LEVERAGING PUPILLOMETRY AND LUMINANCE-BASED MENTAL IMAGERY FOR A NOVEL MODE OF COMMUNICATION

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by
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ABSTRACT

The aim of the present study was to characterize participants' abilities to answer binary yes/no questions by mentally manipulating imagery to produce imagined changes in luminance, which would in turn cause reflexive perturbations in pupil diameter. First, a paired association was established with participants, linking "yes" responses with imagining a "sunny sky" and "no" responses with imagining a "dark room". Participants (N=20) then answered 16 yes/no questions using this response method, in place of providing verbal or gestural (e.g., head nod) answers. Pupil diameters were recorded for a period of 8000 ms following each stimulus question while participants maintained the mental image that corresponded with their answer. We hypothesized that on average, "no" responses would yield a pupil dilation and increased diameter relative to baseline, while "yes" responses would instead result in constrictions and smaller pupil diameters compared to baseline. A 2-factor repeated measures analysis of variance (ANOVA), where time was one factor and response type (i.e., yes or no) was the other, revealed a statistically significant interaction of time and response type, a significant main effect of time, and a trend toward significance for response type in aggregated group data. Item level discrimination consisted of comparing the mean pupil diameter in response to a single item for a single participant (e.g., "yes" response on one trial) to the mean pupil diameter of all contrasting responses for that same participant (e.g., all "no" response trials). This method achieved a 64.5% discrimination accuracy. This investigation affirmed the plausibility of leveraging pupillometry and luminance-based mental imagery in favor of an alternative communication system for individuals who are locked-in, as well as its potential as a screening tool. However, further investigation is warranted prior to its implementation.

This manuscript

is dedicated to Dr. Jamie Reilly.

It’s been real.

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CHAPTER 1

INTRODUCTION

There exist a range of neurological conditions wherein patients retain high order cognition and consciousness in the context of a profound movement disorder. Patients are said to experience total locked-in syndrome (LIS) when they can neither speak, nor reliably initiate volitional motor movements that might serve as an indicator of their intact consciousness or a conduit for communication (e.g., blink twice for yes). Other disorders, such as Amyotrophic Lateral Sclerosis (ALS) may also result in a locked-in state, particularly in advanced stages. The management of locked-in phenomena is complicated by the fact that it is difficult to discriminate from other conditions, such as disorders of consciousness (DOCs), making diagnosis challenging and often significantly delayed. Attempts have been made to develop effective assessments of consciousness and to promote communication in patients with such debilitating motor impairments; however, current methods do not satisfy the needs of patients living in a locked-in state. The pupillary system of the human eye provides the potential to aid in the diagnosis and communication of individuals who are locked-in.

Locked-in Syndrome

Patients experiencing locked-in phenomena are either paralyzed entirely or left with the ability to execute only minimal movements. Among the devastating challenges facing patients with LIS are diagnosis, which may be inaccurate and delayed, and communication, which in some cases is unattainable. Prior to considering these challenges, it is necessary to review the etiology and epidemiology of the patients affected.

LIS is typically associated with bilateral damage to the ventral pons, through vascular pathology, traumatic brain injury, or neurodegenerative disease. The ventral pontine lesion causes supranuclear motor de-efferentation, leading to paralysis of all four limbs and the final cranial nerves, meaning the patient will also experience anarthria. The disorder has been divided into three subtypes. The first subtype, referred to as classical LIS, is characterized by the retention of vertical eye movement in the face of an otherwise complete motor impairment. An individual is considered to have incomplete LIS when s/he maintains some degree of voluntary movement. Incomplete LIS is a less severe version of classical LIS. Finally, total LIS consists of being entirely paralyzed, including loss of eye movement. Total LIS is the most severe form of the syndrome (Bauer, 1979). In all subtypes of the disorder, consciousness is preserved, despite the profound motor impairments.

Certain disorders may evolve into a state that presents with the same characteristics as any one of the three LIS subtypes. For example, ALS causes progressive and fatal neuromuscular degeneration, without an impact on cognition. The degeneration begins in the lower motor neuron of the motor cortex, progresses to the upper motor neuron, and eventually affects the brain stem and spinal cord as well, potentially leading to a locked-in state. Patients experiencing locked-in phenomena, such as those with ALS, are unable to produce observable indications of their consciousness. Consequently, they are difficult to identify and may remain undiagnosed, isolated, and unable to communicate for great lengths of time.

According to a 2002 survey of 44 individuals diagnosed with LIS living in France, the mean time elapsed prior to diagnosis of the syndrome was 78.76 days. Passing more than two months in complete isolation, despite being surrounded by friends, family, and medical professionals, is scarcely imaginable. Moreover, in 54% of cases the first person to recognize that the individual was aware and conscious was not a doctor, but a family member. Surprisingly, physicians were the first to realize the patient's consciousness in only 24% of cases (León-Carrión, Van Eeckhout, Dominguez-Morales, & Perez-Santamaria, 2002). This survey provides a snapshot of the extended diagnostic timeline in this population. Not only are individuals with LIS often misdiagnosed initially, but it also appears as though the physicians are not the ones making the behavioral observations that can serve to correct the diagnostic error. It is evident that a more reliable and concrete method of medical diagnosis would greatly benefit this vulnerable population and in fact, is necessary to ensure their proper care.

Diagnosis

Patients with LIS may be misdiagnosed for a variety of reasons. First, diagnosis will be inaccurate if minor voluntary movements (e.g., vertical eye movement, especially for those with classical LIS) are not examined, missed during examination, or mistaken for involuntary movements (Smith & Delargy, 2005). Furthermore, since observation of volitional movements is the chief indicator of consciousness in this population, those without the ability to execute voluntary movements will be misdiagnosed as well. In such cases as these, patients will likely be diagnosed with a DOC. However, this is not without reason, seeing as LIS presents similarly to DOCs, sharing the inability to respond by conventional means.

DOCs include coma, vegetative states (VS), and the minimally conscious state (MCS). Each of these diagnoses reflects a segment on a continuum of consciousness and cognition. Behaviors are used to gauge an individual's approximate position along this continuum. On one end of the continuum is coma, where patients will show no signs of consciousness and instead remain in a sleep-like state. If the coma endures, it is referred to as chronic. The MCS exists at the other end of the continuum, where patients will show few, inconsistent signs of consciousness. Those diagnosed as being in a VS fall somewhere between individuals in a MCS and those in a coma. Patients in a VS show evidence of a sleep/wake cycle with periodic eye opening, but no purposeful behavioral responses. If this continues for at least four weeks, it becomes considered a "persistent vegetative state" (PVS). After a longer period of time, designated based on the specific etiology (e.g., 12 months following traumatic brain injury), the state is recognized as permanent (Hirschberg & Giacino, 2011).

