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Quantification of gross anatomy learning using gaze tracking and electroencephalography (EEG)

El-Shaar, Ala'a Abdul

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Boston University
QUANTIFICATION OF GROSS ANATOMY LEARNING USING GAZE TRACKING AND ELECTROENCEPHALOGRAPHY (EEG)

by

ALA’A ABDUL EL-SHAAR

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Approved by

First Reader
Ann Zumwalt, Ph.D.
Assistant Professor of Anatomy & Neurobiology

Second Reader
Andrew Budson, M.D.
Professor of Neurology
The goal of medical educators is to teach their students in a manner that is effective for long-term, accurate knowledge retention, but measurement of long-term retention is difficult. Recent work by our lab has explored the use of gaze tracking to document and measure learning in medical gross anatomy students. In this study we combine gaze tracking and EEG to examine knowledge retention by these students. Medical gross anatomy students (n=22) were asked to identify anatomical structures displayed on a computer screen immediately following the gross anatomy course and again six months after the course ended. In this experiment the participants were instructed to visually fixate on the named structure of interest, or to indicate uncertainty by fixating on the upper left corner of the screen. Immediately after the course ended the students correctly fixated on the structures 70% of the time, incorrectly fixated 26.5% of the time, and indicated uncertainty 3.5% of the time. Preliminary results indicate that six months after the end of the course the students’ performance at this task had not diminished (67% correct, 26% incorrect, 7% uncertain). However, the speed with which the students made their final decision was significantly longer 6 months after the course ended. The average time to identify the structure by fixating for the final time on the region of interest was 2.22s immediately after the course and 3.0s at the 6 month follow up (p<0.001). These results indicate that 6 months after the end of the course the subjects
have solid knowledge retention but require more time to think before answering correctly. Visuospatial ability did not significantly correlate with speed to identify the structure ($r = -0.279; \text{ ns}$). Additionally, our results confirm that the students’ correct behavioral responses of a task by visual fixation demonstrate signals associated with familiarity and recollection, 300-500 ms and 500-800ms post-stimulus onset respectively, on waveforms generated from EEG activity.
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LIST OF ABBREVIATIONS

Ag.................................................................................................................. Silver
AgCl........................................................................................................ Silver Chloride
AOI............................................................................................................ Area of Interest
EEG......................................................................................................... Electroencephalography
EOC.......................................................................................................... Electrooculogram
ERP.......................................................................................................... Event Related Potential
fMRI.......................................................... Functional Magnetic Resonance Imaging
LPC........................................................................................................... Late Parietal Component
MRT.......................................................................................................... Mental Rotation Test
ms.............................................................................................................. Milliseconds
N400......................................................................................................... Early Frontal Effect
PET.......................................................................................................... Positron Emission Tomography
SAT.......................................................................................................... Speed Accuracy Tradeoff
SMI.......................................................................................................... SensoMotoric Instruments
ROI........................................................................................................... Regions of Interest
INTRODUCTION

The goal of medical educators is to teach their students in a manner that is effective for long-term, accurate knowledge retention. In this study, we combine the use of gaze-tracking and electroencephalography (EEG) to measure learning in medical gross anatomy students. We devised a paradigm to investigate learning through the lens of two memorial processes of recognition memory, familiarity and recollection, and examined these neural correlates using electroencephalography. We also used eye tracking to investigate whether varied gaze patterns reflect differences between novel and experienced learners. To our knowledge, this series of studies is the first to combine EEG measures of memory and gaze tracking measures of learning to examine long-term retention in a classroom setting.

Learning can be defined as “the process leading to relatively permanent behavioral change or potential behavioral change” (Radin, 2009). Learning is not simply the acquisition of information; rather it plays a role in the development of perception, social cognition, language, mathematical thinking, causal and scientific reasoning, psychomotor skills, problem solving skills, and conceptual knowledge (Anderson, 2009). Theories of learning have had a tremendous influence on educational practice. Learning is thought to bring about cognitive development, and many modern neurocognitive studies have shown the flexibility of the human brain and the effect learning has on brain structure. Education researchers have begun seeking different research methods developed across academic and scientific domains in an attempt to present the process of learning from different angles. Eye tracking has attracted attention from educators and is
considered a promising tool for tracking the cognitive processes of learning. Eye tracking monitoring has also been advocated as a powerful tool to advance the field of cognitive neuroscience as viewers’ eye movements can reveal memory of prior experiences without asking for verbal reports or requiring conscious recollection (Hannula et al., 2010).

Human memory is another complex cognitive function that is intertwined with all aspects of cognition that shape our thoughts, behaviors, and interactions with the world (Buckner et al., 1998). The comprehensive study of memory, therefore, requires an understanding of molecular, neural and cognitive systems, with experimental models to bridge gaps between these levels (Buckner, & Koutstaal, 1998). Functional neuroimaging techniques have emerged as promising methods to analyze the neurological basis of human cognition (Posner, 1994). Memory models conceptualize memory processing in three stages: encoding, storage, consolidation and retrieval. Encoding and retrieval are more active processes occurring at specific points in time. Encoding is the initial processing of information and retrieval refers to processing that accesses the results of prior encoding episodes. Storage and consolidation occur between these two active processes (Tulving, 1983). Currently, the most commonly used neuroimaging techniques used to attempt to observe these processes are positron emission tomography (PET), functional magnetic resonance imaging (fMRI), and electroencephalography (EEG), which is sensitive to physiologic events correlating with specific intervals of time (Buckner et al., 1998).
Electroencephalography and Event Response Potentials

The electroencephalography (EEG) records electrical activity of the brain using noninvasive electrodes that are attached to the scalp (Sur et al., 2009). This recording is generated by the synchronous activity of thousands of millions of neurons in the cortex. Neural networks in the brain are randomly active at any given time and can be synchronized in response to a stimulus. Although EEG has poor spatial resolution, compared to fMRI and PET it provides excellent temporal resolution. (Frokjaer, Olesen, Graversen, Andresen, Lelic, Drewes, 2011). EEG has been used in a wide variety of clinical and human research studies to investigate a wide variety of questions about cognitive function.

