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Stimulus-Specific Cortical Visual Evoked Potential Morphological Patterns

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Cover Letter

The University of Texas at Austin
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CSP
Central Sensory Processes Laboratory

December 13, 2018
To the Editor,
JoVE

Dear Dr. Myers,

Thank you for allowing us to submit a revised version of our methods manuscript, Stimulus-Specific Cortical Visual Evoked Potential Morphological Patterns' to JoVE.

We have revised the manuscript and answered each of the points made by reviewers. These changes include generalization of the protocol, generalization of results, and simplification of methodological steps.

Please do not hesitate to contact me with any questions you may have.

Sincerely,

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TITLE:

Stimulus-Specific Cortical Visual Evoked Potential Morphological Patterns

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KEYWORDS:

Visual Evoked Potentials, Morphological Patterns, Ventral Stream, Dorsal Stream, High-density EEG, EEGLAB

SUMMARY:

In this paper, we present a protocol to investigate differential cortical visual evoked potential morphological patterns through stimulation of ventral and dorsal networks using high-density EEG. Visual object and motion stimulus paradigms, with and without temporal jitter, are described. Visual evoked potential morphological analyses are also outlined.

ABSTRACT:

This paper presents a methodology for the recording and analysis of cortical visual evoked potentials (CVEPs) in response to various visual stimuli using 128-channel high-density electroencephalography (EEG). The specific aim of the described stimuli and analyses is to examine whether it is feasible to replicate previously reported CVEP morphological patterns elicited by an apparent motion stimulus, designed to simultaneously stimulate both ventral and dorsal central visual networks, using object and motion stimuli designed to separately stimulate ventral and dorsal visual cortical networks. Four visual paradigms are presented: 1. Randomized visual objects with the consistent temporal presentation. 2. Randomized visual objects with inconsistent temporal presentation (or jitter). 3. Visual motion via a radial field of coherent central dot motion without jitter. 4. Visual motion via a radial field of coherent central dot motion with jitter. These four paradigms are presented in a pseudo-randomized order for each participant. Jitter is introduced in order to view how possible anticipatory-related effects may affect the morphology of the object-onset and motion-onset CVEP response. EEG data analyses are described in detail, including steps of data exportation from and importation to signal processing platforms, bad channel identification, and removal, artifact rejection, averaging, and

categorization of average CVEP morphological pattern type based upon latency ranges of component peaks. Representative data show that the methodological approach is indeed sensitive in eliciting differential object-onset and motion-onset CVEP morphological patterns and may, therefore, be useful in addressing the larger research aim. Given the high temporal resolution of EEG and the possible application of high-density EEG in source localization analyses, this protocol is ideal for the investigation of distinct CVEP morphological patterns and the underlying neural mechanisms which generate these differential responses.

INTRODUCTION:

Electroencephalography (EEG) is a tool that offers an inexpensive and non-invasive approach to the study of cortical processing, especially when compared to cortical assessment methods such as functional magnetic resonance imaging (fMRI), positron emission tomography (PET), and diffusion tensor imaging (DTI)[1]. EEG also provides high temporal resolution, which is not possible to attain when using measures such as fMRI, PET, or DTI[2]. High temporal resolution is critical when examining central temporal function in order to obtain millisecond-precision of neurophysiologic mechanisms related to the processing of specific input or events. In the central visual system, cortical visual evoked potentials (CVEPs) are a popular approach in studying time-locked neural processes in the cerebral cortex. CVEP responses are recorded and averaged over a number of event trials, resulting in peak components (e.g., P1, N1, P2) arising at specific millisecond intervals. The timing and amplitude of these peak neural responses can provide information concerning cortical processing speed and maturation, as well as deficits in cortical function[3-5].

CVEPs are specific to the type of visual input presented to the viewer. Using certain stimuli in a CVEP paradigm, it is possible to observe the function of distinct visual networks such as the ventral stream, involved in processing form and color, or parvocellular and magnocellular input[6-8], and the dorsal stream, which largely processes motion or magnocellular input[9,10]. CVEPs generated by these networks have been useful not only in better understanding typical neurophysiologic mechanisms underlying behavior but also in the targeted treatment of atypical behaviors in clinical populations. For example, delayed CVEP components in both dorsal and ventral networks have been reported in children with dyslexia, which suggests that visual function in both these networks should be targeted when designing an intervention plan[11]. Thus, CVEPs recorded via EEG offer a powerful clinical tool through which to assess both typical and atypical visual processes.

In a recent study, high-density EEG was used to measure the apparent motion-onset CVEPs in typically developing children, with the goal of examining variable CVEP responses and related visual cortical generators across development. Participants passively viewed apparent motion stimuli[12-15], which consisted of both shapes change and motion, and designed to simultaneously stimulate dorsal and ventral streams. It was found that approximately half of the children responded with a CVEP waveform shape, or morphology, consisting of three peaks (P1-N1-P2, pattern A). This morphology is a classic CVEP response observed throughout the literature. In contrast, the other half of the children presented with a morphological pattern comprised of five peaks (P1-N1a-P2a-N1b-P2b, pattern B). To our knowledge, the robust occurrence and

comparison of these morphological patterns have not previously been discussed in CVEP literature in either child or adult populations, although variable morphology has been noted in both apparent-motion and motion-onset CVEPs[14,16]. Furthermore, these morphological differences would not have been apparent in research using other cortical functional assessment methods, such as fMRI or PET, due to the low temporal resolution of these measures.

To determine the cortical generators of each peak in CVEP patterns A and B, source localization analyses were performed, which is a statistical approach used to estimate the most likely cortical regions involved in the CVEP response[12,13]. For each peak, regardless of the morphological pattern, primary and higher-order visual cortices were identified as sources of the CVEP signal. Thus, it appears that the main difference underlying CVEP morphology elicited by apparent motion is that those with pattern B activate visual cortical regions additional times during processing. Because these types of patterns have not been previously identified in the literature, the purpose of the additional visual processing in those with CVEP pattern B remains unclear. Therefore, the next aim in this line of research is to gain a better understanding of the cause of the differential CVEP morphology and whether such patterns may relate to visual behavior in both typical and clinical populations.

The first step in understanding why some individuals might demonstrate one CVEP morphology versus another is to determine whether these responses are intrinsic or extrinsic in nature. In other words, if an individual demonstrates one pattern in response to a visual stimulus, will they respond with a similar pattern to all stimuli? Or is this response stimulus-dependent, specific to the visual network or networks activated?

To answer this question, two passive visual paradigms were designed, intended to separately activate specific visual networks. The stimulus presented in the initial study was designed to stimulate both dorsal and ventral streams simultaneously; thus, it was unknown if one or both networks were involved in generating specific waveform morphology. In the current methodological approach, the paradigm designed to stimulate the ventral stream is composed of highly identifiable objects in basic shapes of squares and circles, eliciting object-onset CVEPs. The paradigm designed to stimulate the dorsal stream consists of visual motion via a radial field of coherent central dot motion dots at a fixed speed toward a fixation point, eliciting motion-onset CVEPs.

A second question that arose as a result of the initial study was whether differential VEP morphology could be due to participant anticipation of upcoming stimuli[13]. For instance, research has shown that top-down cortical oscillatory activity occurring prior to a target stimulus may predict subsequent CVEP and behavioral responses to some degree[17-19]. The apparent motion paradigm in the first study employed non-randomized frames of a radial star and circle with consistent inter-stimulus intervals (ISIs) of 600 ms. This design may have encouraged the expectation and prediction of the upcoming stimulus, with resulting oscillatory activity affecting later apparent motion-onset CVEP morphology[12,13,19].