It is common for patients with DOCs to retain reflexive movements, such as eye opening and blinking, despite the lack (or low frequency) of intentional movements. When an individual is locked-in, it may appear as though s/he is experiencing a similar state of reduced arousal and/or a lack of consciousness. If any volitional movements are present, they may be mistaken for reflexive ones. The patient's inability to respond to stimuli in a conventional way means that physicians have little information available in order to accurately assess the patient's consciousness.

Making the assessment and diagnostic processes even more challenging is the fact that there exists no universal operational definition for consciousness itself (Owen & Coleman, 2008). Lacking such a definition limits the ability to develop diagnostic criteria for patients with and without DOCs. Typically, consciousness is broken down into the components of wakefulness and awareness. However, the way these components are measured becomes problematic. Wakefulness can be measured in a relatively straightforward fashion using EEG to record a typical waking state. On the other hand, awareness currently does not correlate with any functional biomarkers; therefore, the ability to follow either verbal or behavioral commands is used by proxy (Fernández-Ospejo & Owen, 2013). When an individual demonstrates wakefulness without awareness, s/he will be considered to lack consciousness and will typically be diagnosed as in a VS. It is clear how this presents a profound obstacle for those locked-in individuals who are unable to demonstrate awareness through conventional behavioral means.

Instruments such as the Glasgow Coma Scale (GCS) and Rancho Los Amigos Scale (RLAS) exist to gauge the degree of impairment to consciousness, but they are subjective in nature and provide little utility in detecting consciousness when consistent behavioral responses are absent. The GCS takes into account eye opening, verbal responses, and motor responses (Teasdale & Jennett, 1974). Since those with LIS will not demonstrate any verbal or motor responses and may show only very limited eye opening, the scores obtained using the GCS will not accurately reflect their state. The RLAS also focuses on the patient's response to stimuli, which is clearly insufficient to distinguish LIS from DOCs (Hagen, Malkmus, & Durham, 1972). Ultimately, neither the GCS nor the RLAS will differentiate between a patient with LIS and a patient with impaired consciousness, reinforcing the need for a more effectual tool.

In addition to DOCs, still other diagnoses overlap with LIS in terms of phenotype, compounding the difficulty of diagnosis. A patient in a locked-in state may demonstrate characteristics consistent with akinetic mutism (AM), for example. AM is not a paralyzing disorder, but rather an impaired ability to execute or carry out movements due to problems with initiation, most likely as a result of damage to the frontal lobe, cingulate gyrus, basal ganglia, thalamus, and/or mesencephalon (Nagaratnam, Nagaratnam, Ng, & Diu, 2003). Although AM is not a paralyzing disorder, patients may move very little and experience varying degrees of anarthria. For example, patients may display eye movements used to track a speaker, but no other movement whatsoever. Due to the extremely limited movement and speech characteristics present in the disorder, it is not difficult to see how an individual who is locked-in may be mistakenly diagnosed with AM, or vice versa. However, since the etiology and course of treatment for AM is so distinct from LIS, accurate diagnosis is critical to providing the best care to patients with either disorder. In light of their mutual absence of behavioral responses, being able to ask questions of patients with LIS or AM in order to better characterize their condition has the potential to improve diagnostic outcomes.

In sum, there are many factors involved in preventing the accurate diagnosis of individuals with LIS. An important consideration is that severe motor impairments, such as ALS and LIS, have low incidence and prevalence in the general population (Mehta et al., 2014; Smith & Delargy, 2005). Nevertheless, the profound isolation that results from such conditions necessitates the development of both an effective assessment tool to accurately identify patients who are locked-in and an alternative communication system to allow them to participate in their lives to the greatest extent possible. Attempts have been made to do this, but at present we lack a standardized methodology to diagnose patients with these disorders and provide them with an alternative means of communication.

Communication

Once an accurate diagnosis has been made for individuals who are locked-in, the issue of communication can be addressed. For those with classical or incomplete LIS, it may be possible to utilize a communication system based on the most reliable movement that the patient is able to elicit (Barreca et al., 2003). With training, the patient may be taught to blink once for "no" and twice for "yes," look down for "no" and up for "yes," or raise one finger for "no" and two fingers for "yes," etc. However, for those with total LIS, such communication methods are not possible due to their complete paralysis. Furthermore, communication methods involving movements may turn out to be unreliable even for those with classical or incomplete LIS, due to fatigue or variability in motor function (Oken et al., 2014). One promising alternative communication modality is the use of brain-computer interfaces (BCIs). Both invasive and non-invasive BCI mechanisms are currently under investigation, including electroencephalography (EEG) and electrocorticography methods.

BCI systems aim to directly connect the human brain with computers that can be used to carry out a variety of functions, such as communication, while bypassing the peripheral nervous system entirely. Despite the fact that BCIs may allow for a wide repertoire of responses and broad communicative applicability, there are pitfalls that prevent their present utilization. For example, these systems have achieved low levels of accuracy, particularly among experimental participants with movement disorders in a live or online context (Lesenfants et al., 2014; Oken et al., 2014). Even in invasive systems that boast high levels of accuracy in single-subject studies, it has become evident that factors such as fatigue, infections, the presence of pain, or the use of analgesics can interrupt use of the system for extended periods of time, delaying necessary training and limiting communication (Kennedy, Bakay, Moore, Adams, & Goldwaithe, 2000). Moreover, even the non-invasive systems require up to 45 minutes of preparation time to set up the device, preventing their use in immediate or urgent situations. Finally, the equipment required for BCI systems of all varieties can be extremely costly, reducing the likelihood of patients gaining access to these systems in a timely fashion. Ultimately, the effective and convenient use of BCI systems may be available down the road, but in the mean time, a reliable communication system is still needed for those currently living with LIS.

Mental Imagery

Two separate lines of recent investigation lay the groundwork for developing an effective tool to both diagnose patients who are locked-in and allow them to communicate. What they have in common is mental imagery. The first suggests that it may be possible to determine the consciousness and awareness of a patient through measuring thought modulation, observed via functional magnetic resonance imaging (fMRI) and/or EEG. The studies discussed have also explored the use of thought modulation to respond to binary questions (i.e., providing a yes/no response). The second line of research implicates the human pupillary response as a potentially low-cost and convenient alternative to using fMRI or EEG to accomplish these functions; that is, establishing both a patient's awareness and a system of bedside communication.

