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PRINCIPAL INVESTIGATOR: Randy Kardon M.D. Ph.D.

CONTRACTING ORGANIZATION: University of Iowa
Iowa City IA 52242-1316

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Randy Kardon MD PhD, Pieter Poolman PhD
Andrew Russo, PhD
E-Mail: randy-kardon@uiowa.edu

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14. ABSTRACT
Purpose: to test the whether photosensitivity (photophobia) after traumatic brain injury (TBI) is due to increased sensitivity of the brainstem trigeminal sensory nucleus, as revealed objectively by an exaggerated photoblink reflex (photo-electromyogram). This will be tested in humans and in a mouse strain genetically engineered to be hypersensitive to calcitonin gene related peptide (CGRP), the neurotransmitter modulating trigeminal nerve function.
Scope: objective methods to quantify photo-sensitivity include 1) light evoked potentials (electromyogram) from the blinking and squinting muscles of the forehead 2) the pupil light reflex 3) light evoked changes in sympathetic nerve activity, measured by changes in skin conductance and heart rate.
Major Findings (Year 4): 1) we have designed and assembled a multi-camera platform that will allow us to transition from EMG recording with surface skin electrodes to an electrode-free system that will take advantage of facial feature changes in response to light and pain which is anticipated to be useful in remote settings and home testing, utilizing smart phone devices, 2) we have configured the recording and analysis software to apply Componica's software library to multiple camera video streams simultaneously in order to analyze minute changes in facial features in real time, and 3) we have tested additional normal subjects to better match age and sex of those subjects already tested with photosensitivity in order to define the normative range of values for light induced EMG.
Significance: objective testing of photosensitivity in humans and mice will provide new approaches to finding the underlying mechanisms, classification of photosensitivity, diagnosis and monitoring of new treatments.

15. SUBJECT TERMS
Photophobia, photodynia, photosensitivity, light sensitivity, traumatic brain injury, electromyogram, calcitonin gene related peptide (CGRP), trigeminal

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INTRODUCTION

Two of the most prevalent problems reported by military personnel following traumatic brain injury (TBI) are photosensitivity and headache. Currently, better means are needed for diagnosing and treating post-traumatic light sensitivity and headache. The goal of this project is to establish a clinically translatable assay of photosensitivity to facilitate diagnosis and treatment of light sensitivity and headache. The clinically translatable assay will take advantage of a natural brain reflex, the photic-electromyogram (EMG), a reflex contraction of the eyelid muscles in response to light. The photic EMG is modulated by the thalamus and central sensory trigeminal pain center of the brainstem, which conveys light input to the facial muscles to elicit an eye blink and squinting response. We hypothesize that the hallmark of patients with photosensitivity is abnormal sensitization of the thalamus and brainstem trigeminal neurons to light input. This grant's objective is to show that the trigeminal and photic blink reflex to light, as measured by the photic EMG, is a valid surrogate for assessing central thalamic and trigeminal hypersensitivity as a cause for photosensitivity and headache, which can be treated. The specific aims of this grant are twofold: 1) to objectively characterize the photosensitive response in humans by recording the photic EMG in normal subjects compared to photosensitive patients and assess treatment with blue blocking lenses, and 2) to examine the photosensitive response in awake, un-anesthetized mice by recording the photic EMG in a genetic mouse strain that has trigeminal hypersensitivity and light aversion. The effect of injecting calcitonin gene related peptide (CGRP), the neurotransmitter modulating trigeminal neurons, and an antagonist, olcegepant, will be used to investigate a new medical treatment of photosensitivity in the mouse model. These studies will establish the foundation for future clinical diagnosis and treatment of photosensitivity.

BODY – RESEARCH ACCOMPLISHMENTS ASSOCIATED WITH APPROVED STATEMENT OF WORK FOR YEAR 4:

1) IRB (Human Use)

In Year 4, we were notified of the annual renewal of our human use protocol from the University of Iowa IRB committee.

The HRPO point of contact for this study has been Lori J. Walther, Human Subjects Protection Scientist, at 301-619-2286/lori.j.walther.ctr@us.army.mil.