To address this issue, the visual object and motion paradigms in the current protocol are designed

with both consistent ISIs of the same temporal value and randomized ISIs with different temporal values (i.e., jitter). Using this approach, it may be possible to determine how temporal variation can affect VEP morphology within distinct visual networks. Altogether, the aim of the described protocol is to determine if the visual object and motion stimuli would be sensitive to variations in CVEP morphology and whether the temporal variation of stimuli presentation would affect characteristics of the CVEP response, including peak latency, amplitude, and morphology. For the purpose of the current paper, the goal is to determine the feasibility of the methodological approach. It is hypothesized that both visual objects and motion may elicit variable morphology (i.e., patterns A and B will be observed across subjects in response to both stimuli) and that temporal variation would affect object-onset and motion-onset CVEP components.

PROTOCOL:

All methods described here have been approved by the Institutional Review Board (IRB) for Human Research at the University of Texas at Austin.

1. Stimuli characteristics

1.1 Create object stimuli using open source images available through the Bank of Standardized Stimuli (BOSS). This database consists of standardized images used throughout visual cognitive experiments. Download four images (e.g., ball02, book01a, brick, button03) with a high rate of identification (above 75%)[20,21].

1.2 Create motion stimuli using a modified version of the DotDemo script, which is available through the open source Psychtoolbox-3 set of functions operated via MATLAB, as well as the movie function available in MATLAB (see the **Supplementary File**).

1.2.1 Configure the dot field parameters according to the size of the presentation screen and viewing distance.

1.2.2 Enter 3600 for the number of movie frames.

1.2.3 Enter 80 (in cm) for monitor width.

1.2.4 Enter dot speed at 5°/s.

1.2.5 Enter a dot limited lifetime fraction of 0.05.

1.2.6 Enter 200 for the number of dots.

1.2.7 Enter the minimum radius of the field annulus as 1° and the maximum as 15°.

1.2.8 Enter 0.2° for the width of each dot.

1.2.9 Enter 0.35° for the radius of the fixation point.

1.2.10 Specify that white dots are used on a black background.

1.2.11 Export the movie in .avi format.

2 Visual paradigm design

2.1 Create paradigms via stimulus-presentation software. Generate fixation crosses with Courier New size 18 font, bold, and centered on the presentation screen.

2.2 Design the visual object paradigm without temporal jitter (i.e., consistent ISI values) by creating a black fixation cross on a white background presented for 500 ms, followed by one of four objects presented in randomized order: ball, book, brick, or button.

2.2.1 Present each object for 600 ms (**Figure 1A**). Show all objects 75 times, for a total of 300 trials and a paradigm duration of 5.5 min.

2.3 Design the visual object paradigm with temporal jitter to consist of the same black fixation cross on a white background, shown for a period of 500 or 1000 ms and followed by one of the four objects, lasting for 600 or 1000 ms (**Figure 1B**).

2.3.1 Create four trials using stimulus-presentation software: a fixation cross with a duration of 500 ms, followed by an object for 600 ms; a fixation cross with a duration of 500 ms, followed by an object for 1000 ms; a fixation cross with a duration of 1000 ms, followed by an object for 600 ms; and a fixation cross with a duration of 1000 ms followed by an object for 1000 ms.

2.3.1.1 Randomize these trials. Present each trial 19 times, culminating in 304 trials and resulting in a viewing time of approximately 7.85 minutes.

2.4 Create the visual motion paradigm without temporal jitter by generating a white fixation cross centered on a black background, lasting for 500 ms, followed by the visual motion movie, which is truncated to present for approximately 1000 ms (**Figure 2A**).

2.4.1 Repeat this sequence a total of 300 times, for a viewing duration of approximately 7.5 min.

2.5 Create the visual motion paradigm with temporal jitter using the same fixation cross, lasting for intervals of 500, 750, or 1000 ms.

2.5.1 After each fixation cross, present the visual motion movie with a duration of approximately 600 or 1000 ms (**Figure 2B**).

2.5.2 Create six trials: A fixation cross with a duration of 500 ms, followed by a movie for 600 ms, a fixation cross with a duration of 750 ms, followed by a movie for 600 ms, a fixation cross with

a duration of 1000 ms, followed by a movie for 600 ms, a fixation cross with a duration of 500 ms followed by a movie for 1000 ms, a fixation cross with a duration of 750 ms followed by a movie for 1000 ms and a fixation cross with a duration of 1000 ms followed by a movie for 1000 ms.

2.5.2.1 Randomize these trials, with each shown 50 times. Present a total of 300 trials, for a viewing period of approximately 7.75 min.

3 Participant Consent, Case History, and Vision Screening

3.1 Greet the participant on arrival. Obtain informed consent by presenting the participant with a consent for participation in research form. Explain the consent form to the participant and answer any questions that arise.

3.2 Have the participant fill out a case history form that includes information on native language, handedness, hearing status, vision status, and other diagnoses the participant may have (e.g., psychological and neurological). Exclude participants who report the hearing loss and/or neurological diagnoses, such as traumatic brain injury. Include all other participants.

3.3 Escort the participant out of the lab to complete a vision screening using a Snellen chart to determine visual acuity. Have the participant stand 20 feet away from the chart and begin by covering his or her left eye to determine right eye visual acuity, and then switch eyes to determine left eye visual acuity. Calculate visual acuity based on the smallest line of text that the participant can repeat at least one more than half of the total number of letters.

NOTE: For example, if the participant repeats 5 of the 8 letters on the 20/20 line, the participant is calculated as 20/20 visual acuity for that eye.

3.4 Escort the participant into the EEG recording room. Have the participant sit in the designated chair in the center of a double-walled magnetic-shielded soundproof booth.

4 EEG Preparation

4.1 Measure the head circumference of the participant in centimeters and select the appropriate EEG net size. Measure and mark the midpoint of the scalp (midway between nasion/inion and right and left mastoids) for the placement of the reference electrode.

4.2 Prepare a solution of warm water (1 L) mixed with baby shampoo (5 mL) and potassium chloride (11 g/10 cc), which increases the electrical conductance between the electrodes and scalp, leading to lower voltage impedances and an increased signal-to-noise ratio.

4.3 Place the EEG net in the solution. Allow the net to soak in the solution for 5 min before placing on the participant's scalp.

4.4 Turn on the stimulus-presentation computer and the EEG acquisition computer. Check the

impedance values.

4.5 Place a towel or other absorbent material around the participant's neck to prevent the solution from dripping onto his or her clothes.

4.6 Connect the EEG net to the amplifier. Instruct the participant to close his or her eyes when putting on the EEG net to prevent the solution from dripping into his or her eyes.

4.7 Firmly grip the EEG net with both hands and spread into place onto the participant's head. Ensure that the net is placed symmetrically on the scalp head, with the reference electrode at the scalp midline point that was measured. Tighten the chin and ocular net lines to ensure a secure connection between the scalp and electrodes. Ask the participant if he or she is comfortable and if anything needs to be adjusted.

4.8 Check for the proper electrode impedance values, with an average target of 10 kOhms.

4.9 To reduce impedance values following the placement of the electrode net, use a 1 mL pipette to apply the potassium chloride solution onto the scalp/electrodes that have a high impedance. Continue this process until adequate impedances values across the electrodes are achieved.

5 EEG Recording

5.1 Instruct the participant to focus on the visual stimuli that will appear on the monitor. The viewing distance is approximately 65 inches.