In a series of fMRI and EEG studies, it was revealed that a group of patients thought to be in a VS may actually have been locked-in. In studies by Owen (2006), Monti (2010), and colleagues, examiners prompted patients who were thought to be in a VS to produce mental imagery, which activated specific brain areas that could be observed with fMRI. Several of the immobile patients in the study by Monti et al. (2010) demonstrated cerebral activity consistent with control participants, prompting further bedside investigation. Of the five patients found to demonstrate cerebral activity consistent with controls, three showed further signs of awareness at follow-up bedside testing. One of the five patients answered yes/no questions using the same binary thought modulation task that was used earlier in the study to evaluate consciousness (e.g., imagining hitting a ball back and forth on a tennis court to answer "yes" and navigating through either the streets of a familiar town or the patient's home to answer "no," or vice versa). A study by Cruse et al. (2011) corroborated Monti and colleagues' (2010) findings by using EEG to demonstrate awareness in 19% of patients diagnosed as being in a PVS. By revealing the presence of residual cognitive activity in patients who were thought to be in a PVS, these studies have implicated the use of fMRI and/or EEG as a new diagnostic tool to differentially diagnose PVS and total LIS.

In addition to suggesting a new diagnostic tool to be used in cases of LIS, the above researchers also established the potential for a communication system. However, only one participant successfully used it to communicate. This could be due to the fact that motor imagery may be impaired in some individuals who are locked-in (Conson, Pistoia, Sarà, Grossi, & Trojano, 2010), which may also be the case in individuals with ALS (Fiori et al., 2013). This calls into question the reliability of the motor imagery based diagnostic task and suggests the need for a different thought modulation task for reliable communication. Furthermore, the use of fMRI does not provide the opportunity for bedside communication and the use of EEG may be expensive and time-consuming, highlighting the need for at least a new communicative tool and perhaps a more efficient, cost-effective, and reliable diagnostic tool, as well.

In a study completely unrelated to the diagnosis and communication of individuals with LIS, investigators made a discovery related to the human eye's pupillary reflex that can be leveraged to develop a non-motoric communication system. The pupillary reflex has been extensively studied for several decades. It is well understood that the sympathetic system controls dilation, while the parasympathetic system controls constriction of the pupil. Since these two processes are controlled by distinct systems, they can occur simultaneously to varying degrees. As a result, the size of the pupil is determined by the ratio of activity occurring in both systems: the sympathetic pathway innervating the dilator muscles of the iris and the parasympathetic system innervating the sphincter pupillae muscle, which constricts the pupil (Beatty & Lucero-Wagoner, 2000). These processes function to let in more or less light to protect the eye from ultraviolet damage, while maximizing visual perception, which improves with greater illumination.

Through a series of experiments, Laeng and Sulutvedt (2014) determined that the pupil of the human eye not only reacts to environmental changes in luminance, but also variations in luminance that occur in the mind, during mental imagery tasks. That is to say, when people were cued to imagine scenes that varied by luminance, their pupils involuntarily dilated or constricted. This reaction was most robust when pupils were primed (i.e., very recently exposed to the same bright or dark object that the individual was prompted to imagine), but the reflex was also maintained to a lesser degree during spontaneous imagery generation.

Participants in the final experiment of Laeng and Sulutvedt's (2014) study were asked to spontaneously generate mental images of six different settings or objects (i.e., a sunny sky, night sky, cloudy sky, dark room, face in sunlight, and face in shadow) while their pupil diameters were recorded for a period of 7 seconds during each trial. During the task, the 52 participants observed the same gray screen throughout, so as to maintain consistency of environmental luminance conditions. On average, the most robust pupillary reactions across conditions were an average pupil diameter decrease (constriction) of 0.55 pixels from baseline during the "sunny sky" trials and an average pupil diameter increase (dilation) of 0.6 pixels from baseline during the "dark room" trials. These results demonstrated small, but significant differences in pupil size between imagining bright and dark conditions.

Pupillometry

The pupil dynamics in Laeng and Sulutvedt's (2014) study were readily observable and recorded via infrared eyetracking, a well-recognized and widely accepted method of measuring changing pupil diameter in a variety of experimental applications (Laeng & Endestad, 2012; Piquado, Isaacowitz, & Wingfield, 2010). During infrared eyetracking, rays are emitted from an infrared camera, aimed at the pupil, and reflected by the retina, which generates a high-resolution image of the pupil that can be measured in pixels and converted to millimeters, for more accurate comparisons across studies. Eyetrackers are produced in head mounted and remote variations. In the former, the distance between the camera and pupil is fixed, typically at 10 cm or less, which provides good precision. In the latter, the distance between the camera and pupil can change, allowing participant movement, and may measure up to 100 cm. Although the distance between the camera and pupil will alter when using remote eyetrackers, the device accounts for this change through estimating the distance and calculating the appropriate foreshortening division (Klingner, 2010).

Despite the fact that remote infrared eyetrackers can account for movement that causes a change in the distance between camera and pupil, excessive change still allows room for error and has the ability to alter or invalidate results. This is in part due to the fact that more work is created for the eyetracker: measuring the pupil diameter while simultaneously monitoring the pupil's distance from the camera and adjusting the measured pupil diameter accordingly. A further complication is that the size of the pupil actually fluctuates as a function of distance, in order to focus and clarify objects being perceived at a variety of distances from the eye. This phenomenon is referred to as the accommodation response (Beatty & Lucero-Wagoner, 2000). Asking participants to remain in a seated position and focus on a computer screen that presents stimuli at a close proximity can control this response. The accommodation response can also be controlled by fixing the head position (e.g., by using a chin rest), to ensure a participants' eyes are a constant distance from the screen, and presenting stimuli via auditory recordings, preventing the need for the participants' pupils to focus in order to read on-screen text.

Another factor that influences pupil diameter is cognitive load (Beatty & Lucero-Wagoner, 2000; Piquado et al., 2010). When experiencing an increase in the cognitive demand of a task, an individual's pupils will correspondingly dilate. This cognitive response is not evolutionarily advantageous, as it serves no clear purpose. However, the effect is not harmful either because the fluctuations are so small in amplitude that there is no discernable impact on vision (Beatty & Lucero-Wagoner, 2000).

Cognitive effort is of course difficult to control for, particularly when asking participants to modulate their thoughts in order to answer questions. In Laeng and Sulutvedt's (2014) experiment, beginning the recording period for a given trial after participants had already conjured up the appropriate mental image likely reduced the effect of mental effort on pupil diameter. That is to say, participants were asked to initiate the recording period with a key press after they had already brought the appropriate image to mind. Participants' pupils were then recorded while they simply maintained the mental image, which likely requires less cognitive effort than the initial phase of developing the mental image (D'Esposito, Postle, Ballard, & Lease, 1999). This also resulted in eliminating the need to estimate when in the time course of the recording the participant actually generated the mental image.

