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Practice Makes Perfect: An ERP Analysis of the Effects of Physical Practice on Cortical Signal As Evidenced by the N500

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Practice Makes Perfect: An ERP Analysis of the Effects of Physical Practice on Cortical Signal as Evidenced by the N500

A thesis submitted in partial fulfillment of the requirement for the degree of Bachelors of Science in Neuroscience from The College of William and Mary

by

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Practice Makes Perfect: An ERP Analysis of the Effects of Physical Practice on Cortical Signal As Evidenced by the N500

Jessica Marie Van Sciver

The College of William and Mary
Abstract

The benefits of motor and mental practice on physical performance have been identified for decades. Here, we used event-related potentials to examine the precise effect of motor practice on the cortical signal during physical performance. Participants were scanned before and after learning, having practiced a particular script in between these two sessions. All participants learned a particular hand sequence on their first scanning session and were later scanned again when performing a second hand sequence. The difference between the participant-groups was the hand sequence practiced in between the two scanning sessions. The N500 was examined for both pre and post scans for all participants across the frontal (F3, Fz, F4), frontal-central (FC3, FCz, FC4) and the central (C3, C4, C5) electrodes. Our hypothesis was that there would be no significant difference across the various electrode sites during the N500 between the pre and post-practice sessions for those who were tested twice on novel tasks but that there would be a significant difference for those who had practiced a task for a few days. There was a significant overall main effect of time for pre versus post scan sessions at the N500. There was also a significant interaction between time and group. Our results suggest that the group who had practiced the hand sequence tested at the post session revealed significantly different N500 deflection compared to those who had practiced a different hand sequence. Thus, the physical practice affected the cortical signal, namely it resulted in the presence of the N500.
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Practice Makes Perfect: An ERP Analysis of the Effects of Physical Practice on Cortical Signal as Evidenced by the N500

The cognitive mechanisms and neural components implicated in motor learning has become an increasingly investigated topic, and for good reason. Despite the many studies that have been conducted on this subject, a number of questions regarding the nature of motor learning remain. For example, how precisely, at the cortical level, does motor practice improve performance? The present study seeks to examine this question using electromyography (EEG) measurement of event related potential (ERPs) to characterize changes in cortical activity that occur as a function of motor practice.

Participants were scanned before (pre-practice) and after (post-practice) learning hand sequences; one group became experts on the same sequences tested in the post session, the other group became experts on a different set of hand sequences. We hypothesized unique cortical responses in the post session; specifically, a differential activation for the group that had become experts in the task. This activation, essentially, may serve as a marker for motor consolidation.

Motor learning and resultant response shifts

Most investigators agree that there are two distinct stages of motor learning: the early stage which involves acquisition and the later stage which involves consolidation. Acquisition is characterized as being the fast process that has poor retention and requires more attention while consolidation is known as the slow process that has better retention yet requires little to no attention and can be performed automatically (Hadipour-Niktarash, Lee, Desmond, & Shadmehr, 2007). Others propose that motor learning involves three
stages rather than just two: early, intermediate and late (Mancini, et al., 2009). The early and the later stages are similar to those just described but the intermediate stage involves an overall increase in performance and accuracy without an increase in automaticity-- a feature that remains specific to the late stage.

A shift in cortical signal is essentially anything that has changed in the brain due to the learning of a new motor task. The shift can occur in a variety of areas, including primary motor cortex, premotor cortex, supplementary area cortex, the basal ganglia and the cerebellum (Ziemann, Iliac, Pauli, Meintzschel, & Ruge, 2004). Changes in these motor areas that result from learning include plasticity (Ziemann et al., 2004; Rosenkranz, Kacar, & Rothwell, 2007; Kreitzer & Malenka, 2008), synaptogenesis (Kleim et al., 2004), protein synthesis (Luft, Buitrago, Ringer, Dichgans, & Schulz, 2004), blood flow, and the development of an internal model (Shadmehr & Holcomb, 1997). Moreover, these changes vary in their time of onset (early vs. late stage of motor learning) and in their location within the motor-associated areas of the brain. Nevertheless, their appearance during the learning of a new motor task serves as an indicator that the task is being acquired and consolidated within the brain.

Usually within a discussion of plasticity following a motor task, LTP/LTD (Long-Term Potentiation/Long-Term Depression) mechanisms are considered. However, LTP/LTD may not be the only form that plasticity can take on. Plasticity could also involve the activation of preexisting synapses or a change in excitability in postsynaptic neurons, seen mostly in the primary motor cortex during motor learning (Ziemann et al., 2004). The striatum, which includes the caudate and the putamen, is located within the basal ganglia and has also been shown to exhibit plasticity during motor learning.
Specifically, the dorsomedial striatum seems to be important in early motor learning since LTP is seen here whereas the dorsolateral striatum appears to be involved more in long-term motor memory (Kreitzer & Malenka, 2008). A recent study by Rosenkranz involved training subjects on a thumb abduction task over a period of five days. LTP was induced on days 0, 1 and 5 using paired-associative stimulation (PAS) of the median nerve and motor cortex. On day 0, one week before the start of the experiment, they induced LTP using PAS. On day 1 (one week later) when the task was performed by the participants, the researchers found that the LTP that was induced the week prior had actually reversed and LTD-like plasticity had proliferated even though they saw within just one session that the participants performance on the task had improved. On the last day of the experiment, the investigators saw that both LTP and LTD-induced changes had actually reverted back to pre-experimental levels. The authors suggest that the reversal of LTP seen on day 1 was a result of synaptogenesis. They have stated that LTP is involved in early learning (acquisition) and it serves to strengthen already existing synapses. However, as learning continues (consolidation) synaptogenesis takes over and makes new connections related to this task thereby allowing the LTP to be reduced (and eventually LTD) back down to pre-practice levels since they are no longer needed. The authors concluded that plasticity occurs transiently throughout the early stages of learning and is replaced by synaptogenesis for long-term storage of the task (Rosenkranz et al., 2007).