5.2 Use a pseudorandom number generator to determine the order of presentation for the four visual paradigms.

5.3 Begin the visual tasks and EEG recording.

5.4 Monitor the EEG recording as necessary. If ongoing EEG shows high myogenic or 60 Hz activity. Pause the experiment to recheck electrode-scalp connectivity.

5.5 Repeat steps 5.3 and 5.4 for the visual object paradigm, the visual object with the temporal jitter paradigm, the visual motion paradigm, and the visual motion with temporal jitter paradigm.

5.6 At the conclusion of the experiment, instruct the participant to close his or her eyes in order to prevent the solution from entering his or her eyes when removing the net. Begin by loosening the chin and ocular net lines, then remove the net by gently pulling the chin strap up and over the participant's head, making sure to pull slowly to ensure the net will not get tangled in the participant's hair.

5.7 Disconnect the EEG net from the amplifier. Begin the disinfection process by placing the EEG cap in and out of a bucket filled with water and rinsing under a faucet. Then, create the

disinfectant solution by adding approximately 2 l of water to the disinfectant bucket and mixing 15 ml of disinfectant with the water.

5.8 Immerse the sensor end of the net in the disinfectant. Set a timer for 10 min; for the first 2 min, continuously plunge the net up and down. Leave the net soaking for the remainder of the 10 min.

5.9 Remove EEG cap from disinfectant solution. Place the EEG net in and out of the electrode bucket filled with water and under running water to rinse. Repeat four times. Allow the net to air dry.

6 EEG Analyses

6.1 Export EEG files for analyses in MATLAB via the EEGLAB toolbox using a 1 Hz high-pass filter, segmentation around each trial (or event) of 100 ms pre-stimulus and 500 ms post-stimulus periods, and file exportation with an EEGLAB-compatible format that includes calibrated data and reference channel export options.

6.2 Import data using the EEGLAB toolbox.

6.2.1 Choose the **File** option from the drop-down menu and click on **Import data**. Select **using EEGLAB Functions and plugins** from the menu. Next click on the appropriate export file format.

6.3 Re-assign channel locations based on the type of electrode montage used by choosing **Edit** from the drop-down menu and selecting **Channel locations**. Click on **Look up locs** and select the ellipses to locate the path of the electrode montage file of interest.

6.4 Assign pre- and post-stimulus times to the epoch start and end times. Enter a value of -0.1 seconds in the **Start time** box.

6.5 Baseline-correct data according to the pre-stimulus interval.

6.6 Identify and remove bad channels using probability at a Z-score threshold of 2.5.

6.6.1 Verify successful identification and removal of bad channels by plotting all electrodes. Manually remove channels with mean voltage amplitudes outside of the range of +/- 30 µV.

6.7 Perform artifact rejection by entering values of -100 µV and +100 µV.

NOTE: This method is effective in the removal of ocular activity recorded at ocular electrodes (126, 127). However, it may be necessary to manually remove trials with artifact occurring at small-voltage amplitude (i.e., within the +/- 100 µV range) for certain participants.

6.7.1 Take note of channels that were bad for entire segments (i.e., with voltages outside of

the +/-100 µV range) and highlighted in red. Manually remove these bad channels if they constitute 60% or more of the rejected trials. Repeat this step as many times as necessary.

6.7.2 Follow artifact removal steps as previously described. Ensure that a minimum of 100 sweeps are accepted. Remove trials marked for rejection.

6.8 Plot channel 75 (equivalent to Oz), or the channel(s) of interest, to categorize morphological patterns. Prior to plotting this channel, make sure to perform pre-stimulus baseline correction.

6.9 Choose pattern A if CVEP morphology is characterized by a large positive peak at approximately 100-115 ms (P1), followed by a negative peak at approximately 140-180 ms (N1) and a positive peak at approximately 165-240 ms (P2).

6.10 Choose pattern B if CVEP morphology is characterized by a large positive peak at approximately 100-115 ms (P1), followed by a negative peak at approximately 140-180 ms (N1a), a positive peak at approximately 180-240 ms (P2a), then a negative peak at approximately 230-280 ms (N1b) and positive peak at approximately 260-350 ms (P2b).

6.11 Append individual datasets together according to the morphological pattern visually observed to create a group average. Name and save the newly merged dataset file.

6.12 View appended files as an average by plotting the channel(s) of interest.

REPRESENTATIVE RESULTS:

Figure 3 and **Figure 4** show the representative object-onset and motion-onset CVEP results of five participants, aged 19-24 years, who passively viewed each visual paradigm. This design allowed observation of CVEP responses elicited by visual objects (with and without jitter) and visual motion (with and without jitter) both within and across subjects according to each condition. Participant CVEPs were grouped according to the morphological pattern elicited by visual stimuli and grand-averaged to create an average CVEP pattern. In the objects with no temporal jitter condition (**Figure 3**), two participants were found to present with pattern A, while three presented with pattern B (**Figure 3A**). Similarly, in the objects with temporal jitter condition (**Figure 3B**), two subjects presented with pattern A and three with pattern B. Interestingly, two subjects presented with a different pattern as a result of the jitter paradigm (i.e., one subject presenting with pattern A in the no jitter condition presented with pattern B in the jitter condition, and one subject presenting with pattern B in the no jitter condition presented with pattern A in the jitter condition). It may also be observed that jitter affects amplitude and latency in each object-onset CVEP pattern (**Figures 3C,D**).

For the motion condition (**Figure 4**), two subjects demonstrated pattern A morphology and three subjects presented with pattern B. However, in contrast to the object-onset CVEPs, motion-onset CVEP morphological patterns for each participant were consistent across jitter condition. Furthermore, the pattern B group average shows no clear evidence of the multiple peak components typically present. This lack of differential morphology occurred in both motion

paradigms without and with temporal jitter (**Figures 4A,B**). Similar to the object's paradigm, jitter in the motion paradigm appears to affect motion-onset CVEP characteristics in both morphological patterns (**Figures 4C,D**).

FIGURE AND TABLE LEGENDS:

Figure 1. Example of Visual Object Stimuli Paradigms Without and With Temporal Jitter. A. Without temporal jitter: A fixation cross is presented for 500 ms, followed by a randomized presentation of one of four objects from the BOSS database (button, book, ball, brick). Each object presentation is 600 ms in duration. B. With temporal jitter: A fixation cross is presented for 500 or 1000 ms, values which are randomized across trials, and then one of four objects from the BOSS database (button, book, ball, brick). Each object is presented for randomized values of 600 or 1000 ms.

Figure 2. Example of Visual Motion Stimuli Paradigms Without and With Temporal Jitter. A. Without temporal jitter: A fixation cross is presented for 500 ms, followed by a visual motion movie of a radial field of dots moving inward toward a central fixation point (denoted by white arrows) for 1000 ms. B. With temporal jitter: A fixation cross is presented for 500, 750, or 1000 ms, values which are randomized across trials. A visual motion movie is then presented for either 600 or 1000 ms, values which are randomized across trials.

