



From qualia to quantia: A system to document and quantify phosphene percepts elicited by non-invasive neurostimulation of the human occipital cortex

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ABSTRACT

The stimulation of the occipital cortex induces transient visual percepts, known as phosphenes. The characterization and analysis of the features of these visual qualia can provide a window into the physiology and neuroanatomy of cerebral visual networks of humans. Phosphenes can be reliably elicited in humans by a variety of invasive and non-invasive techniques that depolarize visual cortex neurons. Nonetheless both research into their neural basis and categorization of their features are ultimately reliant on subjective self-reports. A variety of methods have been employed to provide a more objective means of recording the localization and morphology of neurostimulation-induced phosphenes. In spite of these attempts, phosphenes remain difficult to measure. A standard technique able to both document the myriad of features characterizing phosphenes in a flexible manner and allow a systematic quantitative comparison across groups or repeated measures is lacking. We hereby provide detailed instructions on how to use off-the-shelf components to construct and implement the LTaP (laser tracking and painting system) system for a relatively objective and real-time documentation of the presence, shape, area, spatial location and distribution of phosphenes in visual space. We further provide experimental data demonstrating the feasibility and reliability of the LTaP system to accurately capture established features of phosphenes induced by transcranial magnetic stimulation (TMS) of occipital cortex.

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1. Introduction

Stimulation of the human occipital cortex is known to elicit circumscribed, salient and brief percepts appearing in the visual field, referred to as ‘phosphenes’. Phosphenes can be purposefully elicited by noninvasive brain stimulation technologies such as transcranial magnetic stimulation (TMS) (Kammer, 1998; Marg, 1991; Meyer et al., 1991) or alternate current stimulation (tACS), but not by subthreshold stimulation techniques such as transcranial direct current stimulation (tDCS) (Kanai et al., 2008) that do not directly depolarize neurons. Phosphenes typically appear as a flash of light and can take different sizes, shapes, textures, and in some cases even colors (Silvanto et al., 2007a). They are normally static percepts, although when induced by the stimulation of visual motion areas such as MT/V5, phosphenes can appear as being briefly in motion (Pascual-Leone and Walsh, 2001). The

presence of a phosphene can immediately precede a short period in which a transient scotoma prevents visual perception (Kammer, 1999). Elicited phosphenes and subsequent transient scotomas are thought to be a result of direct depolarization of neurons within the visual cortex and the phosphene location is thought to be associated with the aggregated receptive fields of the stimulated visual cortical neurons, likely within areas V2 and V3 (Kammer et al., 2005a; Thielscher et al., 2010).

Phosphenes represent a direct and intensity-dependent physiological response to stimulation, and can therefore be utilized as an independent and robust measure of the excitability of the visual cortex (Amassian et al., 1989; Deblieck et al., 2008; Kammer, 2007; Silvanto and Muggleton, 2008; Silvanto et al., 2009). TMS-induced phosphenes have been used to investigate a large array of questions involving: the functional organization and plasticity of visual regions (Kammer et al., 2005b); the cortico-cortical interactions between visual cortices (Pascual-Leone and Walsh, 2001; Silvanto and Cattaneo, 2010; Silvanto et al., 2006, 2009); the susceptibility of the visual cortex to pathology (Afra et al., 1998; Aurora et al., 1998; Silvanto et al., 2007c); different aspects of visual processing (Silvanto and Cattaneo, 2010; Silvanto et al., 2007a; Silvanto and Pascual-Leone, 2008), and more recently, the state-dependent nature of neurostimulation methods such as TMS

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(Silvanto et al., 2007a,b), and tACS (Kanai et al., 2010). Overall, the use of phosphenes have been widely used to study the human visual system and its functional interactions with other brain networks.

An inherent hurdle in using phosphenes is the limited ability to record and quantify a subjective phenomenological experience in a quantifiably meaningful way. This is made even more difficult as the observed properties of phosphenes vary in size, shape, texture and spatial location. To date, a variety of methodologies have been employed in an attempt to accurately record the appearance, location and different qualitative characteristics of a phosphene. Many studies have simply relied upon a short verbal report from their participant acknowledging the presence of any visual sensation in their visual field (Pascual-Leone and Walsh, 2001; Silvanto et al., 2007a,b). Often this is followed by requesting the participant to trace the location and extent of the phosphene with their finger in the air within an imaginary visual space. Slightly more sophisticated methods use the elaboration of a hand-drawing captured on a piece of paper or translated into a computer coordinates using a mechanical digitizing pen from memory (Cowey and Walsh, 2000; Kamitani and Shimojo, 1999; Kammer, 1998; Kammer et al., 2005a,b). While successful in documenting phosphenes, those approaches have the potential to introduce several confounding variables that could compromise an accurate documentation of a subjective experience such as a phosphene. For example, research questions regarding spatiotopic or retinotopic cortical organization are intimately coupled with head orientation and gaze direction, and it is well known that phosphenes are retinotopically anchored and thus they change position with eye movements (Kammer et al., 2005a,b). Hence, phosphene recordings may often become inconsistent due to inevitable shifts in eye, head and body positioning, and spatial translations constrained by the mechanical requirements involved in drawing percepts on a sheet of paper (Bolognini and Maravita, 2007).

Thus in order to keep track of changes in phosphene features in response to neurostimulation, a technique capable of recording in real-time, subtle changes in the subjective qualities of phosphenes is required. To fill this gap and to provide a tool for the neurostimulation community, we developed the laser tracking and painting system (LTaP), an open source, low-cost, easy to build hardware and software system that is customizable for different research aims. This system allows participants to record information on the phosphenes in real-time with minimal effort, while simultaneously documenting related data such as visual field position, shape, perimeter and surface area. We designed the LTaP to use low-cost, off-the-shelf components which can be assembled in 24–48 h by a researcher with modest electronic skills. We believe that the LTaP system, is an affordable, and easy to operate piece of equipment that would benefit the toolkit of any neurostimulation researcher. Although we created the LTaP in response to our own research needs, we aimed at developing a system with broad utility and appeal. With this in mind, we provide the research and clinical community with a description of the real-world applications and reliability of this system. We encourage other researchers to use our design for the LTaP (provided in the supplemental materials) and to modify it as needed to suit their own experimental needs.

2. Material and methods

2.1. Overview

The LTaP system was developed because there was no inclusive system capable of (1) objectively recording the characteristics of subjectively reported phosphenes evoked by TMS in our experiments; (2) logging in real time (i.e., immediately after

the phosphene had been evoked), the appearance, spatial location, shape, and perimeter of such percepts for the purpose of quantitative and qualitative analysis; and (3) providing a fairly naturalistic environment in which to document the features of phosphenes, such that the subject's perception is minimally impacted by the process of depicting the percept. Additional requirements were for the system to be open source, low-cost, robust, portable and easy to modify or adapt to every researcher's needs.

While the utility of the LTaP system was developed and tested in response to our specific projects, through modifications of its original design, its application can be far-reaching in both related and unrelated fields of study. We thus envisioned a system in which subjects could manually outline their visual percepts on a flat surface with the minimal possible amount of spatial translation. We hypothesized that the device would provide progress over past strategies, obtaining more detail than verbal reporting and more accuracy than drawing on a piece of paper placed on a desk, which forces subjects to divert eyes and head from their original position, and requires also the translation of a wide visual field in the confined space of a sheet of paper. Further advantages provided by our system would be the capture of phosphenes in real size, the capacity to easily calculate a series of numerical parameters characterizing those visual percepts, and the ability to study how these characteristics change across different experimental conditions and longitudinally over time. The software we developed (LTaP) uses a standard webcam to detect a laser pointer beam and records the position using X–Y coordinates. As points are recorded they are redisplayed as being connected using a visible line, which allows both the participant and research to observe the performance and accuracy of the outlined phosphene.

2.2. Software overview and interface

Software for the LTaP system was elaborated on the basis of AForge.NET framework ("AForge.NET:: Framework," 2010), published under LGPL v3 license (gnu.org, 2010). It was developed in C# using Microsoft's Visual Studio 2008, because the express edition is freely available for download and the abundance of resources available for the .NET framework (<http://www.microsoft.com/express/Windows/>). The LTaP software serves to detect the brightest pixel in the webcam field of view and then records the location as an X–Y coordinate. As coordinate points are recorded they are connected in real-time and displayed as a visible line on the projection screen. The source code and executable are provided (see supplemental materials).

When viewing the LTaP's graphical-user-interface (GUI) (Fig. 1) the examiner is presented with several user controls and a window displaying a live-feed from the webcam. The live webcam feed serves a number of useful functions (Fig. 1a); participant performance can be observed remotely, and the state of the LTaP system can quickly be determined. In addition to webcam's live-feed, the X–Y coordinate of the detected laser beam are presented in real time.

The examiner interacts with the software via a series of user controls (Fig. 1b). These are used to control options to record and then project the coordinates for either the brightest point (see Section 2.8) or a designated color within the webcams field of view (see Section 2.9), as well as the ability to control and clear projected images. Additional controls, presented as track bars (Fig. 1c) can be used to adjust the detection threshold. For example depending on the ambient luminance and the intensity of the laser pointer the threshold may require adjustment to ensure consistent tracking and detection of the laser point. Other useful information such as the webcam's rate of recorded frames per second (fps) or acquisi-

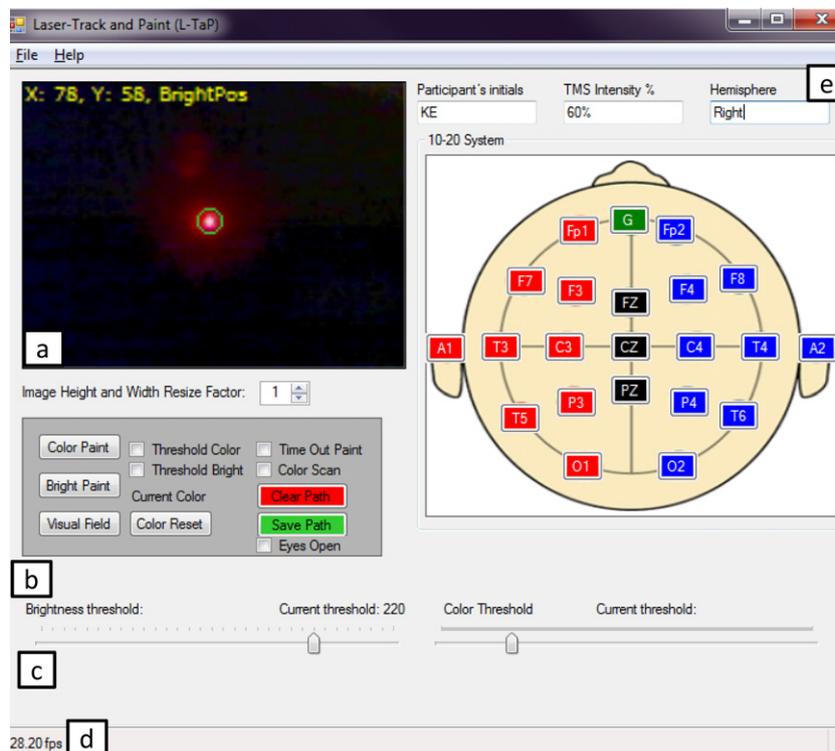


Fig. 1. Screen capture of the L-TaP system's graphical user interface (GUI). The window in the top left corner provides a live feed from the webcam, as well as the current X–Y coordinates of the detected laser point (a). The controls settings (within in the dark gray box) are used to select the type of tracking (brightness, or color), to begin recording and projecting data points, and to clear data (b). The two track bars along the bottom portion of the GUI adjust threshold levels (c). The number of recorded frames per second is presented in the bottom right corner (d) (as seen in the screen capture).

tion frequency is also visible in real-time. This information provides the experimenter with useful feedback to help calibrate the L-TaP system. For instance, if the frame rate is lower than required, the examiner may adjust the webcam settings for example in an effort to increase the image acquisition rate. Lastly there are options to indicate the brain area being stimulated as well as fields to enter key demographic and other pertinent information about the phosphenes (Fig. 1e).

2.3. Hardware

The L-TaP system consists of five essential hardware components; a standard USB webcam (1), a front-and-rear projection screen (2), a standard projector (3), a laser pointer (4) and a computer running custom made L-TaP software (5) (in our case a Lenovo X60 tablet with Intel Core Duo CPU L2400 at 1.66 GHz) running MS Windows Vista was used (Fig. 2). The standard webcam (1) is set into position so that its field of view (FOV) is entirely filled by the projection screen (2). A rear-view projection screen was chosen because it allowed for the webcam and the projector (3) to be placed behind the projection screen, so that no part of the projection surface was occluded by the participant. The participant is then provided with a laser pointer (4) (532 nm 50 mW green) and instructed to outline phosphenes as perceived in the visual field as soon as they are evoked by neurostimulation (i.e., TMS). In a dimly lit room (~ 0.5 cd/m²) the laser pointer is visible through the projection screen and serves as the brightest point within the webcam's field of view. The computer (5) running the L-TaP software rapidly scans and detects the brightest point of the image provided by the webcam. When a bright point is detected the X–Y coordinates of the maximally illuminated pixel is saved. In this fashion, the entire path of the laser point is tracked and recorded. A complete phosphene can be drawn by moving the laser pointer from site to site, outlining the shape of the percept.

2.4. The webcam

A standard off the shelf USB-webcam was used to capture real-time video; the images were then analyzed using the L-TaP software to locate the “brightest spot”. The webcam had to be capable of recording at up to 30 frames per second (fps). The high frame rate provided the ability to smoothly track and detect

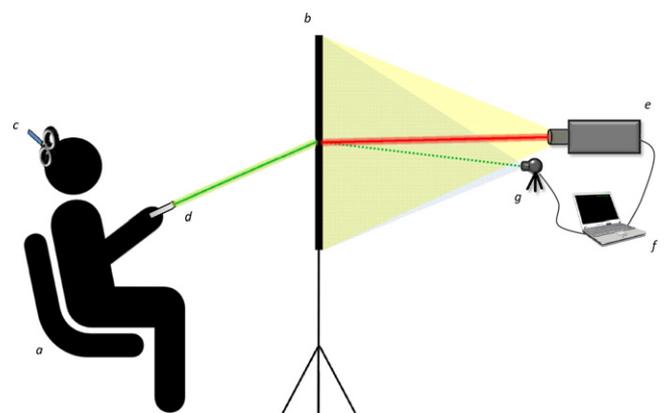


Fig. 2. Schematic illustration of the L-TaP system. Schematic drawing of the materials and procedure allowing the L-TaP (laser tracking and painting system) to document phosphenes. A participant (a) sits in front of a rear projection screen (b). After a pulse of noninvasive neurostimulation given in our case by means of a TMS coil placed on the right occipital pole (c), the participant experiences the perception of a phosphene and uses a laser pointer (d) to draw the percept on the projection screen by outlining the outer perimeter of its shape (d). A rear-facing webcam located behind the screen (e) records images of that screen at 30 fps. A computer (f) running the L-TaP software receives the video input. The coordinates of the detected laser beam are then stored. Individually detected coordinates are used to draw solid line, projected onto the projection screen via a rear-facing projector, in order to provide the participant with feedback of their drawing (e).

the path of the laser pointer as it was used to outline the perceived phosphene path. According to our observations, when lower frame acquisition rates were used (<15 fps) the recorded laser path appeared jerky and missed critical data points. Thus the high frame rate (at least >25 fps) is an important factor when the duration of an individual laser path can be less than 1 s. A webcam featuring pan, tilt, zoom (PTZ) capabilities facilitated the task of filling the entire FOV with the projection screen. The USB-webcam used in the current experiment was the Logitech Quickcam Orbit AF (Logitech, Fremont, CA). It was selected because of its technical specifications and low-cost, nonetheless there is no reason to believe that other webcams with similar capabilities and frame rate acquisitions would not work equally well. An important note is that the Quickcam Orbit AF includes proprietary software (Logitech® RightLight™ 2 Technology) that adjusts the image based on current lighting conditions. This feature or similar features on other cameras should be turned off as they make the detection of a single bright point on a dark background more difficult.

The webcam was positioned on the opposite side of the projection screen from where the participant was seated. This allowed for a direct projection without occlusion (Fig. 2). The webcam was placed on top of the projector approximately 200 cm away from the projection screen at a height of 80 cm from the ground. Since the webcam faced the projection screen opposite the participant, the image was required to be mirrored. The Quickcam Orbit AF has a setting to mirror the image; however this can be accomplished through software if necessary. Before starting the real experiments, subjects were familiarized with the system and encouraged to practice how to draw so that their laser traces were correctly recorded. Nonetheless, the learning curve was rapid and ~5–10 min of training proved enough for them to understand how to efficiently use the system.

2.5. Rear projection screen

A front-and-rear projection screen (127 cm × 127 cm) (Da-View fast-fold® deluxe screen, Warsaw, IN) was chosen over a standard projection screen because it permitted for the projector and the webcam to be placed on the opposite side of the projection screen from where the participant was located (Fig. 1). This arrangement allowed for a clear line of site of the projection screen, without occluding either the webcam or the participants line of site. As the projection screen was semi-transparent the beam of the laser pointer is clearly visible from the reverse side (see Section 2.4). In our tests, the rear projection screen viewing area equaled 127 cm × 127 cm, although the image projected onto the screen only occupied 127 cm × 95 cm. With the participant seated directly in front of the projection screen (~45 cm from the nasion to the center of the screen) the projected image occupied 108° (horizontal) and 93° (vertical) of visual angle. A wider field of view, encompassing a larger portion of the visual field could be achieved using several methods; placing the projector further away from the projection screen in addition to using a larger projection surface, or by positioning subject's closer to the screen.

For labs without access to a rear projection screen or the space behind which to setup the webcam and projector, a standard front projection screen may be used with the webcam and projector positioned in front of the screen, offset from the center and adjacent to the participant. This approach would require additional calculations to infer the correct position and path of the drawings taking into account the webcam's oblique point of view. If neither front nor rear projection screens are available, any large flat and semi-transparent surface could serve as an acceptable substitute.

2.6. The projector

The projector used for the LTaP system (Epson PowerLite 7900p Epson America, Inc., Long Beach, CA) (4000 ANSI lumens) (1200 × 1600 pixels) was connected to the computer running the software via a VGA port. It was used to display the laser tracing onto the projection screen to allow the participant (under the scrutiny of an examiner) to receive feedback and judge the accuracy of the outlined phosphene. Additionally, the projector could be used to display onto the projection screen computer generated landmarks, such as a central fixation cross, a set of horizontal and vertical meridians dividing the visual space or any other type of static or moving landmark or visual pattern of scientific interest. Projected landmarks may also be used to guide participant's performance when learning to use the LTaP system (see Section 3 for more details on an example using such a feature).

For our purposes, the projector was positioned approximately 200 cm behind the projection screen at a vertical height of ~70 cm. Because the projector was behind the projection screen, the image was reversed, therefore from the prospective of the participant, the image was correctly oriented. Any projector should suffice provided that it is capable of mirroring/reversing the image.

2.7. Laser pointer

A modified green laser pointer (532 nm, 50 mW) was used by the participant for tracing the observed phosphene. The use of a 50 mW laser was justified by the high luminance value (974 cd/m²) compared to a standard (5 mW) red laser (189 cd/m²), when measured from behind the projection screen, under ambient light conditions of ~0.5 cd/m². As the webcam was positioned behind the projection screen and directly facing the participant, the laser point must be visible through the projection screen. Although the rear projection screen is semi-transparent and the detection threshold value can be adjusted via the LTaP software, the increased luminance of the green laser compared to the red laser made detection easier and more consistent overall.

The recommended duty cycle for the green laser pointer we used is 20 s on, 10 s off. Despite following these recommendations and the use of fresh batteries, the beam would quickly dim. The rapid dimming made it difficult to consistently detect the beam throughout the entire duration of a phosphene tracing. This issue motivated us to modify the laser pointer by fitting it with a DC transformer (3VDC 100 mA). This ensured that the laser pointer would be provided with a consistent power supply throughout the duration of use. The laser pointer power source was modified by first obtaining a suitable DC transformer and cutting the male plug to expose the negative and positive leads. The case of the laser pointer was then unscrewed and the batteries were removed. The body of the laser pointer case was placed in a vice and a drill press was used to drill a small hole in the bottom end (the end opposite to the laser diode). The two leads of the wire were then threaded through the hole that we had drilled. A knot was made in the wire so that the wires could not be pulled out of the laser case. The negative wire was then soldered to the negative lead of the laser circuit board (the poles may differ in other models) and the positive wire was grounded to the case by hot-gluing the exposed wire to the inside of the case. The laser pointer was then screwed back together. It should be noted that using a DC transformer to power a laser pointer may significantly shorten the life of the laser diode or even destroy it. If attempting to follow this method, possibly destroying the laser pointer should be an acceptable outcome. Despite the power provided by the DC transformer being equivalent to the output of 2 × 1.5 V AAA batteries there was some noticeable dimming of the laser. The small decrement of luminance output was not an issue however because it was consistent throughout the use of the

laser and could therefore be countered by increasing the sensitivity of the system (see Section 2.8 for more details).

2.8. Laser point detection (brightness)

The laser beam serves as the bright point, which is detected by scanning each pixel in each captured frame. In a for-loop beginning at $[X_0-Y_0]$ (top left corner), the red, green, and blue (RGB) values for each pixel is successively determined line by line. Based upon the RGB values for each pixel the color brightness value is calculated using the following equation: $\text{brightness} = ((299 \times \text{red}) + (58 \times \text{green}) + (114 \times \text{blue})) / 1000$. The calculated color brightness (0–255) for each pixel is then compared to the threshold value set up by the user (Fig. 1c). The user defined threshold value (0–255) can be adjusted in response to current lighting conditions or laser intensity. For example the luminance value of the laser point captured by the webcam is a function of several factors, including the power of the laser pointer, the material of the projection screen, webcam software or hardware filters, and ambient luminance. The ability to adjust the threshold value is thus an important feature as experimental conditions may vary. Nonetheless the LTaP system is still capable of recording neurostimulation induced phosphenes in a well-lit environment.

2.9. Laser point detection (on the basis of color)

In place of detecting the brightest point (i.e., the laser point), the LTaP system is also capable in a well-lit environment of detecting and tracking a user defined color value [RGB (0–255, 0–255, 0–255)]. In the color detection condition, the webcam and projector may be positioned in front of the projection screen (as indicated in Section 2.5), that is, on the same side as the participant. Therefore in this configuration, the webcam would face the participant and the projector would face the projection screen. Similar to setting the color brightness threshold, the color threshold for example may be set to red (255, 0, 0). In this condition, a participant would be seated in front of a monochromatic background (not red) with a red sticker placed on the tip of the index finger. Therefore when the participant is instructed to trace a neurostimulation induced phosphene with their index finger, the LTaP system will be able track and record the position of the red sticker.

2.10. Data coordinates

When a pixel calculated RGB value is above threshold, its X – Y coordinate is recorded. Therefore upon the completion of a single traced phosphene, a set of X – Y coordinates will be obtained. Each individual traced phosphene will result in a set of X – Y coordinates. At this point, two events are simultaneously occurring in real-time. First, the path of the X – Y coordinates is projected or displayed onto the projection screen. Second, the X – Y coordinates are being saved as an XML (extensible markup language) file. Therefore, the traced phosphenes can be observed in real-time, as well as having the ability to perform post-analyses using the exact size and spatial location of the recorded path for each traced phosphene.

3. Results

To showcase the capabilities of the LTaP system we implemented an experimental protocol in which transcranial magnetic stimulation (TMS) was used to evoke occipital phosphenes while simultaneously using the LTaP system to document their feature shifts under several experimental conditions. More specifically, we gauged the LTaP system's ability to reliably and accurately record observed phosphene tracings. We predicted that phosphene tracings would be consistent with the small receptive field size and

organization of the occipital cortex. Furthermore, we aimed at documenting a well-established phenomenon, that phosphenes are retinotopically anchored percepts. Hence the spatial location of phosphenes should shift with changes in directed gaze, while the perimeter and bounded area remain constant (Meyer et al., 1991).

A total of 9 participants were recruited from the Boston University Medical Center (BUMC) community (7 female, average age: 24.8; range: 21–36). All participants were pre-screened prior to admission into the study against the exclusionary criteria of personal or family history of epileptiform disorders, metallic implants, and neuroleptic medications (Rossi et al., 2009). The use of TMS in the present study was approved by the BUSM Institutional Review Board.

Stimulation was performed using a hand-held figure-eight coil (70 mm wing diameter) attached to a single pulse monophasic magnetic stimulator (Monopulse Magstim, Carmarthen, Wales, UK). Visual phosphenes were induced by single TMS pulses (<0.2 Hz) and documented by the LTaP system. Each session began by determining the scalp location where vivid phosphenes could be reliably elicited (phosphene hotspot) and the intensity at which phosphenes were detected in 50% of pulses (phosphene threshold). Participants were comfortably seated in front of the projection screen and fitted with a tight-fitting lycra swimming cap. The TMS coil was initially positioned 2 cm dorsally and 2 cm laterally to the right of theinion, corresponding to the approximate location O2 of the 10–20 EEG system (Herwig et al., 2003). The coil was manually held tangentially to the surface of the scalp with the handle pointing laterally away from the midline. TMS pulses were initially delivered at 60% of TMS maximal output intensity. Following each TMS pulse, participants were asked to report if they had perceived a phosphene and if so, to describe the qualities of the percept and its relative location in the visual space. If phosphenes were not reported, the sequence was repeated with intensity increased by ~10% of the TMS machine output until a phosphene was reported that met universally accepted criteria (Kammer et al., 2005a,b): namely, phosphenes should be located pericentrally in the left visual field following right occipital stimulation and should appear regardless of whether the recipient's eyes are open or closed. Sham TMS pulses were delivered by tilting the coil stimulation surface away from the scalp or by placing the coil over non-primary visual areas such as the vertex or the anterior and superior parietal cortex. Sham pulses were randomly interleaved to insure reliability of reported phosphenes. Coil position and intensity were adjusted as needed until phosphenes were reliably and consistently reported at a similar location in visual space. Then the exact location in which the TMS coil was marked on the swim cap for further reference. Phosphene threshold values were determined by delivering a series of TMS pulses (<0.2 Hz) while varying the intensity. This was done in order to find the minimum intensity necessary to produce an unambiguous phosphene report according to our criterion at least five out of ten times (Elkin-Frankston et al., 2010). In our 9 participants the mean phosphene values were $59 \pm 6\%$, of total machine output.

Once the phosphene hotspot and threshold for occipital cortex and had been determined, three sets of five TMS pulses were applied at an inter-pulse frequency of 0.1–0.2 Hz (1 pulse every ~7 s), which largely avoids cumulative effects. In particular, we delivered in a pseudo-randomized order three non-sham and two sham pulses (total of 15 pulses, 4 sham and 9 real TMS pulses) at 120% of the previously determined phosphene threshold value (the mean TMS intensity in our population was $70 \pm 6\%$). Participants were then instructed to fixate their gaze on one of three potential sites within the screen: (1) on a centrally located cross hair, presented briefly prior to stimulation, at 0° ; (2) at a point located 45° above the fixation cross; and (3) on a site located 45° to the right of the central fixation cross. Immediately following the delivery

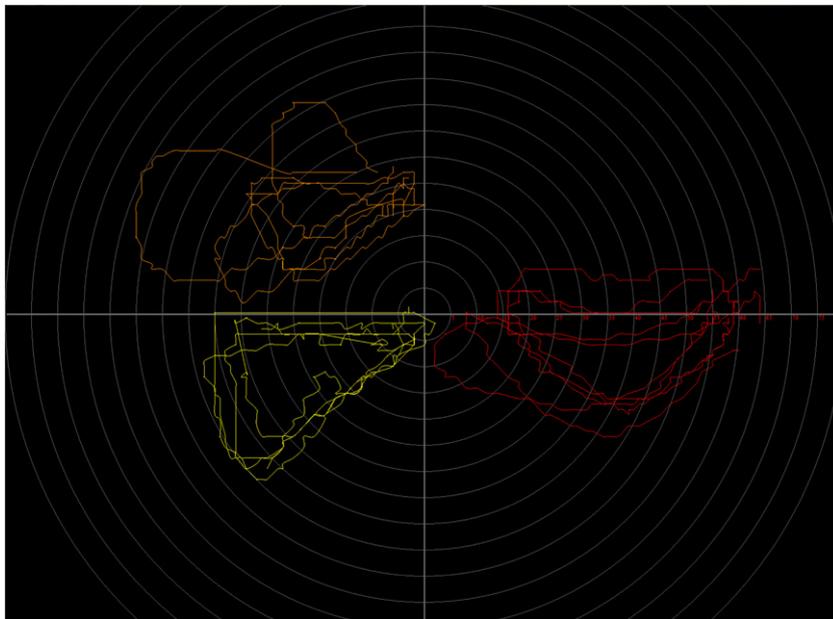


Fig. 3. Real data from 3 different directions of gaze. Figure displaying the LTaP output raw data (in pixels) of the phosphenes drawn by a representative participant. Each set of colored outlines represent a series of individual phosphene tracings obtain at each of the three given directions of gaze (central fixation, yellow; upward fixation, orange and rightward fixation, red). As indicated above, notice that the mean center of gravity but not the average surface of phosphenes shifted according to eye positions. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of each pulse, participants were instructed to verbally report the occurrence of a TMS-evoked phosphene and use the laser pointer to outline its perceived shape (Elkin-Frankston et al., 2010). We predicted that the location of the reported phosphene in visual space would be gaze-dependent as documented by the LTaP system. In other words, the location of phosphenes would shift with the location of fixation (Meyer et al., 1991) (Fig. 3). All three locations were tested in blocks and the order of each was counterbalanced across the nine subjects. The output of the LTaP system consisted of an

XML file containing time stamped X–Y coordinates for each location recorded by LTaP. Analysis was performed using Microsoft Excel 2007. Since the number of data points depended upon the duration of the recorded tracing, each individual phosphene consisted of anywhere between 10 and up to 200 data points.

The spatial location for each phosphene was determined by calculating the center of gravity. This was achieved by averaging the X–Y coordinates of each of the points characterizing the profile for a given phosphene. In addition the total spatial area and perimeter for each phosphene was also calculated. Phosphenes were typically characterized as being a closed shape, although the borders may sometimes appear to be diffused without a circumscribed border. Consequently those participants who traced open shapes were later queried about the veracity of their drawing compared to the properties of the perceived phosphene. Participants univer-

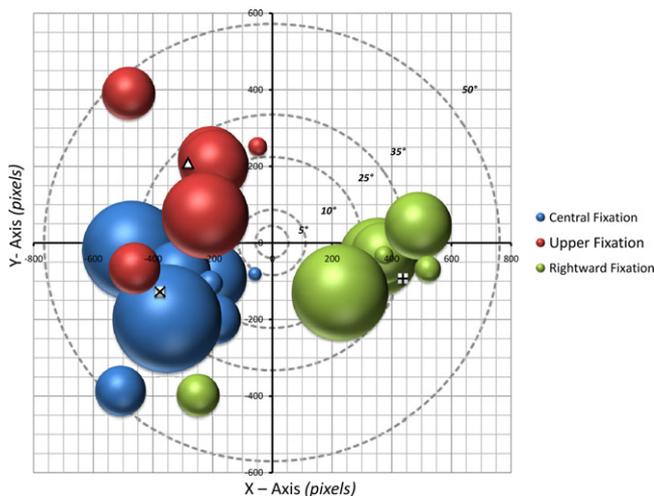


Fig. 4. Bubble graph representing the average location and mean area of phosphenes. Bubble graph representing the average location and mean area of phosphenes for each of the 9 participants. The location of each 'bubble' is the averaged X–Y coordinate for each of the three direction of gaze (central fixation: 0°, 0°; upward: 0°, 45°, and rightward 45°, 0° fixation point). The size of each 'bubble' represents the mean phosphene area calculated using the methods described in Sections 2 and 3 of the article. The averaged center of gravity, or averaged X–Y coordinate for each of the three gaze locations is represented by the symbols [x, Δ, +]. Notice that as hypothesized, the LTaP system documented that the mean center of gravity but not the average surface of phosphenes shifted according to eye positions, confirming that such visual percepts are retinotopically anchored.

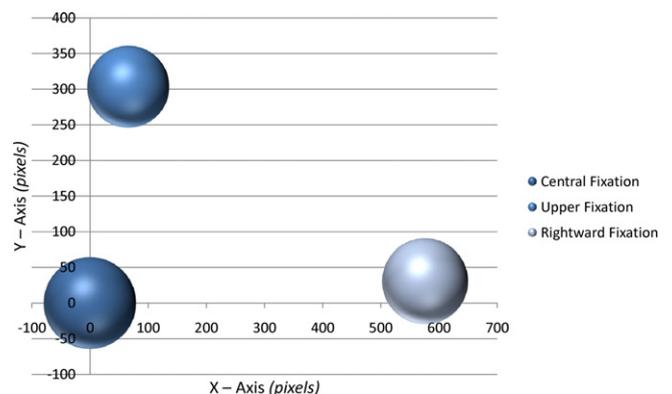


Fig. 5. Normalized difference of phosphene location for each direction of gaze. Figure displaying the normalized group differences ($n=9$ participants) in spatial location and total area with respect to the central fixation across the three gaze directions tested in our experiment. The center of gravity coordinates of each subject were normalized to the initial coordinates of the central fixation condition so that the X and Y coordinates of the latter equals $X=0$, $Y=0$ and shifts in the remaining conditions are all relative differences (in pixels). Notice the differences in the average location for the upper and rightward conditions, which display similar surface.

sally reported that the perceived phosphenes were indeed closed shapes as opposed to an open shape. Therefore, in order to accurately compute the total area the values for the first X – Y coordinate were also set as the last X – Y coordinate values. Artificially “closing” the shape enabled us to calculate phosphene area. A variant of Green’s Theorem: $\text{area} = (1/2) \sum_{i=1}^n (x_i y_{i+1} - x_{i+1} y_i)$, was used to calculate the total area of the closed shape phosphene. With both a center of gravity and total area, phosphene location and area were represented in a bubble graph (Fig. 4).

Visual inspection of the location phosphenes were outlined confirmed it was dependent on the direction of fixed gaze: with central fixation phosphenes were drawn in the left visual hemifield, just below the horizontal meridian; with gaze directed 45° above and to the right of fixation, respectively, phosphene location was shifted ~45° up and rightward. A one-way analysis of variance (ANOVA) revealed no significant differences of the total area of phosphenes across any of the three gaze directions, $F(2,9) = 0.71$, $p < 0.49$. Participants were categorized in accordance with their phosphene geometry as being either linear or ellipsoid. Two of the nine participants tested were categorized as having predominately linear shaped phosphenes. No significant interaction were observed between the averaged phosphene perimeter for any of the three gaze directions (ANOVA $F(2,9) = 0.32$, $p < 0.72$). The current results are a confirmation that the LTaP system is robust enough to document multiple neurostimulation-induced phosphenes within a single session yet sensitive enough to capture subtle nuances such as variance in morphology or spatial location with respect to eye movements. Furthermore, the system can be easily implemented in an experimental setting and is capable of yielding data that is both accurate and straight forward to analyze.

4. Discussion

In view of the growing use of neurostimulation evoked-phosphenes to study the organization and function of the visual systems in humans, we designed the LTaP system and offer it to the research community. We aimed to address the lack of an accurate, low-cost and portable system able to objectively document phosphene features (such as spatial location, shape, surface, and perimeter) in a quantitative manner, with minimal spatial transformations and effort by the participants. We demonstrated that the LTaP system can detect and store outlined drawings made by participants using a laser pointer on a screen. The output of this system accurately represents subjective visual percepts induced by occipital noninvasive stimulation, perceived as appearing in circumscribed areas of the visual field. This was achieved by using hardware that can be put together with minimal skills, and made of components, which are easily available in research labs (laptop computer, webcam, projector, projection screen and a laser pointer) or purchased and customized at a relatively low-cost. We developed the software executable and source code (freely available from our website: <http://www.bumc.bu.edu/anatneuro/research/research-labs/laboratory-of-cerebral-dynamics/>) to control and optimize the recording of phosphene traces; the code can be modified to accommodate individual research needs and novel applications. Finally, we have provided a sense of the capabilities of the LTaP system by describing an experimental protocol. In particular, focal noninvasive stimulation by TMS was applied to evoke occipital phosphenes, while we simultaneously used our newly developed tool to document their location, features and changes under several experimental conditions.

To the best of our knowledge this is the first published report of computer vision technology used for the purposes of documenting and quantifying not only the appearance rate, and thresholds, but

also the localization and geometrical properties of TMS induced-phosphenes. This advance provides the possibility to follow the modification of phosphenes over time or across experimental conditions. Prior phosphene research has primarily relied on verbal reports by participants, documented and translated into treatable information, or used subject-made schematic drawings, which were later processed qualitatively or quantified numerically. All those approaches have mainly relied on quantifying changes in phosphene perception rates, and used such information to extract information about potential shifts of their thresholds (i.e., the intensity of the magnetic field at which phosphenes are evoked in at least half of the attempts) at a given location under different experimental circumstances. By doing so, they have provided extremely insightful information on the organization of the visual areas (Kammer et al., 2005a,b), the pattern of cortico-cortical interactions held with extrastriate, parietal and frontal regions in both intact (Pascual-Leone and Walsh, 2001; Silvanto and Cattaneo, 2010; Silvanto et al., 2006, 2009) and brain damaged patients (Silvanto et al., 2007c). More recently, some of those studies have contributed highly to the exploration of the underlying mechanisms of classical and novel neurostimulation techniques such as TMS (Silvanto et al., 2007a,b), tDCS (Antal and Paulus, 2008), and more recently tACS (Kanai et al., 2010). Notwithstanding, the original subjectivity of phosphene measures and their high sensitivity to involuntary biases during the process of translating a percept into treatable and interpretable data threatens to unfairly limit the scope and the credibility of such valuable research. One could ultimately argue that every behavioral response, recorded in terms of performance, response reaction times, or as a verbal report to an experimenter is in essence a subjective correlate. Nonetheless, any attempt to limit the nature and duration of the phosphenes translation process in time and space (and thus the likelihood of biases and interferences by which such percepts are to be documented), advances objectivity and is thus worth the effort. The task at hand is not simple, since as it has been well documented that phosphenes are extremely brief percepts of light, which are characterized by the richness and high inter-individual variability of their features. Moreover phosphenes have been shown to be highly dependent on attentional allocation (Blakemore et al., 2007) and can be modulated by sensory tactile and auditory sensations (Ramos-Estebanez et al., 2007; Romei et al., 2007). Furthermore, in spite of the growing attempts to capture the objective correlates of such percepts through neuroimaging techniques such as fMRI or EEG (Taylor et al., 2010), the subjective report of its presence and characteristics (location, shape, size etc.) remains to date necessary and the most reliable correlate researchers have been counting on.

Our LTaP system proved to be successful in documenting phosphenes and facilitating a rapid transfer from the visualized percept into an “electronic drawing”. This was done on a surface, which was ultimately a full size representation of the visual field in which those phosphenes were perceived. As expected according to the retinotopic organization of the visual system, our system recorded phosphenes as being located in the left pericentral visual field following stimulation of the right occipital pole. This can be easily explained by the preferential depolarization of striate neurons of the right occipital pole. Naïve participants were satisfied with the ease of use of the system and the procedure, which they learned to command after only a few minutes of laser drawing practice. Similarly, naïve experimenters learned quickly to set up the components and to navigate and operate the settings and special features provided in the software interface. In testing the LTaP several technical advantages became evident. The system was capable of passively recoding at 30 fps and processing data indefinitely or at least for as long as computer storage resources remained available. It recorded phosphene information over multiple trials and wrote them into a single XML file, which could be then easily ana-

lyzed off-line using standard or customized data analysis software. In our experiment, for example, a logically structured, comprehensive database able to quickly treat the output files generated by the LTaP system facilitated the execution of standardized quantitative analyses that would have proved much more difficult if relying on verbal reports or handmade phosphene drawings executed on individual paper sheets. Crucially, the documentation of phosphenes did not require any significant change in body position or eye movement during or in between trials that could have clearly compromised the accuracy of the percept and the consistency of the testing conditions. We were able to evoke and to record a phosphene at least every 7–10 s (shorter intervals were avoided to limit risks of TMS cumulative and modulatory effects on the occipital pole), thus providing experimenters with the ability to collect a high number of quantifiable data in a relatively short period of time. Such an interval was also sufficient to allow the experimenter to document additional verbally reported phosphene features such as motion, color or texture, and store them into a LTaP-linked data base associated with the laser drawings.

The reliability of the of the LTaP system was also further determined by means of a simple and robust experimental paradigm, which has been used to date to demonstrate that the location of cortically evoked visual percepts is visuotopically anchored, and hence they change position, but not features, with eye movements (Meyer et al., 1991). Accordingly, as the TMS coil was held in a consistent right occipital position over three conditions; participants eyes directed toward a central (0° , 0°) fixation, an upward (0° , 45°) site or rightward (45° , 0°) fixation point presented in the screen. As expected, the LTaP objectively documented shifts in phosphene location within the visual field which followed the direction of gaze (Fig. 3). Furthermore, our data provided numerical evidence of spatial translation of the center of gravity across the three conditions without changing the mean area or the total perimeter of the phosphenes (Fig. 5). This is just an example of the type of studies in which cortically induced phosphenes recorded by the LTaP system would be useful to researchers and help in probing and understanding the organization of the visual systems. Nonetheless, based on its ease of use and spatiotemporal accuracy, the utility of the LTaP system could be easily extended to more complex paradigms. In the current report we have mainly devoted the use of this new system to the documentation of quantitative phosphene features such as surface area, perimeter or eccentricity, which emerge from the laser traces made by subjects. Nonetheless, phosphenes constitute a rich visual experience with potentially interesting and sometimes unexpected qualitative features (color, texture, intensity, motion direction and radial diffusion if any) that might not allow a direct quantification through laser drawings, unless very specific applications are developed. To accommodate this, the database associated with the LTaP system allows for documentation of associated phosphene features the subject may report spontaneously or upon the request of the experimenter. Furthermore, customized versions of the current system could easily include keyboard, mouse, or laser pointer operated context menus projected onto the screen, in which participants could choose from a list of associated qualities (e.g., colors, textures and shapes) for subjects to choose from that fit their perception". Future applications of the LTaP system could also help in further exploring the potential modulation of occipital phosphene location, area and shape by altering activity in connected regions in extrastriate (MT/V5), parietal (IPS) or frontal (FEF) locations in intact subjects and patients (Silvanto et al., 2006, 2007c, 2009). Finally, due to its ability to project computer generated stimuli to the whole field or in specific areas of the visual hemifields, the LTaP system could serve to shed further light on the spatial resolution and sensitivity of phosphene features to brain state dependency, as modulated by visual adaptation strategies (Silvanto et al., 2007a,b, 2008).

As indicated above, the relative immediacy of the LTaP and the minimal spatial translation in collecting phosphenes reduces potential interferences and biases. Furthermore, it provides an efficient method to store and longitudinally follow such visual percepts across time and study the influence of experimental conditions. It should be noted that no system, the LTaP included, completely eliminates every potential source of interference and bias. Subjects will still have to use their hands to draw the phosphenes, whether by using a laser pointer projected on a screen, or in an alternate configuration by moving a trackable object or even their own finger. It might be argued the use of manual methods for translating a mental "picture" of a visual percept into treatable electronic data is still susceptible to biases related to handedness, motor planning, spatial and manual abilities, and that the later three could eventually be modified over time by experimental conditions set up to specifically influence phosphenes. Nonetheless, those biases are pervasive in any method of documenting phosphenes, and are addressed by control experiments. To completely circumvent these issues, the LTaP system could be modified to rely on other types of input. For example, a second web-cam based tracking system could allow subjects to operate a drawing tool through head or eye movements (e.g., through the use of eye tracking or a laser pointer mounted on spectacle frames). Such alternatives might be more intuitive for patients or healthy subjects, but is not exempt from new sources of reporting biases that would also need to be addressed on a case by case basis.

As it is currently designed, the LTaP system is affected by the following limitations. Nonetheless, these limitations do not curtail its ability to capture phosphenes and can eventually be overcome by customizing or further expanding the features of the current system. First, it is not easily compatible with the use of blindfolds. Blindfolds are commonly worn by naïve participants. Our experience has been that with training, subjects can perceive phosphenes without the use of a blindfold in a dimly lit room. By reducing ambient light and assuring that the projected image is of minimal-luminance the perception of phosphenes with eyes open can be easily facilitated. Interestingly, the projector can be used to integrate a grid of white concentric circles of increasing diameter from fixation outwards at steps of 20° , which with our subjects proved extremely useful in guiding the accurate location of the phosphenes and in guiding direction gaze. Second, for our purposes we assumed the visual field to be a flat two-dimensional surface. However the use of cylindrical or semispherical surface would represent a more realistic model of real-world visual space. Since a flexible projection screen could be modeled at will, this is entirely possible. Nonetheless additional calculations and changes in hardware would be required to compensate for the differences in angles when drawing at different positions of the space. For example, the use of a web-cam offers a means to capture data that is both fast and reliable. However, a web-cam only gathers data from a selective point of view, requiring the participant to face the camera. A possible solution would be to incorporate the use of additional web-cams, thereby increasing the LTaP systems total field of view. However a more efficient solution may be replacing the web-cam altogether with a large array of photoresistors secured to the surface that is being used to trace phosphenes.

By way of the LTaP system, what was once a highly subjective measure or a series of visual qualia, difficult to quantify numerically, and thus highly prone to participant's and observer's biases, can now be captured and analyzed as series of perceptual quantia. In particular, due to the system's ability to objectively document, analyze and compare phosphenes under various experimental conditions or across time, studies using phosphenes as a measure of visual excitability can now be more consistently undergone with lower risk of biases. Finally, although the LTaP has been presented as a finished product it has been created as an open and expand-

able tool, which must be customized to integrate new applications and features according to rapidly changing research needs and ever evolving technological solutions. In the current manuscript, we have advanced a series of functions, which in our opinion could prove extremely useful in the near future. It is now the turn of any interested researcher to openly contribute their ideas and solutions.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jneumeth.2011.02.013.

References

- AForge.NET.: Framework <<http://www.aforogenet.com/framework/>>; 2010 [accessed 18.01.10].
- Afra J, Mascia A, Gérard P, Maertens de Noordhout A, Schoenen J. Interictal cortical excitability in migraine: a study using transcranial magnetic stimulation of motor and visual cortices. *Annals of Neurology* 1998;44(2):209–15, doi:10.1002/ana.410440211.
- Amassian VE, Cracco RQ, Maccabee PJ, Cracco JB, Rudell A, Eberle L. Suppression of visual perception by magnetic coil stimulation of human occipital cortex. *Electroencephalography and Clinical Neurophysiology* 1989;74(6):458–62.
- Antal A, Paulus W. Transcranial direct current stimulation and visual perception. *Perception* 2008;37(3):367–74, doi:10.1068/p5872.
- Aurora SK, Ahmad BK, Welch KM, Bhardhwaj P, Ramadan NM. Transcranial magnetic stimulation confirms hyperexcitability of occipital cortex in migraine. *Neurology* 1998;50(4):1111–4.
- Blakemore C, Driver J, Thilo KV. Spatial attention changes excitability of human visual cortex to direct stimulation. *Current Biology* 2007;17:134–9.
- Bolognini N, Maravita A. Proprioceptive alignment of visual and somatosensory maps in the posterior parietal cortex. *Current Biology* 2007;17(21):1890–5, doi:10.1016/j.cub.2007.09.057.
- Cowey A, Walsh V. Magnetically induced phosphenes in sighted, blind and blind-sighted observers. *Neuroreport* 2000;11(14):3269–73.
- Deblieck C, Thompson B, Iacoboni M, Wu AD. Correlation between motor and phosphene thresholds: a transcranial magnetic stimulation study. *Human Brain Mapping* 2008;29(6).
- Elkin-Frankston S, Fried PJ, Pascual-Leone A, Rushmore RJ, Valero-Cabre A. A novel approach for documenting phosphenes induced by transcranial magnetic stimulation. *Journal of Visualized Experiments: JoVE* 2010;38(1), doi:10.3791/1762.gnu.org. <<http://www.gnu.org/licenses/lgpl.html>>; 2010 [accessed 20.09.10].
- Herwig U, Satrapi P, Schönfeldt-Lecuona C. Using the international 10–20 EEG system for positioning of transcranial magnetic stimulation. *Brain Topography* 2003;16(2):95–9, doi:10.1023/B:BRAT.0000006333.93597.9d.
- Kamitani Y, Shimojo S. Manifestation of scotomas created by transcranial magnetic stimulation of human visual cortex. *Nature Neuroscience* 1999;2:767–71.
- Kammer T. Phosphenes and transient scotomas induced by magnetic stimulation of the occipital lobe: their topographic relationship. *Neuropsychologia* 1998;37(2):191–8, doi:10.1016/S0028-3932(98)00093-1.
- Kammer T. Phosphenes and transient scotomas induced by magnetic stimulation of the occipital lobe: their topographic relationship. *Neuropsychologia* 1999;37(2):191–8.
- Kammer T. Masking visual stimuli by transcranial magnetic stimulation. *Psychological Research* 2007;71(6):659–66.
- Kammer T, Puls K, Erb M, Grodd W. Transcranial magnetic stimulation in the visual system. II. Characterization of induced phosphenes and scotomas. *Experimental Brain Research* 2005a;160(1):129–40.
- Kammer T, Puls K, Strasburger H, Hill NJ, Wichmann FA. Transcranial magnetic stimulation in the visual system. I. The psychophysics of visual suppression. *Experimental Brain Research* 2005b;160(1):118–28.
- Kanai R, Chaieb L, Antal A, Walsh V, Paulus W. Frequency-dependent electrical stimulation of the visual cortex. *Current Biology* 2008;18(23):1839–43, doi:10.1016/j.cub.2008.10.027.
- Kanai R, Paulus W, Walsh V. Transcranial alternating current stimulation (tACS) modulates cortical excitability as assessed by TMS-induced phosphene thresholds. *Clinical Neurophysiology* 2010;121(9):1551–4, doi:10.1016/j.clinph.2010.03.022.
- Marg E. Magnetostimulation of vision: direct noninvasive stimulation of the retina and the visual brain. *Optometry and Vision Science: Official Publication of the American Academy of Optometry* 1991;68(6):427–40.
- Meyer BU, Diehl R, Steinmetz H, Britton TC, Benecke R. Magnetic stimuli applied over motor and visual cortex: influence of coil position and field polarity on motor responses, phosphenes, and eye movements. *Electroencephalography and Clinical Neurophysiology* 1991(Suppl. 43):121–34.
- Pascual-Leone A, Walsh V. Fast backprojections from the motion to the primary visual area necessary for visual awareness. *Science* 2001;292(5516):510–2, doi:10.1126/science.1057099.
- Ramos-Estebanez C, Merabet LB, Machii K, Fregni F, Thut G, Wagner TA, et al. Visual phosphene perception modulated by subthreshold cross-modal sensory stimulation. *Journal of Neuroscience* 2007;27(15):4178–81, doi:10.1523/JNEUROSCI.5468-06.2007.
- Romei V, Murray MM, Merabet LB, Thut G. Occipital transcranial magnetic stimulation has opposing effects on visual and auditory stimulus detection: implications for multisensory interactions. *Journal of Neuroscience* 2007;27(43):11465–72, doi:10.1523/JNEUROSCI.2827-07.2007.
- Rossi S, Hallett M, Rossini PM, Pascual-Leone A. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clinical Neurophysiology* 2009;120(12):2008–39, doi:10.1016/j.clinph.2009.08.016.
- Silvanto J, Cattaneo Z. Transcranial magnetic stimulation reveals the content of visual short-term memory in the visual cortex. *NeuroImage* 2010;50(4):1683–9, doi:10.1016/j.neuroimage.2010.01.021.
- Silvanto J, Muggleton NG, Cowey A, Walsh V. Neural adaptation reveals state-dependent effects of transcranial magnetic stimulation. *The European Journal of Neuroscience* 2007a;25(6):1874–81, doi:10.1111/j.1460-9568.2007.05440.x.
- Silvanto J, Muggleton NG, Cowey A, Walsh V. Neural activation state determines behavioral susceptibility to modified theta burst transcranial magnetic stimulation. *The European Journal of Neuroscience* 2007b;26(2):523–8, doi:10.1111/j.1460-9568.2007.05682.x.
- Silvanto J, Cowey A, Lavie N, Walsh V. Making the blindsighted see. *Neuropsychologia* 2007c;45(14):3346–50, doi:10.1016/j.neuropsychologia.2007.06.008.
- Silvanto J, Lavie N, Walsh V. Stimulation of human frontal eye fields modulates sensitivity of extrastriate visual cortex. *Journal of Neurophysiology* 2006;00015.
- Silvanto J, Muggleton N. Testing the validity of the TMS state-dependency approach: targeting functionally distinct motion-selective neural populations in visual areas V1/V2 and V5/MT+. *NeuroImage* 2008;40(4):1841–8.
- Silvanto J, Muggleton N, Lavie N, Walsh V. The perceptual and functional consequences of parietal top-down modulation on the visual cortex. *Cerebral Cortex* 2009;19(2):327–30, doi:10.1093/cercor/bhn091.
- Silvanto J, Muggleton N, Walsh V. State-dependency in brain stimulation studies of perception and cognition. *Trends in Cognitive Sciences* 2008;12(12):447–54.
- Silvanto J, Pascual-Leone A. State-dependency of transcranial magnetic stimulation. *Brain Topography* 2008;21(1):1–10.
- Taylor PC, Walsh V, Eimer M. The neural signature of phosphene perception. *Human Brain Mapping* 2010., doi:10.1002/hbm.20941.
- Thielscher A, Reichenbach A, Ugrasbil K, Uludag K. The cortical site of visual suppression by transcranial magnetic stimulation. *Cerebral Cortex* 2010;20(2):328–38, doi:10.1093/cercor/bhp102.