because the “synesthetic experience” which Bor et al. (2014) claimed to have given non-synesthetic adults, faded away approximately two months at the conclusion of the study.

Anecdotally, one participant in the current study mentioned that they had begun to associate the color orange with high-pitched sounds due to their training. Upon asking the other participants whether they too had begun to associate letters and colors or sounds and colors together, none of the other participants mentioned that they were able to do so.

Based on the results for both the Synesthetic Stroop and Chromesthesia Tasks, the key finding is that the participants became much better at the tasks they were given, because their brains put less cognitive effort into processing the stimuli they were presented with, as indicated by the significantly reduced P3 component. Since the P3b subcomponent of the P3 is often used to determine the cognitive workload (Kok, 2001) and a person’s response to unexpected stimuli, the smaller the P3b amplitude is the less, the more unexpected the stimuli are (Kok, 2001). The P3a on the other hand is usually elicited to novel stimuli, the less novel the stimulus, the smaller the P3a amplitude (Luck 2005). In the data, Figures 8-11 demonstrate that the lower P3 amplitude (and therefore also a lower P3b and P3a amplitude) shows that after a month of training for both tasks, the stimuli ceased to seem improbable or novel for the participants. A

learned association essentially means someone that someone associates to unrelated stimuli together, until it is no longer surprising them to associate them together. However, the lack of significant difference between the auditory and visual N1 component even after training, is indicative of the participants not registering or identifying the stimuli any differently than they did during the pre-test. Because both the auditory and visual N1 components are ERP components that show one's ability to identify auditory or visual stimuli, respectively (Luck, 2005). If the participants had gained synesthetic experiences after training, then not only should they have a greatly reduced (or even nonexistent) P3b—as the participants in the current study had—they should have also been significantly better at identifying the stimulus itself. Better identification of a stimulus is key to synesthesia because a synesthete's identification of a stimulus as being linked to different sensory modalities is the basis of synesthesia itself (Hubbard, 2007; Hubbard & Ramachandran, 2005; Schweizer et al., 2013).

An important question that one may ask is why this study provides no behavioral data. The main reason is that the buttons pressed by the participants were mainly to keep them focused on the Synesthetic Stroop and Chromesthesia tasks. Reaction time, and correct or incorrect button presses do not matter in understanding the nature of synesthesia, as much as understanding a participant's ability to identify or process a stimulus. As a result, there was no need to present any behavioral measures.

None of the results of the current study are currently able to inform about which hypothesis: the neonatal, neural pruning, or local cross-activation model is more correct about the nature of synesthesia. The current results also show that, as tested in this study, synesthesia is not a mainly learning phenomenon. A replicated study looking at either topography or utilizing functional Magnetic Resonance Imaging (fMRI) might help to tease out what hypothesis of

synesthesia's origins is more likely the correct one (or even remove one of the hypotheses from consideration), and potentially lend credence to the idea of hyperconnectivity which Zamm et al. (2013) describes.

The current study does affirm the notion that synesthesia is, in fact a neural phenomenon, and that non-synesthetic adults cannot develop synesthesia or synesthetic experiences after a lengthy training period (in this case four weeks), which would have lasted longer than a learned association. The results of this current study, while they do somewhat counteract the claims put forth by Bor et al. (2014), are also built upon their research by looking at a neural analysis of the data, instead of using behavioral measures and self-reports to determine whether participants had synesthetic experiences.

Limitations and Future Directions

There were several limitations in this study that may have affected the procedure and outcomes of this study. First, the number of participants in this study were relatively low, compared to Bor et al. (2014)—this study had 15 total participants, where Bor et al. had 33 participants. Second, the amount of time that was available to conduct this study was limited due to the academic Spring Semester, and so it was only possible for training to occur over a month, instead of the three months used by Bor et al. (2014). Another limitation is the available EEG caps being used were relatively old, and as a result, they did not always work as expected. Should this study be replicated with both more participants, and greater time between pre- and post-testing, it would strengthen the findings of the current study and reduce the mentioned limitations. Additionally, one could also run the study utilizing fMRI or magnetoencephalography (MEG) technology to help monitor the learning (or potential synesthetic) changes in non-synesthetes. Additionally, the current study could also be replicated

with significantly more trials for the Synesthetic Stroop and Chromesthesia Tasks (e.g., around 500 plus trials for each task) to help clear out some of the noise in the current data, and reduce the large error bars seen in the Bar graphs (Fig. 9, 11).

This study is also limited by the technology available to train participants for synesthetic experiences in chromesthesia. A future study could also utilize technology such as p5.js to program a website to flash colors on demand with particular sounds. This was not possible in the current study, because no such technology has yet been designed and the expected timeline for development was longer than it took to complete the current study. In the future, it would also be much more preferable to bring in true synesthetes. Their participation in a study similar to the current one, could help to further corroborate the idea that synesthesia cannot be trained.

Summary

The overall findings of the current study are contrary to the results previously uncovered by a similar study (Bor et al. 2014). The current findings also help to expand the literature studies on how ERPs can be used to study and monitor synesthesia—something which is relatively limited in the current available literature. Taken together these findings indicate that synesthesia is not only a neural phenomenon, but also one that, under the conditions of the current study, is more likely to create a learned association rather than synesthetic experiences, if non-synesthetes are trained on synesthesia based tasks.