Figure 3. Representative Object-onset CVEP Data Without and With Temporal Jitter. A. Pattern A morphology (i.e., a P1-N1-P2 response) was observed in two participants (solid black line) in response to the object paradigm without jitter. Pattern B morphology (i.e., a P1-N1a-P2a-N1b-P2b response) was observed in 3 participants (dashed red line) in response to the object paradigm without jitter. Amplitude in microvolts is depicted on the vertical axis and time in milliseconds on the horizontal axis. B. Pattern A morphology was found in two participants (solid black line) elicited by the object paradigm with jitter. Pattern B morphology was found in 3 participants (red dashed line) elicited by the object paradigm with jitter. C. Pattern A morphology comparison in the same three participants in response to the object paradigm without jitter (solid black line) and the object paradigm with jitter (red dashed line). D. Pattern B morphology comparison in the same two participants as elicited by the object paradigm without jitter (solid black line) and the object paradigm with jitter (red dashed line).

Figure 4. Representative Motion-onset CVEP Data Without and With Temporal Jitter. A. Pattern A morphology (i.e., a P1-N1-P2 response) was observed in two participants (solid black line) in response to the motion paradigm without jitter. Pattern B morphology (i.e., a P1-N1a-P2a-N1b-P2b response) was observed individually in 3 participants (dashed red line) in response to the motion paradigm without jitter. Note, however, the typical pattern B morphology is not observed in the CVEP group grand average. Amplitude in microvolts is depicted on the vertical axis and time in milliseconds on the horizontal axis. B. Pattern A morphology was found in two participants (solid black line) elicited by the motion paradigm with jitter. Pattern B morphology was found individually in 3 participants (red dashed line) elicited by the motion paradigm with jitter. Again, the pattern B morphology is not apparent in the CVEP grand average. C. Pattern A morphology

comparison in the same three participants in response to the motion paradigm without jitter (solid black line) and the motion paradigm with jitter (red dashed line). D. Pattern B morphology comparison in the same two participants as elicited by the motion paradigm without jitter (solid black line) and the motion paradigm with jitter (red dashed line).

DISCUSSION:

The goal of this methodological report was to evaluate the feasibility in recording differential CVEP morphology by using visual object and motion stimuli specifically designed to separately stimulate ventral and dorsal streams in passive viewing tasks[6-8], both with and without variation of ISIs (jitter)[19]. Conditions were not designed to be directly compared, rather, observations were made as to whether variable CVEP morphology was present in either condition, and whether temporal jitter within that condition affected morphology. Object-onset and motion-onset CVEP responses were recorded and time-locked to the onset of visual object and motion stimuli, presented in four paradigms, via 128-channel high-density EEG. Five young adults participated in passive viewing of each visual paradigm, and resulting CVEP responses were visually categorized, subjectively, according to CVEP pattern A (P1-N1-P2) morphology and CVEP pattern B (P1-N1a-P2a-N1b-P2b) morphology, a method used in previous research upon which this approach is based[12,13].

Representative data suggest that the described visual stimuli are sensitive to differential CVEP morphology. In addition, jitter appears to affect specific characteristics of the CVEP response, such as latency and amplitude, rather than the overall morphology of the waveform. No further conclusions may be drawn due to the small sample size and lack of statistical comparisons. Therefore, these data show that the experimental design may be useful in the study of variable CVEP morphology and associated visual behavior. Future research is planned to focus on significantly enlarging the number of participants to verify whether CVEP patterns elicited by a variety of stimuli are an intrinsic or extrinsic phenomenon and whether certain visual cortical networks may be more involved than others in generating specific morphology. Future studies will also include temporal variation in visual paradigms for further assessment of possible anticipatory effects on CVEP responses, including greater variability in jitter values, as the limited jitter intervals included in the current approach may not completely eliminate predictability. Finally, source localization analyses on CVEP peak components will be performed for qualitative information on visual cortical networks involved in the generation CVEP morphological patterns, including verification that the presented stimuli activate the intended visual networks.

Although the methods described show an effective approach to the investigation of object-onset and motion-onset CVEP morphology, critical steps should be noted. For instance, in visual stimuli creation, it is important that factors such as luminance be consistent and controlled for, as these lower-order changes may affect CVEP characteristics[22]. In EEG preparation, it is imperative that close attention is paid to electrode impedance values. The high-density EEG system used in the current study is a high-impedance system, meaning that EEG activity can be successfully recorded with electrode impedance values of up to 50 kOhms. However, in our laboratory, we aim to maintain these values under 20 kOhms, and ideally around 10 kOhms. Lower impedance values greatly affect the overall quality of the recording and result in faster analyses and a higher

number of accepted trials. In addition, it is important to monitor the subject state, especially as these paradigms are passive in nature. It can be a challenge for some participants to remain alert, resulting in alpha oscillations and ocular artifact that can contaminate the recording. In EEG analyses, it is critical to remove bad electrode channels prior to artifact rejection to ensure that the maximum number of trials are accepted into the average. The greater the number of trials, the better the signal-to-noise ratio of the CVEP response. Furthermore, a large number of trials are necessary for source localization analyses. In our laboratory, a minimum of 100 accepted trials is typical for visual studies[12,13,22]. The EEG analysis method described in this study may also be modified according to the researcher's discretion. There are many approaches to successful EEG analysis, and the one provided has been developed in our laboratory. Other approaches that may be useful can be reviewed through various tutorials provided by the creators of the EEGLAB toolbox.

While EEG methodology does have limitations, specifically in spatial resolution for imaging purposes[2], the benefits of a low cost, non-invasive approach, and high temporal resolution make this an ideal tool for the investigation of CVEP morphological patterns. For example, the latency and amplitude of the specific peak components which constitute the CVEP waveform would not be identifiable using a different approach, except possibly with magnetoencephalography (MEG). Furthermore, source localization analyses, which are possible with high-density EEG recordings, have advanced to such a level that estimation of cortical generator location has been accepted in a multitude of studies[12,13,23-26]. If spatial localization remains a concern for the researcher, a multi-modal approach may be used to combine the temporal resolution of EEG with the spatial resolution of other measures, such as fMRI[27]. It is important that a large amount of trials is collected in each paradigm for future source localization analyses, which requires a high EEG signal-to-noise ratio for accurate estimation of cortical generators[12,13,23].

Overall, the described protocol is useful and effective for the observation and study of CVEP morphological patterns. Similar methodologies have been presented in the literature[14,15,28,29], but have not focused on the categorization of group participant responses according to morphology, as described in the EEG analyses section. Future research may benefit from examining CVEP morphology more closely, as distinct visual processes have been shown to underlie specific patterns[12,13]. While additional work is necessary to clarify whether CVEP morphology elicited by various stimuli and underlying visual function are related to visual behavior, the experimental paradigms and EEG analyses discussed in this pilot study provide an initial point from which to better understand basic visual cortical processes.

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DISCLOSURES:
The authors have nothing to disclose.