The following DOD Human Use Officer has been informed, was sent documentation and has acknowledged the annual renewal:

CARYN L. DUCHESNEAU, BS, CIP
Chief, Human Subjects Protection Review
Human Research Protection Office
Office of Research Protections
US Army Medical Research and Materiel Command

We have continued to identify patients with photosensitivity to add to our tested subject group. We have access to a TBI database from the Iowa City VA Medical Center as a source for military-associated TBI. Patients with photosensitivity after TBI have also been identified as they are referred to the Iowa City VA Eye Clinic and seen by the PI (Randy Kardon MD PhD) in his VA and University of Iowa neuro-ophthalmology clinics. In addition, we have accessed the University of Iowa Hospital patient database by diagnosis, allowing us to obtain an Excel spreadsheet listing patients with TBI and also those with photosensitivity as a diagnosis. We also have a list of normal subjects that we have used as research subjects for other studies and have continued to recruit normal subjects from this pool, since their visual system has already been well characterized. Finally, we have also been recruiting migraine patients since they commonly report light sensitivity between headaches and have been recruiting migraine subjects in the immediate 25-mile radius using email announcements and also the UIHC database by diagnostic category and patient location.

2) Optimization of a novel method to objectively assess photosensitivity in humans

During the past year, we have made progress on the following tasks:

Task 1: Optimization of a novel method to objectively assess photosensitivity in humans. This task has been completed. During completion of Task 1, we discovered that contraction of the facial muscles mediating the response to light could be quantified from a video recording that is capable of replacing the direct surface skin recording of the electrically evoked response from the underlying facial muscles (facial EMG). Importantly, the video analysis of facial muscle activation by light has provided us the opportunity to translate this measurement to remote, mobile testing devices that already have video recording capabilities, such as a smartphone in combination with an LED light stimulator. This has tremendous positive implications for testing of military and veteran patients with photosensitivity and monitoring their treatment from remote sites.

In order to take advantage of this unique finding, we have continued the effort to enhance our testing system to enable us to record and analyze facial responses to light stimuli:

During the first quarter, we have enlisted Componica, LLC to configure our software to be able to analyze minute changes in facial features in response to light in real time so that these responses can be evaluated immediately at the time of testing. Componica, LLC is a software development entity with specific expertise and track record of successfully implementing facial feature tracking algorithms in real time on mobile devices and other low-powered CPU platforms. The software includes the following features and benefits:

1. Facial Detection: Algorithms for detecting a face in a video frame are readily available, such as solutions commonly found in image processing libraries such as OpenCV. However, these methods are CPU intensive processes and therefore, require higher powered computer devices and would not be suitable for use on small portable devices in real time. Componica's solution is based on a Local Binary Pattern face detector, which they have found to be significantly faster on mobile devices with imperceptible loss of accuracy, and can be sped up by reducing the search and scale space based on facial detection results from previous frames. This approach is ideal for remote testing of military and veteran patients with subjective complaints of photosensitivity.

2. Facial Feature Detection and Derived States: Convolutional Neural Networks (CNNs) and other related neural networks have recently been proven successful for deriving a multitude of computer vision tasks ranging from optical character recognition to facial characterization such as pose estimation and finding facial landmarks. Inspired by the biological processes of the visual cortex, the Componica solution has the advantage of directly operating on raw pixel images with minimum preprocessing, learning low-level shift-invariant features and other higher level representations of facial features. Additionally, CNNs can estimate orthogonal classifications like object recognition and pose detection simultaneously. Therefore, Facial Action Coding System (FACS) Action Units (AUs), affective and pain states, pose, and landmarks can be simultaneously assessed with a single network, allowing all determinations to be made simultaneously on a low processing power CPU residing in a mobile device. **Figure 1** summarizes the image analysis steps in deriving the facial features of interest which are affected by light. **Figure 2** shows an example of the type of facial feature landmarks and analysis that will be derived from a smartphone device after completion of this grant.