In addition to distance and cognitive load, pupillary reactions can be stimulated by arousal. Bradley, Miccoli, Escrig, and Lang (2008) observed that both pleasant and unpleasant emotionally arousing pictures produced pupil dilations, following an initial pupillary constriction that reflexively responded to increased illumination on the screen where stimuli were presented. The results of their study imply that stimuli must be carefully chosen to be the least arousing possible when examining the effect of other variables on the pupillary response. This means refraining from using particularly enjoyable or particularly noxious concepts in stimuli because both extremes will produce a pupillary reaction. Relatively neutral or mundane concepts, however, will not trigger the pupillary system's response to arousal or emotional valence and therefore may be used in such experiments.

Considering the several variables that may impact pupil diameter in addition to luminance-based mental imagery, it may not be immediately obvious why luminance-based imagery should be utilized for a communication system and diagnostic tool over the other variables that could be manipulated. For example, it would be possible to ask participants to calculate answers to simple math problems to answer "yes" and to calculate answers to challenging math problems to answer "no". Likewise, participants could be asked to imagine a scene of low valence to answer "yes" and high valence to answer "no". However, in these conditions the difference between the two responses might not be as great as when luminance-based mental imagery is used. Although in both examples the "no" response would likely produce a pupil dilation, calculating a simple math problem and imagining a scene of low valence will not produce a pupillary constriction in the way that imagining a bright scene has been shown to do (Laeng & Sulutvedt, 2014). As a result, previous studies have had to provide multiple choice options, such as a yes option and a no option, and allow participants the opportunity to respond to each option with a "yes," read as a pupil dilation (i.e., by increasing cognitive load while calculating the solution to a difficult math problem) or "no," read as no change from baseline (i.e., by not calculating the solution to the math problem) (Stoll et al., 2013). Our method, using luminance based imagery would allow participants to actually answer a binary question by producing a change from baseline pupil diameter in opposing directions (i.e., increase in diameter for "no" and decrease in diameter for "yes"), thereby increasing the efficiency over a system where participants have to respond to each choice available to answer a question.

Aim of Present Study

Although BCI systems, including the use of fMRI, show great promise for the future, the human pupillary system offers an alternative mode of assessing consciousness that can double as a communication modality. In this thesis we will explore a novel communication paradigm that could also potentially be used for diagnostic purposes. More specifically, we will investigate the ability of participants to modulate mental imagery and, in turn, a physiological response that is outwardly recognizable and measureable. Importantly, these pupillary responses are reflexive and occur independent of volitional oculomotor control. Thus, the pupillary system offers a potential avenue to circumvent speech motor control, directly connecting the thoughts of individuals lacking volitional movements with their communication partners.

The aim of the present study was to characterize participants' abilities to answer binary yes/no questions by mentally manipulating imagery to produce imagined changes in luminance. We used Laeng and Sulutvedt's (2014) finding that the pupil responds to imagined changes in light conditions in order to fill the need for an easily measured and reliable non-motoric response modality. Participants responded to yes/no questions by willfully modulating their mental activity, in the same way that participants in Owen's (2006) and Monti's (2010) studies did so. However, rather than imagining playing tennis or wandering familiar streets to respond to yes/no questions, participants imagined scenes of differing luminance conditions. We hypothesized that, on average, "no" responses, produced by imagining a "dark room," would yield a pupil dilation and increased diameter, relative to baseline, while "yes" responses, produced by imagining a "sunny sky," would instead result in constrictions and smaller pupil diameters compared to baseline.

CHAPTER 2

METHODS

In the experiment to follow, which took place on the campus of Temple University, we presented a set of simple yes/no questions to healthy young adults with the aim of potentially leveraging reflexive pupillary dilation dynamics as a nonverbal response modality. More specifically, we trained participants to associate affirmation ("yes") with imagined brightness (i.e., a sunny sky) and negation ("no") with imagined darkness (i.e., a dark room). We hypothesized that these contrastive luminance-based imagery conditions would produce opposing physiological effects. That is, perturbations in imagined brightness would result in reflexive pupillary constriction in contrast to shifts in imagined darkness that would result in involuntary pupil dilation. These distinct response profiles (down for "yes," up for "no") could then act as a proxy signal for verbal responses. We monitored such pupillary dilation dynamics in response to a set of simple yes/no questions using a remote infrared eyetracking system.

Participants

Participants included healthy young adults (N=29) [mean age = 21.79, range=18-33] from the Temple University undergraduate community. Participants were by self-report free of prior neurological injury, ocular trauma, or learning disability. Participants passed an auditory screening (detection of pure tones binaurally presented at 0.5, 1, and 4 kHZ @ 25 dB SPL) and vision screening using a Snellen eye chart with a corrected visual acuity of 20/30 or better. We excluded participants with hard contact lenses or anti-glare eyeglasses. Participants removed eye-makeup with complimentary make-up removing wipes upon arrival, if necessary. Prior to the experiment, participants first provided informed consent and completed a demographic survey.

Equipment

Participants were tested in a closed, windowless room with no changing light source. We turned off all overhead fluorescent lights such that the only light sources were the computer monitor and any residual ambient light passing under the door. We continuously tracked pupil size at a sampling rate of 120 Hz using a remote infrared eyetracker (Sensorimotoric Instruments, RED-M). Participants were seated at an optician's chin rest at a distance of 60 cm from an infrared eyebar coupled to the base of a 17" monitor. We first calibrated the eyetracker to each individual using a 5-point calibration and validation sequence and only initiated the experiment for participants whose calibration accuracy exceeded 0.5 degrees in the X and Y planes. We used the eyetracker's proprietary software (i.e., Experiment Center) to program the experiment and present stimuli.

Experimental Procedures

Participants were seated at the eyetracker and positioned in the optician's chin rest. Following calibration and validation, participants completed a sequence of training followed by testing. Training consisted of exposure to a set of written instructions, teaching participants to associate affirmative responses with imagining brightness and negative responses with imagining darkness. Participants observed these directions on the computer screen, and received reinforcement from the examiner. Each participant was asked to sit quietly and respond to simple, factual yes/no questions (e.g., "Is a banana yellow?") presented via the auditory modality. In order to answer "yes," participants were asked to imagine a "sunny sky," and in order to answer "no," participants were asked to imagine a "dark room". Participants were also asked to refrain from producing verbal or overt motoric responses (e.g., head nod) when answering the questions.

Following instructions, participants were permitted to ask questions to clarify any misunderstandings, and then began with practice items (N=4). After each practice item, the examiner asked what the participants were imagining in order to determine whether they were imagining the scene (sunny sky or dark room) associated with the correct answer (yes or no, respectively).

Once the examiner concluded that the participant comprehended the task, the eyetracker was re-calibrated and they proceeded with the experiment. The experimental trials consisted of pre-recorded "yes" (e.g., "Does a clock tell time?") and "no" (e.g., "Is three an even number?") questions (N=8 each). These questions were designed to be simple, and universally known in order to reduce executive demands. A handsome adult male recorded the stimuli in a sound booth at a constant distance from the microphone. We subsequently trimmed silences from each audio file using the Audacity waveform editor. We then batch amplitude matched the audio files using the GoldWave waveform editor. Trimmed and volume-matched stimuli were presented over stereo speakers in a fixed pseudorandom order. The appendix reflects the test stimuli.