One method of observing memory processes using EEG involves the examination of event related potentials (ERPs), electrical potentials specifically related to time-locked events (Luck, 2014). These signals are small voltages generated in response to specific stimuli and their waveforms are typically described according to their latency and amplitude (Sur & Sinha, 2009). ERPs reflect ongoing brain activity without delay, making them especially useful for answering questions about the timing of mental processes. They are also commonly used to determine which cognitive process is being influenced given a certain experimental manipulation. An example of this includes an investigation of reaction times when subjects perform two tasks simultaneously compared to performing one task. Subjects’ reaction times are slowed during the simultaneous tasks, and ERPs show that this does not reflect a delay in identifying the stimuli (Luck, 1998) but rather a longer time to determine which response is appropriate for the stimulus.
(Osman & Moore, 1993). ERPs are more appropriately used to answer questions related to timing rather than those correlating with specific brain regions. They can, however, monitor mental activity in the absence of behavioral response. Luck and colleagues report that ERPs have been used to show that stimuli that cannot be reported, due to inattention or subliminal presentation, have been processed to the point of activating semantic information (Luck, Vogel, & Shapiro, 1996).

Many ERP components are elicited by a stimulus, and the sum of these components together produces an observed ERP waveform. These components are made up of a voltage deflection that is produced when a specific neural process occurs in a specific brain region (Luck, 2005). The positive and negative peaks making up the waveform relate to the underlying components. It is important, however, to understand that voltage recorded at, for example, 170 milliseconds (ms) does not reflect a single component. Instead, it is a sum of all components active at that point in time. Naming conventions for these ERP components generally indicate whether the component is positive or negative, beginning with the letter P or N, respectively, followed by a number indicating the peak latency of the waveform. For example, the N400 ERP is indicative of a negative component peaking at 400 ms after the onset of a stimulus, but not all ERPs are named accordingly (Luck, 2005).

ERPs can be divided into two categories: sensory and cognitive. Sensory waves peak early, within the first approximately 100 milliseconds after the stimulus. Sensory waves indicate responses based largely on the physical parameters of the stimulus (e.g.
shape, sound, etc). Cognitive waves reflect the way in which a subject evaluates a stimulus and processes information related to the stimulus. ERPs that represent ongoing cognitive processes occur slightly longer after the stimulus as compared to sensory waves (Rugg & Curran, 2007).

There are, however, issues that are not easily studied using the ERP technique. ERPs are extracted from the EEG by averaging together many trials time-locked to the onset of a particular stimulus. If a mental process being studied is not reasonably time-locked to an observable event, the averaging process is ineffective. Additionally, hundreds of trials are generally averaged for each study condition to generate enough artifact free trials to produce appropriate results, and some experimental paradigms are not appropriate to reasonably expect to be able to include that many trials per condition. Finally, ERPs tend to be most sensitive to processes unfolding within 2 seconds or less, and slower processes are more difficult to observe in the ERPs (long-term memory consolidation) (Luck, 2012).

**ERPs and Memory**

The dual process theory of recognition memory suggests that individuals use two mechanisms for active memory retrieval (Atkinson & Juola, 1973, 1974; Hintzman & Curran, 1994; Jacoby, 1991; Jacoby & Dallas, 1981; Mandler, 1980). Familiarity and recognition are two cognitive processes associated with memory that can be dissociated at the neural level. Familiarity-based recognition has been described as a fast-acting, relatively automatic response. Recollection, on the other hand, is a slower cognitive
process that gives rise to more consciously accessible information. This information is retrieved in the context with which it was previously experienced. For example, an individual may remember the color of an article of clothing an individual was wearing and location in which they previously encountered them (Rugg, & Curran, 2007). Familiarity would represent not being able to remember contextual details of the interaction and only remember having encountered that individual.

The N400 ERP component has been shown to be associated with familiarity (Curran, 2000; Friedman & Johnson, 2000; Rugg et al., 1998). The N400 has been the subject of extensive investigation as it has the potential to reveal aspects of cognitive processing in areas such as language comprehension and processing, which involve both long-term memory representations and their integration into semantic and syntactic structures (Lau et al., 2008). The N400 component presents as a broad negative deflection that starts at about 200-300ms after a stimulus has been presented either aurally or visually and peaks after approximately 400ms at bilateral frontal electrode sites. This component has been elicited by stimuli such as isolated words, pseudowords, faces, and pictures (Lau et al., 2008). Other research has also shown that in the context of recognition memory testing, familiar test items elicit a larger early frontal effect compared to unfamiliar test items (Hintzman & Caulton, 1997; Hintzman et al., 1998; Hintzman & Curran, 1994; McElree, Dolan, & Jacoby, 1999).

Recognition memory tests have shown that ERPs differ between old (previously studied) and new (non-studied) stimuli. The ERP component associated with recollection,
the late parietal effect (also referred to as the late parietal component or LPC) differs both spatially and temporally from the N400 component, which is associated with familiarity. The LPC presents as a positive deflection occurring maximally over left parietal regions from 400-800 ms after stimulus onset. The voltage recorded over parietal sites within the 400-800 ms interval post-stimulus onset is more positive for old stimuli than new stimuli, previously studied versus non-studied, respectively. This parietal old/new effect has been suggested to depict recollection (reviewed by Allan, Wilding, & Rugg, 1998) and is thought to reflect recollection more than familiarity (Paller & Kutas, 1992; Paller, Kutas, & McIsaac, 1995; Rugg, Cox, Doyle, & Wells, 1995).