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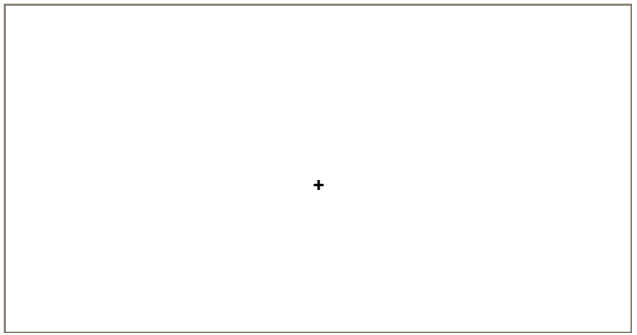
A



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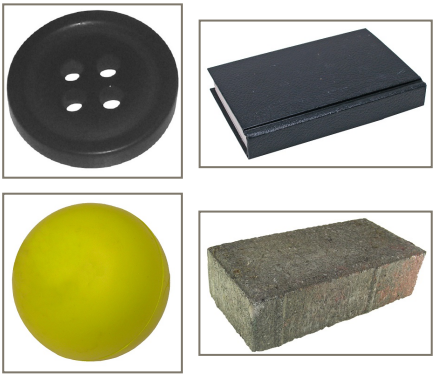

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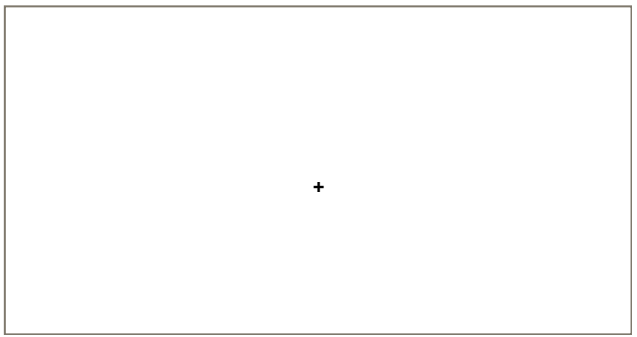

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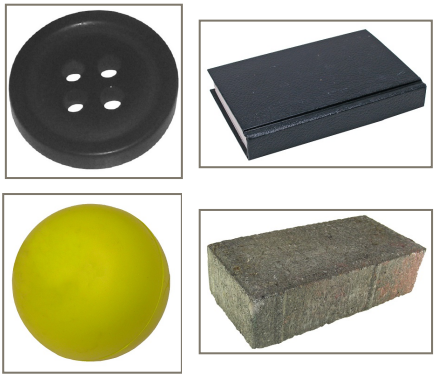

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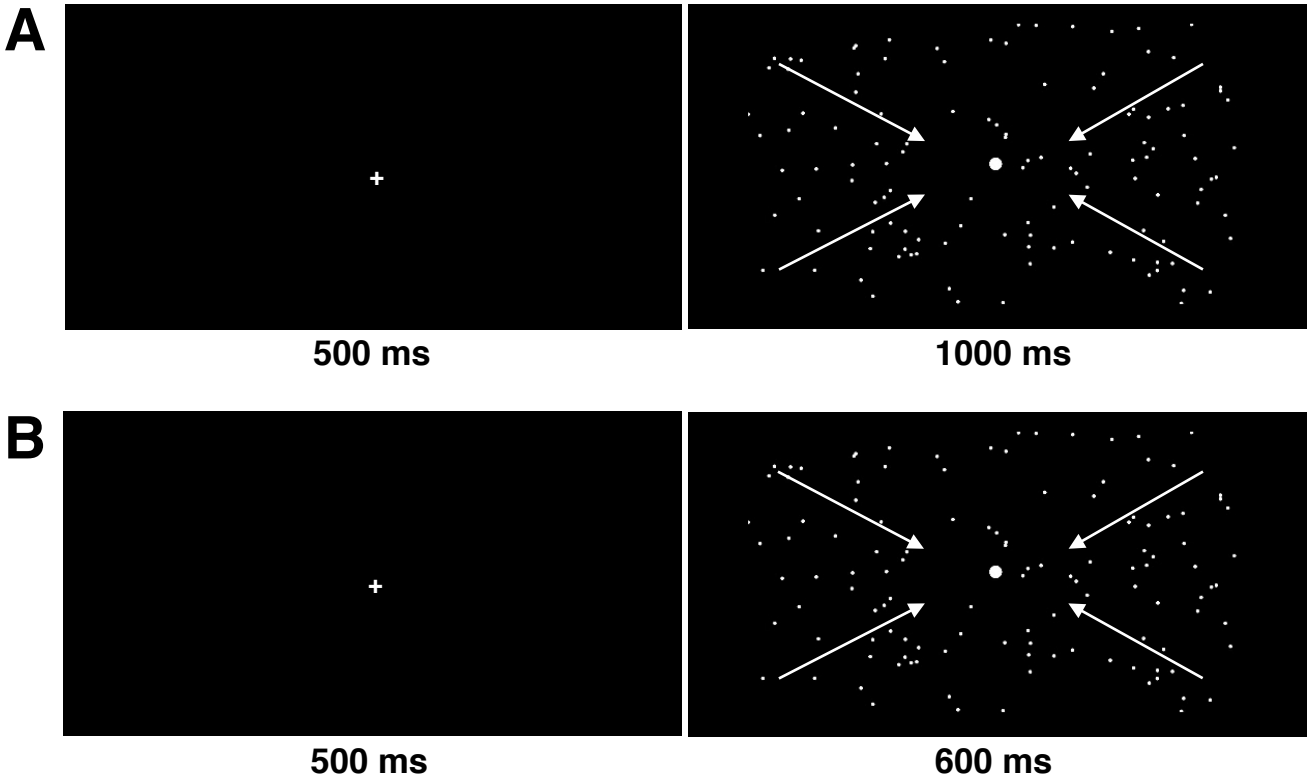
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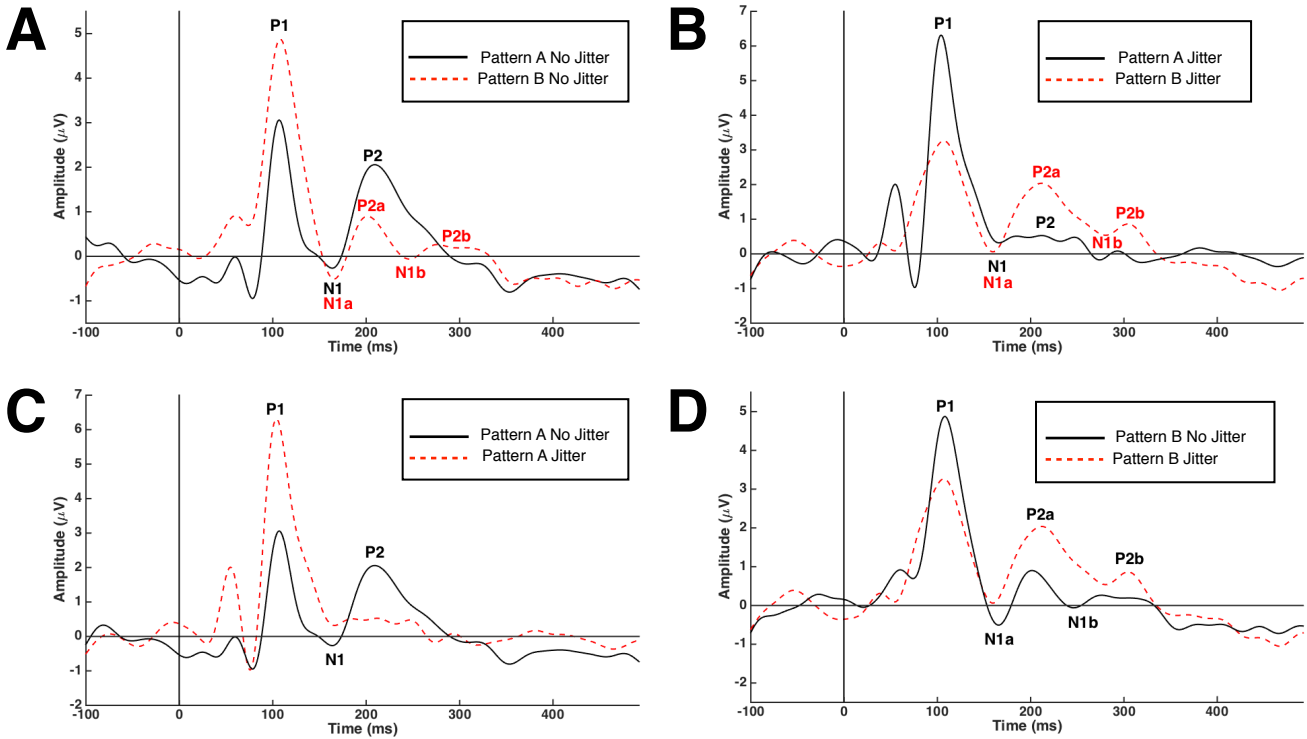
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Pattern B Jitter
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Pattern A No Jitter
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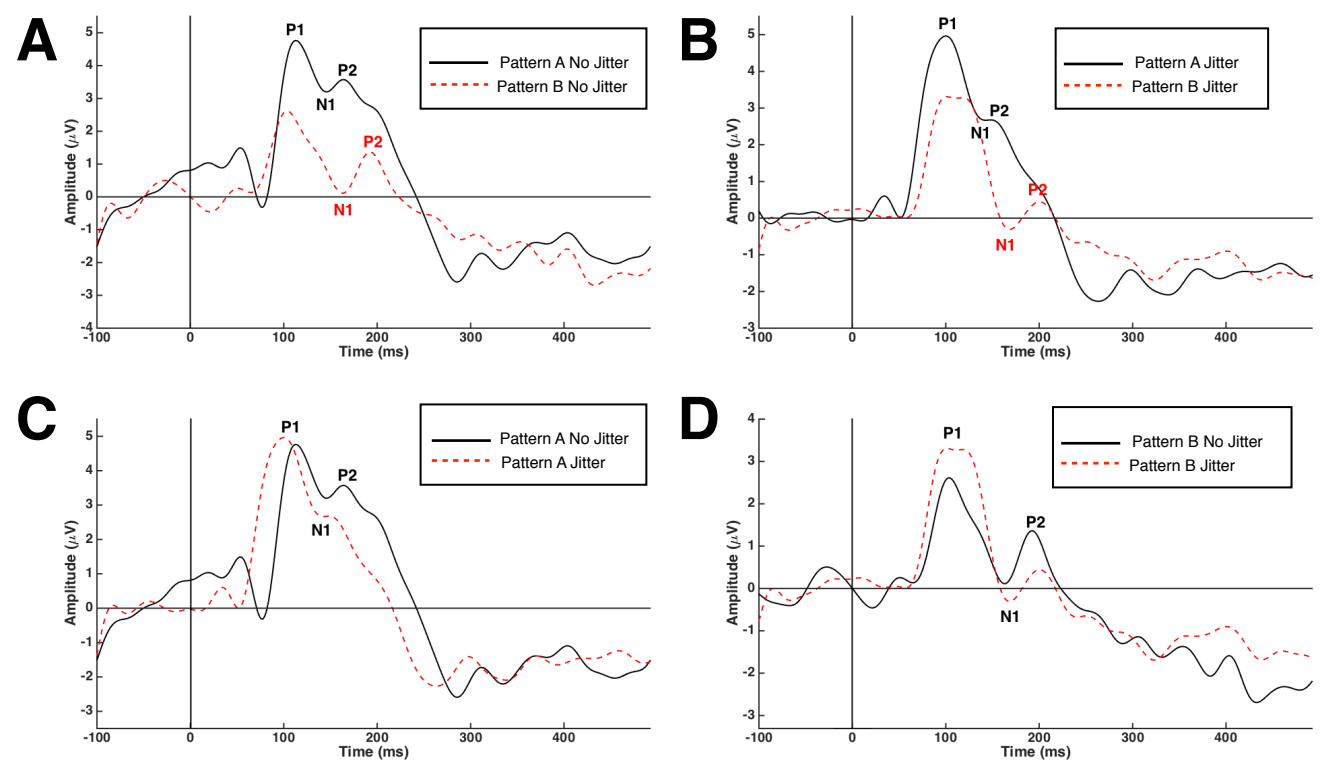
Amplitude (μV)
Time (ms)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Pattern B Jitter
Amplitude (μV)
Time (ms)