As the previous study has shown, it is almost impossible to talk about plasticity without having to talk about synaptogenesis since the appearance of one usually predicts the appearance of the other. It has already been established that early learning involves LTP/LTD whereas late learning involves synaptogenesis. During the learning process, the
associated cortical areas must reorganize in order to support what has just been learned. This reorganization is dependent upon the brain’s ability to form new synapses. This occurs during the consolidation of a motor task, which is a much slower process. Data has indicated that the generation of new synapses result in increased synapse number within the cortical area involved in the specific motor task (Kleim et al., 2004). Accordingly, this number is variable and can increase further or decrease depending on what motor skills are being used. The timing aspect of reorganization in this manner appears to make sense since the forming of new synapses would require more time than shuttling receptors to pre and post-synaptic neurons. These differing mechanisms are indicative of the brains ability to allow us to begin acquiring information almost immediately, rather than having to wait for the formation of synapses to begin to learn a new task.

The synthesis of proteins within the motor cortex has also been shown to be important in motor skill learning. For example, it has been found that the interruption of protein synthesis within the motor cortex interrupted motor learning (Luft et al., 2004). And, these results were dependent upon the learning phase; the interruption of protein synthesis during the late phase of learning did not reveal the same disruption as that which resulted from disruption during the early phase, indicating protein synthesis is involved in acquisition and not consolidation of motor skill learning (Luft et al., 2004).

Changes in blood flow during the acquisition of a motor skill, specifically around the synapses, also play an important role in motor skill learning. Within one session of practice, an increase in blood flood has been observed in the sensorimotor cortex and the putamen. However, when participants returned for their second practice session, five and
a half hours later, there was actually a decrease in blood flow in these areas (Shadmehr et al., 2007). This learning effect essentially reveals a decrease in the amount of resources needed for the performance of the task after motor learning had occurred. When researchers then compared blood flow between the second practice session and the participant’s recall of the task, they observed an increase in blood flow in several other areas: left posterior parietal cortex and the left dorsal premotor cortex. These changes were a result of recalling the motor task, for when a comparison was made between blood flow during session 1 and blood flow during practice session 2, there appeared to be no significant changes.

The authors interpret their work as evidence of the development an internal model (IM) of the task (Shadmehr et al., 1997). An internal model (IM) is representational map of a movement as a whole which includes its trajectory and muscle strength. One useful example of an internal model would be a task as simple as picking up a bottle of full milk, as expected; the muscle movements are appropriately adjusted and we are successful when the bottle is actually full of milk and not empty but painted white as if it had been full of contents. IM’s are developed upon our first practice with a movement, but continue to develop throughout time and with additional practice. The development of an IM involves a change in cortical signals because it involves reorganization of the brain, as previously discussed. It is reasonable to assume that every task that one learns, from simple to complex, has its own IM and its own set of cortical networking, with some areas of overlap.
The effect of matched and mismatched practice on motor learning

Looking at the effects of matched versus mismatched practice has proven to be a valuable tool in the study of motor learning. Matched practice constitutes practicing a particular motor skill throughout all sessions whereas mismatched practice involves practicing one motor skill and then practicing another motor skill within a relatively short amount of time (usually up to 24 hours). The stages of motor learning, acquisition and consolidation, proceed naturally in matched practice paradigms. It is in these paradigms that we learn how normal motor learning occurs. This is exemplified in any athlete who practices certain skills in order to embed them so that attention to their movements is no longer required. Motor skill acquisition and consolidation are dependent upon time. Within one session of practice, improvements can be seen in the first practice session alone (Classen, Liepert, Wise, Hallett, & Cohen, 1997). The importance of time dependence is shown more concretely with mismatched practice for in matched practice paradigms, all practices lead to acquisition and consolidation even when only given a few minutes in between practices (Muellbacher, Ziemann, Wissel, Dang, Kofler, Facchini et al., 2002).

Mismatched practice involves practicing one motor skill and then practicing another motor skill within a certain amount of time. The time between the performance of one task and the performance of a new task appears to be an important factor in the acquisition and consolidation of the two motor skills. Throughout various studies, it has been shown that the consolidation of the first motor task can be disrupted if the practice of a second motor skill immediately follows, a process known as retrograde interference (Brashers-Krug, Shadmehr, & Bizzi, 1996; Muellbacher et al., 2002; Krakauer, Ghez, &
Ghilardi, 2005; Criscimanga-Hemminger, Shadmehr, 2008; Song, 2009). However, stable learning of both tasks can be obtained if a minimum of four hours is spaced between the two tasks (Brashers-Krug et al., 1996; Muellbacher et al., 2002; Song, 2009).

The timing between the consolidation of a previous task and acquisition of a new task appears to have critical periods. When researchers trained participants on task A and then immediately trained participants on task B with no break in between tasks, it was found that not only was performance on task B much worse than on task A, but when these participants were tested on their performance on task A there appeared to be no difference between days 1 and 2 (Brashers-Krug et al., 1996). These findings demonstrate that task A was not consolidated because it was interrupted by the training of task B. Also, a phenomenon known as negative transfer, prior exposure to one task causes worsening on a similar task, caused task B not to be acquired. However, there appeared to be no interference of consolidation of task A if four hours passed between the practice of task A and that of task B (Brashers-Krug et al., 1996). In a similar study, it appears that after a four hour separation between tasks A and B, consolidation of both tasks were allowed to develop (Criscimanga-Hemminger et al., 2008).