Other studies have used response signal speed–accuracy tradeoff (SAT) procedures to separate fast familiarity from slower recollection processes (Hintzman & Caulton, 1997; Hintzman et al., 1998; Hintzman & Curran, 1994; McElree, Dolan, & Jacoby, 1999). Behavioral evidence has indicated that ERP effects of familiarity should occur before effects of recollection. Subjects were asked to study lists of singular and plural words (jar, cats). The recognition test then included the studied words, highly similar words (jars, cat), and completely new words. Subjects were prompted to recognize studied words and reject similar and new words. Results of this study indicate that ERP effects relating to familiarity occur earlier in processing than do those that reflect recollection.
Gaze tracking and memory

There is also significant evidence that memory influences eye movement patterns and that these patterns reflect an individual’s level of expertise with respect to a specific task. Using eye movements as a measurement of memory is powerful because the movement of the eyes across the visual world is not random. It is dictated by two factors, stimulus characteristics and previous experience. The stimulus characteristics are the physical properties in which the visual stimulus is embedded, such as luminance and hue (e.g., Buswell, 1935; Mackworth and Morandi, 1967). The second factor is related to the observers’ episodic and semantic memory, or the prior knowledge they bring to a viewing situation (Hannula et al, 2010).

The purpose of eye movements is to extract new information from a visual field. When an individual has had exposure to that field in the past the amount of new information available is reduced (Parker, 1978). The amount of visual processing needed to evaluate the stimulus decreases, which in turn increases the speed of efficiency by which this processing occurs. Studies by Heisz & Shore have shown that repeated exposure to faces decreases overall number of eye movements directed toward the faces (Heisz & Shore, 2008). Numerous studies have shown that gaze patterns differ in expert versus novice individuals, including studies of sports (Savelsbergh, Williams, Van der Kamp, 2002; Piras et al., 2010), surgery (Kocak, Ober, Berme, and Melvin, 2005), and involvements in crime (Peth, Kim, & Gamer, 2013).
In order to better understand the effect of classroom learning and memory storage of anatomical stimuli, we devised a paradigm that investigated Medical Gross Anatomy students’ anatomy knowledge as they progressed through their learning. This study investigates the gaze and EEG patterns of students when they attempt to identify anatomical structures at time points spanning the Medical Gross Anatomy course: prior to the course, immediately after the course, and six months after completion of the course. The purpose of this study is to investigate whether EEG and gaze tracking methods are sensitive enough to indicate a physiological response at the time when a behavioral (correct) identification through fixation is made.

We anticipate that students will demonstrate an improved knowledge of anatomy at the end of the course with an increased percentage of correct identifications during our task. This is expected because the students are exposed to lectures and labs throughout the course, increasing their knowledge of the subject matter. We also hypothesize that students who perform better on the identification task will also demonstrate a stronger performance in the Gross Anatomy course as a whole; therefore we predict a positive correlation between the two (task performance and course performance). Students’ gaze and EEG patterns were recorded during this identification task. As participants are learning, we expect to see more focused eye movement patterns. That is, fewer eye fixations on certain features within the image as opposed to a scattered gaze pattern with a larger number of shorter duration fixations. A strong student, therefore, would be able to identify anatomical features faster than a student who is not as comfortable with the material. These measures would then be associated with ERP measures of strength, where
stronger students would present with a stronger waveform amplitude signal of either the N400 or late parietal components, in their respective time intervals. We hypothesize that correct identifications of anatomical structures in the task will be associated with the N400 (representing familiarity), and the LPC (representing recollection). We also predict the speed with which participants make a decision about the identification of the structure will correlate positively with the strength of ERP presence: the faster the response, the stronger we predict the ERP signal will be. Finally, we predict that both ERPs will be more pronounced in students who score higher on a test of visuospatial ability.
METHOD

The protocol for this study was approved under the Boston University Medical Campus IRB protocol #H-32308. All procedures ensured that the Medical Gross Anatomy course director had no knowledge of the identity of the students who participated in the study before, during, or after the course.

Participants

Students enrolled in the first year Medical Gross Anatomy course at Boston University School of Medicine (BUSM) participated in the experiment. Participants were excluded if they were left-handed, did not have normal or corrected to normal vision, were non-native English speakers, or if they had previously taken a formal anatomy course. Recruitment methods included email messages sent to all students enrolled in the Medical Gross Anatomy Course, one month and two weeks before the course began. Students volunteered for the study by emailing a laboratory email account that was monitored by a research assistant. Those accepted were assigned a random subject number with which all their data was associated. Participants were reimbursed $30 per session for the first three sessions and $40 for the final session.

Design

The BUSM Gross Anatomy course is divided into three sections: Back & Limbs, Thorax-Abdomen-Pelvis, and Head & Neck. The sections are taught consecutively, with a non-cumulative examination following each section. Sections range from 4-6 weeks long, with approximately equal lecture and laboratory contact hours across each. Course examinations consist of a 90 - 100 question multiple-choice examination and a 40 item
practical examination. Students are also separately assessed on the quality of their dissections.

The experiment consisted of four sessions. The first session (baseline) occurred during the ten-day time period before the Gross Anatomy course began. The second session occurred within a week after the second anatomy exam was administered. The third session occurred after the course ended, during the ten-day time period after the third exam was administered. The fourth session will occur six months after completion of the course. All subjects completed the same two experimental tasks (described below, under section: Experimental tasks) at sessions 1, 3 and 4, and completed a test of visuospatial ability, the Mental Rotation Test (Vandenbergh & Kuse 1978), at session 2.

*Stimuli*

During each session, subjects viewed 83 stimuli consecutively. These stimuli were a conglomeration of cadaver dissection images and also images from anatomy atlases. The images the subjects viewed were the same at baseline and revisits. They included an equal proportion of images from each of the three sections of the course. The images were selected to mimic the style and content of a BUSM Gross Anatomy practical exam. Images were taken from the internet and also from the BUSM laboratory, and were chosen to contain as few visually salient background distractions as possible. All images were presented in color, and not distorted to fit the monitor.

*Experimental Design*

Subjects completed two tasks upon arrival to Session 1 (prior to the course), Session 3 (immediately after course completion), and Session 4 (6 months after course
completion). The first task will be referred to as *Gaze Experiment*, and the second as *Combination Gaze-EEG Experiment*.