The pupillary response function is strongly influenced by shifts in luminance (Beatty & Lucero-Wagoner, 2000); therefore, control of the luminance of experimental stimuli is an essential prerequisite for the validity of controlled experimentation in pupillometry. Throughout the experiment, we collected event-related baseline and experimental data while participants viewed a constant, neutral gray screen. Thus, observed results cannot be attributed to actual shifts in luminance.

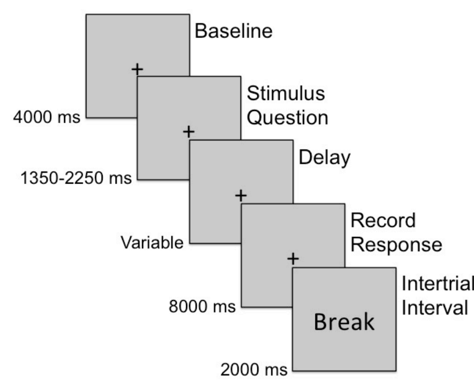

Figure 1. Trial structure including durations.

Figure 1 represents the trial structure and according timing. Participants viewed black crosshairs in the center of a neutral gray screen while the eyetracker recorded their pupils. Following the baseline period, the participants heard a pre-recorded probe question that lasted between 1350 and 2250 ms. After the question, a delay allowed the participants to conjure up the appropriate mental image (i.e., either a "sunny sky" or a "dark room") while they continued to observe the crosshairs at the center of the neutral gray screen. Once the participants had the image in mind, they pressed the space bar to initiate the response recording period, which lasted for an interval of 8000 ms. The gray screen and crosshairs remained constant until the end of the trial period, at which point the word "Break" appeared on the screen before the onset of the next trial. The approximate experiment duration was 5 min (16 questions x ~18 seconds).

Data Analysis

We first exported each participant's raw time series and proceeded with a series of steps to process these data. We eliminated data from any participant with sampling error on any single question (N=9). From participants with complete data sets, we first averaged the pupil diameter measurements for both eyes to generate a single discrete time series for each participant. Raw time series data contain a significant amount of random noise, along with blocks of missing data corresponding to blinks. We applied a linear interpolation across trials corresponding to blinks using the Zoo package in R (R Core Team, 2013). This interpolation step imputed missing values. We then dealt with random variability by applying a smoothing algorithm to the raw time series in the form of a 6-point moving average using the TTR package in R (R Core Team, 2013). These processing steps yielded a smoothed and complete time series which we then downsampled using a binning procedure in the computer program Python. For this step, we aggregated 15 trials into a single bin, corresponding to a time window of 125 ms per bin. We analyzed bins from the onset of each event outward to 6000 ms (creating a total of 48 bins). We then converted the bin means to difference scores by subtracting the mean of the baseline from each. This procedure allowed us to standardize difference scores that were contrastable across different events.

CHAPTER 3

RESULTS

Group Level Analysis

To follow we report group and item-level statistics. Figure 2 reflects group level results for 20 participants aggregated across 16 questions per participant. We first conducted a 2-factor repeated measures analysis of variance (ANOVA). The dependent variable across this analysis was the raw pupillary change score (in mm) from baseline. The first factor was time, reflecting 48 equally spaced intervals spanning the time series from 0-6 seconds after each event. The second factor was response type (yes or no).

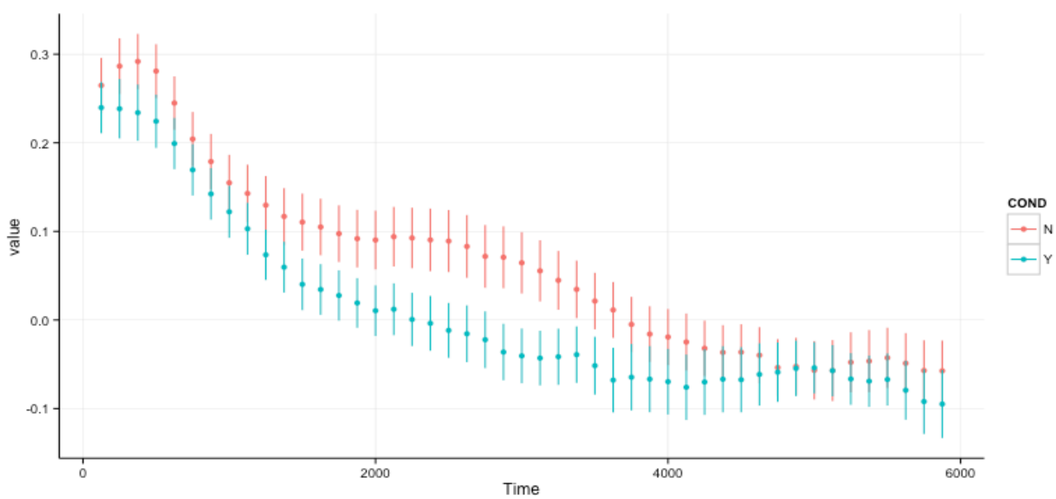

Figure 2. Group analysis of the time series corresponding to yes/no responses. The Y-axis represents raw change scores in the diameter of the pupil. Y-error bars reflect the standard error of the mean. Bins reflect aggregated samples over 125 ms increments.

The time * response interaction was statistically significant [F(19,47)=1.67, p=.003]. In addition there was a significant main effect of time [F(19,47)=21.32, p<.0001] and a trend toward significance for response type (yes/no) [F(19,1)=3.09, p=.09]. These effects were evident in the hypothesized direction (i.e., negation responses showed a higher amplitude of pupil dilation than affirmation responses). However, the time series was non-linear. Visual inspection of Figure 2 illustrates that "no" responses evoked a higher amplitude pupillary response relative to the "yes" responses exclusively in the window from 2000-4000 ms. Table 1 reports statistical confirmation of these differences via paired t-tests contrasting the "yes" and "no" pupillary response functions at each successive time bin.

Table 1. Tests of pupillary differences across time bins for yes/no response conditions.