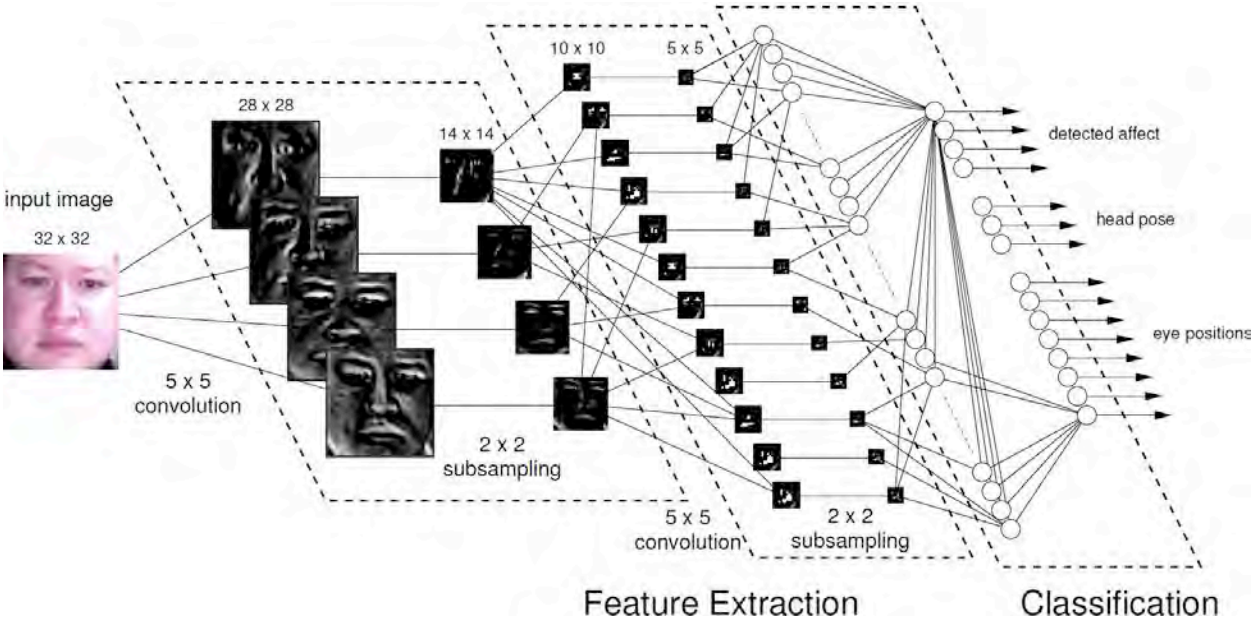

Figure 1. Flow diagram of Componica's Convolutional Neural Network (CNN) implementation for facial feature tracking and state classification for use on mobile devices, like smartphones. Input video frame is show as the input image on the left and output features are derived on the right.

3. Event Aggregation and Reporting: The proposed process of detecting a subject's facial responses to light, affective and pain states, etc. is based on a machine learning approach, applied on a frame-by-frame basis. Because frame-by-frame analysis does not take into account frames in the past, spurious outputs like eye blinking or glancing off screen can give results that can be mistaken as affect events and could be taken out of context. To resolve this problem, an event aggregation method is now being implemented in year 5 of the grant extension to buffer detected affective/pain states over a short time window in order to output events that are consistent over time. This will allow dynamic responses of the face to light to be assessed.

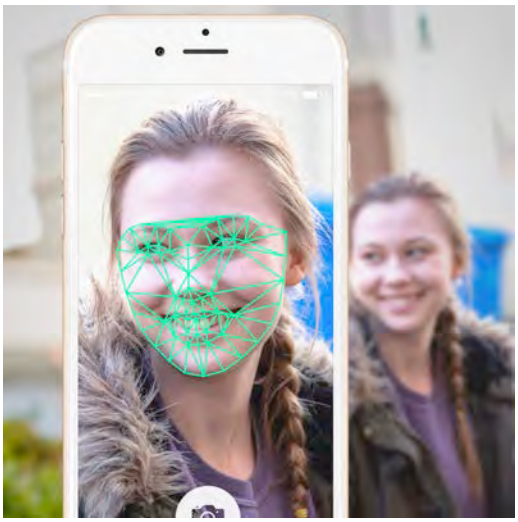

Figure 2. Example of facial feature landmarks automatically derived from single video frames that will be collected on a smartphone device as a function of light intensity for remote determination of photosensitivity and monitoring of treatment.

During the second quarter, we have acquired a unique camera, JAI's AD-130GE, which is a prism-based 2-CCD progressive area scan camera capable of simultaneously capturing visible and near-infrared light spectrums through the same optical path using two individual channels in one camera, as shown in **Figure 3**. The first channel has a Bayer mosaic color imaging sensor that captures visible light, while the second channel has a monochrome sensor for capturing near infrared light. The AD-130GE camera features a resolution of 1.3 megapixels per channel, can be triggered externally, runs at 31 frames per second in continuous operation, and supports separate exposure times for each channel, allowing mutual adjustment without changes in gain to maintain a consistent signal-to-noise ratio. The motivation for acquiring facial images in both color and infrared wavelengths is to take full advantage of all facial features that could be revealed using different wavelengths of light. In addition, a neural network trained to this unique data set collected with both sensors will be more robust at defining the same facial features under lighting conditions that might be suboptimal in the field. In addition, common methods of obtaining and analyzing facial features, including pupil borders, eyelid borders and eye position mostly use infrared cameras and the analysis approaches are not translatable to images collected under highly variable lighting conditions recorded with color camera sensors that would be encountered in wide usage in different environments by military and civilian applications. Using the combined, simultaneous imaging of both color and infrared sensors and training an analysis algorithm to both will provide a very robust analysis that will be valid under many different lighting environments.