The Gaze Experiment included 22 images: 7 Back & Limbs images, 8 Thorax-Abdomen-Pelvis images, and 7 Head & Neck images. Images consisted of photographs of dissections of cadavers, images from anatomical atlases, and cross sections in which a structure was tagged with an arrow. Subjects observed paired slides in sequence: (1) on the first was an image with a tagged structure that could be viewed for a maximum of 60 seconds before advancing automatically, then (2) on the second was an answer slide, where subjects could identify the tagged structure by typing their answer into a designated box. The answer slide was self-paced. Subjects could then advance to the next pair of slides using spacebar. A gaze tracking camera was programmed to record eye movements on image slides, but not on answer slides. The purpose of the gaze tracker for this experiment was to record measurements of eye movement (fixations, duration time, etc.), which could be analyzed with respect to course and task performance.

The Combination Gaze-EEG Experiment included 83 trials, with an approximately equal proportion of images from each course section (Back & Limbs, Thorax-Abdomen-Pelvis, Head & Neck). Images consisted of photographs of dissections of cadavers, images from anatomical atlases, and cross sections. Subjects observed pairs of slides in sequence: (1) on the first slide they were instructed to find a particular anatomical feature in the following slide, then (2) on the second slide they viewed an image that contained that feature. This experiment was completely self-paced. Subjects indicated their answer by visually fixating on the feature for one second before advancing to the next slide.
When they did not know the answer they were instructed to fix their gaze on the upper left corner of the slide.

Gaze and electrophysiological data were recorded during this experiment using a gaze tracking camera and EEG. Slides in this experiment were assigned respective trigger numbers that were read by the EEG apparatus. Gaze fixation patterns were used to identify correct, incorrect, and uncertain responses. These responses were then compared to the ERP signals of familiarity and recollection to determine whether physiological activity present at each trigger matched behavioral response.

Table 1: Experiment Timeline

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<th>Year 1: 2013-14</th>
<th>Session 1: Baseline</th>
<th>Session 2: Midcourse</th>
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<td>Gaze, Combination</td>
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<td>MRT</td>
<td>Gaze, Combination</td>
<td>Gaze, Combination</td>
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*Due to technical error, combination gaze-EEG data for years 1 and 2 were not analyzed. **Data to be collected May, 2015.
Mental Rotation Test

During Session 2, subjects completed a Mental Rotations Test (MRT), a test of visuospatial ability. MRT scores were correlated with task and course performance (Vandenbergh & Krus 1978). This test is described in detail below.

Apparatus

Gaze Tracking Equipment and Software. The gaze tracking camera and software used in this study were designed by SensoMotoric Instruments (SMI; www.smivision.com; Boston, MA) and have been featured as particularly useful for noninvasive psychological research. The Red-m camera used in the experiment collects infrared light reflecting from the subject’s eyes and records both gaze and pupil data. The camera allows for free head movement by the participant during data collection and has an accuracy to 0.5° and a spatial resolution to 0.1°. Data were collected at a sampling rate of 120 hertz and a spatial resolution of 1280x720 for the monitor. The camera was posted at the bottom of a 22-inch LG stimulus monitor, angled at about 20 degrees. The SMI Experiment Center software was used for the experiment creation, planning (e.g., order of images, instructions, timing of transitions, etc.) and execution. SMI iView XTM software acquired the actual gaze data from the camera, and SMI BeGazeTM software suite was used for initial data filtering and analysis.

EEG Equipment and Software. Subjects were fitted with an Active Two electrode cap (Behavioral Brain Sciences Center, Birmingham, UK). A total of 128 Ag-AgCl BioSemi electrodes were connected to the cap (Figure 1A), broken down into four strips of A (1-32), B (1-32), C (1-32), and D (1-32) electrode bunches (Figure 1B), each of which was
inserted into a respective letter-number well on the cap. Prior to placement, electrodes were soaked in warm sodium chloride solution that served as a conductor for electrical currents between the scalp and electrodes themselves. In addition to the scalp electrodes, two mini-biopotential electrodes were placed on each mastoid process, and vertical and horizontal electrooculogram (EOC) activity was recorded from bipolar electrodes placed below the left eye and on the outer canthus of the left and right eye.

**Figure 1. EEG Hardware:** (A, left) Active Two Electrode Cap, 128 electrodes. (B, right) 32 Electrode strip labeled by letter & number.

EEG data cleaning and analysis was performed using EMSE Suite Data Editor from Source Signal Imaging, Inc. EEG and electrooculogram (EOG) activity was amplified with a bandwidth of 0.03 – 30 hertz (3 decibel points) and digitized at a sampling rate of 2048 hertz. Recordings were referenced to a vertex reference point, but were later re-referenced to a common average reference to minimize the effects of reference-site activity and accurately estimate the scalp topography of the measured electrical fields.

The sampling epoch for each EEG test trial lasted for a total of 2000 ms, which included a 200 ms pre-stimulus baseline period. This pre-stimulus period was used to
baseline the averaged 1800ms ERP epochs. Trials were corrected for excessive EOG activity using the EMSE Ocular Artifact Correction Tool, in which artifact data (e.g. eye blinks), were manually identified and removed from the clean data by the investigator. Individual channels with poor recording were corrected with the EMSE spatial interpolation filter.

Procedure

The experimental procedure was described to the subjects when they arrived for the baseline visit and their consent was obtained for the procedure. Subjects who require corrective eyewear for normal vision were advised to wear contact lenses instead of glasses, if possible. After each subject was fitted with the EEG apparatus they completed the experiment task in a windowless room, with the door closed to prevent light and noise interference. Subjects were seated in a chair adjusted to their preference. Subjects’ heads were positioned on a chinrest that was pre-adjusted approximately 60cm from the experiment monitor. The chin rest was sanitized prior to each subject. The subject had a keyboard and mouse in front of them for advancing slides and answering questions. Overhead lights were turned off during the experiment.