		Mean	St. Deviation	Std. Error	t	df	P-Value
Pair 1	NBIN1 - YBIN1	.010	.120	.027	.376	19	.711
Pair 2	NBIN2 - YBIN2	.025	.130	.029	.860	19	.401
Pair 3	NBIN3 - YBIN3	.048	.137	.031	1.565	19	.134
Pair 4	NBIN4 - YBIN4	.058	.138	.031	1.869	19	.077
Pair 5	NBIN5 - YBIN5	.057	.156	.035	1.618	19	.122
Pair 6	NBIN6 - YBIN6	.046	.158	.035	1.290	19	.213
Pair 7	NBIN7 - YBIN7	.035	.164	.037	.946	19	.356
Pair 8	NBIN8 - YBIN8	.037	.165	.037	.992	19	.334
Pair 9	NBIN9 - YBIN9	.033	.164	.037	.895	19	.382
Pair 10	NBIN10 - YBIN10	.040	.167	.037	1.069	19	.299
Pair 11	NBIN11 - YBIN11	.056	.171	.038	1.468	19	.159
Pair 12	NBIN12 - YBIN12	.057	.166	.037	1.538	19	.140
Pair 13	NBIN13 - YBIN13	.070	.151	.034	2.081	19	.051
Pair 14	NBIN14 - YBIN14	.071	.140	.031	2.251	19	.036
Pair 15	NBIN15 - YBIN15	.070	.1470	.033	2.123	19	.047
Pair 16	NBIN16 - YBIN16	.073	.138	.031	2.360	19	.029
Pair 17	NBIN17 - YBIN17	.080	.136	.030	2.621	19	.017
Pair 18	NBIN18 - YBIN18	.082	.144	.032	2.541	19	.020
Pair 19	NBIN19 - YBIN19	.092	.152	.034	2.709	19	.014
Pair 20	NBIN20 - YBIN20	.094	.158	.035	2.670	19	.015
Pair 21	NBIN21 - YBIN21	.101	.169	.038	2.668	19	.015
Pair 22	NBIN22 - YBIN22	.099	.170	.038	2.594	19	.018
Pair 23	NBIN23 - YBIN23	.094	.168	.038	2.507	19	.021
Pair 24	NBIN24 - YBIN24	.107	.1737	.039	2.758	19	.013
Pair 25	NBIN25 - YBIN25	.105	.170	.038	2.765	19	.012
Pair 26	NBIN26 - YBIN26	.098	.178	.040	2.477	19	.023
Pair 27	NBIN27 - YBIN27	.086	.177	.039	2.185	19	.042
Pair 28	NBIN28 - YBIN28	.074	.177	.040	1.864	19	.078
Pair 29	NBIN29 - YBIN29	.073	.168	.038	1.944	19	.067

Table 1. Tests of pupillary differences across time bins for yes/no response conditions, continued.							
Pair 30	NBIN30 - YBIN30	.079	.155	.035	2.285	19	.034
Pair 31	NBIN31 - YBIN31	.060	.144	.032	1.849	19	.080
Pair 32	NBIN32 - YBIN32	.051	.150	.033	1.521	19	.145
Pair 33	NBIN33 - YBIN33	.050	.161	.036	1.405	19	.176
Pair 34	NBIN34 - YBIN34	.051	.162	.036	1.408	19	.175
Pair 35	NBIN35 - YBIN35	.038	.175	.039	.970	19	.344
Pair 36	NBIN36 - YBIN36	.030	.181	.040	.747	19	.464
Pair 37	NBIN37 - YBIN37	.031	.187	.042	.745	19	.466
Pair 38	NBIN38 - YBIN38	.022	.194	.043	.500	19	.623
Pair 39	NBIN39 - YBIN39	.005	.198	.044	.119	19	.907
Note: Cells containing statistically significant contrasts are shaded in gray.							

Item Level Response Discrimination

Item level reliability is a key consideration for the development of an effective communication system. We evaluated several potential metrics of scoring yes/no at the level of individual questions. First, we isolated time bins 15-30, roughly corresponding with the period of 2000-4000 ms after the trial onset. We then evaluated whether the bin mean for that particular question fell either below or above the median for all of that particular individual's observed responses. We scored responses that fell above the median as "no" (indicative of dilation). In contrast, we scored responses that fell below the median as "yes" (indicative of a constriction). We then assessed the correlation of these physiologically generated responses with the nature of the original question (yes/no). The median method produced discrimination accuracy only marginally higher than chance (55% prediction accuracy). We also attempted to score item level responses using the mean as a cutpoint, and this, too, produced poor prediction accuracy (53% accuracy).

As an alternative method, we scored individual "yes" or "no" questions relative to each participant's mean for the alternative answer. That is, for all "yes" questions we scored a response as correct if the mean across all of the time bins was less than the average for the mean of all the "no" responses. In contrast, we scored individual "no" questions as correct if the pupil diameter was above the mean for all “yes” questions. This method produced trial level discrimination accuracy of 64.5%.

CHAPTER 4

DISCUSSION

The results of the present investigation act as proof of concept for utilizing pupillometry and luminance-based mental imagery as a novel mode of communication and potential diagnostic tool for individuals who are locked-in. Despite a relatively small sample size and occasional sampling errors during testing, a group level effect was demonstrated in the data analysis. In addition, we obtained item level discrimination above chance across participants. Importantly, all participants produced some degree of pupil diameter shift relative to baseline, implicating this protocol as a potential screening measure for those believed to have LIS. Further investigation, including identifying responders vs. non-responders, modifying the training procedure, and developing superior discriminant functions to be used in analysis, may produce more reliable item level results. Testing individuals with LIS will characterize the unique pupillary responses that may be observed in this population.

Group Level Effect

The most notable effect in the averaged group data occurred between roughly 2000 ms and 4000 ms post initiation of participant response. As expected, the "yes" and “no” response conditions were significantly different across participants during the majority of this time window (Table 1). Completing a group analysis was useful for multiple reasons. First, the group analysis allowed us to demonstrate that, on average, our sample produced a different pupil response pattern when responding to questions in the negative than when responding in the affirmative. Having demonstrated this fact, it became viable to look for differences between single items across participants: between those items eliciting a “yes” vs. “no” response. The group analysis also focused the scope of the item level analysis. Since the most contrastive effects of the “yes” vs. “no” conditions could be observed during the 2000 ms to 4000 ms window in the pupillary response, we used the data from these bins (15-30) to perform the item level analysis.

Item Level Effect

Contrary to the group level effect, the item level effect, even within the 2000 ms to 4000 ms window, is not currently reliable enough to successfully implement the present system for the purpose of communication. Although comparing the mean pupil diameter for a single response (e.g., a "yes" response) to the mean pupil diameter across opposing trials (e.g., across all of the same participant's "no" trials) produced a prediction accuracy better than chance (64.5%), the level of accuracy is not sufficient to warrant applying this system to the intended population of individuals with LIS. Especially considering that this potential communication modality would be implemented in order to aid patients and families in making critical medical decisions, a higher level of accuracy is necessary in a control sample prior to proceeding with testing in the patient population.