With the assistance of our external software developer and Componica, LLC, we have integrated the JAI AD-130GE camera into our existing recording and analysis software suite, and configured the software to apply Componica's software library to multiple camera video streams simultaneously in order to analyze minute changes in facial features in real time as shown in **Figure 4**. This capability will allow us in the future to track and monitor responses of patients to light stimuli immediately at the time of testing with digital video from either infrared and visible camera sensors. Componica, LLC is continuing their effort to enhance facial feature tracking algorithms for real-time use on mobile devices and other low-powered CPU platforms that use color sensors and infrared sensors.

Figure 3. The JAI AD-130GE camera (left) combines both a color video sensor and an infrared video sensor mounted on the same optical axis (right), enabling one to easily locate and compare the image parameters of different facial features as seen in both the visual and infrared spectra. For example, the contrast at the pupil border between the a blue iris and the pupil is low in the infrared spectrum, but significantly higher in the visible spectrum.

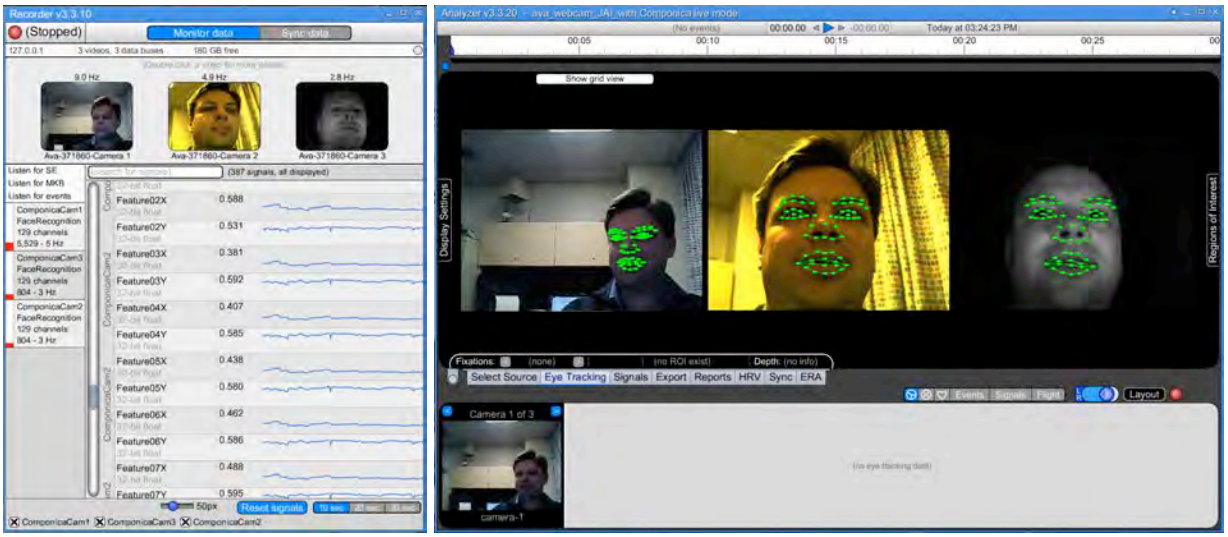

Figure 4. Left: As shown in the Recorder GUI, we have incorporated the video streams from the JAI camera into our recording software. The Recorder GUI shows 3 video streams: a USB webcam video on the left ("Camera-1"), the JAI color video in the middle ("Camera-2"), and JAI infrared video on the right ("Camera-3"). Below the video window, the facial feature locations (i.e., 2D X and Y coordinates), calculated by the Componica facial feature library analysis for each camera's video frames individually in real time, are displayed as tracings of feature changes in real time. Right: In the Analyzer GUI, the Componica facial feature locations are overlaid on top of the associated camera video stream. The color and infrared video streams from the JAI camera are displayed as the middle and right video views respectively. Note that the JAI color and infrared cameras share the same optical axis.

Based on the need to enable remote, mobile monitoring and assessment of light sensitivity in subjects, we have also purchased an off-the-shelf, pocket-sized, portable LED light panel that has been developed for use with smartphones and camcorders as shown in **Figure 5**. This LED light panel, called KICK and manufactured by Rift Labs, is lightweight and enables one to adjust its color brightness and temperature over a Wi-Fi connection through either a smartphone app or a software development kit (SDK), available for desktop applications. The KICK light is battery powered, and can be recharged via USB. Based on the SDK, we have developed a Matlab GUI interface in order to control the KICK light during experiments. This portable wireless LED light source will be incorporated into our testing in Year 5 to provide a well characterized light stimulus from which we will record facial feature responses.