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Supplemental Materials:

Table 1	
<i>Letters and their Corresponding Colors for the Synesthetic Stroop Task</i>	
<u>Letter</u>	<u>Color</u>
A	Red
B	Yellow
C	Blue
D	Orange
E	Purple
F	Green
G	Red
H	Yellow
I	Blue
J	Orange
K	Purple
L	Green
M	Red
N	Yellow
O	Blue
P	Orange
Q	Purple
R	Green
S	Red
T	Yellow
U	Blue
V	Orange
W	Purple
X	Green
Y	Red
Z	Yellow

Table 1

Table 2		
<i>Musical Pitches, their Corresponding Frequencies and Colors for the Chromesthesia Task</i>		
<u>Musical Pitch</u>	<u>Frequency (Hz)</u>	<u>Color</u>
C ₄	261.63	Red
D ₄	293.66	Yellow
E ₄	329.63	Blue
F ₄	349.23	Green
G ₄	392	Orange
A ₄	440	Purple
B ₄	493.88	White
C ₅	523.25	Red

Table 2

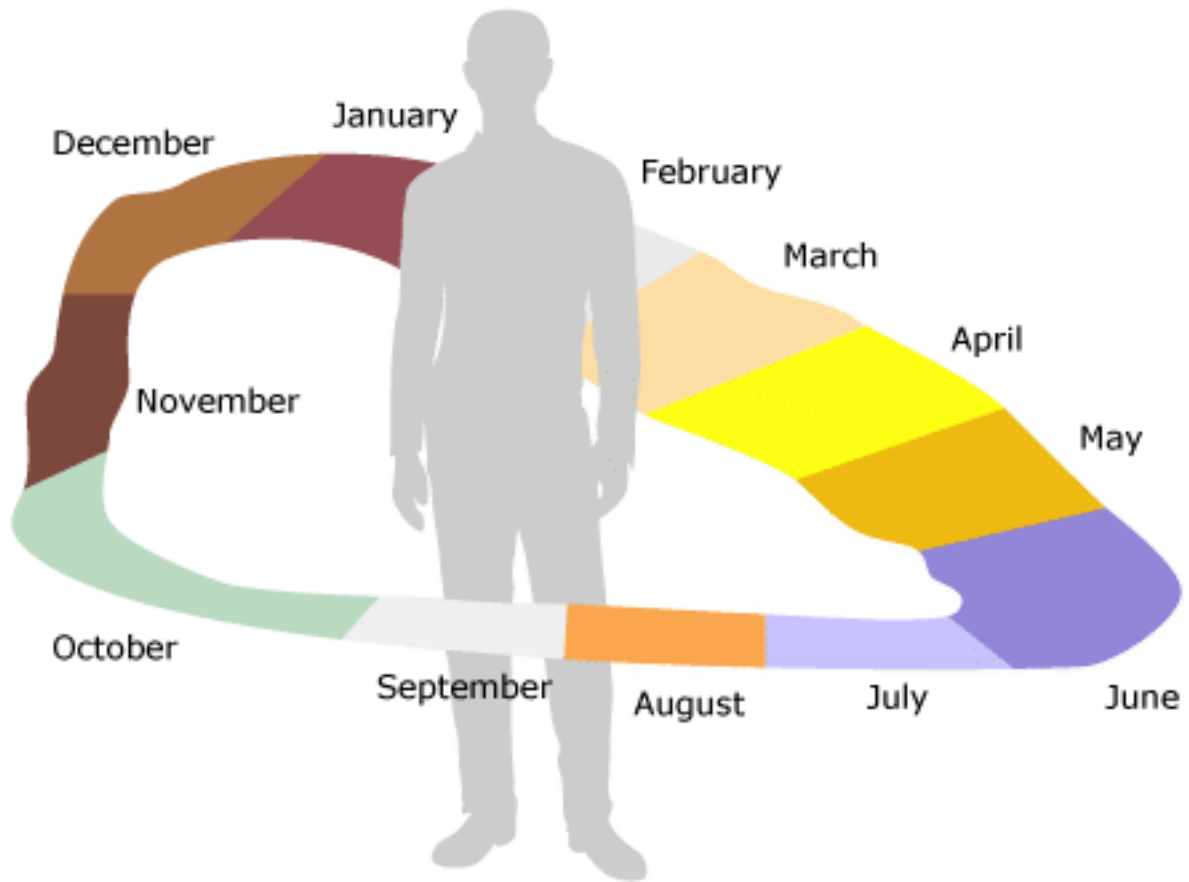


Figure 1. Image of a potential temporo-spatial synesthete's visualization of his/her synesthesia. (Gill, 2009)