Gaze Experiment. In this task the subjects examined images of anatomy dissections and identified the tagged structure on the subsequent slide by typing in their answer. The task began with an instructions slide that described the details of the procedure. The subsequent two slides were practice stimulus-response slides. The subjects were then given the opportunity to ask the researcher questions to ensure they understood the procedure. The next step was a five-point camera calibration and
validation procedure to ensure accurate spatial representation of the subject’s gaze over the area of the stimulus screen. A calibration test receiving a maximum dispersion value of less than one degree was considered sufficiently accurate, as recommended by SMI.

The experiment began once calibration was complete. Subjects viewed the 22 stimuli consecutively. A cross hair appeared in the center of the screen for 3 seconds before each image. Subjects were instructed to focus on that cross hair to standardize where their gaze began on each image. Subjects were instructed to examine each image on which a structure was tagged (Figure 2A), and on the next slide name or describe the indicated structure as precisely as possible by typing in their answer (Figure 2B). Subjects were instructed to type “I don’t know” for structures they were unable to identify. Subjects were allowed up to 60 seconds to examine each image before the slide automatically advanced, but could proceed to the answer slide sooner than that by pressing the space bar. The answer slide was self-paced.
Figure 2: (A, above) Gaze Experiment Prompt Slide – Subjects were prompted to identify the structure indicated by the arrow. (B, below) Gaze Experiment Answer Slide.

*Combination Gaze-EEG Experiment.* The experiment began with an instructions slide that gave the details of the procedure. The subjects were then given the opportunity to ask the experimenter questions to ensure they understood the procedure. The next step
was a five-point calibration and validation test to ensure accurate spatial representation of the subject’s gaze over the area of the stimulus screen. A calibration test receiving a maximum dispersion value of less than one degree was considered sufficiently accurate, as recommended by SMI.

The experiment began once calibration was complete. Subjects viewed 83 stimuli consecutively. These stimuli consisted of photographs of dissections of cadavers, images from anatomical atlases, and cross sections of the human body. Subjects observed pairs of slides in sequence: (1) on the first slide they were instructed to find a particular anatomical feature in the following slide (Figure 3A), then (2) on the second slide they viewed an image that contained that feature (Figure 3B). Subjects indicated their answer by visually fixating on the feature for one second before advancing to the next slide. If the feature was bilateral, they could fixate on either side. If they did not know where the feature was located, subjects were instructed to look to the upper left corner of the screen for at least one second before advancing to the next prompt by pressing the space bar. This experiment was self-paced.
Figure 3: (A, above) Prompt slide for Combination Gaze-EEG Experiment. (B, below) Image slide for Combination Gaze-EEG Experiment

Locate the **deltoid tuberosity** on the following image. Fixate on the feature for at least one second, then press spacebar when finished.
**Other Data Collection.** Subjects’ course practical and written examination grades were collected to document the subjects learning process over time, and students’ score on the Mental Rotation Test was used to assess their visuospatial ability.

**Course grades:** After the course was over, the subjects’ practical and written exam grades for all three sections of the course were linked to the de-identified subject numbers. To protect the subjects’ identities and grade information, a research assistant who is unaffiliated with the School of Medicine or the Gross Anatomy course in any way performed this process.

**Visuospatial ability:** All subjects completed the Mental Rotation Test (MRT), a validated tool that measures visuospatial ability by assessing participants’ ability to mentally manipulate objects in three dimensions (Vandenberg and Kuse, 1978). The test was administered according to its protocol at Session 2. In this test subjects are asked to identify which two of four comparison images are rotated versions of a criterion image (see Figure 4). To score the MRT, each item was given 2 points if both comparison stimuli were correctly identified, no points if one correct and one incorrect answer was given, and one point if only one answer was chosen and it was correct. Raw scores are then adjusted by subject age and gender as required by the validated tool and adjusted scores are reported.

**Figure 4:** Mental Rotations Test, example. The criterion image is the first image on the left, and the four comparison images are on the right.
ANALYSIS

Gaze Experiment

Subjects’ answers were scored as follows: 0 = incorrect or “I don’t know”; 1 = descriptively correct (for example, “artery in the hand”); 2 = correct identification and naming of structure, as dictated by the standards used during the Gross Anatomy course practical exam grading. For the purposes of this study scores of 0 and 1 were grouped as ‘incorrect’ and only fully correct answers (i.e., graded as 2) were considered correct.

Combination Gaze - EEG Experiment

Gaze data. Prior to analysis, areas of interest (AOIs) were delineated on each stimulus image, outlining the feature requested for identification. An additional AOI was delineated on the top left corner of each image screen to account for “don’t know” fixation responses. Preliminary processing of the gaze data occurred in the BeGaze software. This software analyzes the raw gaze data in the context of the experimental images to document the duration, timing, and location of gaze data such as fixations, saccades, and dwell time both on the entire image and specifically on the delineated AOIs. These data were then exported to Microsoft Excel (Microsoft Excel for Mac 2011; Microsoft Corp., Redmond, WA) for filtering and reorganization for statistical testing.

The following two variables were recorded for this part of the experiment: identification accuracy and time to answer. To determine identification accuracy the location of the subjects’ final fixation of more than 1 second was assessed. A fixation is when a viewer focuses on a single point of interest for a given duration of time while not moving the eye or gaze location (Humphrey & Underwood, 2009). Correct and incorrect
fixations were defined as follows: If the last fixation made on the image fell within the
delineated AOI of the feature, and was more than or equivalent to one second, it was
recorded as correct. If the last fixation made on the image fell outside the parameters of
the AOI of the feature being requested, or if the subject indicated uncertainty by fixating
in the AOI on the top left corner of the screen, they were recorded as incorrect or “Do
Not Know,” respectively, for that trial. Time to answer is defined as the time of entry into
the particular area of interest in which the participant fixated for more than one second.