Not all types of motor learning exhibit retrograde interference. It may be that certain motor tasks, such as visuomotor tasks exhibit a different kind of interference. A study involving mismatched practices used a visual stimulus that was rotated at different angles to which participants were instructed to manipulate using a hand-held device in order to reach a specific target on a computer screen (Krakauer, Ghez, & Ghilardi, 2005). The first task utilized this paradigm with the visual stimulus rotated at a 30° angle whereas the second task involved a counter-rotation of the same visual stimulus.
Interestingly, they discovered that even after allowing a 24 hour lapse in time between the first and the second task involving these rotations, the counter-rotation task appeared to interfere with the consolidation of the 30° rotation task. What seems to be the most astonishing result is that as the time between the two different rotational tasks increased, so did the measure of interference. These results are indicative of anterograde interference because the more time that passed between the learning of the two tasks, the more the counter-rotation task interfered with the 30° rotation task. This is not what is seen in other motor tasks that involve retrograde interference for in these situations the more time that passes between the learning of two different tasks, the less the learning of the second task interferes with the consolidation of the first.

Motor transfer and neural activation

Motor transfer is the brain’s ability (or inability) to transfer certain aspects of one motor skill to the learning of another (Seidler & Noll, 2008). As has been mentioned earlier, negative transfer is the phenomenon whereby the learning of one motor task disrupts the consolidation of a subsequent motor task. This type of transfer manifests itself as a below average performance on one motor task because of previous engagement in a prior motor task (Brashers-Krug et al., 1996). Conversely, positive motor transfer is the phenomenon whereby the learning of one motor task assists in the learning of a later motor task. However, an interesting adjunct to this phenomenon is that positive transfer does not necessarily require the prior task being a motor task. According to one review, mental practice of a motor movement can lead to positive transfer when the motor movement is actually performed (Seitz, Matyas, & Carey, 2008).
There appear to be limits to motor transfer. A different study trained subjects on a particular finger taping sequence on their non-dominant hand for several weeks and observed normal motor learning consolidation and acquisition on this trained hand. However, when the sequence was repeated on the opposite (dominant) hand after the fifth week, there appeared to be no significant transfer of the motor task (Karni, Meyer, Jezzard, Adams, Turner, & Ungerleider, 1995). Perhaps this type of motor skill only involves one hemisphere of the brain and there is no transfer to the other hemisphere, possibly by interhemispheric inhibition (Romei, Thut, Ramos-Estebanez, & Pascual-Leone, 2009). Perhaps in skills that involve both hemispheres of the brain, positive transfer can be delivered to other parts of the body that also involve both hemispheres. Researchers examined juggling a football with both of their feet and then with juggling with both of their knees. Significant positive transfer was observed when this juggling skill was transferred to the knees (Weigelt, Williams, Wingrove, & Scott, 2000).

Any study of motor skill learning without the mention of the associated activated neural components involved in the task would be incomplete. While several conflicts and areas of overlap exist in this area of research, there appear to be several major areas within the brain that become activated during early motor learning (acquisition): the supplementary motor area (Ziemann et al., 2004; Lacourse, Orr, Cramer, & Cohen 2005; Mancini et al., 2009; Xiong, Ma, Wang, Narayana, Duff, Egan et al., 2009), the motor cortex (Classen et al., 1997; Lacourse et al., 2005; Seidler et al., 2008; Mancini et al., Xiong et al., 2009), the ventral premotor area (Lacourse et al., 2005; Poldrack, Sabb, Foerde, Tom, Asarnow, Bookheimer et al., 2005), the frontal and prefrontal cortices (Jueptner, Stephan, Firth, Brooks, Frackowiak, & Passingham, 1997; Ziemann et al.,
2004; Lacourse et al., 2005, Poldrack et al., 2005), the cerebellum (Ziemann et al., 2004; Lacourse et al., 2005; Seidler et al., 2008; Mancini et al., 2009) and the basal ganglia (Ziemann et al., 2004; Lacourse et al., 2005).

Many lines of evidence point to the primary motor cortex as being involved in early motor acquisition (Lacourse et al., 2005; Seidler et al., 2008; Mancini et al., Xiong et al., 2009), however, one study found that the primary motor cortex was not involved in early motor learning and so was postulated to be involved in later motor learning (Agostino, Lezzi, Dinapoli, Suppa, Conte, & Berardelli, 2008). This study used intermittent theta-burst stimulation (iTBS) to stimulate the motor cortex, hoping to affect practice-related plasticity after training of a motor task. However, what they found was surprising. When this stimulation was applied to the primary motor cortex, there appeared to be no change in the practice-related affects in the cortex, meaning that primary motor cortex remained unchanged by the stimulation (Agostino et al., 2009). They concluded from these results that the primary motor cortex was not involved in early motor acquisition. While this study stands in opposition of many studies showing that the motor cortex is indeed involved in early motor skill acquisition, it could be possible that this particular task did not involve the primary motor cortex in its acquisition. This serves as an example of the many conflicts that arise in the primary literature involving this evasive subject.

Based on what is agreed upon, the neuronal areas that do become activated during early motor acquisition can be detected using various recording devices such as PET (positron emission tomography) and fMRI (functional Magnetic Resonance Imaging). During the early stages of learning, usually within the first session alone, these areas
become activated and depending on the task can remain activated over many practice sessions for several weeks (Xiong et al., 2009) or can begin to decrease after a few practice sessions within less than an hour (Mancini et al., 2009). However long it takes for these areas to go through the acquisition stage, overall these cortical and subcortical areas will decrease in activation in normal subjects. This deactivation of anterior regions of the brain is followed, or even overlapped by, the increase in activation of more posterior regions of the brain.