Screening Tool

Despite the fact that item level analysis revealed modest levels of accuracy, the protocol utilized in the present study does provide the potential for use as a screening tool. All participants produced some change in pupil diameter compared to baseline. We can draw the conclusion that this would not have occurred without some kind of thought modulation in response to stimuli because other potential factors that might impact pupil dilation and constriction were controlled. Obtaining a change from baseline pupil diameter across all participants in all trials introduces the possibility of using pupillometry as a way to screen for consciousness in those patients who demonstrate little to no volitional movement. Even unreliable responses do not preclude the use of pupillometry as a screening tool because any change from baseline would denote a reflexive pupillary response resulting from modulation of mental imagery, as long as other potentially confounding factors were controlled. That is to say, if a patient with questionable consciousness is able to listen to and follow a clinician’s instructions to willingly generate mental imagery on command, yielding a reflexive pupil dilation or constriction, then that patient could be identified as potentially conscious and locked-in.

Future Directions

Future iterations of the present investigation should address identifying "responders" vs. "non-responders", improved and more extensive training protocols, applying more sophisticated discriminant functions to single item responses, and testing patients with known movement disorders, including LIS. However, incorporating these additions may incur various challenges.

During item level analysis, it became apparent that two distinguishable groups may exist among our sample of participants: a group of relatively consistent "responders" and a group of inconsistent "non-responders". Individual responses of the "responder" group were more consistently accurate than the individual responses of the "non-responder" group, according to the procedures mentioned above where a single response (e.g., "yes" response) was compared with the mean of the alternative responses (e.g., all "no" responses) for the same participant. It was outside the scope of the present investigation to analyze these groups separately; however, this is a potential avenue for future inquiry as is determining screening procedures to identify members of these two groups prior to testing. If "non-responders" could be screened out of the experimental sample in future studies, perhaps higher item level accuracy could be achieved.

Improving upon the training procedures may benefit future studies as well by potentially converting a portion of the "non-responders" to "responders". Checking a participant's understanding of the task more thoroughly and providing each participant with more explicit instruction may ensure that a subset of participants who would otherwise be considered “non-responders” instead perform as “responders”. One way of adding to the training procedure would be to provide exemplars of a “sunny sky” and “dark room” so that all participants visualize scenes with relatively equal levels of illumination. In the present study, participants may have been imagining scenes with variations in luminance. For example, one participant may have imagined a cloudless “sunny sky” with bright rays of sunshine while another participant may have imagined a “sunny sky” including clouds that blocked part of the sun’s radiation. These two distinct images may have elicited varying degrees of pupillary constriction.

Providing exemplars is also supported by a different experiment in Laeng and Sulutvedt's study (2014). The authors showed that the most robust effects of mental imagery on pupil diameter occurred when participants were primed. Participants were shown one of a variety of shapes with varying levels of illumination on the hue, saturation, and lightness (HSL) scale, followed by a delay where they observed a neutral gray screen. Participants were then asked to imagine the shape they had just been presented with, therefore having a clear recent memory of what they were supposed to be visualizing. Even if participants in future versions of the present study were not provided with an image of a "sunny sky" and "dark room" prior to the onset of each trial, providing such exemplar images during training may aid in their consistent visualization throughout the experiment by allowing them to replicate the previously viewed imagery rather than requiring them to spontaneously conjure up a scene that is in no way standardized.

Another way of improving upon the training protocol would be to add a narrative while teaching the visualization technique. This verbal imagery could be presented when participants first view the "sunny sky" and "dark room" exemplars and again when they are asked to visualize the scenes in their minds' eyes, prior to being taught the paired association between mental imagery and responding to questions. The benefit of adding a narrative would be attaching more semantic information to each condition (i.e., "sunny sky" and "dark room") so that the scene could be recalled in greater detail or perhaps with greater accuracy. However, the risk of adding a narrative would be influencing arousal or cognitive load, which would in turn have unwanted effects on pupil diameter (Beatty & Lucero-Wagoner, 2000; Bradley et al., 2008; Piquado et al., 2010). Utilizing verbal imagery during training would require emotionally neutral and simple characterizations of the exemplar scenes. For example, when training the "dark room," it would be preferable to state "You are entering a dark room in your house. You can't see any furniture," rather than making statements such as "You are entering the attic. You can't see where you are walking," because the latter might induce a sense of fear for the participant, thus potentially causing a pupil dilation due to emotional and/or arousing factors rather than the imagined darkness.

In addition to adapting the training protocol, applying more sophisticated discriminant functions to single item analysis may result in higher accuracy across participants. Using measures other than mean and median to compile responses (e.g., all "no" responses for a participant) may provide a starker contrast with alternative answers (e.g., a "yes" response). However, identifying the most appropriate measure(s) will be the challenge.

Although it would be ideal to obtain higher levels of item discrimination accuracy in control samples first, a final concept that needs to be addressed in future iterations of this experiment is the potential for differences to arise when studying the pupillary reflex of those with LIS. To better understand the functioning of the pupillary system in individuals with LIS, and how it may be different from healthy adults, studies must be conducted with participants that have a confirmed diagnosis. For example, those who are locked-in may experience a delay in the response time of their pupillary reflex. If the pupil response latency were shifted in patients with LIS, this would mean the window identified in the present study showing the greatest promise of distinguishing a “yes” from a “no” response in neurotypical adults (approximately 2000 ms to 4000 ms post onset) might not apply to those with LIS. Rather, it may take longer for the pupil reflex to react to visualized imagery.

Furthermore, it must be kept in mind that the 2000 ms to 4000 ms window discovered in the present study will inherently be shifted when testing a sample of patients with LIS due to their inability to produce a key press to initiate the recording period. When testing patients with LIS, recording will need to begin at the offset of the stimulus question and will most likely need to extend beyond the 8 seconds recorded in the current experiment. If participants have been well trained and can conjure up the appropriate image rapidly when responding to questions, we can hope that a discriminating window (equivalent to the 2000 ms to 4000 ms window documented here) will be revealed among their responses. Because individual differences in visualization time may occur, it would be useful to compare a high volume of "yes" and "no" responses within individual participants prior or in addition to a group comparison.

Conclusion

This investigation affirmed the plausibility of leveraging Laeng and Sulutvedt's discovery in favor of an alternative communication system for individuals who are locked-in. The benefit of such a system would be greater convenience, cost-efficiency, and potentially greater reliability than utilizing the technologies (i.e., fMRI or EEG) currently being targeted for development of diagnostic and communicative tools for those who are locked-in. Although BCI systems may be available in the future, they are currently unreliable and fall short of providing accurate diagnostic and communicative information, making their immediate utilization inadvisable. Moreover, using luminance-based visual imagery could provide a more accurate diagnostic picture of the number of misdiagnosed individuals who are currently locked-in as a result of its potentially increased reliability in this population over the motor-based imagery that has been previously utilized (Conson et al., 2010; Fiori et al., 2013). Further investigation, including identification of "responders" and "non-responders," the use of a more extensive training protocol, development of superior discriminant functions for analysis, and testing patients with LIS, will determine the ultimate utility of leveraging pupillometry and luminance-based mental imagery for not only a novel mode of communication, but also a diagnostic tool.