It is important to note that we have been using the Diagnosys Ganzfeld bowl for delivering controlled light stimuli to patients over the course of this research effort to date. However, the Diagnosys bowl geometry limits the ability to install and record multiple color and infrared cameras simultaneous while delivering light stimuli to the subject. Therefore, as we are also interested in developing and evaluating portable and low-cost systems that can be used in the field, we proceeded during the third quarter of Year 4 to integrate the JAI camera and KICK lights as an alternative platform for collecting facial responses to light stimuli. This is a progression of the technology to provide a portable smart phone like device for recording facial features to increasing light stimuli for diagnosis and monitoring treatment of abnormal light sensitivity.

Figure 5. The KICK light device contains a LED panel that can produce different colors of light at different intensities, and can be controlled wirelessly from a smartphone app (picture on left), or through an SDK from a laptop or desktop device (middle picture). We have developed a Matlab GUI (picture on right) to control the KICK light from a desktop PC.

Apart from the JAI AD-130GE camera, we also integrated two additional cameras into the new platform: a USB 3.0 color camera and a USB 3.0 infrared camera, both manufactured by Imaging Development Systems GmbH (IDS). Similar to the JAI camera, the IDS cameras feature a resolution of 1.3 megapixels, can be triggered externally, and can sample up to 60 frames per second in continuous operation, if needed. As shown in **Figure 6**, we have integrated the JAI and IDS cameras into our recording software. In order to ensure synchronous triggering of the cameras shutters, needed for 3D operation, we have adapted an Arduino Nano device to generate user-specified sequences of TTL signals delivered to each camera. The resulting four different facial views with infrared and color sensors are shown in **Figure 7**.

The motivation for adding additional cameras is to provide a multi-view camera system to give synchronized camera views of the face using both infrared and color video cameras, and to support the anticipated development of 3D facial action coding system (FACS) recording and analysis of facial feature responses. This multi-camera platform will enable us to collect data in our final year of funding (Year 5), which will inform us of the best recording approach and views needed to enable 3D assessment of facial features in response to light level. With 3D image analysis, it becomes possible to extract facial features that correlate with light sensitivity even when the head changes position by extracting geometrically invariant features (thus bypassing the problem of 2D pose estimation). This overcomes the major problem inherent in 2D facial feature analysis. For example, it has been shown that facial feature tracking algorithms that use 2D data, are prone to failure when trained with frontal face poses that can change as the subject moves their head, while 3D based facial feature recognition performance remains constant. Substantial distortion and occlusion of some features caused by out-of-plane rotations of the face result in pose estimation difficulties when only 2D analysis is used. In terms of facial action unit (AU) coding, results show that 3D analysis offers significant advantages in AU detection and performs overall better than the 2D under the same feature extraction and classification algorithms. In general, lower face AU detections benefit more from 3D as compared to 2D. A case in point is the considerable improvement in the detection rate of AU 23 (Lip Tightener), which is difficult even for certified coders. This AU is useful, for instance, in telling a genuine expression of pain from feigned pain as might occur when monitoring photosensitivity for migraine treatment. 3D also proves its value for expressions of low magnitude.

Figure 6. Different views of the multi-camera platform system to support the development of 3D FACS recording and analysis of facial feature responses. The subject's face is monitored synchronously from different viewpoints by a JAI AD-130GE camera (center camera), as well as from USB 3.0 color and infrared cameras from IDS to the left and right of it. Note that the JAI color and infrared cameras share the same optical axis. In order to ensure synchronous triggering of the cameras shutters, needed for 3D operation, we have adapted an Arduino Nano device to generate user-specified sequences of TTL signals delivered to each camera. The system also includes 2 infrared illuminators, as well as 2 KICK LED light panels that can produce different colors of light at different intensities, and can be controlled wirelessly from a smartphone app, or through an SDK from a laptop or desktop device.

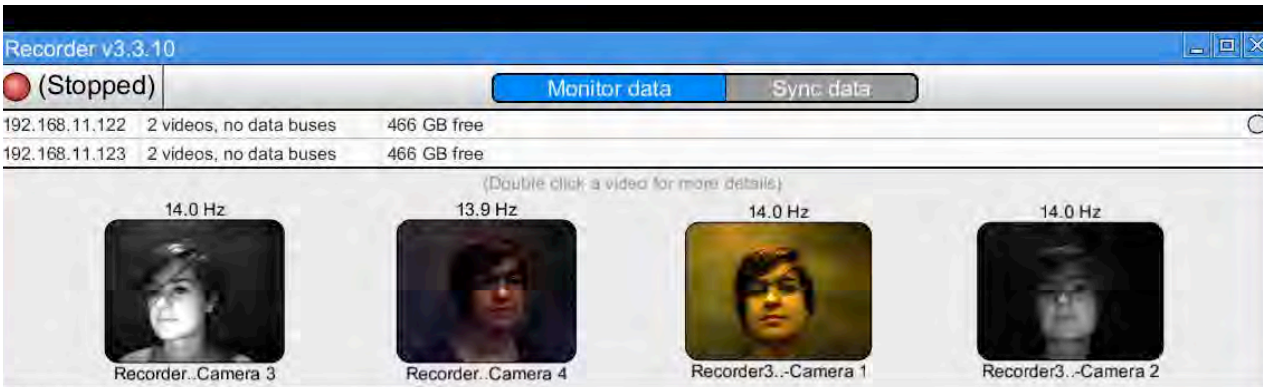

Figure 7. Multi-view platform for development of 3D facial feature analysis. Four different video camera frames from the USB infrared camera (1st view), the USB color camera (2nd view), and the prism camera providing both color (3rd view) and infrared (4th view) simultaneously with the system shown in Figure 6.

Tasks 2 and 3 - In normal humans without photosensitivity and in patients with self-reported photosensitivity, collect and define the normative range of values for light induced EMG: We have tested 5 additional normal subjects to better match age and sex of those subjects already tested with photosensitivity. The updated facial EMG responses from our control vs. photosensitive patient are shown in **Figure 8** and demonstrate a significant difference between the EMG responses between the normal and photosensitive groups. The results were presented by Dr. Andy Russo at the 17th Congress of the International Headache Society (IHC 2015) in Valencia, Spain during May, and received very favorable comments.

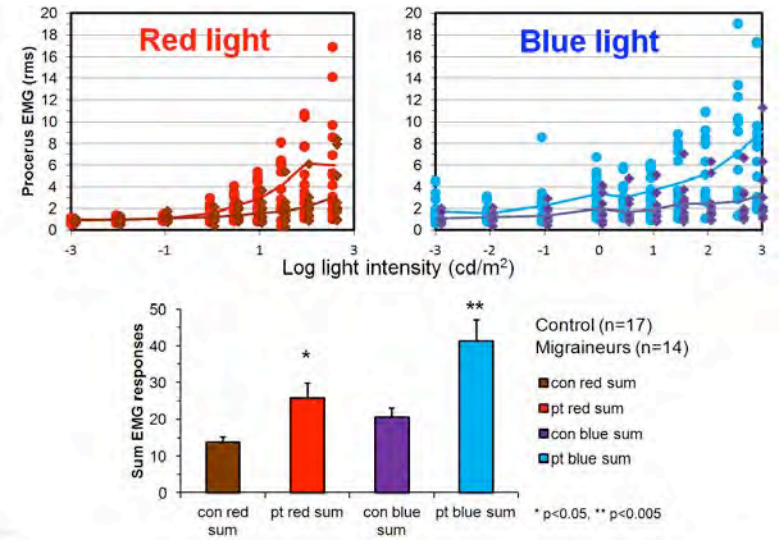

Figure 8. Data using electromyogram (EMG) recording of procerus muscles from surface skin EMG electrodes showing increasing activation of the muscle with increasing light intensity, using red light (top left) and blue light (top right), matched for photopic intensity. Note the significant increase in muscle activation by light in the patients with light sensitivity (bright red and bright blue markers) compared to normal subjects (darker red and blue markers) for both red and blue light, but more with blue light (bottom bar graph). The difference in response was the greatest at the higher levels of light intensity.

KEY RESEARCH ACCOMPLISHMENTS (SUMMARY)

- We have designed and assembled a multi-camera platform, including a JAI AD-130GE color/IR camera as well as IDS color and IR cameras, with synchronous triggering of cameras shutters and KICK LED lights for presenting stimuli. This will allow us to transition from EMG recording with surface skin electrodes to an electrode-free system that will take advantage of facial feature changes in response to light and pain which is anticipated to be useful in remote settings and home testing, utilizing smart phone devices.
- We have integrated the camera feeds of the multi-camera platform into our existing recording and analysis software suite, and configured the software to apply Componica's software library to multiple camera video streams simultaneously in order to analyze minute changes in facial features in real time.
- We have tested additional normal subjects to better match in age and sex of those subjects already tested having photosensitivity in order to define the normative range of values for light induced EMG.

REPORTABLE OUTCOMES

Presentation of facial EMG responses from our control vs. photosensitive patients were presented at the following meetings and invited talks from the standpoint of either

1) new approaches for diagnosis of light sensitivity in migraine and its treatment or
2) new approaches for the diagnosis of light sensitivity in the context of a manifestation of traumatic brain injury (TBI)

Podium session at the 17th Congress of the International Headache Society (IHC 2015) in Valencia, Spain, May 2015 (Andrew Russo PhD)

Visiting Professor (Randy Kardon MD PhD), University of Indiana Ophthalmology Department, April 2-3, 2015, Indianapolis, IN.

Visiting Professor (Randy Kardon MD PhD), Albert Einstein Department of Neurology, April 16, 2015, New York, NY.

Visiting Professor (Randy Kardon MD PhD), University of Michigan Ophthalmology Department, April 23, 2015, Ann Arbor, MI.

Visiting Professor (Randy Kardon MD PhD), Michigan State University Neurology Department and School of Veterinary Medicine, April 24, 2015, East Lansing, MI.

Invited Speaker (Randy Kardon MD PhD), Special Interest Group, "Vision and Traumatic Brain Injury in Veterans and Athletes", Association for Research in Vision and Ophthalmology, Denver, CO, May 2, 2015.

Visiting Professor (Randy Kardon MD PhD), University of Illinois Ophthalmology Department, June 16, 2015, Chicago, IL.

Visiting Professor (Randy Kardon MD PhD), Dean's lecture, City University of London, London, England, September 18, 2015.

Invited lecture (Randy Kardon MD PhD), Nebraska Academy of Eye Physicians and Surgeons, University of Nebraska Medical Center, Omaha, Nebraska, October 2-3, 2015.

CONCLUSIONS

The research work that we are carrying out has important implications for the greater public good, in addition to its military relevance. Light sensitivity and migraine headaches following traumatic brain injury are the two most commonly reported symptoms in military personnel exposed to direct trauma to the brain or indirectly from blast injury. Similar symptoms can also occur in the civilian population from TBI resulting from motor vehicle accidents and also from head injury due to contact sports at both the school and professional level. At present there are no biological markers or tests that can be used to objectively diagnose and monitor treatment of photo-- sensitivity or migraine headaches. This would be the first research to facilitate investigations of the mechanisms in humans using controlled, photic stimuli with monitoring of physiological reflexes in response to the light stimuli. In order to accomplish this task, it is required that a sophisticated software and hardware integration be in place to accurately measure light evoked reflexes that can be used in research and in a clinical setting.

REFERENCES – a literature search was performed to update the previous literature on photophobia associated with migraine and pain, as well as facial feature detection algorithms, and yielded the following relevant references:

1. Yezierski RP. The effects of age on pain sensitivity: preclinical studies. Pain Med. 2012;13 Suppl 2:S27-36.
2. Berge OG. Predictive validity of behavioural animal models for chronic pain. Br J Pharmacol. 2011; 164(4):1195-206.
3. Mujagic Z, Keszthelyi D, Aziz Q, Reinisch W, Quetglas EG, De Leonardis F, et al. Systematic review: instruments to assess abdominal pain in irritable bowel syndrome. Aliment Pharmacol Ther. 2015.

4. Salaffi F, Sarzi-Puttini P, Atzeni F. How to measure chronic pain: New concepts. Best Pract Res Clin Rheumatol. 2015; 29(1):164-86.
5. Eaton M. Common animal models for spasticity and pain. J. Rehabil. Res. Dev. 2003; 40(4 Suppl 1):41-54.
6. Cornelius R, The Science of Emotion. Upper Saddler River, NJ: Prentice-Hall, 1996.
7. Hadjistavropoulos T, Craig K. Social influences and the communication of pain. Pain: Psychological Perspectives. New York: Erlbaum, 2004.
8. Williams A, Davies H, Chadury Y. Simple pain rating scales hide complex idiosyncratic meanings. Pain. 2000; 85(3):457–463.
9. Wong D, Baker C. Pain in children: Comparison of assessment scales. Pediatr. Nurs. 1988; 14(1):9–17.
10. Turk D, Melzack R. The measurement of pain and the assessment of people experiencing pain. Handbook of Pain Assessment, D. Turk and R. Melzack, Eds. New York: Guildford Press, 2001, pp. 1–11.
11. Hill ML, Craig KD. Detecting deception in pain expressions: the structure of genuine and deceptive facial displays. Pain. 2002; 98:135-144.
12. Williams AC. Facial expression of pain: an evolutionary account. Behav. Brain Sci. 2002; 25:439–55.
13. Craig KD, Prkachin KM, Grunau RVE. The facial expression of pain. In: Turk DC, Melzack R, editors. Handbook of pain assessment. 2nd ed. New York: Guilford; 2001. p. 153–69.
14. Kunz M, Scharmann S, Hemmeter U, Schepelmann K, Lautenbacher S. The facial expression of pain in patients with dementia. Pain. 2007; 133:221–8.
15. Lehr VT, Zeskind PS, Ofenstein JP, Cepeda E, Warrier I, Aranda JV. Neonatal facial coding system scores and spectral characteristics of infant crying during newborn circumcision. Clin. J. Pain. 2007; 23:417–24.
16. Kunz M, Lautenbacher S, LeBlanc N, Rainville, P. Are both the sensory and the affective dimensions of pain encoded in the face? Pain. 2012; 153:350-358.
17. Ekman P, Friesen WV. Facial action coding system. Palo Alto, CA: Consulting Psychologists Press; 1987.
18. Lucey P, Cohn JF, Matthews I, Lucey S, Sridharan S, Howlett J, Prkachin KM. Automatically detecting pain in video through facial action units. IEEE Transactions on Systems, Man, and Cybernetics—Part B: Cybernetics. 2011; 41(3):664-674.
19. Ekman P, Davidson RJ, Friesen WV. Duchenne's smile: emotional expression and brain physiology II. J. Pers. Soc. Psych. 1990; 58:342–53.
20. Matsumiya LC, Sorge RE, Sotocinal SG, Tabaka JM, Wieskopf JS, Zaloum A, King OD, Mogil JS. Using the Mouse Grimace Scale to reevaluate the efficacy of postoperative analgesics in laboratory mice. J Am Assoc Lab Anim Sci. 2012; 51(1):42-9.
21. Sotocinal SG, Sorge RE, Zaloum A, Tuttle AH, Martin LJ, Wieskopf JS, Mapplebeck JC, Wei P, Zhan S, Zhang S, McDougall JJ, King OD, Mogil JS. The Rat Grimace Scale: a partially automated method for quantifying pain in the laboratory rat via facial expressions. Mol Pain. 2011; 29;7:55. doi: 10.1186/1744-8069-7-55.
22. Hamm J, Kohler CG, Gur RC, Verma R. Automated Facial Action Coding System for dynamic analysis of facial expressions in neuropsychiatric disorders. J Neurosci Methods. 2011; 200(2):237-56.
23. Krumhuber EG, Tamarit L, Roesch EB, Scherer KR. FACSGen 2.0 animation software: generating three-dimensional FACS-valid facial expressions for emotion research. Emotion. 2012; 12(2):351-63.
24. Markuš N, Frljak M, Pandžić IS, Ahlberg J, Forchheimer R. Object Detection with Pixel Intensity Comparisons Organized in Decision Trees. http://arxiv.org/pdf/1305.4537.pdf
25. Zhou E, Fan H, Cao Z, Jiang Y, Yin Q. Extensive Facial Landmark Localization with Coarse-to-fine Convolutional Neural Network. ICCV workshop on 300 Faces in-the-Wild Challenge, 2013.

26. Cao XD, et al. Face Alignment by Explicit Shape Regression. International Journal of Computer Vision, 2014. 107(2): p. 177-190.
27. http://opencv.org/
28. Ahonen T, Hadid A, Pietikainen M. Face recognition with local binary patterns, in Computer Vision - ECCV 2004, Pt 1, T. Pajdla and J. Matas, Editors. 2004. p. 469-481.
29. Viola P, Jones MJ. Robust real-time face detection. International Journal of Computer Vision, 2004. 57(2): p. 137-154.
30. Cootes TF, Taylor CJ. Statistical Models of Appearance for Computer Vision., http://personalpages.manchester.ac.uk/staff/timothy.f.cootes/Models/app_models.pdf
31. Lecun Y, et al., Gradient-based learning applied to document recognition. Proceedings of the IEEE, 1998. 86(11): p. 2278-2324.
32. Matsugu M, Mori K, Mitari Y, Kaneda Y. Subject independent facial expression recognition with robust face detection using a convolutional neural network. Neural Networks 16 (2003) 555–559.
33. Russo AF. CGRP as a neuropeptide in migraine: lessons from mice. Br J Clin Pharmacol. 2015; 80(3):403-14.
34. Davidson EP, Coppey LJ, Kardon RH, Yorek MA. Differences and similarities in development of corneal nerve damage and peripheral neuropathy and in diet-induced obesity and type 2 diabetic rats. Invest Ophthalmol Vis Sci. 2014; 55(3):1222-30.

APPENDICES – none

SUPPORTING DATA – all figures including in body of report