The neuronal areas that become activated during later consolidation of a motor task and to the point of automaticity include: the precentral and postcentral gyri, the superior temporal lobe and the cerebellum (Lacour et al., 2005). These could be the areas that store the long-term memories that become consolidated during extended motor practice since these areas are involved during later stages of learning and studies of automaticity. Another possible area of contingency is when the cerebellum becomes activated. Within his study, Lacourse found no change in activation levels between the early stage and the later stage of learning, the cerebellum remained activated equally during both conditions. He mentions that other studies have found both increases and decreases in cerebellar activation during extended motor practice involving late learning (Lacour et al., 2005). This obviously presents a problem in attempting to ascertain the cerebellum’s role in motor skill learning. Despite the conflicts, it is agreed upon that the cerebellum is involved in motor learning but its time of activation remains unclear.
The ERP signal

The event-related potential (ERP) serves as a useful and important tool in the examination of the changes in cortical signal, particularly temporal shifts that occur. Resultant waveforms can be epoched over small (e.g., 300ms) or large (e.g., 1000ms) time frames, providing a time-dimensional window into neural processing. There are several common components in an ERP waveform. For example, the P1-N1-P2 complex appears early in the ERP waveform, within the first 60-90ms post-stimulus. This complex is elicited by visual stimuli and is modulated by attention (Luck, 2005).

Later components, those typically occurring after 300ms, are indicative of higher order processing. For example, the N400 is a maker of linguistic processing. The N400 is elicited during apparent violations of semantic expectancies, such as replacing an expected word within a sentence with an unexpected word (Luck, 2005) The N500 has been associated with analogy formation: the formation of a schema for an analogy is associated with more negative deflection in the ERP waveform over the frontal-central scalp regions and in bilateral activation of prefrontal regions (Qiu, Li, Chen, & Zhang, 2008). This aligns with other ERP studies indicating that the acquisition of a new motor skill involves the prefrontal cortices (Jueptner et al., 1997; Ziemann et al., 2004; Lacourse et al., 2005, Poldrack et al., 2005).

In order to obtain cortical activity during a task, electrodes are placed above and around specific brain regions. For example, the supplementary/premotor areas are recorded with the FCz electrode, while the C3 and C4 electrodes record activity of the primary motor cortex (Romero, Lacourse, Lawrence, Schandler, & Cohen, 2000).
The present study

The present study uses aspects of both matched and mismatched practices to observe the effects of these practices on the cortical signal. Our study also uses the ability of human participants to make analogies between position numbers and hand signals. We examined the changes in cortical signal, particularly looking at the N500 (400-600ms post-stimulus) (Luck, 2005). This was observed between the two groups of participants, between the pre and post sessions. One study looked at activity within the premotor/supplementary area and the primary motor cortex, M1 in which they used the FCz electrode and the C3 and C4 electrodes to record the electrical activity (Romero, Lacourse, Lawrence, Schandler, & Cohen, 2000).

We were interested in the activity of these areas in addition to the activity within the prefrontal cortex. We recorded activity within the premotor/supplementary motor area using the FC3, FCz and FC4 electrodes, activity within the motor cortex using the C3, Cz and C4 electrodes and activity in the frontal area using the F3, Fz and F4 electrodes. Our hypothesis was that there would be no significant difference across these electrodes during the N500 between the pre and post-practice sessions for those in group A, for in both sessions they were presented with a new hand script, script A during the pre-practice session and script B during the post-practice session. We also hypothesized that there would be a significant difference across the electrodes during the N500 activity for those in group B, for they received script A on the first day which is their pre-practice session, script B during their practice sessions on the second, third and fourth days and again script B on their last day during the post-practice session.
Methods

Participants

Eighteen college-aged students (10 females, 8 males) from the College of William and Mary’s Research Participation Pool voluntarily signed up and participated in this study for 4 course credits. Due to data recording problems, we were only able to use data from fourteen participants (7 females, 7 males). They signed up for two ERP sessions through the Research Participation Pool, 2 course credits per ERP session, and three practice sessions in between when they came in for their first ERP session. All participants gave informed consent; the protocol was approved by the William & Mary Internal Review Board (IRB), protocol number PHSC-2008-03-12-5240-wgcole.

Hand Signal Scripts

Two hand signal scripts were presented to participants in this study: Superlab hand signal Script A and Superlab hand signal Script B. Script A consists of four novel hand signals while Script B consists of a different set of four novel hand signals: eight in total were presented throughout the study. See Figures 1 and 2 for the hand signal sequence scripts. Both scripts first introduced the novel hand signals in an introduction block by presenting four right-handed hand signals with a position number (1, 2, 3, or 4) individually for one trial each. In the next block of trials, these four hand signals along with their position number were presented randomly and individually for twenty-four trials. After each hand signal was presented, a black screen appeared where the participant was instructed to replicate the hand signal that was just presented. During the next block of trials, hand signals with position number were randomly presented in a series of four followed by a black screen where instructions indicated to the participant
that they were to replicate the four hand-signals in the order they were just presented. These sets of four hand signals repeated for twenty-four trials. Finally, the participant was presented with position numbers only, four position numbers were presented in a series of four followed by a black screen. After the fourth position number, the black screen appeared, at which point the participant was to generate in order the four hand signals that corresponded to the four positions presented. This repeated for twenty-four trials and concluded the Superlab script. Script A is given to every participant on their first day of the study and Script B is given to every participant on their last day of the study.