Name of Material/ Equipment	Company	Catalog Number
E-Prime 2.0	Psychology Software Tools, Inc	
Net Amps 400	Electrical Geodesics, Inc	
Net Station Acquisition V5.2.0.2	Electrical Geodesics, Inc	
iMac (27-inch)	Apple	
Optiplex 7020 Computer	Dell	
HydroCel GSN EEG net	Electrical Geodesics, Inc	
1 ml pipette	Electrical Geodesics, Inc	
Johnson's Baby Shampoo	Johnson & Johnson	
Potassium Chloride (dry)	Electrical Geodesics, Inc	
Control III Disinfectant Germicide	Control III	
32-inch LCD monitor	Vizio	
Matlab (R2016b)	MathWorks	
EEGlab v14.1.2	Swartz Center for Computational Neuroscience, University of California, San Diego	https://sccn.ucsd.edu/eeglab/index.php
BOSS Database	Bank of Standardized Stimuli	https://sites.google.com/site/bosstimuli/
Psychtoolbox-3	Psychophysics Toolbox Version 3 (PTB-3)	http://psychtoolbox.org/

Comments/Description
Used in data acquisition
Used in data acquisition
Used in data acqusition
Used in data acquisition
Stimulus computer
Used in data acqusition
Used to lower impedances
Used in impedance solution
Used in impedance solution
Used in disinfectant solution
Used to present stimuli
Used in data analysis
Used in data analysis
Used in generation of visual object stimuli
Used in generation of visual motion stimuli

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Institution:	University of Texas at Austin
Title:	Assistant Professor
Signature:	
Date:	9/25/2018

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Author Response to Reviewers

Editorial comments:

Changes to be made by the author(s) regarding the manuscript:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

Thank you; we have done so.

2. Please provide an email address for each author.

We have added the email addresses for Alison LaBrec and Connor Bean.

3. Keywords: Please provide at least 6 keywords or phrases.

We added the keyword 'EEGLAB' for a total of 6 keywords.

4. Please revise the protocol (steps 1, 2 and their substeps, 6.1,etc.) to contain only action items that direct the reader to do something (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as "could be," "should be," and "would be" throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a "Note." Please include all safety procedures and use of hoods, etc. However, notes should be used sparingly and actions should be described in the imperative tense wherever possible. Please move the discussion about the protocol to the Discussion.

Thank you for pointing this out. We have edited the protocol to be in the imperative tense and moved the protocol discussion to the Discussion section.

5. 3.1: What are the inclusion/exclusion criteria for recruiting the participants?

This is now described as the following: Exclude participants who report hearing loss and/or neurological diagnoses, such as traumatic brain injury. Include all other participants.

6. 5.5: Please specify the four visual tasks.

The tasks have been clarified in the following sentence: Repeat steps 3 and 4 for the visual object paradigm, the visual object with temporal jitter paradigm, the visual motion paradigm, and the visual motion with temporal jitter paradigm.

7. 6.2.7, 6.2.8, 6.2.8.1, 6.2.9, 6.2.11, etc.: The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections. Please simplify the Protocol so that individual steps contain only 2-3 actions per step and a maximum of 4 sentences per step. Use sub-steps as necessary. Please move the discussion about the protocol to the Discussion.

We apologize for this oversight and have made the listed corrections.

8. Please remove commercial language and use generic terms instead: MATLAB, E-Prime, etc.

This has been attempted throughout the paper, although MATLAB and similar terms are used sporadically as deemed necessary.

9. Please include single-line spaces between all paragraphs, headings, steps, etc.

This has been corrected.

10. After you have made all the recommended changes to your protocol (listed above), please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol.

Thank you, we have done this.

11. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense.

We have now done so.

12. Please include all relevant details that are required to perform the step in the highlighting. For example: If step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be highlighted.

All relevant steps have been highlighted in full.

Reviewers' comments:

Reviewer #1:
Manuscript Summary:
The paper outlines an EEG methodology for the examination of cortical visual evoked potentials (CVEPs) with the goal of examining the underlying mechanisms driving observed differences in CVEP morphology, specifically in relation to dorsal versus visual stream processing, and anticipatory top-down processing.
The methodology described seems generally sound, however, in some parts the methodology could be more generalised to ensure it is a research methodology that could be transferred across different labs.

Major Concerns:
1. The jitter visual object paradigm has two fixation cross intervals (500, 1000ms), while the

visual motion has three (500, 750, 1000ms). What is the reason for the additional intermediary interval in the motion paradigm? Could this make the two paradigms (object versus motion) difficult to compare?

This is an extremely valid point. An additional fixation interval was added to the motion jitter paradigm to decrease predictability. The object paradigm inherently includes decreased predictability due to the presentation of four different objects. Therefore, another fixation interval was not included in that paradigm. Regardless, we are not currently directly comparing motion versus object conditions. The overall goal is to observe whether either condition elicits pattern A or pattern B morphology. Thus, the jitter conditions are for within-condition comparison only (e.g., motion versus motion with jitter), controlling for possible effects of an additional fixation interval across motion and object conditions.

We have included a sentence in the Discussion to clarify: 'Conditions were not designed to be directly compared, rather, observations were made as to whether variable CVEP morphology was present in either condition, and whether temporal jitter within that condition affected morphology'.

2. Sections 4-6 of Procedure - sometimes the key aspects get lost in the highly specific details of the instructions. These sections may benefit if there was some greater highlighting of the key methodological aspects more generally to aid replication regardless of EEG system/analysis software etc.

Thank you for this suggestion; we have endeavored to simplify this section for generalization purposes.

3. Section 4.7 of Procedure - it states that the reference electrode should be at scalp midline, and that this midline point may be verified by measuring. I would argue that it should always be verified by measuring , and this should include some instruction on how to obtain the measurement (e.g., measuring between nasion and inion).

We agree and have added this instruction in 4.1.

4. Section 5.2 - you argue for use of a random number generator to determine order of presentation. With large numbers of participants this will work, however, it may be optimal to use a pseudorandom order that is controlled by the experimenter to ensure that all orders are carried out evenly.

This is a great suggestion and we have added it to Section 5.2.

Minor Concerns:
1. In the introduction (lines 107-110 of manuscript), it states that the 'paradigm designed to stimulate the dorsal stream is composed of highly identifiable objects . . . The paradigm designed to stimulate the ventral stream consists of visual motion . . .' This is the incorrect way

around.

We apologize for this oversight and have corrected the description.

Reviewer #2:

Manuscript Summary:

According to the title of the manuscript I have expected that a new contribution to analysis of the „ventral and dorsal visual cortical networks" activity, using visual evoked potentials, will be based on a good knowledge of existing literature to this topic and that adequate precise methods will be used.

However, authors ignore already well known generally accepted facts concerning characteristics (morphology) of VEPs and needed parameters of visual stimulation for activation of either magno/parvocellular systems or ventral/dorsal streams of the visual pathway.

Major Concerns:

1. Authors use uniform term CVEPs for all cortical visual evoked potentials irrespective of the particular kind of visual stimulations which evoke different types of VEPs (e.g. flash VEPs, pattern related VEPs - pattern-reversal or pattern onset/offset, motion-onset VEPs.

Thank you for pointing this out. We have now used the terms 'motion-onset CVEPs' and 'object-onset CVEPs' to differentiate the responses to specific stimuli throughout the manuscript.

2. They claim that they use „visual object stimuli" and „visual motion stimuli" and they do not mind that both types of the recorded VEPs are almost identical (with a dominant positive peak at about 100ms) and do not display standard characteristics of any known type of VEPs.

We do not believe the responses to look identical, especially when considered within each condition. For example, in the motion condition (Figure 4), the same subjects who presented with a pattern A response presented with similar morphology when jitter was added to the condition (this was also the case for pattern B). However, amplitude and latency of the waveform was different. In other words, if subject 1 presented with a motion-onset CVEP response with pattern A morphology, when jitter was added they still presented with pattern A morphology but different amplitude and latency of peaks, as evaluated qualitatively. We also believe the waveforms to appear qualitatively different between object and motion conditions, with varying morphology.

In addition, these CVEPs (particularly the peak at 100 ms), are similar to previous CVEP research we have conducted (Campbell & Sharma, 2014, 2016a,b) and those reported in other studies (Allison et al., 1999; Coch et al., 2005; Doucet et al., 2005, 2006; Schulte-Korne et al., 2004).

Since all their visual stimuli do not have constant luminance (change at the trigger time) and include some pattern appearance, it looks that their VEPs will represent probably some mixture of reactions to luminance changes and pattern-onset VEPs.

We agree that luminance may be an important factor and have added this consideration to the manuscript in the Discussion section. Pattern-related processing may occur during both conditions, but we remained consistent with literature that used similar approaches for object and coherent dot motion presentation (Allison et al., 1999; Prieto et al., 2007; Schulte-Korne et al., 2004; Yamasaki et al., 2017).

In „motion stimulation", they speak only about „apparent motion" (in fact almost any motion on a PC monitor is only apparent, since it is created from many discrete static pictures) and they do not consider that their motion VEPs should be specified as „motion-onset VEPs" (but they are not because parameters of stimulation are not adequate).

Thank you for this comment. We have now termed the CVEPs in the motion condition as motion-onset CVEPs throughout the manuscript. We believe that these are motion-onset responses as they were time-locked to the onset of each presentation of centrally-directed coherently moving dots.

It is true that all visual motion presented on-screen is apparent motion to some degree. In that respect, all typical visual experiments involve this type of motion. However, we remained consistent with the literature, specifically Doucet and colleagues (2005, 2006) and Bertrand and colleagues (2012), which terms apparent motion as distinct frame changes between radially-modulated shape changes versus fluid motion of dots in a coherent direction (Prieto et al., 2007; Schulte-Korne et al., 2004; Yamasaki et al., 2017).

The presented morphology does not correspond to the existing nomenclature (the dominant positive peak at about 100 ms cannot represent the motion-onset).

Thank you for this comment. However, we do not state that the P1 in the Representative Data represents the motion-onset. Indeed, earlier activity may be viewed in several waveforms. However, it is of interest that one study found that the P1 measured via MEG in response to coherent motion of dots reflected onset of motion activation in V5+, a common cortical region associated with visual motion processing (Prieto et al., 2007).

Authors did not pay any attention to the existing methodology - although they cite (non-adequately) one of the existing review articles (Kuba et al. - review by S. Heinrich provides the same recommendations) they do not comment the discrepancies in results.

Many approaches to recording CVEPs have been presented in the literature. Ours is comparable to several studies (Allison et al., 1999; Doucet et al., 2005, 2006; Schulte-Korne et al., 2004). In addition, we refrained from interpretation of result comparisons due to the small sample size (Representative Data).

3. Although multichannel recordings were done, the only information is about 0z results (not optimal for the motion related activity). Data from more channels could help to identify the recorded potentials.