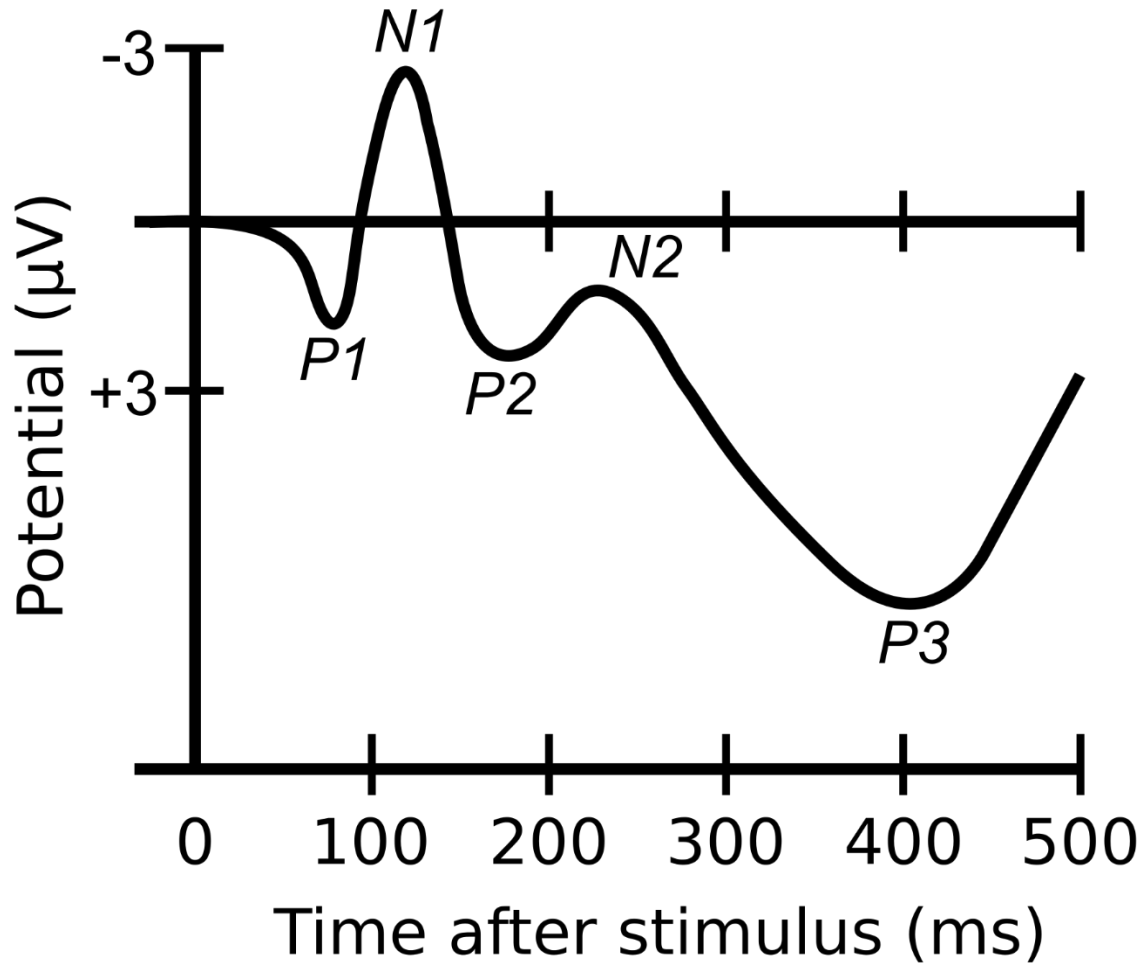


Figure 2. A simplified example of a typical EEG waveform showing the P100, N100, P200, N200, and P300 ERP components. (An EEG waveform...)

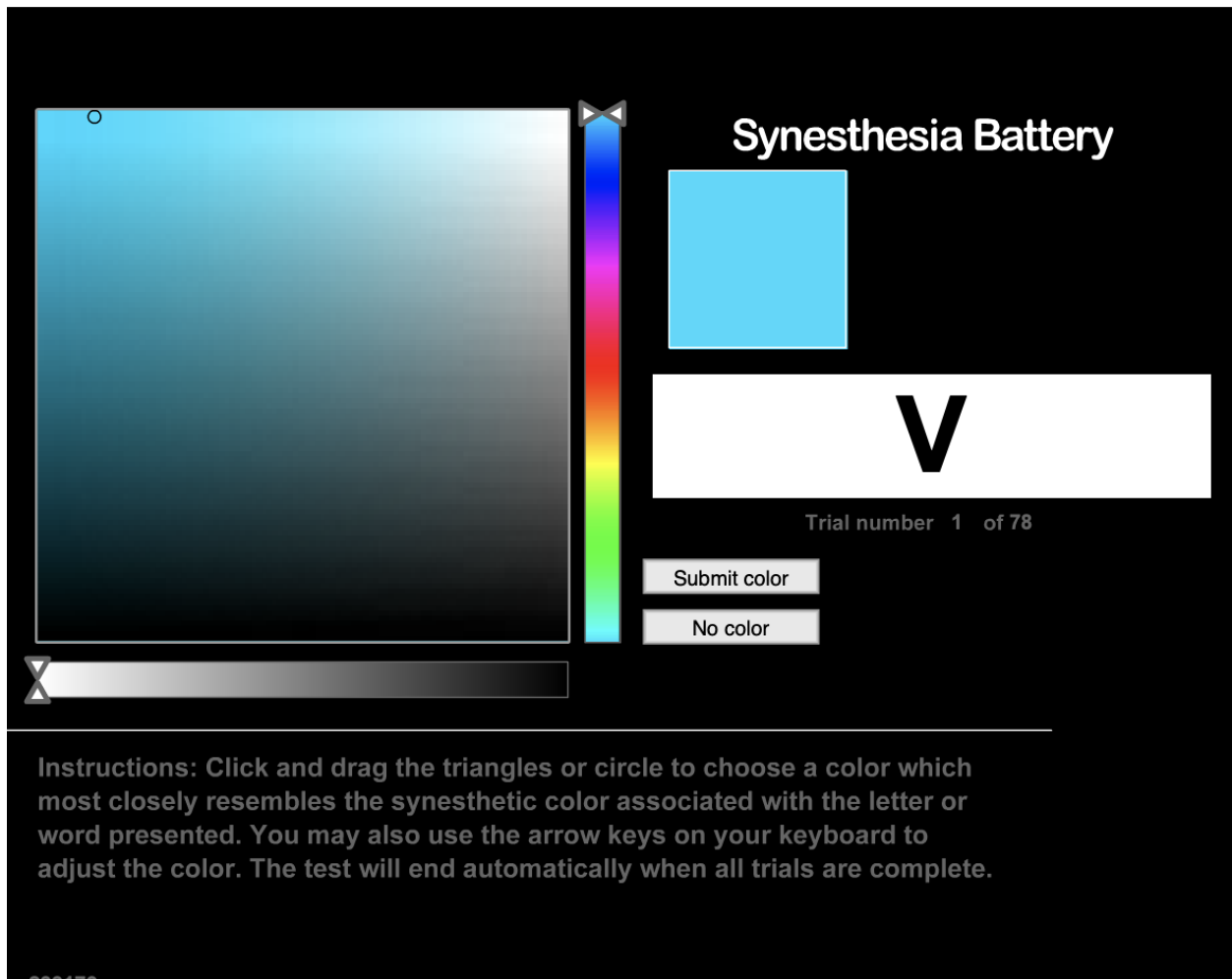


Figure 3. *Synesthetic Stroop Task Instructions.* This is a screen shot from synesthete.org's "Letter→Color" Task (Eagleman et al., 2007). This image shows both the instructions the participant was given, as well as a sample of the color gradient that the participants can use to select.

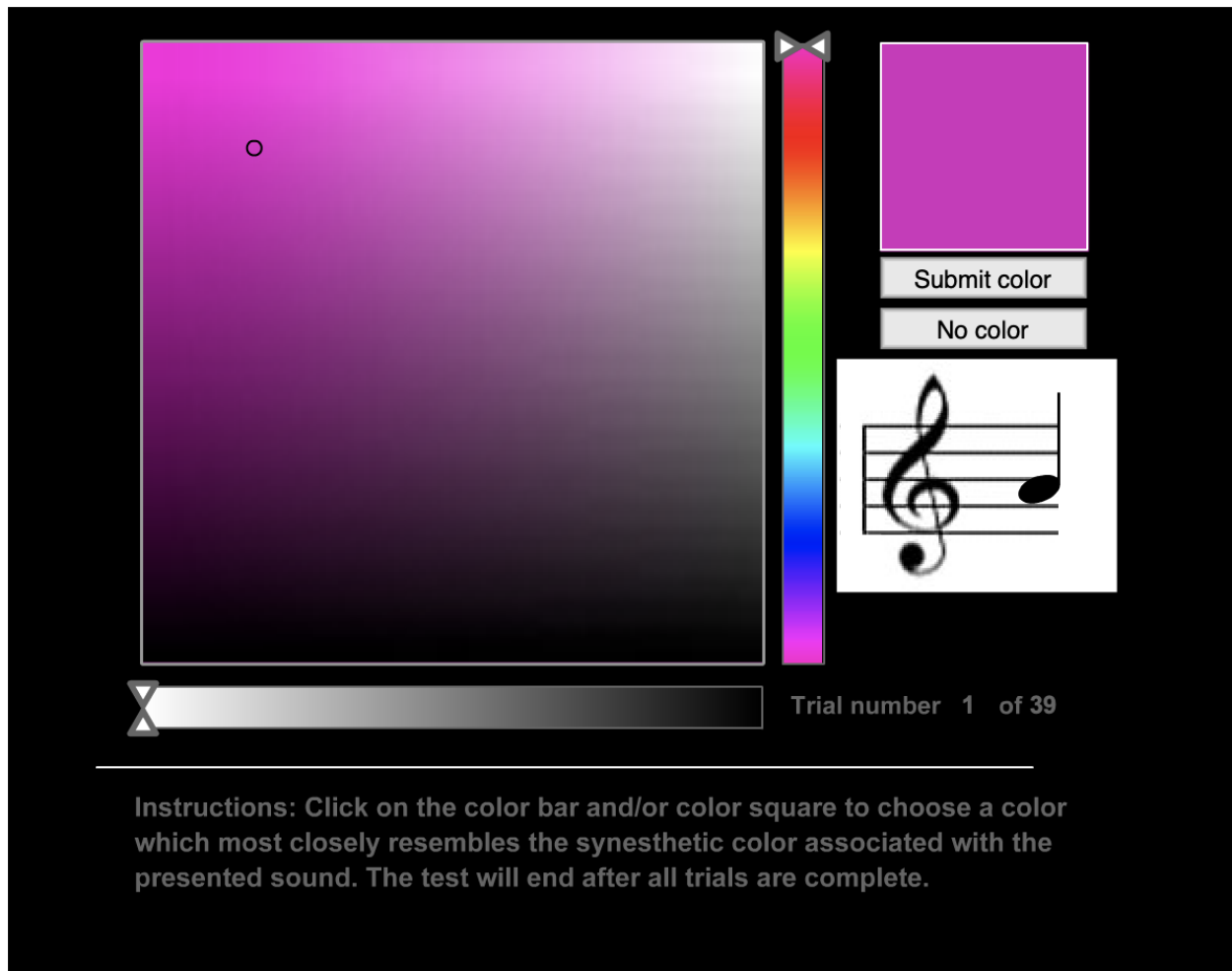


Figure 4. *Synesthetic Stroop Task Instructions.* This is a screen shot from synesthete.org's "Musical Pitch→Color" Task (Eagleman et al., 2007). This image shows both the instructions the participant was given, as well as a sample of the color gradient that the participants can use to select.

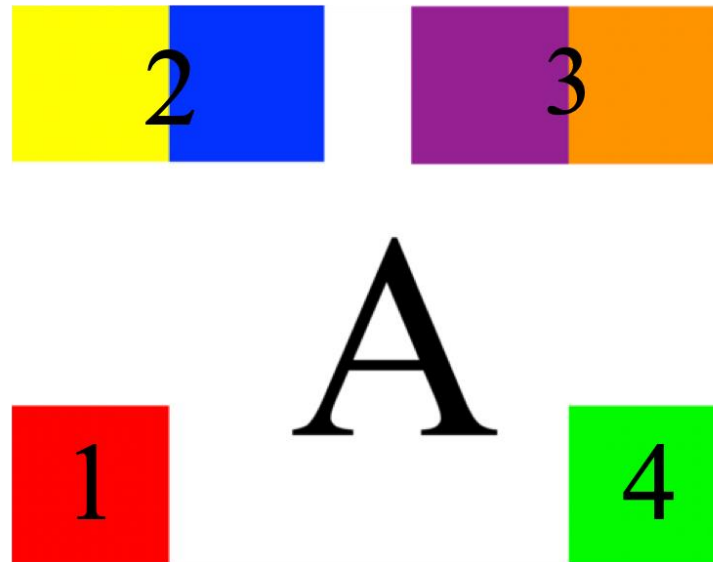


Figure 5. *Example Image of a Letter During The Synesthetic Stroop Task.* Each letter was presented as above (without the presence of numbers on each color rectangle). The above numbers indicate which color(s) was associated with each button on the response pad.

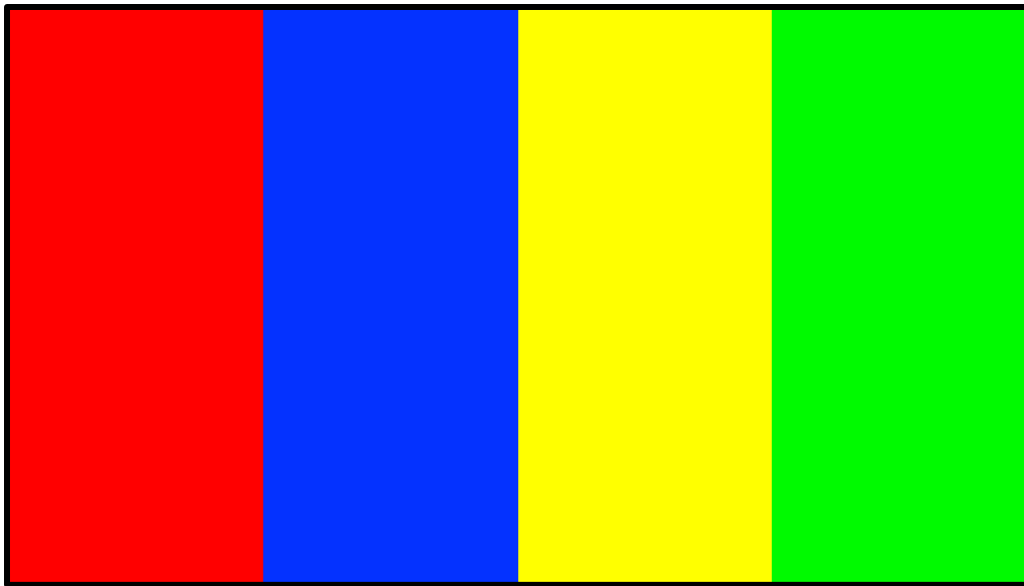


Figure 6. An image of multiple colors, presented on the computer screen to the participants. The participants were asked to press button 1 for red (musical note C₄ and C₅), button 2 for blue (musical note D₄), button 3 for yellow (musical note E₄), and button 4 for green (musical note F₄).

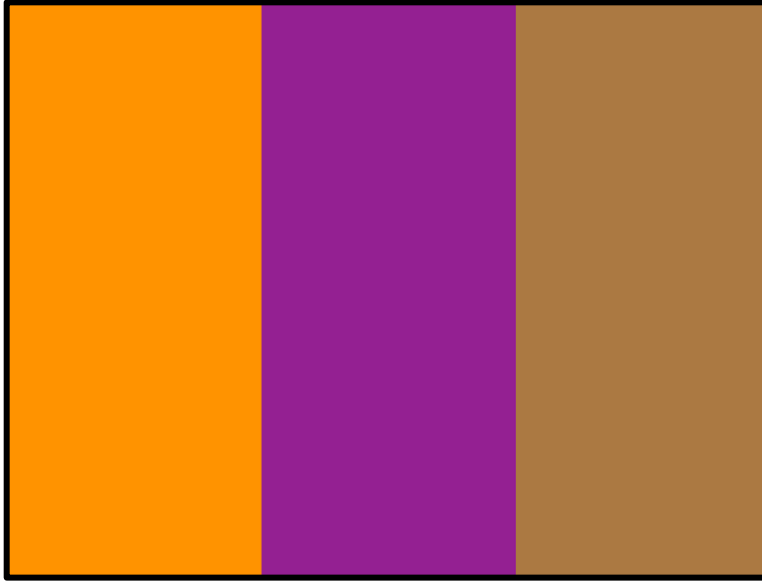


Figure 7. An image of multiple colors, presented on the computer screen to the participants. In Block 1: The participants were asked to press button 4 for any coll for orange (musical note G₄), button 2 for purple (musical note A₄), and button 3 for brown (musical note B₄).



Figure 8. QR Code for High Hopes. By accessing through a code scanner application, this will take the accessor to the flat.io page that contains the color-coded music sheet for High Hopes by *Panic! at the Disco*



Figure 9. QR Code for High Hopes. By accessing through a code scanner application, this will take the accessor to the flat.io page that contains the color-coded music sheet for High Hopes by *Panic! at the Disco*

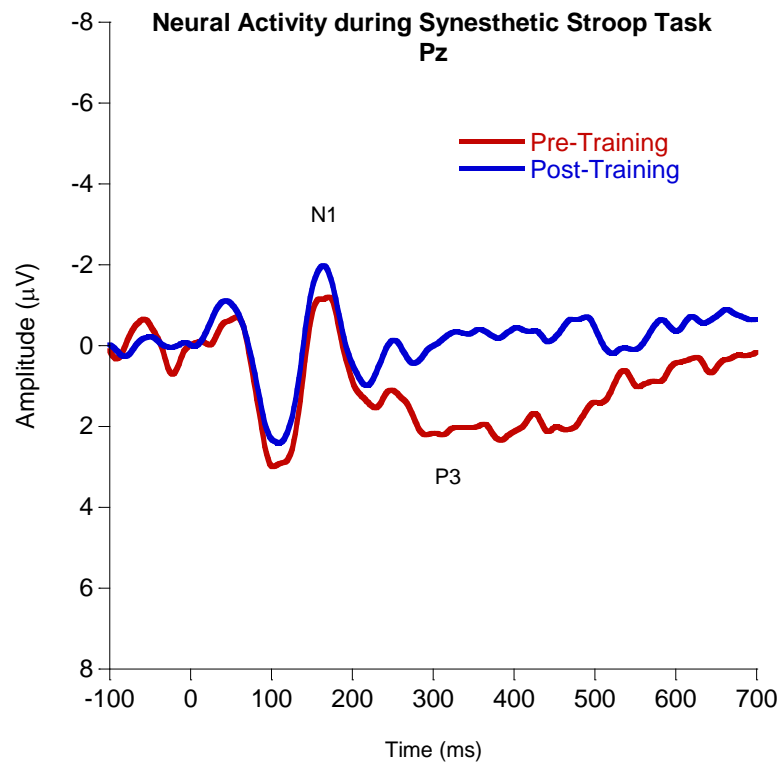


Figure 10. Grand-averaged stimulus locked N1 and P3b waveforms at Pz during the Synesthetic Stroop Task, both pre- and post-training. There is a non-significant difference for N1 pre- and post-training, but a significant difference between the pre-training P3 and post-training P3.

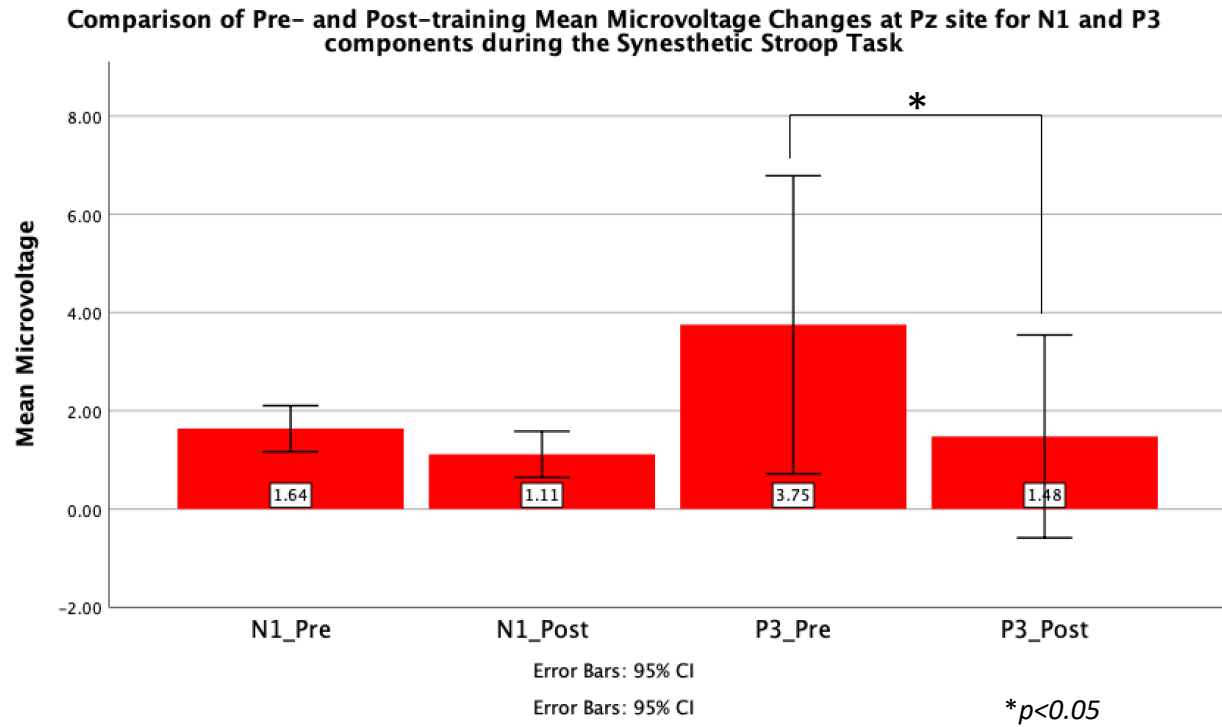


Figure 11. Comparisons of the mean microvoltage amplitude for the N1 and P3 components at Pz during the Synesthetic Stroop task. $p=0.040$

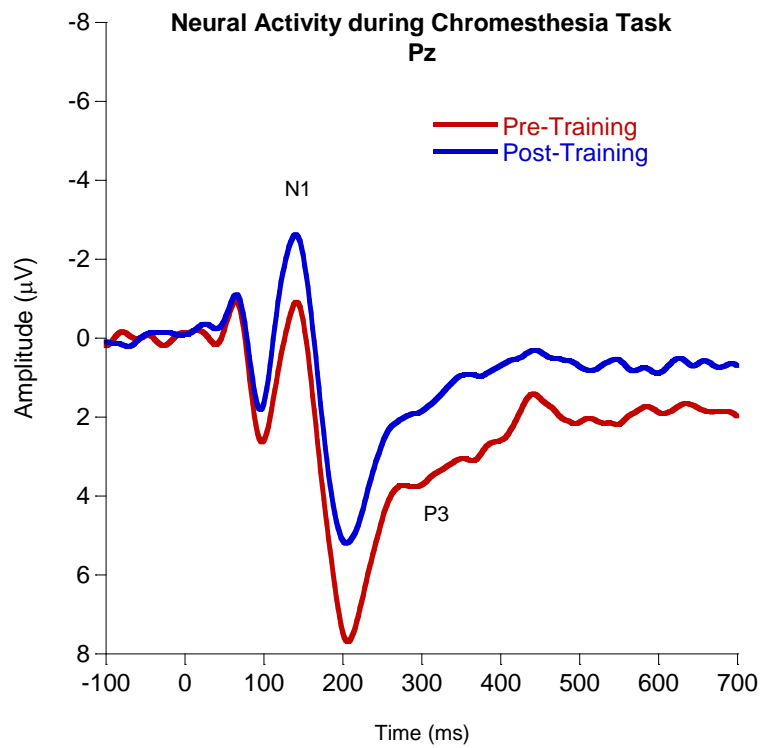


Figure 12. Grand-averaged stimulus locked N1 and P3b waveforms at Pz during the Chromesthesia Task, both pre- and post-training. There is a non-significant difference between the average amplitude (μV) for the N1 pre- and post-training, but a significant difference between the average amplitude (μV) pre-training P3 and post-training P3.

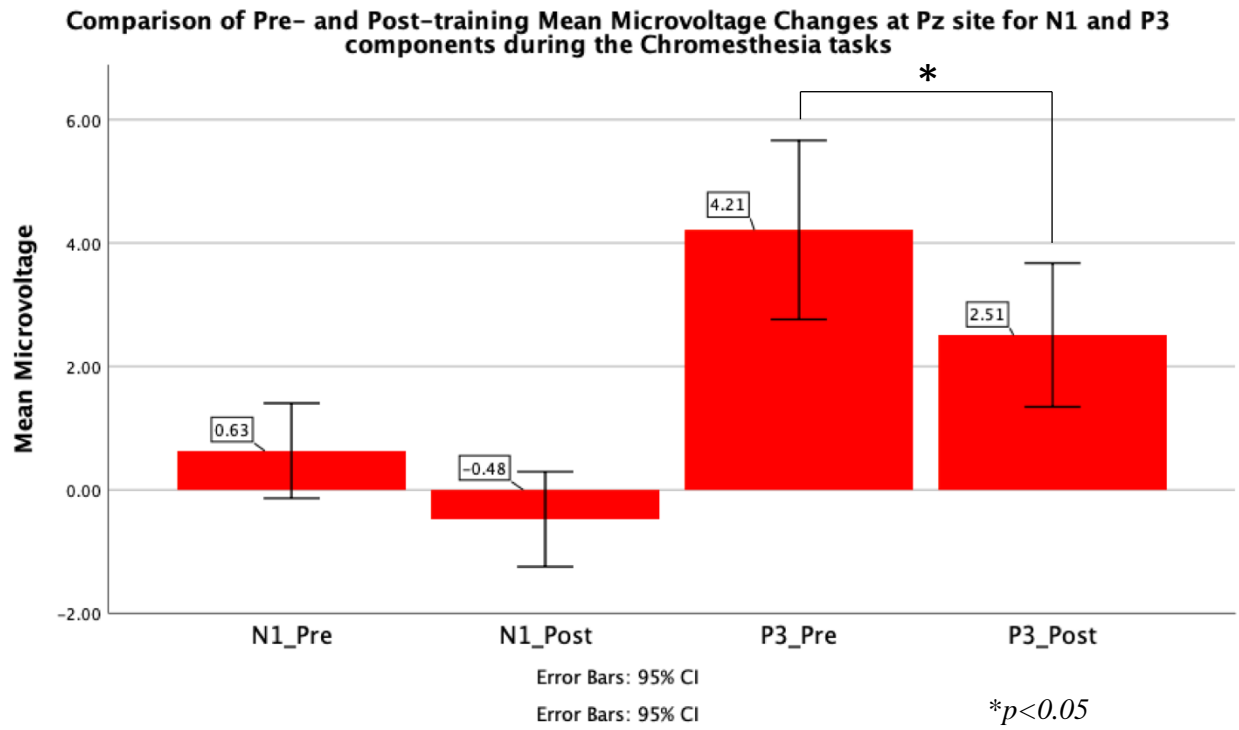


Figure 13. Comparisons of the mean microvoltage amplitude for the N1 and P3 components at Pz during Chromesthesia task. $p=0.018$

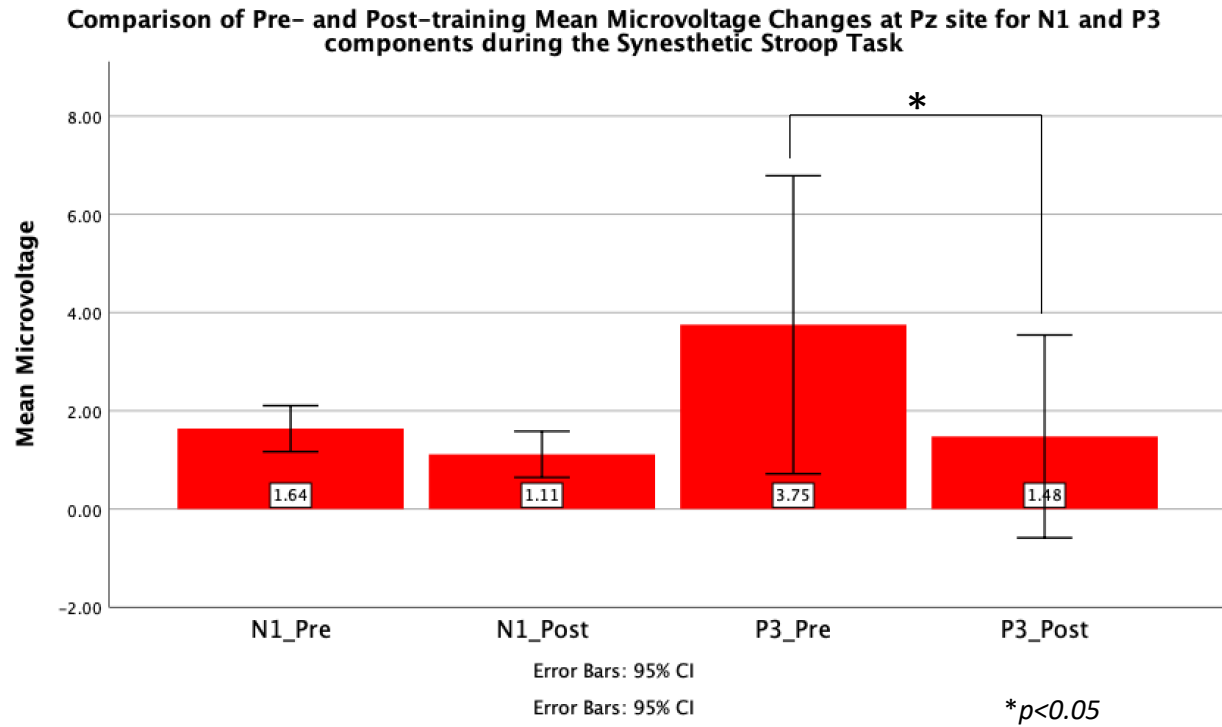


Figure 11. Comparisons of the mean microvoltage amplitude for the N1 and P3 components at Pz during the Synesthetic Stroop task. $p=0.040$

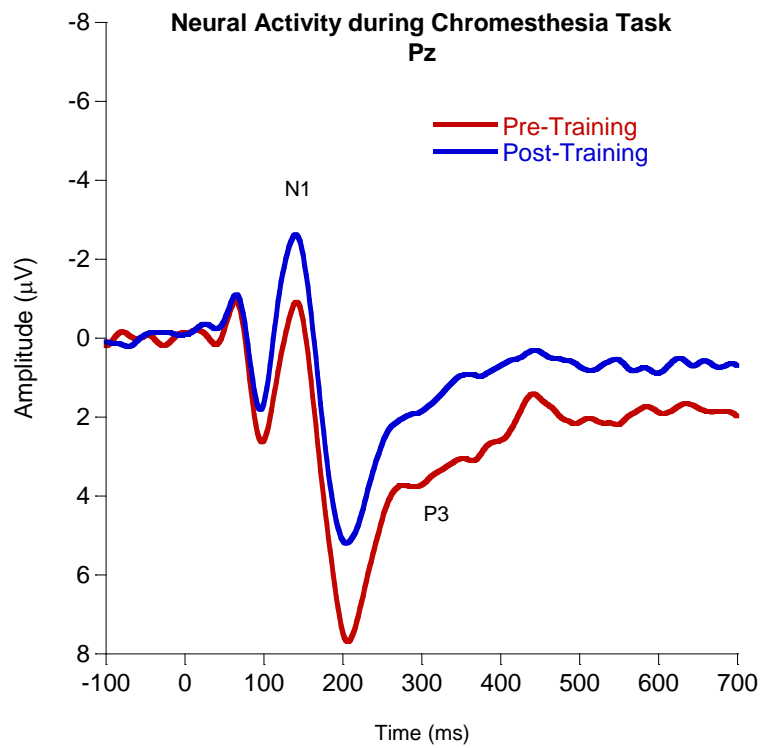


Figure 12. Grand-averaged stimulus locked N1 and P3b waveforms at Pz during the Chromesthesia Task, both pre- and post-training. There is a non-significant difference between the average amplitude (μV) for the N1 pre- and post-training, but a significant difference between the average amplitude (μV) pre-training P3 and post-training P3.

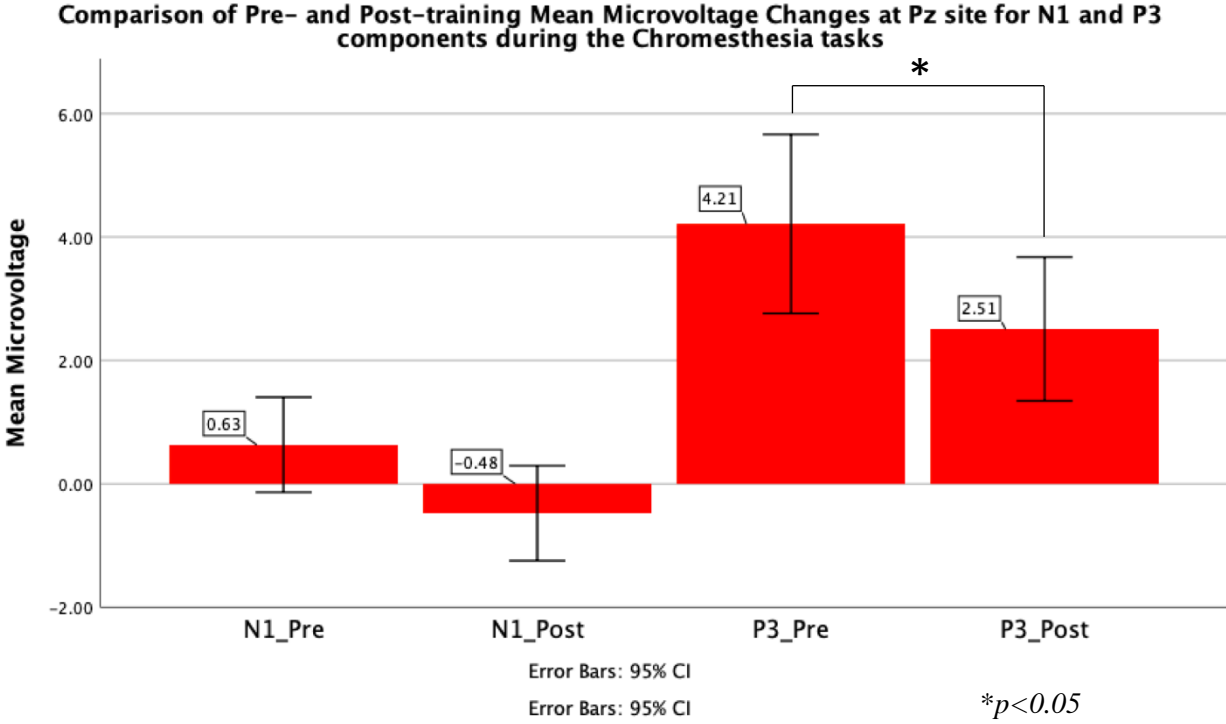


Figure 13. Comparisons of the mean microvoltage amplitude for the N1 and P3 components at Pz during Chromesthesia task. $p=0.018$