**EEG data** Topographical maps were created in EMSE. Datasets for various conditions
were generated based on behavioral response to allow comparisons of these conditions.
All correct behavioral responses (correct fixations) were grouped as “Correct,” and all
incorrect behavioral responses (incorrect fixations and fixations on upper left corner to
indicate “I don’t know”) were grouped as “Incorrect.” Grand average files, or the mean of
the means of several subsamples, were produced from correct and incorrect triggers. The
minimum number requirement in each epoch was 15 trials (i.e. subjects had to have at
least 15 correct and 15 incorrect answers).

The grand averages were investigated for the two event related potentials relevant
to this study (N400 and LPC). An early frontal effect is maximal over frontal electrodes,
generally appearing in the 300-500ms interval post-stimulus. The late parietal effect is
maximal over parietal electrodes, generally on the left hemisphere, and appears 500-
800ms post-stimulus. Figure 5 below illustrates the locations of the 128 electrodes with
respect to the scalp, and different regions of interest.
Figure 5: ROI Map: Figure of 128 electrodes on the Bio-Semi Active 2 cap, with ROI’s relevant to our experiment identified as 1, 5, 6, and 8. ROI1 represents frontal activity, relevant to the N400 signal. ROI’s 6 & 8 represent parietal activity, on the left and right hemispheres respectively. These are relevant to the late parietal effect. ROI 5, present on the right, is also thought to influence the late parietal effect and recollection.

Waveforms were generated accounting for all correct responses of all subjects across all ROI’s of interest (1, 5, 6, and 8). A negative deflection was looked for in the frontal regions, associated with ROI1, in the 300-500ms interval post-stimulus. A positive deflection was looked for in parietal regions, associated with ROI’s 5, 6, & 8, in the 500-800ms interval post-stimulus.

Statistical analysis. Statistical tests were performed in SPSS Statistics, version 20 (SPSS Statistics, IBM Corp., Armonk, NY). T-tests were used for analyses in which the same individuals were tested more than once. These tests include the comparison of task performance to speed of response. Speed of response was indicated by the time of entry into the final fixation >1000ms. Correlations were performed comparing gaze (average
entry time into correct AOI) and EEG (Regions of interest 1, 5, 6, 8) variables, as well as overall task performance, final course performance, average practical score performance, and MRT scores. ROI1 is maximal over frontal electrodes, coding for frontal activity of familiarity in the N400 response, between 300-500ms post-stimulus. ROI’s 6 & 8 are maximal over parietal electrodes, left and right hemispheres, respectively. These two ROIs are used to correlate for late parietal effect, in the 500-800ms interval post-stimulus. ROI5 is used due to its relative proximity to ROI 8, and its prospective contribution to any activity representative of a late parietal effect. Also, EEG activity & data was analyzed with respect to image slides in this experiment only, and not prompt text slides (Figure 3A).
RESULTS

Gaze Experiment

Performance in Anatomy: Identification Accuracy

It was hypothesized that students would demonstrate an improved knowledge of anatomy at the end of the course with an increased percentage of correctly recorded answers. At baseline, subjects correctly identified 2.1% of the stimuli and incorrectly identified 97.9%. Following completion of the course, subjects correctly identified stimuli 67.9% of the time, and incorrectly identified 32.1%.

Table 2: Gaze Experiment – Identification of Anatomical Structures

<table>
<thead>
<tr>
<th>2014 – 2015: n=22</th>
<th>Baseline</th>
<th>End of Course</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correct Identification</td>
<td>2.10%</td>
<td>67.90%</td>
</tr>
<tr>
<td>Incorrect Identification</td>
<td>97.90%</td>
<td>32.10%</td>
</tr>
</tbody>
</table>

Figure 6: Gaze Experiment – Identification of Anatomical Structures

It was also hypothesized that subjects who performed better on the experimental task would demonstrate a better understanding of the course as a whole by scoring higher on
their practical examinations (averaged score of three practical examinations). This was not significant with a correlation coefficient of 0.308 (p > 0.05).

**Figure 7: Gaze Task Performance vs Average Practical Performance**

![Gaze Task Performance vs Average Practical Performance](image)

Combination Gaze-EEG Experiment

*Performance in Anatomy: Identification Accuracy*

It was hypothesized that subjects who performed better on the experimental task would demonstrate a better understanding of the course as a whole by scoring higher on their practical examinations (averaged score of three practical examinations). This was found to be not significant with a correlation coefficient of -0.030 (p > 0.05).
It was hypothesized that subjects would identify a larger percentage of structures correctly at the end of the course compared to six months after course completion (between sessions 3 and 4). These data are not yet available for the 2014-2015 academic year, so these results focus solely on the 2013-2014 year (year 1) data. No significant differences were seen in percentages of correctly and incorrectly identified structures between the two time points. Immediately after the course ended the students correctly fixated on the structures 70% of the time, incorrectly fixated 26.5% of the time, and indicated uncertainty 3.5% of the time. Preliminary results indicate that six months after
the end of the course the students’ performance at this task had not diminished (67% correct, 26% incorrect, 7% uncertain). However, the speed with which the students made their final decision was significantly longer 6 months after the course ended. The average time to identify the structure by fixating for the final time on the region of interest was 2.22 seconds immediately after the course and 3.0 seconds at the 6 month follow up (p < 0.001).

**Table 3: Combination Experiment – Identification of Anatomical Structures**

<table>
<thead>
<tr>
<th>2013 - 2014</th>
<th>End of Course</th>
<th>Six Months after Course</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correct Identification</td>
<td>70%</td>
<td>67%</td>
</tr>
<tr>
<td>Incorrect Identification</td>
<td>26.5%</td>
<td>26%</td>
</tr>
<tr>
<td>Indicated Uncertainty</td>
<td>3.5%</td>
<td>7%</td>
</tr>
</tbody>
</table>

**Figure 9: Combination Experiment – Identification of Anatomical Structures**
Correlates of Learning & Memory

It was hypothesized that the correct identification of anatomical features by fixation would result in the presence of a late parietal effect, which has been associated with the recollection of the stimulus. A topographical map (Figure 10) averaging all correct responses (end of course, year 2) illustrated a strong late parietal effect over right brain regions between 500 and 800 ms post-stimulus.