This is an excellent point. We clarify in the Protocol that a different channel or channels may be utilized to view the data. Oz was chosen for the current representative data as an example, and is a common electrode selected in studies to illustrate CVEP responses (Campbell & Sharma, 2014).

4. It is rather strange that multiple peaks in „pattern B" of VEPs are classified, although they represent probably only a small non-significant oscillation of about 1 uV. All peaks designated as negative are positive (over zero - splitting of positive peaks). Both „patterns" rather seem to represent about the same type of VEPs with some inter-individual variability (grand averages from 3 and 2 subjects only!!!).

We completely understand the concern with such a small sample size. The sample size is reduced due to the publication journal's request for representative data, and several methodological studies have been published in this journal using single-subject data (see Sadeh & Yovel, 2014; Tardif et al., 2018). We also now state that only feasibility of the approach can be estimated from the representative data, rather than conclusions reached via statistical analyses, and have generalized sentences in the Representative Data section referring to group differences.

Classification of peaks was made based upon previous work (Campbell & Sharma, 2016a,b) with larger sample sizes and found to be consistent with this research.

In regards to the designation of negative peaks as being positive (according to absolute value): We have used this nomenclature in past evoked potential studies (Campbell & Sharma 2013; 2016a,b; Sharma et al., 2014, 2016) and aimed to remain consistent with this work.

5. Large parts of the manuscript look like a manual for a laboratory assistant or some other staff - e.g. lines 188 - 267, 281 - 360 (it was intentionally included??).

We agree and have edited the Protocol to be more generalized. The publication journal requested steps to allow others to replicate described methodology accurately, and thus we endeavored to be clear in each step.

There are much more problems in Methods and thus, it is not possible (I did not dare to do it) to interpret and discuss Results (it is not clear which activity represent the recorded VEPs). Moreover, I do not consider results from 5 subjects sufficient for the made conclusions.

Due to the small sample size, we have added sentences in the Introduction and Discussion sections to clarify that the goal of the manuscript was to demonstrate feasibility of the methodological approach rather than draw definitive conclusions from group comparisons. We have also generalized the Representative Data so that group comparisons were avoided.

I do not thing that it is necessary to specify more particular problems when MY GENERAL OPINION IS THAT THE MANUSCRIPT IS NOT SUITABLE FOR PUBLICATION.
(I am rather surprised that similar electrophysiological data were successfully published by the first author in Frontiers in Human Neurosci.

We are sorry to hear that you feel this way and welcome further comments.

Reviewer #3:
Manuscript Summary:
The MS concerns using EEG measurement in order to investigate possible different morphologies of CVEP. The outline of EEG protocol for recording is fairly standard for the EGI system, therefore the emphasis on methodology is on the appropriateness of the stimuli and use of jitter etc in order to test dorsal vs ventral processing effectively.

Minor Concerns:
The analysis steps are also fairly standard, and as such should be fine. However, the authors mention other influences that might affect the data, such as alpha oscillations during recording. It would be useful to have a consideration of using ICA in the pre-processing of the data and why visual inspection was preferred.

This is a great suggestion. For this specific methodologic approach, we were building on previous research (Campbell & Sharma, 2016a,b) which utilized visual identification for differential morphology.

We have included a sentence in the Discussion to clarify this point: 'resulting CVEP responses were visually categorized, subjectively, according to CVEP pattern A (P1-N1-P2) morphology and CVEP pattern B (P1-N1a-P2a-N1b-P2b) morphology, a method used in previous research upon which this approach is based[12'].

Although the ventral stream is linked to form processing, it would be interesting to have a little more detail on the task. It would appear that it was a passive viewing task, but was there any indication from participants that they may have been naming the (BOSS) stimuli too?

You are correct in stating that the tasks were passive, a point we attempt to emphasize throughout the manuscript. Therefore, we did not have participants naming the object stimuli, nor did we control for this possibility. If this did occur, it was performed silently as participants were not noted to speak during the tasks.

Although the results support the previous work of the authors, the sample size is very small. It would be good to see the different patterns with a larger sample and also to have a statistical test of the difference between the patterns. It would be great to see some statistical analysis of the later components of pattern B -peaks compared to the general trend of the timeline.

We completely agree. The only reason the dataset is small is due to the request of a 'representative sample size' by the publication journal. Previous articles with similar methodology published by this journal include single-subject data (see Sadeh & Yovel, 2014; Tardif et al., 2018). The small sample size is why we conclude, in an edited Discussion, that: 'Representative data suggest that the described visual stimuli may be sensitive to differential CVEP morphology' rather than state that only the ventral stream is responsible for producing specific CVEP morphology. This is to reinforce that the goal of the manuscript was to observe if the methodology was feasible, rather than draw conclusions from findings.

We also state in the Discussion: 'Future research is planned to focus on significantly enlarging the number of participants to verify whether CVEP patterns are an intrinsic or extrinsic phenomenon and whether certain visual cortical networks may be more involved than others in generating specific morphology'.

Although implied, in the description of the representative results, it would be good to clearly express whether it is the same participants with pattern A/B in each stimulus condition. Further, the discussion of whether the difference in the motion conditions is really due to the CVEP being exclusive to the ventral stream or whether it is due to noise or a small sample size does rather undermine the aim of the study to distinguish between dorsal/ventral processing.

This is an excellent point. We have removed the sentence which speculated as to why pattern B morphology may be absent in the CVEP motion condition. In addition, it has been made explicit which participants presented with specific morphology.

Reviewer #4:

Manuscript Summary:

The article describes a technique for recording high density VEPs elicited by motion stimuli.

Major Concerns:

There are several concerns about the publication.

1. The waveforms appear quite different than those described by others, notably Kuba and also work by Bach and by Spileers.

We agree that the waveforms appear different from the research by the authors mentioned. This is likely due to methodological differences in the presented stimuli, as well as analytical differences. For example, Kuba et al. (2007) utilized radial motion instead of centrally-directed coherent dot motion. Furthermore, these studies tend to use single or linked-mastoid references, while we use common-average reference (due to future source localization analyses). Reference location greatly affects evoked potential waveform characteristics. Finally, in a study using methodology similar to ours (although in children), waveforms appear to be comparable (Schulte-Korne et al., 2004).

2. The jitter described appears to be a fixed sequence, although a variable one, so that concerns of predictability may not have been eliminated.

Thank you for pointing this out. This is completely true and we now mention this point in the Discussion: 'Future studies will also include temporal variation in visual paradigms for further assessment of possible anticipatory effects on CVEP responses, including greater variability in jitter values, as the limited jitter intervals included in the current approach may not completely eliminate predictability'.

3. The major advantage of high density recording is presumably source localization. No source localization is presented in the paper. Therefore it is not clear that there is a separation of dorsal and ventral pathways.

Thank you for this comment. We agree that high-density EEG is mostly advantageous for source localization, which is why we state that future research will utilize this approach to include such analyses in the Discussion. Although we did not include source localization in this specific methodologic approach, we believe that, based on previous fMRI and intracortical studies, the stimuli presented are indeed stream-specific (Allison et al., 1999; Grill-Spector, 2003; Grill-Spector & Malach, 2004; Prieto et al., 2007).

We have now included a statement in the Discussion that source localization will also serve to confirm that these stimuli activate the intended networks: 'Finally, source localization analyses on CVEP peak components will be performed for qualitative information on visual cortical networks involved in the generation CVEP morphological patterns, including verification that the presented stimuli activate the intended visual networks'.