REFERENCES CITED

Barreca, S., Velikonja, D., Brown, L., Williams, L., Davis, L., & Sigouin, C. S. (2003). Evaluation of the effectiveness of two clinical training procedures to elicit yes/no responses from patients with a severe acquired brain injury: A randomized single-subject design. *Brain Injury*, *17*(12), 1065-1075.

Beatty, J., & Lucero-Wagoner, B. (2000). The pupillary system. In J. T. Cacioppo, L. G. Tassinary, & G. Berntson (Eds.), *Handbook of psychophysiology* (pp. 142-162). Cambridge, MA: Cambridge University Press.

Bradley, M. M., Miccoli, L., Escrig, M. A., & Lang, P. J. (2008). The pupil as a measure of emotional arousal and autonomic activation. *Psychophysiology*, *45*(4), 602-607.

Conson, M., Pistoia, F., Sarà, M., Grossi, D., & Trojano, L. (2010). Recognition and mental manipulation of body parts dissociate in locked-in syndrome. *Brain and Cognition*, *73*(3), 189-193.

Cruse, D., Chennu, S., Chatelle, C., Bekinschtein, T. A., Fernández-Espejo, D., Pickard, J. D., ... & Owen, A. M. (2012). Bedside detection of awareness in the vegetative state: A cohort study. *The Lancet*, *378*(9809), 2088-2094.

D'Esposito, M., Postle, B. R., Ballard, D., & Lease, J. (1999). Maintenance versus manipulation of information held in working memory: An event-related fMRI study. *Brain and Cognition*, *41*(1), 66-86.

Fernández-Espejo, D., & Owen, A. M. (2013). Detecting awareness after severe brain injury. *Nature Reviews Neuroscience*, *14*(11), 801-809.

Fiori, F., Sedda, A., Ferrè, E. R., Toraldo, A., Querzola, M., Pasotti, F., ... & Bottini, G. (2013). Exploring motor and visual imagery in Amyotrophic Lateral Sclerosis. *Experimental Brain Research*, *226*(4), 537-547.

Hagen, C., Malkmus, D., & Durham, P. (1972). Rancho Los Amigos levels of cognitive functioning scale. Downey, CA: Professional Staff Association.

Hirschberg, R., & Giacino, J. T. (2011). The vegetative and minimally conscious states: Diagnosis, prognosis and treatment. *Neurologic Clinics*, *29*(4), 773-786.

Kennedy, P. R., Bakay, R. A., Moore, M. M., Adams, K., & Goldwaithe, J. (2000). Direct control of a computer from the human central nervous system. *IEEE Transactions on Rehabilitation Engineering*, *8*(2), 198-202.

Klingner, J. (2010). *Measuring cognitive load during visual tasks by combining pupillometry and eyetracking* (Unpublished doctoral dissertation). Stanford University, Stanford, CA.

Laeng, B., & Endestad, T. (2012). Bright illusions reduce the eye's pupil. *Proceedings of the National Academy of Sciences*, *109*(6), 2162-2167.

Laeng, B., & Sulutvedt, U. (2014). The eye pupil adjusts to imaginary light. *Psychological Science*, *25*(1), 188-197.

León-Carrión, J., Eeckhout, P. V., Domínguez-Morales, M. D. R., & Pérez-Santamaría, F. J. (2002). Survey: The locked-in syndrome: A syndrome looking for a therapy. *Brain Injury*, *16*(7), 571-582.

Lesenfants, D., Habbal, D., Lugo, Z., Lebeau, M., Horki, P., Amico, E., ... & Noirhomme, Q. (2014). An independent SSVEP-based brain–computer interface in locked-in syndrome. *Journal of Neural Engineering*, *11*(3), 035002.

Mehta, P., Antao, V., Kaye, W., Sanchez, M., Williamson, D., Bryan, L., Muravov, O., & Horton, K. (2014). Prevalence of amyotrophic lateral sclerosis-United States, 2010-2011. *Morbidity and Mortality Weekly Report Surveillance Summary*, *63*(7), 1-14.

Monti, M. M., Vanhaudenhuyse, A., Coleman, M. R., Boly, M., Pickard, J. D., Tshibanda, L., ... & Laureys, S. (2010). Willful modulation of brain activity in disorders of consciousness. *New England Journal of Medicine*, *362*(7), 579-589.

Oken, B. S., Orhan, U., Roark, B., Erdogmus, D., Fowler, A., Mooney, A., ... & Fried-Oken, M. B. (2014). Brain–computer interface with language model–electroencephalography fusion for locked-in syndrome. *Neurorehabilitation and Neural Repair*, *28*(4), 387-394.

Owen, A. M., & Coleman, M. R. (2008). Functional neuroimaging of the vegetative state. *Nature Reviews Neuroscience*, *9*(3), 235-243.

Owen, A. M., Coleman, M. R., Boly, M., Davis, M. H., Laureys, S., & Pickard, J. D. (2006). Detecting awareness in the vegetative state. *Science*, *313*(5792), 1402-1402.

Piquado, T., Isaacowitz, D., & Wingfield, A. (2010). Pupillometry as a measure of cognitive effort in younger and older adults. *Psychophysiology*, *47*(3), 560-569.

R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Retrieved from http://www.R-project.org/.

Smith, E., & Delargy, M. (2005). Locked-in syndrome. *British Medical Journal*, *330*(7488), 406.

Stoll, J., Chatelle, C., Carter, O., Koch, C., Laureys, S., & Einhäuser, W. (2013). Pupil responses allow communication in locked-in syndrome patients. *Current Biology*, *23*(15), R647-R648.

Teasdale, G., & Jennett, B. (1974). Assessment of coma and impaired consciousness: A practical scale. *The Lancet*, *304*(7872), 81-84.

APPENDIX

SUMMARY OF PRESENTED STIMULI

Condition	Question	Duration (ms)
Yes	Does a clock tell time?	1531
Yes	Is a circle round?	1405
Yes	Is a penny less than a dollar?	2042
Yes	Is the current president Barack Obama?	2226
Yes	Can you eat bread?	1385
Yes	Do birds have wings?	1577
Yes	Are there 12 months in a year?	1685
Yes	Is a dog an animal?	1530
No	Do turtles run fast?	1600
No	Is it 1983?	1773
No	Does 1 + 1 = 7?	2042
No	Do heaters make you cold?	1577
No	Are you 2 years old?	1481
No	Is 3 an even number?	1873
No	Is L a number?	1481
No	Are there 10 days in a week?	1802