Figure 10: Topographical Map – Year 2, End of Course topographical map representing all correct responses, n=21. Late parietal effect present in interval 500-800ms post-stimulus, associated with recollection.

It was also hypothesized that this activity would significantly correlate with the time it took subjects to correctly answer the task prompt. No significance was seen between correlations of speed of fixation and the average amplitude of ROIs 1, 5, 6, or 8.
**Figure 11**

![Graph showing correlation between Speed of Fixation vs Average Waveform Amplitude of ROI1 with r = 0.114 (p > 0.05)]

**Figure 12**

![Graph showing correlation between Speed of Fixation vs Average Waveform Amplitude of ROI6 with r = -0.187 (p > 0.05)]
Waveforms were generated for all correct responses for all subjects with respect to ROIs 1 and 6, reflective of the early frontal effect and late parietal effect, respectively.
As predicted, the waveform representative of all subjects showed a negative deflection in the 300-500 ms post-stimulus interval for ROI 1 (see Figure 15), which corresponds to the N400 signal of familiarity. However, contrary to our predictions, there was no significant correlation between the strength (average amplitude) of this N400 signal and speed to answer.

Additionally, when all subjects are pooled there is a positive deflection in ROI 6 (see Figure 16) approximately 500-800ms post-stimulus onset, which has been associated to signal recollection. However, there was no significant correlation between the amplitude of this signal and behavioral gaze data.

**Figure 15:** ROI 1, representative of all subjects’ correct answers. Time: End of course, year 2.
Figure 16: ROI 6, representative of all subjects’ correct answers. Time: End of course, year 2.

Visuospatial Ability

Contrary to our predictions, students who performed better in the course as indicated by average practical grades did not perform better at the mental rotations task (r = -0.328; p > 0.05). Visuospatial ability did not significantly correlate with speed to identify the structure (r = -0.279; p > 0.05).
DISCUSSION

The purpose of this study was to investigate learning through the use of two ERP components, the N400 and LPC, and through our knowledge of gaze pattern measures of learning. Education researchers are constantly looking for different research methods in an attempt to approach learning from all angles, and while this is the first series of studies to combine EEG and gaze-tracking, there were some variations from our predictions. These variations may be a result of this studies novelty, and the need for a more thorough examination of additional variables that can be assessed within gaze tracking and ERP measures.

Performance in Anatomy

One of the goals of this study was to study student retention of knowledge over time. Based on our subject population of Medical Gross Anatomy students, it was observed that students retained about the same amount of information six months out of course as they did immediately following course completion as indicated by performance in the experimental task. This is contrary to our prediction that subjects’ anatomy knowledge retention would decrease. However, subjects took a significantly longer time to answer task questions six months after the course than they did immediately after the course ended. Previous studies of gaze patterns of experts suggest that students’ visual exploration of images change with their exposure to the material. While naïve individuals exhibit scattered gaze patterns, experts focus their attention on few relevant regions of a field of vision. Additionally, experts are more likely to focus on regions within the image for longer periods of time with fewer fixations.
(Ryan et al., 2007; Heisz & Shore, 2008; Hannula et al., 2010; Heisz & Ryan, 2011; Schwedes & Wentura, 2012; Peth et al., 2013). It is likely that a six month period away from the material caused the subjects to more thoroughly examine the details of the image to re-familiarize themselves with them. So while their accuracy was equivalent at the time points, their expertise of the material was strongest at the end of the course as compared to 6 months later.

It is important to note that students participating in this experiment had not taken any prior anatomy courses. Although they were able to complete the course successfully, it is difficult to say that they were/are an expert in the material. While this is true compared to an average individual, it is not compared to an expert within the field. Most previous studies relate the gaze patterns of true experts to those of less experienced individuals. They document that experts explore their visual field using few, long duration fixations when engaging in a challenging task (Harvey et al, 2014). That is not to say that there are not shifts in visual perception in short-term scales, too. Short-term exposure to images, such as photographs of faces, over several days showed that observers made fewer fixations over-all and that their eye movements changed in ways similar to those of experts (Heisz & Shore, 2008, Heisz & Ryan, 2011). It may just be that participants in this study are still on their way towards being labeled an expert, and are therefore demonstrating stages of learning as they progress.

Correlates of learning & memory

Another goal of the study was to determine if we can measure learning through using EEG/ERPs and see if they are reflective of learning in a course. Additionally, we
wanted to determine if these can be used as physiologic correlates of learning as measured by the six-month delay. We hypothesized that the correct identification of anatomical features by fixation would be correlated with the presence of a late parietal effect, indicating recollection of the stimuli. Prior studies have suggested that the late parietal effect has maximum activity over the left hemisphere, as opposed to the right hemisphere (Rugg, 2007). This has been attributed to the verbal nature of the materials employed in most studies (Rugg et al, 1998). When examining the grand averaged waveforms across all subjects, a positive deflection in ROI6, maximal over left parietal regions, is observed 500-800ms post-stimulus onset (see Figure 10). This appeared to be corresponding to signals of recollection. We further investigated whether the strength (amplitude) of this signal is correlated with the speed of response during the task, which would further bolster the idea that the ERP signal is indicative of confidence in response. However, the averaged amplitudes of activity present in ROI 6, maximal over left parietal brain regions, did not correlate with the time it took subjects to correctly answer the task prompt. It is possible that this deflection was seen within this ROI because images are associated with their verbal counterparts.

A topographical map averaging all correct epochs of all subjects illustrated a strong late parietal effect over right brain regions between 500 and 800 ms post-stimulus. This is the correct timing and brain location for the late parietal effect of recollection except for the fact that this signal is typically seen on the left. Our results could be due to the prompt being composed of images as opposed to verbal stimuli. We hypothesized that the strength of this signal (as indicated by amplitude) would significantly correlate with
the time it took subjects to answer the task prompt. Correlations were run between the
average time it took subjects to answer their prompts and ROI 5, as well as ROI 8,
maximal over right parietal regions, but no significance was found.