**Practice Sessions**

All participants received the Superlab hand signal script A on their first day of the study, immediately prior to the first ERP session and all participants received the Superlab hand signal script B on their final day of the study. Which Superlab hand signal script the participant practiced during the three days in between for their practice sessions depended on which group they fell into: Group A or Group B. Participants assigned to Group A received the Superlab hand signal script A on their first day, practiced the same Superlab script for the following three days and then finally received the Superlab hand signal script B on their last day. Those individuals who were assigned to Group B received the Superlab hand signal script A on their first day, practiced the Superlab hand signal script B for the following three days and then finally received the same Superlab script on their final day.

**Gentask**

The Gentask program using STIM software was presented to the participant while
ERP data was being acquired in an adjacent room using Neuroscan 4.3 software. Gentask was only presented on a participant’s first and last days of the study, for this program was only used for ERP data acquisition. Gentask presented to the participant a series of fixation points, followed by a series of number sequences that was then followed by a “GO” screen in a period of 24 cycles with each number sequence possibility appearing once. These cycles of 24 were repeated several times. The number sequences were given four at a time (i.e. 1 2 3 4) and at the “GO” screen, the participant was to recall and generate the hand signal positions that the numbers corresponded to. ERP data was acquired while the participant worked through this Gentask program.

**ERP Acquisition**

Data was collected on a participant’s first and last days of the study using Neuroscan 4.3.1 software and according to The College of William and Mary Cognitive Neuroscience Lab ERP lab manual. Upon arrival to the lab on their first day, the participant was asked to complete several forms: a consent form, an Edinberg’s Handedness Questionnaire, to ensure that all participants were right-handed, and a schedule form to schedule their practice sessions. A small amount of Quik-Gel conductive gel was then tested on a participant’s hand to determine if the participant was allergic to the conductive gel. After verifying that there was no allergy present, the participants head was measured from between the participant’s eyes to the back of their occipital bone, or their naison to their inion. Ten percent of this value was taken and placed above the naison up through the forehead using a pen. The front of the NuAmps 40-Channel Quik-Cap was then placed at this mark.

Areas where an electrode was to be placed directly on the skin was first sanitized
with an alcohol wipe. Reference electrodes A1 and A2 were then filled with Quik-gel and placed on the back of the participants ears on their mastoid bones and then secured with medical tape, A1 being placed on the participants left mastoid bone and A2 being placed on the participants right mastoid bone. Electrodes to be placed around the participant’s eyes, electrodes X1, X2, X3 and X4, were also filled with Quik-gel, placed around the left and right eyes and secured with medical tape. These electrodes were to measure a participant’s vertical and horizontal eye movements. Electrode X1 was placed on the outer corner of a participant’s left eye and X2 on the outer corner of the participant’s right eye. Electrodes X3 and X4 were placed above and below the participants left eye. The chin-strap was then secured.

Researchers then proceeded to fill the electrodes across the participants scalp with Quik-gel, first mildly abrasing the skin with a syringe to ensure maximum conductance and surface area of the scalp under the electrode and then administering the gel into the electrode onto the scalp with the syringe. After every electrode was filled with gel, impedances were checked using Neuroscan software. Researchers did everything possible to ensure the lowest impedances were obtained, with efforts including wrapping the cap and the participants head with an ace bandage to increase contact of the electrode with the scalp. Researchers then presented the participant with either Superlab script A or B (depending on whether it was their first or last day of the study) and instructed the participant to let them know when they had finished with the script. This Superlab script also allowed for additional time for the gel to sit on the scalp, thereby improving impedance ratings. Once the participant was finished with the Superlab script, the Stim software and Gentask program were then loaded. Researchers instructed the participants
that they were to look at the number sequences that were presented, and to generate the
hand-signal positions that corresponded to the numbers in the order that they were
presented at the “GO” screen. Upon completion of this task the participant was done for
the day and instructed to come back into lab the next three days for practice sessions that
consisted only of the Superlab script.

**ERP data analysis**

All twenty-eight data files (2 for each participant with 14 participants) were
manually cleaned by rejecting blocks of data that included undesired artifacts such as
channel drift or body movement. Some participant’s data were relatively clean and
required little artifact rejection while others had more undesired artifacts that needed to
be taken out. New files were created for the clean data, resulting in 80 data files (40
original, uncleaned data and 40 cleaned data). The clean data files were then processed
further prior to analysis.

Clean data files were re-referenced to an average of the linked right and left
mastoid electrodes. This was achieved by running a “linked-mastoids” file which ran a
linear derivation of the electrodes. Another linear derivation was ran on the data using
the file “v-h-eog,” which assessed all vertical and horizontal eye movements and
averaged them. After these files were ran, low and high pass filters were applied to the
data. The low pass filter (zero phase shift, 45 Hz, 6 dB/oct) filtered out all frequencies
that were higher than 45 Hz while frequencies lower than 45 Hz passed through. The
high pass filter filtered out frequencies that were lower than .5 Hz and allowed
frequencies higher than .5 Hz to pass through. Then, the data was corrected for ocular
artifacts. Both artifact reduction and artifact reject was run on the data in order to
eliminate or smooth out ocular artifacts caused by extraneous eye blinking or head movement. Also, some files needed to be changed from 100 to 500Hz, and this was done so using the Spline function on Scan.

After application of the filters, any “dead” channels were removed from further analysis. These “dead” electrodes varied for each data file since several different NuAmp caps were used. Averages of neighboring channels were taken to replace values of the “dead” channel. Ocular channel drifts and static were leveled out with ocular artifact reduction transform. Epoching of the data involved taking 100 milliseconds before to 1500ms after stimulus presentation. This was used in further analysis to examine specific waveforms. These data files were then baseline corrected, or set to the same zero point, using a standard baseline. Averages for every event were created for each participant’s two data files. Then grand averages were compiled for pre and post sessions for both groups A and B. Grand averages were then compiled for pre and post sessions for those in group A and pre and post sessions for those in group B. These various averages were compared against each other to look for significant affects within the N500 component.

Results

Electrophysiological Data

Inspection of the grand averages of all pre-practice trials compared against all post-practice trials revealed a possible significant difference between their N500’s. Figures 4, 5 and 6 depict this. Using a repeated-measures ANOVA, significant differences were found in several frontal electrodes (F3, Fz, F4). Specifically, a repeated-measures ANOVA was performed on the data at the peak of the N500 between
groups A and B and it was found that there was a significant overall main affect of site, meaning between the electrodes F3, Fz and F4, \( F(2,11) = 5.214, p < .05 \). There was also a significant overall main affect of time, meaning between the pre and post sessions, \( F(1,12) = 3.856, p < .10 \). This statistic reached stronger significance when the data was doubled, \( F(1,26) = 8.354, p < .01 \). A significant interaction of time by group was also observed, meaning between groups A and B and between the pre and post sessions, \( F(1,12) = 4.323, p < .10 \). This statistic was also strengthened when the data was doubled, \( F(1,26) = 9.366, p < .01 \). See Table 1 and Figure 7 for the means and depiction of the means.

A repeated measures ANOVA was performed on the frontal-central electrodes (FC3, FCz, FC4) around the N500. Within these sites, an overall significant main affect of time was also found, \( F(1,12) = 6.322, p < .05 \). There also appeared to be an overall significant interaction between time and group, \( F(1,12) = 6.179, p < .05 \). There appeared to be an overall affect of site for it was approaching significance, \( F(2,11) = 2.126, p < .2 \). Due to our lower subject numbers, we looked at the data as if the group were twice as large and with this increase in power there was a significant main affect of site, \( F(2,25) = 4.832, p < .05 \). See Table 2 and Figure 8 for the means and depiction of the means.

Finally, a repeated-measures ANOVA was also performed on the central electrodes (C3, Cz, C4) around the N500. The only significant overall main affect was that of time, \( F(1,12) = 4.956, p < .05 \). However, the overall affect of site did appear to approach significance, \( F(2,11) = 1.828, p = .2 \). Again the data was doubled to examine how an increase in power would affect this result and a significant overall main affect of
site was then found, $F(2,25)=4.155, p<0.05$. Initially, it also appeared that time by group was approaching significance. Again, when the data was doubled, time by group became significant, $F(1,26)=4.957, p<0.05$. See Table 3 and Figure 9 for the means and depiction of the means.

When an ANOVA was run on all nine electrodes together, there was an overall main affect of time, $F(1,12)=5.470, p<0.05$. There was a significant interaction of time by group as well, $F(1,12)=4.375, p<0.10$. It also appeared that the overall main affect of site was approaching significance, $F(8,5)=2.054, p=0.222$. Also, the interactions between time, site and group, $F(8,5)=3.173, p=0.110$ and between time by site, $F(8,5)=1.384, p=0.375$, also appeared to be approaching significance. When the data was doubled, the significance for the interaction between time, site and group was reached, $F(8,19)=12.056, p<0.01$. The interaction between time and site also reached a level of significance, $F(8,19)=5.257, p<0.01$. What is an interesting and important thing to note was that the overall main affects of time by site, the interaction of time by group, the interaction of time by site and the interaction of time by group by site all reached a significance of .01 or better upon doubling the data.

Upon running a paired samples t-test on the frontal electrodes, it appears that across both groups, the mean N500 value of the F4 electrode during the post-practice trial was notably smaller than that of the F3 and the Fz electrodes during the post trial. The mean N500 value of F4 during the pre-practice trials across groups remains positive while the means of the F3 and Fz electrodes remained negative. The t-test reveals that there were no significant differences across these three electrodes between the pre-practice and post-practice tasks, however they did appear to approach significance; F3:
Upon doubling the data, F3, Fz and F4 all reached significance between the pre-practice and post-practice trials; F3, $t(27)=2.152, p<.05$, Fz, $t(27)=2.116, p<.05$, F4, $t(27)=2.367, p<.05$.

Within group A, there appeared to be no significant difference across these frontal electrodes between the pre and post trials. However, there was a significant difference between the pre-practice and post-practice sessions within group B of the Fz electrode, $t(6)=1.907, p=.10$. This was also true of the F4 electrode, $t(6)=2.292, p<.10$. F3 appeared to be approaching significance, $t(6)=1.868, p<.15$. Upon doubling the data, F3 reached significance, $t(13)=2.749, p<.05$.

A t-test run on the frontal-central electrodes also revealed several significant findings. Overall between both groups, there appeared to be a significant difference between the FCz electrode between the pre-practice and post-practice sessions, $t(13)=2.748, p<.05$. There also was a significant difference between the pre-practice and post-practice sessions of the FC4 electrode, $t(13)=2.669, p<.05$. However, there appeared to be no significant difference between the pre-practice and post-practice sessions across the two groups in the FC3 electrode. This electrode still remained to be insignificant even when the data was doubled. When the pre and post trials were compared within group A, there was no significant difference between any of the frontal-central electrode. However, there were significant differences within group B both in FCz, $t(6)=2.850, p<.05$, and in FC4, $t(6)=2.792, p<.05$. Using our original data set, FC3 appeared to be approaching significance, $t(6)=1.761, p=.129$. When the data was doubled, FC3 reached a significance better than .05, $t(13)=2.592, p<.05$. In fact, when the data
was doubled for all three frontal-central electrodes, the significance of all was better than a .05.

Finally, a t-test was run on the central electrodes. The means of the three electrodes across both pre-practice and post-practice sessions were obtained and several interesting aspects of the means are worth noting. The mean of the N500 value for the post-practice trials of the C4 electrode possessed a positive value while the means of the post-practice sessions of the C3 and Cz electrodes maintained a negative value. Also, the mean for C3 during the pre-practice session appeared to be notably smaller than that for the Cz and C4 electrodes during the pre-practice trials. The t-test revealed that there was a significant difference between the pre-practice and post-practice sessions of the Cz electrodes, \( t(13)=2.663, p<.05 \). The same holds true for the C4 electrodes during the pre-practice and post-practice sessions, \( t(13)=2.237, p<.05 \). While the C3 electrode across the pre-practice and post-practice sessions did not render a significant value, it did appear to approach significance, \( t(13)=1.242, p=.236 \). So, when the data was doubled to account for the small sample size, there was a significant difference between the pre-practice and post-practice sessions of the C3 electrode, \( t(27)=1.790, p<.10 \). A comparison of the pre-practice and post-practice trials across these electrodes within group A revealed no significant difference. However, when a comparison was made between the pre-practice and post-practice trials across these electrodes within group B did reveal significant differences. There was a significant difference between the pre-practice and post-practice sessions of the Cz electrode, \( t(6)=2.294, p<.10 \). There also was a significant difference between the pre-practice and post-practice sessions of the C4 electrode, \( t(6)=2.025, p<.10 \).
C3 appeared to be approaching significance, $t(6)=1.378, p=.217$. When the data was doubled, C3 reached significance, $t(13)=2.028, p<.10$.

Discussion

The N500 is a phenomenon within the event-related potential that has been related to the formation of analogies (Qiu et al., 2008). In our study, the analogy for all pre-practice sessions was between position number (1, 2, 3 or 4) and hand signal (script A only). The post-practice sessions made a slightly different analogy using the same position numbers but to a different set of hand signals (script B only). All participants received the pre-practice hand sequence script A and post-practice hand sequence script B. What varied was which script was practiced during the three-day interlude between the pre and post ERP sessions. Analogy formation activates the same area as the acquisition of a new motor skill, the prefrontal cortex (Jueptner et al., 1997; Ziemann et al., 2004; Lacourse et al., 2005; Poldrack et al., 2005; Qiu et al., 2008). This suggests that the prefrontal cortex was activated in our study when participants were asked to learn and execute both scripts A and B and make the analogy between the position number and the different hand signals.

Our study also involved aspects of matched and mismatched practice. For those in group A, the matched practice came into play when they were given hand signal script A on their first day and was asked to practice that same script for the next three days. The mismatched practice came in when on their last day they were asked to perform a new hand signal task, script B. The reverse is true for those in group B. The mismatched practice was utilized between their first session where they were given hand signal script
A and the rest of the study where they practiced hand signal script B for the next four days, their three practice sessions and their post-practice session where they performed the same Script B. The matched practice was during their practice sessions of script B and their post-practice session of the same script B. Several studies show that if learning of two different tasks is separated by at least 4 hours then interference is not observed (Brashers-Krug, et al., 1996; Muellbacher et al., 2002; Song, 2009; Criscimagna-Hemminger, et al., 2008). Our data does not appear to contain any form of interference, for all participants had more than 24 hours in between the learning of Script A and Script B, regardless of what group they were in.

The repeated measures ANOVA of the frontal and frontal-central electrodes indicate that there were overall main affects of site, meaning that the cortical signal was different within the frontal and the frontal-central electrodes. This phenomenon was also found, when the data was doubled, for the central region. The main affect of time, pre-practice versus post-practice, was seen for the frontal, frontal-central, and central regions. This indicates that we have significant differences between the two pre-practice and post-practice sessions. The ANOVA analysis of all nine electrodes shows that there was an overall affect of time by group, meaning that there is a difference between the two groups and between their pre-practice and post-practice sessions. So, there is an interaction of time and group. There was also a significant interaction of time, site and group, meaning that the electrodes differed from pre-practice and post-practice sessions and across the two different groups.

The t-tests revealed, for all neural sites examined, that there was no difference between the pre-practice and post-practice sessions in the neural activation for those in
group A. This was in line with our original hypothesis. So, there was no difference in neural activation within the frontal, premotor/supplementary and primary motor areas for those in group A. Despite the fact that those in group A practiced, on both their pre-practice and post-practice scan sessions they were presented with a new hand signal sequence. So, what they practiced for the three days in between the scan sessions was irrelevant. The N500 activation for both their pre-practice and post-practice sessions is relatively the same because they were presented with something new at both of these sessions. This is not true for those in group B. Those in group B received something new on their first day but on their last day they were presented with something that they had already seen and had been practicing for several days. This indicates that there was a change in neural activation within the frontal, premotor/supplementary and primary motor areas due to the practice of what they were to be later tested on. This also supports our original hypothesis that there would be a difference between the pre-practice and post-practice scan sessions for those in group B.

In our study, there appears to be little or no motor transfer of what was learned by participants on their first day to what was learned on their last day. This is evidenced by the pre-practice and post-practice scan sessions for those in group A. What they had learned on their first day and practiced all week did not transfer over to their learning and performance on the new sequence they were presented with on their last day. Perhaps the difference between the activation of those in group A and those in group B is that those in group B had formed a more stable and learned analogy between the script B hand sequence signal and the position numbers. Those in group had received a novel hand sequence script on their first and last scan sessions. So, the analogy they formed on their
first session did not transfer to their last session, even though the position numbers remained the same (1-4) but they were now being presented with a different set of hand signal sequences.

A very interesting trend emerged from the t-test comparisons of those in group A versus those in group B across the various electrodes. Throughout the three regions of interest, the frontal region, frontal-central and central regions, there appears to be a difference in N500 activity on the central and right sides of the brain when comparisons were made between the electrodes of those in group B when looking at the differences between the pre-practice and post-practice sessions. This was not seen in those who were in group A. This is evidenced by the odd electrodes being located on the left side of the head, the z electrodes located along the center and the even electrodes located on the right. Figure 3 shows this schematic of the cap and electrode placement. So, the frontal regions for those in group B showed a difference in activation between their pre-practice and post-practice scan sessions of the Fz and F4 electrodes. F3 did reach significance when the data was doubled. The same holds true for the frontal-central and central regions with the z electrodes and the even electrode exhibiting a significant difference between the pre-practice and post-practice sessions for those in group B only. Again, the odd electrodes became significant when the data was doubled.

This finding is very interesting but difficult to interpret. Doubling the data helps researchers understand if their affects would be seen if there were more data available. If this were true, then the odd electrodes, F3, FC3, and C3 should naturally reach significance on their own without having to be doubled. However, it is possible that there may be some underlying neural activation differences going on between the two
hemispheres of the brain. It is important to note that all participants were right handed and were shown pictures of right hands to imitate.

In conclusion, our original hypotheses were supported by the data we obtained. We expected that those in group A would not differ in neural activation, as measured by the N500, between their pre and post-practice sessions for they received novel hand signal sequences at both of their ERP scan sessions. We also expected that those in group B would differ in neural activation between the pre and post-practice sessions because they had received something on their last scan session that they had already been practicing for several days. This extended practice modulated their neural activation of the frontal, premotor/supplementary and primary motor areas. The N500 differential activation between groups A and B could be due to the differences in the group’s analogy formation between their first and last scan sessions.

The study by Qui involving analogy formation revealed that during analogy formation, a more negative trend of the N500 can be seen (Qui et al., 2008). Our data reflects this in all of our post-practice sessions for those in group B. Figures 4, 5, and 6 show this most easily. The pre-practice sessions for both groups A and B are identical because it is the same script given to both and they are all learning these hand signals for the first time. The analogy that they do form is weak compared to if they were to practice it for several days, group B shows this. So, as the analogy is further learned, the N500 becomes stronger, or more negative. Our data reflects this. This is not seen in group A because what they practice for the three days in between the scan sessions does not help them learn the hand signal script B on their last day. So, they are learning new things on
their first and last days, so their analogy formation of these two different scripts is weak and does not elicit a strong or visible N500.

Several limitations for this study include the need for a larger sample size. Due to uncontrollable factors, several participants were lost, causing our sample size to shrink. More participants should reveal stronger evidence of what was already found in this study. Also, some of the hand signal sequences that were presented were apparently either words or numbers used in the American Sign Language. This was not known by the researchers until after the completion of the study. This presents a potential problem for had anyone been exposed to these hand signals prior to our study, this could affect their performance on the various hand-signal learning tasks. Finally, within our study, it was hard to determine if those within group B had reached a level of automaticity within their execution of the hand signal script B. Several steps would need to be taken in order to obtain this. One might be to video record the participants hand movements and compare execution times as they progress throughout the practice sessions to determine if they are indeed becoming “experts” at this hand signal script.

Possible topics for future study could involve looking at those who are left-handed, and present left hands in the hand signal sequences in addition to those who are right-handed, continuing to present them with right hand signal sequences. This might better explain the phenomenon seen between the differential activation between the left and right hemispheres of the brain. Also, video recording and time recording might prove beneficial to gain a better understanding of the expertise that those in group B might be exhibiting.
References


Table 1

Mean amplitudes for Frontal (F) sites (µV)

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<tr>
<th>Group</th>
<th>Site</th>
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Table 2

Mean amplitudes for Frontal-Central (FC) sites (µV)

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Table 3

Mean amplitudes for Central (C) sites (µV)

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Figure Captions

**Figure 1.** Hand signals for script A

**Figure 2.** Hand signals for script B

**Figure 3.** A map of the electrodes used in the NuAmps 40-Channel Quik-Cap

**Figure 4.** Comparison across the frontal electrodes (F3, Fz, F4) between all pre sessions including both groups A and B against post trials of groups A and B separately

**Figure 5.** Comparison across the fronto-central electrodes (FC3, FCz, FC4) between all pre sessions including both groups A and B against post trials of groups A and B separately

**Figure 6.** Comparison across the central electrodes (C3, Cz, C4) between all pre sessions including both groups A and B against post trials of groups A and B separately

**Figure 7.** Mean Amplitudes for Frontal (F) Sites in Groups A and B

**Figure 8.** Mean Amplitudes for Frontal-Central (FC) Sites in Groups A and B

**Figure 9.** Mean Amplitudes for Central (C) Sites in Groups A and B
Figure 1

Position 1

Position 2

Position 3

Position 4
Figure 2

Position 1

Position 2

Position 3

Position 4
Figure 4

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Grand Average of Pre Trials for Groups A and B
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Grand Average of Post Trials for Group A
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Grand Average of Post Trials for Group B
Figure 5

**FC3**

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**FCz**

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**FC4**

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Grand Average of Pre Trials for Groups A and B
Grand Average of Post Trials for Group A
Grand Average of Post Trials for Group B
Figure 6

C3

Cz

C4

Grand Average of Pre Trials for Groups A and B
Grand Average of Post Trials for Group A
Grand Average of Post Trials for Group B
Figure 7

Mean Amplitudes for Frontal (F) Sites in Group A

Mean Amplitudes for Frontal (F) Sites in Group B

Pre
Post
Figure 9

Mean Amplitudes for Central (C) Sites in Group A

Mean Amplitudes for Central (C) Sites in Group B

Symbols: Pre, Post