Waveforms were generated for all correct responses for all subjects with respect
to ROI 1, maximal over frontal brain regions, and these waveforms demonstrated a
negative deflection in the 300-500ms post-stimulus interval. The timing and location of
these waveforms correspond to the N400 signal associated with familiarity. No
significance was found between the strength in this N400 signal and the average speed
with which students answered their prompt.

The results of the current study confirm that the students’ correct behavioral
responses do demonstrate signals of recollection and familiarity on waveforms generated
from EEG activity. Although speed to answer the task prompt is being used as indicative
of strength of expertise and should hence correspond to a stronger feeling of knowing, it
is possible that other measures of eye movement correlating with expertise are more
useful as our task is not time-constricted.

For future studies it would be beneficial to use number of fixations subjects make
and the durations of these fixations in addition to speed of response, pinpointing experts
as those correctly answering the prompt with fewer, longer duration fixations. It may also
be possible that our subjects are in a state where they have not yet transitioned from naïve
to expert learning, and that their perception of correct versus incorrect and their
knowledge and focus with respect to the material still be developing in stages.
**Visuospatial Ability**

Anatomy is an inherently visual topic. It is therefore reasonable to predict that students with stronger visuospatial abilities would perform more strongly both in the anatomy course and in our experimental task. In the gross anatomy course the students are assessed partially via practical exams, a task that requires a large degree of visuospatial ability to complete successfully. We therefore predicted that students with strong visuospatial abilities would correspondingly be strong performers on the practical exams. However, there was no significant correlation between practical exam performance and the mental rotations task. It is possible that the mental rotations test is not the most appropriate assessment in this particular scenario, as structures are presented in the task in a common and identifiable orientation and therefore the participants are not required to mentally rotate the images to complete the task.

Visuospatial ability did not correlate with course performance or task performance. It was suspected that a student with strong visuospatial ability would be able to orient themselves faster within the task, and therefore, identify their answer more quickly. It may be that the MRT is not the best test for visuospatial ability compared to our task. Additionally, when correlating visuospatial ability with course performance, a students’ grades are not measuring spatial ability, per se, while eye movements do. It may be that the gaze variable of speed to fixation not be the most effective form of testing expertise in this paradigm, as the experiment itself is not time-constrained.
CONCLUSIONS

The predictions of this study were driven by our knowledge of gaze tracking and EEG variables associated with progressive learning and memory. Our results indicate that student retention of knowledge over a six month period does not decrease, but the time with which it takes students to indicate their answers increases significantly. Additionally, our results confirm that the students’ correct behavioral responses of a task by visual fixation demonstrate signals associated with recollection and familiarity on waveforms generated from EEG activity. This, in itself, aligns a new framework of inquiry where behavioral gaze patterns are seen to correspond with physiologic correlates associated with EEG. Finally, learners in our study demonstrating stronger visuospatial skills were able to identify structures in the task more quickly. Overall, the students’ demonstrate learning in their proficiency of completing our experimental tasks, and while a fine line has not been drawn between novelty and expertise, we do see the process with which a student embarks on these stages to reach expertise and valid retention of material.
REFERENCES


VITA
Ala’a El-Shaar B.S.
aelshaar@bu.edu
1992

Academic Training:
August 2013 – Present  Master of Science Candidate, Boston Univ. School of Medicine, Boston, MA. Anatomy & Neuroscience
January 2013  B.S.  Bridgewater State University, Bridgewater, MA; Cum Laude
B.S. in Biology, Concentration: Medical/Molecular Science, Minor: Psychology
May 2009  H.S.  Al-Noor Academy, Mansfield, MA; Valedictorian

Employment:
January 2009 – Present  Office Manager, Medical Associates of New England, Norwood, MA
May 2009 – Present  Dual Enrollment Coordinator/Counselor, Al-Noor Academy, Mansfield, MA
May 2009 – Present  Marketing Director, Al-Noor Academy, Mansfield, MA
Sept 2010 – Dec 2011  Teacher’s Assistant, Freshman Success Community (FSC) Gateway Science Seminars; UMASS Boston, Boston, MA

Honors:
January 2013  Cum Laude, Bridgewater State University, Bridgewater, MA
2011 – 2013  Beta Beta Beta (Tri-Beta) National Biological Honor Society
2011 – 2013  Dean’s List

Teaching Experience and Responsibilities:
January 2015 – Present  Masters Student Representative, BUSM, Boston, MA
Oct 2014 – Dec 2015  Teacher’s Assistant, Medical Gross Anatomy, BUSM, Boston, MA
Sept 2010 – Dec 2011  Teacher’s Assistant, Freshman Success Community (FSC) Gateway Science Seminars; UMASS Boston, Boston, MA
Sept 2010 – Jun 2011  Teacher’s Assistant, Middle & High School English Al-Noor Academy, Mansfield, MA

Major Mentoring Activities:
Sept 2012 – Present  Massachusetts Institute of Technology Muslim Student Association College Application & Preparation Mentorship
Jan 2013 – Present  ‘Cure Syrian Kids’ Campaign Mentor, Shriner’s Hospital for Children, Boston, MA
Sept 2007 – Present  Editor, Writing Studio, Easton, MA
June 2014 – July 2014  Psychosocial Mentor, Killis Refugee Camp, Killis, Turkey
June 2013 – July 2013  Psychosocial Mentor, Zaatari Refugee Camp, Irbid, Jordan
June 2012 – July 2012  Psychosocial Mentor, Killis Refugee Camp, Killis, Turkey

Professional Societies: Memberships, Offices, and Committee Assignments:
Oct 2014 – Present  American Association of Anatomists
June 2007 – Present  Syrian American Medical Society
Presentations:


