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Reliability of Chronically Recorded Visually Evoked Potentials in Awake Mouse Visual Cortex: Effect of Restraint Habituation

A senior thesis submitted to
The Department of Math & Science
College of Arts & Sciences

In partial fulfillment of the requirements
for a Bachelor of Arts degree in Biology

by

McKenna D. Stocker

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Dr. Michael A. Thomas **Date**

Provost_____ _____
Dr. Michelle Cowing (Interim Provost) **Date**

Abstract

Visual function in mice can be quantified using electrophysiological methods. This can be done using chronically implanted electrodes that record visually evoked potentials (VEPs) from a population of neurons in the primary visual cortex (V1) in order to estimate visual acuity. The inherently noisy environment of the brain presents a challenge, as the VEP signal is very small. Our goal is to optimize VEP recording procedures to produce the highest signal-to-noise ratio possible by investigating the role of restraint habituation. The approach we designed included three experimental groups: one in which the animals received regular stimulus exposure and no habituation, one in which the animals received 10 days of restraint habituation prior to 10 days of stimulus exposure, and one in which the animals received habituation only and no stimulus exposure. We found that restraint habituation is necessary in order to produce reliable VEPs. Furthermore, we discovered that over time there is an increase in VEP amplitude that is dependent upon visual experience. This experience-dependent effect is driven by repeated exposure to specific stimuli.

KEY WORDS: Visually evoked potential, visual acuity, habituation, stimulus exposure, visual experience, stimulus-selective response potentiation.

ACKNOWLEDGEMENTS

I would like to acknowledge the people who have played an influential role in my academic career and accomplishments. I would like to thank my parents, Brooke and Kevin, brothers, Logan and Zachary, and other family members who have supported me and loved me throughout my academic career. Their support has been unwavering and has helped me overcome many obstacles.

I also want to thank my thesis supervisor and advisor, Dr. Wayne Tschetter, for leading me through the research process. Thank you to my thesis committee, Dr. Jeanette Eggert and Dr. Bert Coltman, who have mentored me through the writing process.

Thank you to Nicole Patterson, M. J. Murdock Charitable Trust, Dr. Michael A. Thomas, Dr. Mihail Iordanov, Grant Landy, and countless others who have played a significant role in my career at Concordia University and made all of this possible. I cannot thank you enough for helping to shape me into the person I am today.

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Reliability of Chronically Recorded Visually Evoked Potentials in Awake Mouse Visual Cortex: Effect of Restraint Habituation

McKenna D. Stocker and Wayne W. Tschetter[1]

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Introduction

Electrophysiological and behavioral methods are used to quantify the function of the visual system in animal models. Behavioral methods, such as testing visual perceptual thresholds in the visual water task (VWT) and virtual reality tasks that rely on optomotor reflexes, are commonly used to quantify visual acuity (Hosang, Yusifov, & Löwel, 2017; Prusky, West, & Douglas, 2000a; Prusky, West, & Douglas, 2000b; Tokashiki, Nishigucci, Fujita, Sato, Nakagawa, & Nakazawa, 2018; Young, Brennan, Wang, & Tian, 2018). Electrophysiological methods include optical imaging of intrinsic signals and recording of visually evoked potentials (VEPs) from the primary visual cortex (V1) to estimate visual acuity (Cooke & Bear, 2010; Cooke, Komorowski, Kaplan, Gavornik, & Bear, 2015; Heimel, Hartman, Hermans, & Levelt, 2007; Porciatti, Pizzorusso, & Maffei, 1999; Tschetter, Govinidaiah, Etherington, & Neill, 2018). VEPs are a neural population response that is evoked by visual stimuli (Ridder III, & Nusinowitz, 2006). Estimating visual acuity by recording directly from the brain is challenging because the brain is an intrinsically noisy environment, directing a multitude of activities and functions at all times, and the VEP signal is characteristically small. Our goal here is to optimize VEP recording procedures in order to have the highest signal-to-noise ratio possible. This will improve the accuracy of data and help limit the effect of background noise. The factors that affect the VEP signal and have a direct effect on the signal-to-noise ratio are not well

understood. This may include types and placement of recording and reference electrodes, visual stimuli, repeated exposure to the same stimuli, recovery time after surgery, and restraint habituation. These factors could be crucial to producing consistent, reliable, and accurate data, yet they are not reported in the literature, to our knowledge.

We sought to characterize the VEP signal and explore the effect of restraint habituation on said signal. We hypothesized that the physical restraint may have a significant effect on generating reliable VEP signals that can be reproduced daily. For example, an animal unsettled in the restraint will resist, possibly shifting the electrodes, which can affect the signal. To mitigate these effects and noise in the VEP signal we developed a restraint habituation protocol. We predicted that habituating the animals to the restraint would help reduce intrinsically generated noise, therefore producing a more reliable and consistent signal by improving the signal-to-noise ratio.

When a visual stimulus enters the eye, it follows a pathway to a midbrain relay, the lateral geniculate nucleus (LGN), and then continues on to V1 where higher order processing occurs. V1 is a structure that has been shown to be necessary for visual acuity testing (Heimel, Hartman, Hermans, & Levelt, 2007; Prusky, et. al., 2000a; Prusky, et. al., 2000b). As a result, we recorded VEPs by chronically implanting electrodes into V1 (Campbell & Wu, 2018; Cooke & Bear, 2010; Frenkel, Sawtell, Diogo, Yoon, Neve, & Bear, 2006; Tokashiki, Nishiguchi, Fujita, Sato, Nakagawa, & Nakazawa, 2018; Tschetter, et. al., 2018). Our approach was to create three experimental groups to investigate the effects of habituation on VEPs. We tested the effect of stimulus exposure only that included no restraint habituation (E Only), restraint habituation followed by stimulus exposure (H + E), and restraint habituation only with no stimulus exposure (H

only), which allowed us to see the effects of visual experience on VEPs. We report two major findings: (1) habituation is necessary to produce a reliable VEP signal (2) we saw an incremental increase in VEP amplitude that was the result of repeated visual stimulus exposure and experience. Habituation alone did not produce the increase. This finding is consistent with previously published literature on a form of perceptual learning, called stimulus-selective response potentiation (SRP) (Cooke & Bear, 2010; Cooke & Bear, 2014; Cooke, et. al., 2015; Fischer, Aleem, Zhou, & Pham, 2007; Frenkel, et. al., 2006; Hosang, et. al. 2017).

Materials and Methods

Methods previously outlined by Cooke and Bear (2010), Frenkel, et. al., (2006), and Tschetter, et. al. (2018), were used as a reference for surgical techniques, lab setup, and VEP testing equipment.

Animals

Twenty-one-day-old, male, C57BL/6 mice from Jax Laboratories were housed according to International Animal Care and Use (IACU) standards. Food and water were provided *ad libitum*. Light and dark cycles were regulated, with the lights on from 6:00 AM to 6:00 PM, and the lights off from 6:00 PM to 6:00 AM.

Electrophysiology

Methods outlined by Tschetter, et. al. (2018) and Cooke and Bear (2010) were used as a reference. Mice were anesthetized with inhaled isofluorane (initial inhalation at a rate of 4-5%, while maintenance was at 2-3%) and then placed into a stereotaxic frame. The head was shaved and cleaned with iodine and ethanol, and a topical anesthetic was applied to the scalp. A midline incision was made to expose the skull, which was then

dried with acetone. Major sutures on the skull were used as references for electrode placement. Holes were drilled in the skull and a 0.0005-inch diameter insulated platinum recording electrode was implanted in V1 in the right hemisphere using stereotaxic coordinates (0.0 mm lambda anteroposterior, +3.0 mm mediolateral, -0.45 mm dorsoventral). Figure 1B shows the approximate location of the placement of the recording electrode. Twenty-four-gauge silver reference electrodes were implanted in the right hemisphere of the frontal cortex (-1.0 mm bregma anteroposterior, +2.0 mm mediolateral, 0.0 mm dorsolateral). A head post was secured to the anterior part of the skull using cyanoacrylate. Cyanoacrylate was also used to secure the skin around the skull to prevent exposure.

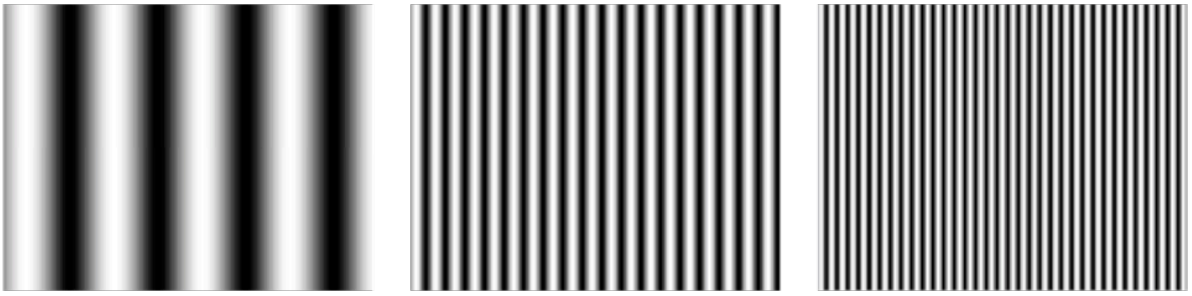
Stimulus Exposure

After a recovery period of 48 hours animals were placed into a plastic tube, intended to support their body during VEP testing, with their head exposed (Fig. 1C). The head post was secured via a fixed arm, which aligned their gaze perpendicular to the computer monitor at a distance of 20 cm. Leads were placed onto the recording and reference electrodes which transmitted the neural signal to an amplifier. Counter-phasing sine-wave gratings were generated and displayed on the monitor using custom MATLAB script. Mice were exposed to spatial frequencies of 0.05, 0.15, 0.30, 0.45, 0.6, 0.75, and 1.0 cycles per degree (cpd) in ascending order at 100% contrast. Figure 1A shows exemplary visual stimuli and Figure 1C shows the experimental setup and physical restraint. Each spatial frequency was presented for 300 seconds (s), the time that was observed to consistently produce the best VEP signal, and signal acquisition was constant throughout the duration of the stimulus exposure. To obtain the VEP, signals were

Figure 1. VEP Recording and Visual Acuity Testing Apparatus

The visual stimulus, electrode implant location, and physical restraint are shown. A) Counterphasing sinusoidal gratings, increasing in cpd from left to right, were used as visual stimuli. B) A recording electrode was surgically implanted in V1 in the right hemisphere using stereotaxic coordinates (0.0 mm lambda anteroposterior, +3.0 mm mediolateral, -0.45 mm dorsoventral). A reference electrode was surgically implanted anterior to the recording electrode also in the right hemisphere using stereotaxic coordinates (-1.0 mm bregma anteroposterior, +2.0 mm mediolateral, 0.0 mm dorsolateral). C) Restrained, head-fixed mice were exposed to the counter-phasing sine-wave grating stimulus. Their gaze was fixed perpendicular to the computer monitor at a distance of 20 cm via a metal rod into which the head post was inserted and secured. The body was secured by a narrow tube. (Figure 1C adapted from Frenkel, et. al., 2006).

A



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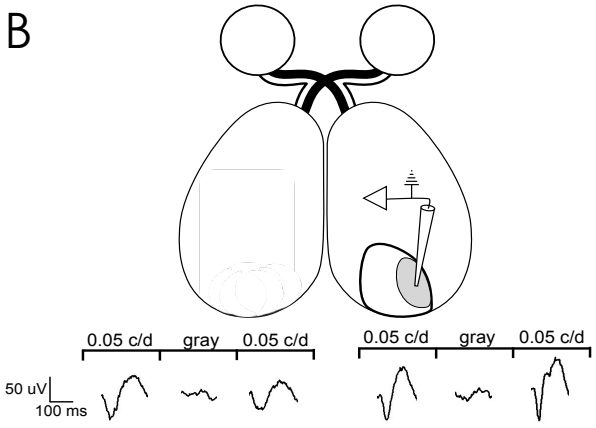

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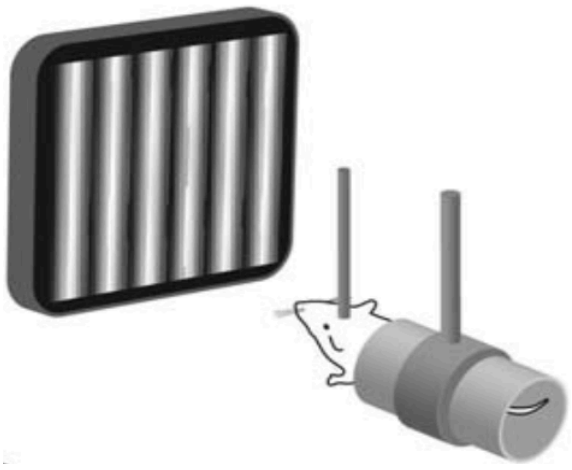


averaged at the reversal of each grating cycle. A control gray screen was used to determine noise and was presented for 300s.

Restraint Habituation

Methods outlined by Cooke & Bear (2010), Frenkel et. al. (2006), and Tschetter et. al. (2018) were used as a reference for the restraint setup. To measure visual acuity, the mouse must be fully restrained and head-fixed. The restraint is comprised of a metal arm to which the animal's head post is secured to ensure that their gaze is fixed perpendicular to the computer monitor displaying the visual stimulus. During habituation the animals were secured in the restraint via the surgically attached head post. Leads were placed on both the recording and reference electrode and a gray screen was presented instead of the sine wave gratings that functioned as the visual stimulus. The gray screen mimics the recording environment but is without a visual stimulus, which allows us to control for the noise created by the screen. Recordings were taken for 35 minutes, equivalent to the time required for one stimulus exposure recording session including exposure to all spatial frequencies, and signal acquisition was constant throughout the duration of the habituation period. The average signal amplitude was determined at the reversal of each grating cycle.

Experimental Groups

To examine the effects of restraint habituation on the VEP signal, the animals were divided into three different experimental groups. Day 1 of the experimental timelines was consistently 48 hours after surgery across all three groups. When the animal is said to have undergone restraint habituation (H), the animal was secured in the restraint and presented with a gray screen. When the animal is said to have undergone

stimulus exposure (E), the animal was secured in the restraint and exposed to the visual stimuli at spatial frequencies of 0.05, 0.15, 0.3, 0.45, 0.6, 0.75, and 1.0 cpd and VEPs were recorded. The characterization of the VEP signal involved examination of VEP amplitudes within and across individuals as well as the three different experimental groups.

Stimulus Exposure Group (E Only)

The Stimulus Exposure Group (E Only) (n = 4) received stimulus exposure on days 1-33 and received no restraint habituation.

Habituation and Stimulus Exposure Group (H + E)

The Habituation and Stimulus Exposure Group (H + E) (n=4) was habituated and received no stimulus exposure from days 1-10. The animals then received stimulus exposure with VEP testing and no habituation on days 11-20.

Habituation Group (H Only)

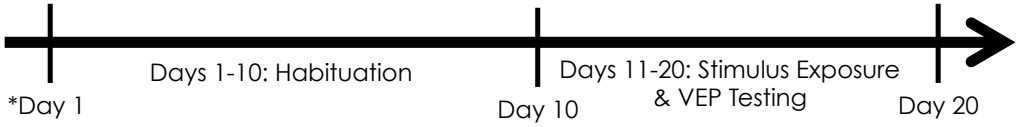
We used the Habituation Group (H Only) (n=3) to test the effects of habituation only on VEP amplitude. This group received stimulus exposure and VEPs were recorded on days 1 and 2. For days 3-9 the animals received habituation and no stimulus exposure, and on day 10 the animals received stimulus exposure and VEPs were recorded. We could then compare the VEP amplitude on day 10 to the baseline amplitudes recorded on days 1 and 2. This data from H Only showed the effects of habituation on VEP amplitude by controlling for a potentiation effect (Cooke & Bear, 2010; Frenkel, et. al., 2006; Guo, et. al., 2017).

Figure 2. Three Experimental Group Timelines Were Used

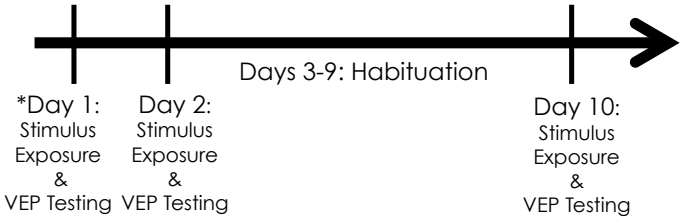
The three experimental group timelines are shown. A) The Group (E Only) timeline is pictured, in which animals underwent no habituation and went straight into stimulus exposure and VEP testing from days 1-33. B) The Habituation and Stimulus Exposure Group (H+E) timeline is pictured, in which animals underwent a habituation period from days 1-10 and then received stimulus exposure and VEP testing from days 11-20. C) The Habituation Group (H Only) timeline in which the animals received stimulus exposure and VEP testing on days 1 and 2 to get a baseline VEP Amplitude, then had a habituation period from days 3-9, and then received stimulus exposure and VEP testing again on day 10.

A **STIMULUS EXPOSURE GROUP (E Only)**

B **HABITUATION AND STIMULUS EXPOSURE GROUP (H+E)**



C **HABITUATION GROUP (H Only)**



*Day 1 = 48h post-op

Results

Our findings indicate that restraint habituation is necessary to produce a reliable VEP signal and that an increase in VEP amplitude over time is dependent on visual experience. Figure 3 shows the VEP amplitudes over time for E Only, including data for an individual animal and averages for the whole group. The VEP amplitudes for E Only were highly variable and inconsistent. Variability is seen at both the individual (Fig. 3A) and group (Fig. 3B) levels. Figure 4 displays a qualitative comparison of VEPs from day 1 to day 10 for an individual animal from H + E. The signal improves and amplitude increases over the 10 days of daily stimulus exposure and VEP testing. Figure 5 shows the VEP amplitudes over time for the individual (Fig. 5A) as well as averages for all animals in H + E (Fig. 5B). The trend is positive across all spatial frequencies and there is an evident SRP effect, as expected. SRP is observed as an increase in VEP amplitude as a result of repeated stimulus exposure, or visual experience, and is an example of perceptual learning (Cooke & Bear, 2010; Cooke & Bear, 2014; Cooke, et. al., 2015; Fischer, et. al. 2007; Frenkel et.al, 2006; Hosang, et. al., 2017; Prusky, et. al., 2000b). This group received habituation only for 10 days prior to stimulus exposure and VEP testing, as opposed to E Only that did not receive any habituation. Figure 6 shows VEP amplitudes over time for H Only. The VEP amplitude increased over time and the trend was positive across all spatial frequencies. This group received stimulus exposure and VEP testing for two days, then received only habituation from days 3-9, and received stimulus exposure and VEP testing again on day 10. At each spatial frequency, there is a significant and observable increase in amplitude following the habituation period.

Figure 7 compares the changes in VEP amplitude from day to day for E Only (Fig. 7A) and H + E (Fig. 7B) at 0.05 cpd. The data clearly show that the change in amplitude from day to day for E Only was highly variable and the change for H + E, which received habituation, was much more consistent and reliable. The magnitude of average amplitude change (Fig. 7C) was greater for E Only when compared with that of H + E, showing that E Only is more variable than H + E.

Figure 8 shows the average changes in VEP amplitude from day 1 to day 10 of stimulus exposure for H + E and H Only. The change in amplitude was greater for H + E, which received consistent stimulus exposure, than for H Only, which received no stimulus exposure and habituation only from days 3-9. This shows that stimulus exposure, or visual experience, has a greater effect on the increase in VEP amplitude than habituation alone. But, this also raises questions concerning the effects of habituation versus SRP effect: is the increase in amplitude due to habituation or to SRP? Would we expect to see a SRP effect after not being exposed to the stimulus for 7 days?

Figure 3. VEP Amplitude Over Time for E Only is Highly Variable

VEP amplitudes for E Only, that received no habituation, was highly variable. A) VEP Amplitudes over time for an individual animal in E Only at 0.05, 0.15, 0.3, and 0.45 cpd are shown. B) Average VEP Amplitudes over time for all animals (n = 4) in E Only at 0.05, 0.15, 0.3, and 0.45 cpd are shown. Amplitudes were highly variable at both the individual and group level.

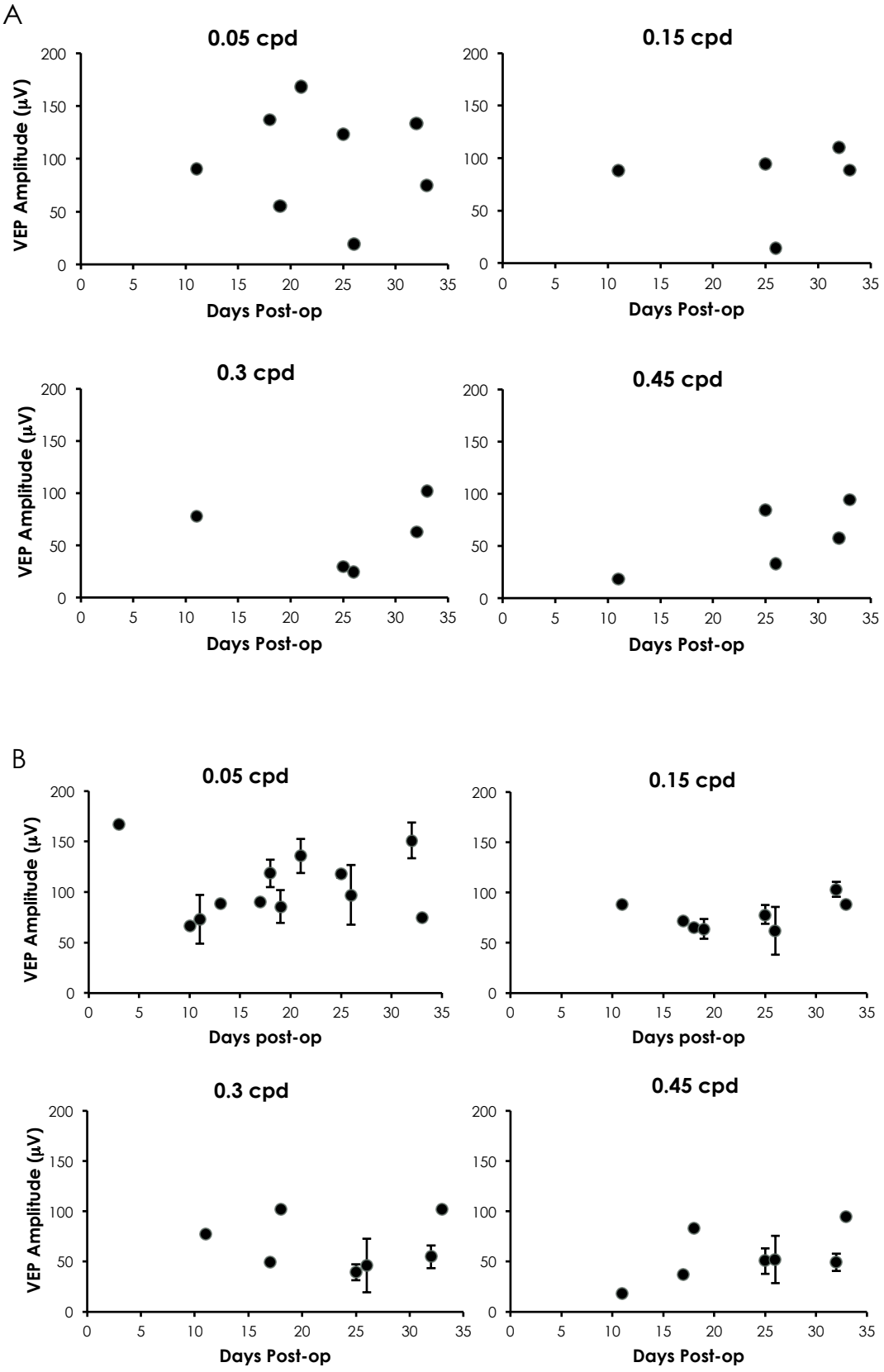
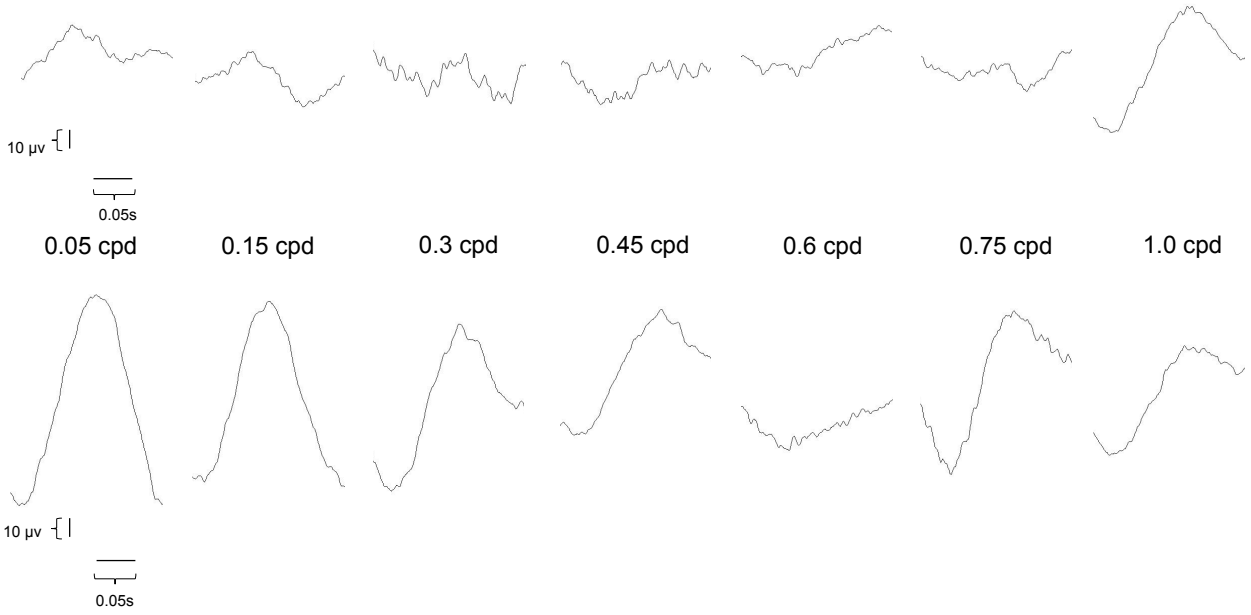

A
0.05 cpd
0.15 cpd
0.3 cpd
0.45 cpd
VEP Amplitude (µV)
Days Post-op
B
0.05 cpd
0.15 cpd
0.3 cpd
0.45 cpd
VEP Amplitude (µV)
Days post-op

Figure 4. Qualitative Comparison of VEPs after 10 Exposure Days Shows Improved VEP Signal

After being exposed to the visual stimuli for 10 days, the quality and amplitude of the VEP signal improved significantly. A qualitative comparison of VEPs from day 1 versus day 10 of stimulus exposure for an individual animal in H + E is shown. The VEPs shown are a depiction of a 1.0 second average, at the reversal of each grating cycle, from each corresponding recording session. There is a significant increase in amplitude and the noise level is reduced on day 10 when compared to day 1.

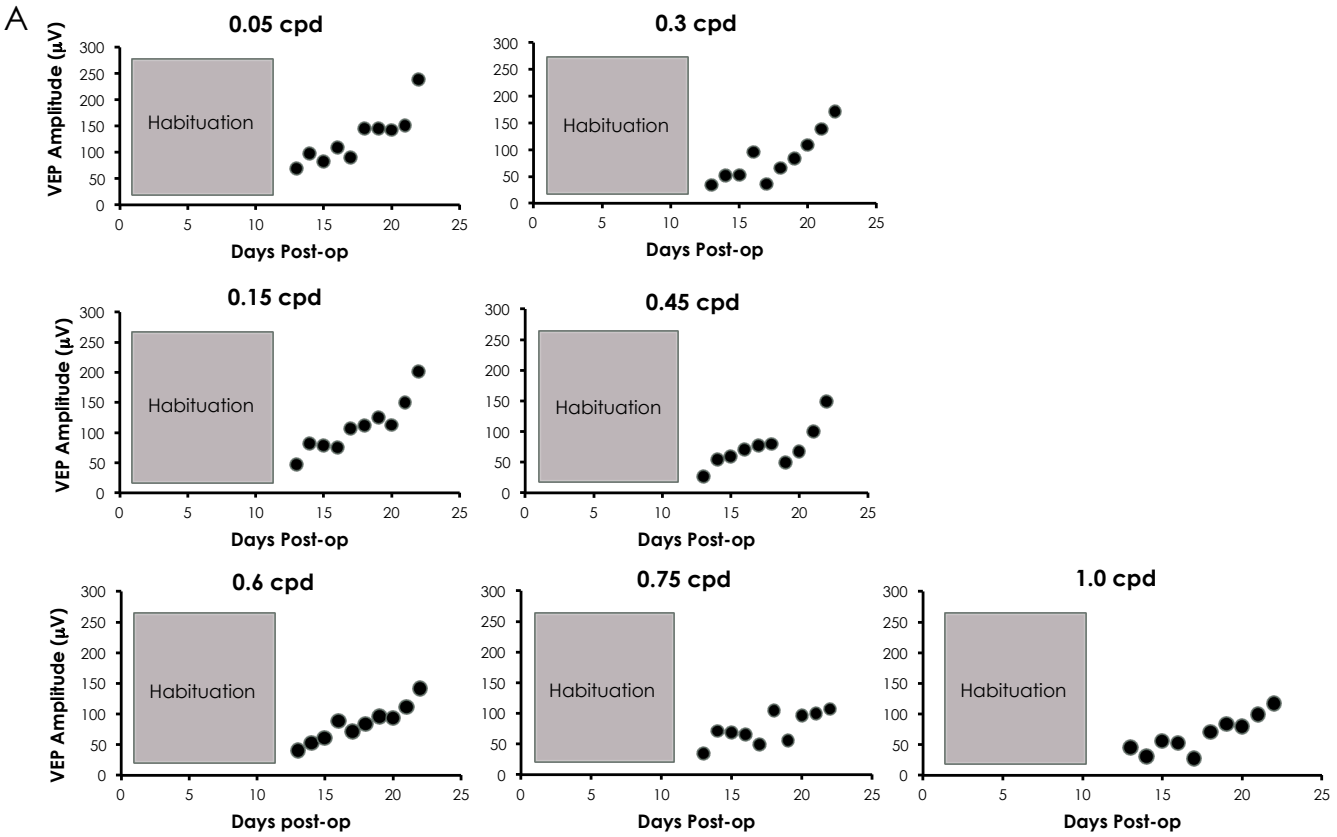
Day 1


10 µv
0.05s
0.05 cpd
0.15 cpd
0.3 cpd
0.45 cpd
0.6 cpd
0.75 cpd
1.0 cpd
10 µv
0.05s

Day 10

Figure 5. VEP Amplitude Over Time for H + E Has a Positive Trend

VEP amplitudes over time for H + E have a positive trend, showing that habituation helps to produce reliable VEPs. A) VEP amplitudes over time are shown for an individual animal from H + E at 0.05, 0.15, 0.3, 0.45, 0.6, 0.75, and 1.0 cpd. Amplitudes were taken from an average 1.0s window, at the reversal of each grating cycle, from the corresponding spatial frequency. B) Average VEP amplitudes over time are shown for all animals (n = 4) from H + E at 0.05, 0.15, 0.3, 0.45, 0.6, 0.75, and 1.0 cpd. There is a positive trend seen in both the individual and group data.


A
0.05 cpd
0.3 cpd
0.15 cpd
0.45 cpd
0.6 cpd
0.75 cpd
1.0 cpd
Habituation
VEP Amplitude (µV)
Days Post-op
Days post-op

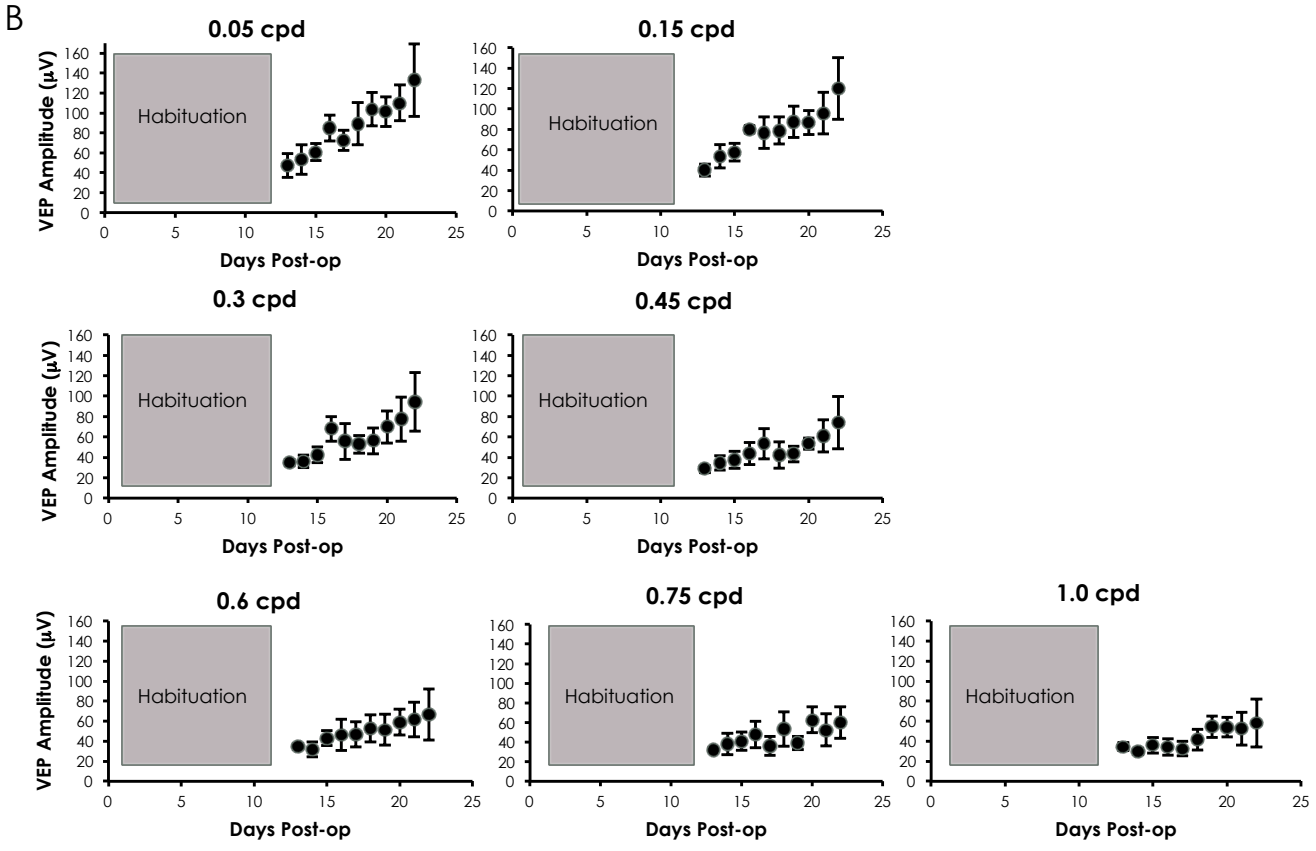
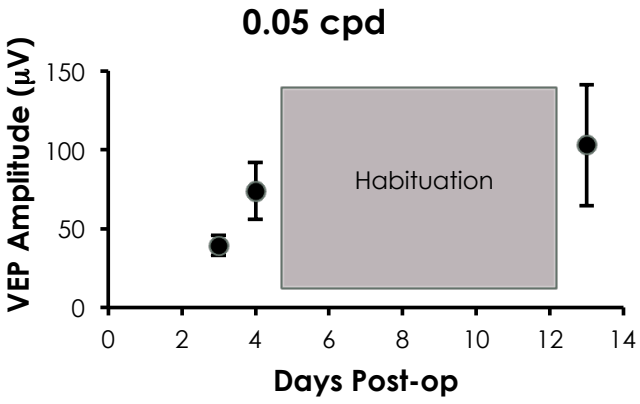
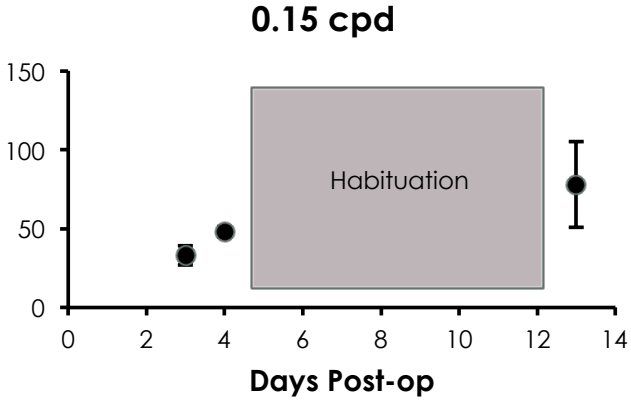
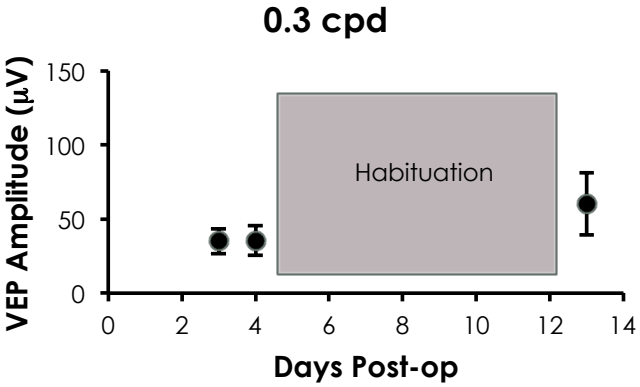

B
0.05 cpd
0.15 cpd
0.3 cpd
0.45 cpd
0.6 cpd
0.75 cpd
1.0 cpd
Habituation
VEP Amplitude (µV)
Days Post-op

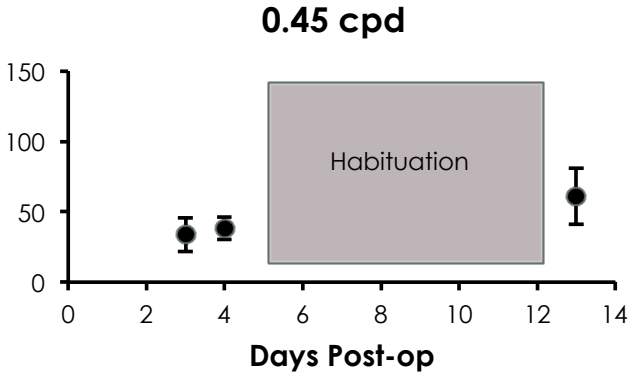
Figure 6. VEP Amplitude Over Time for H Only Shows an Amplitude Increase After Habituation

VEP amplitude over time for H Only shows that after a habituation period there is an increase in amplitude. Average amplitudes over time are shown for all animals (n=3) in H Only at 0.05, 0.15, 0.3, 0.45, 0.6, 0.75, and 1.0 cpd.


0.05 cpd
VEP Amplitude (μV)
150
100
50
0
Habituation
0 2 4 6 8 10 12 14
Days Post-op


0.15 cpd
150
100
50
0
Habituation
0 2 4 6 8 10 12 14
Days Post-op


0.3 cpd
VEP Amplitude (μV)
150
100
50
0
Habituation
0 2 4 6 8 10 12 14
Days Post-op


0.45 cpd
150
100
50
0
Habituation
0 2 4 6 8 10 12 14
Days Post-op

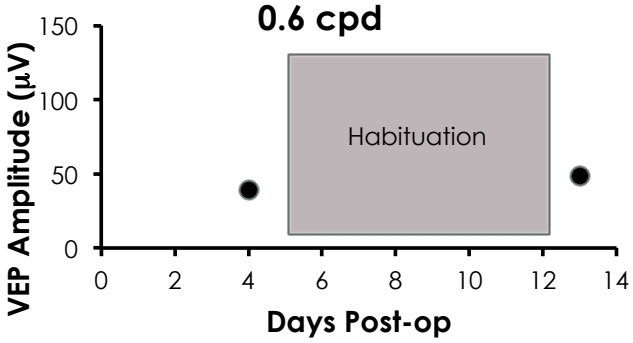
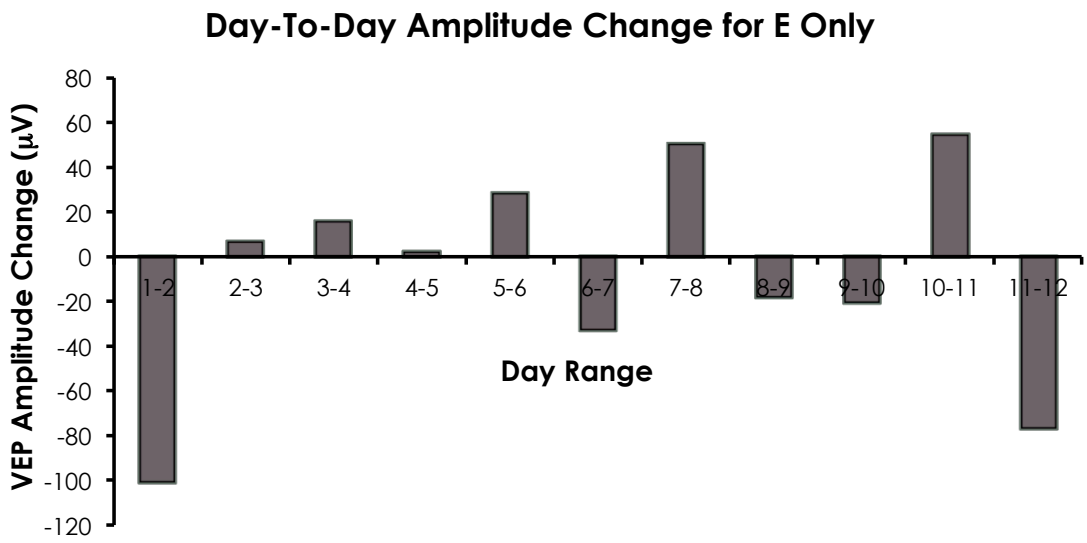

0.6 cpd
VEP Amplitude (μV)
150
100
50
0
Habituation
0 2 4 6 8 10 12 14
Days Post-op

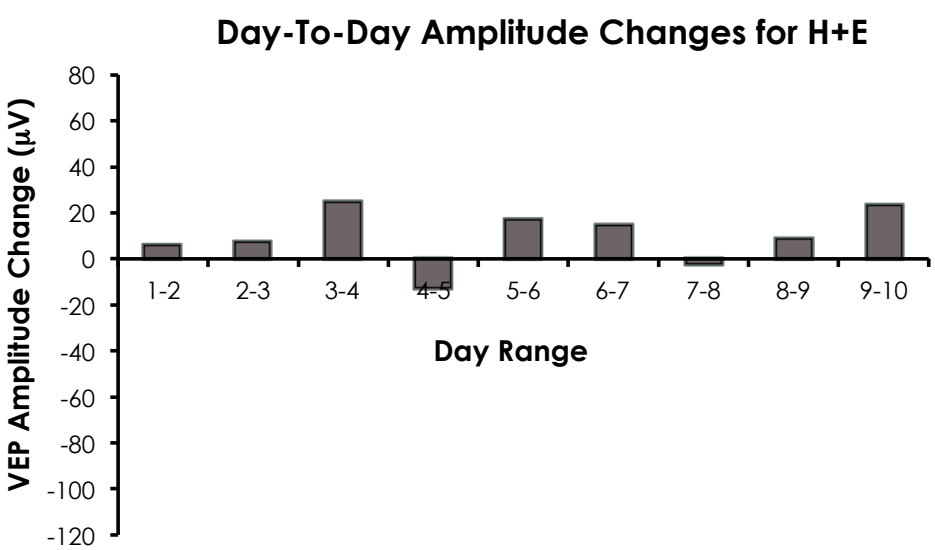
Figure 7. Comparison of Amplitude Change Between E Only and H+E Shows E Only is More Variable

The magnitude of average amplitude change for E Only is much greater than that of H+E, which indicates that VEPs for E Only are more variable. A) Average VEP amplitude change from day to day for E Only at 0.05 cpd is shown. B) Average VEP amplitude change from day to day for H+E at 0.05 cpd. C) The magnitude of average VEP amplitude change over all exposure days for E Only versus H+E is shown at 0.05 cpd.

A


Day-To-Day Amplitude Change for E Only
VEP Amplitude Change (μV)
80
60
40
20
0
-20
-40
-60
-80
-100
-120
1-2
2-3
3-4
4-5
5-6
6-7
7-8
8-9
9-10
10-11
11-12
Day Range

B


Day-To-Day Amplitude Changes for H+E
VEP Amplitude Change (μV)
80
60
40
20
0
-20
-40
-60
-80
-100
-120
1-2
2-3
3-4
4-5
5-6
6-7
7-8
8-9
9-10
Day Range

C

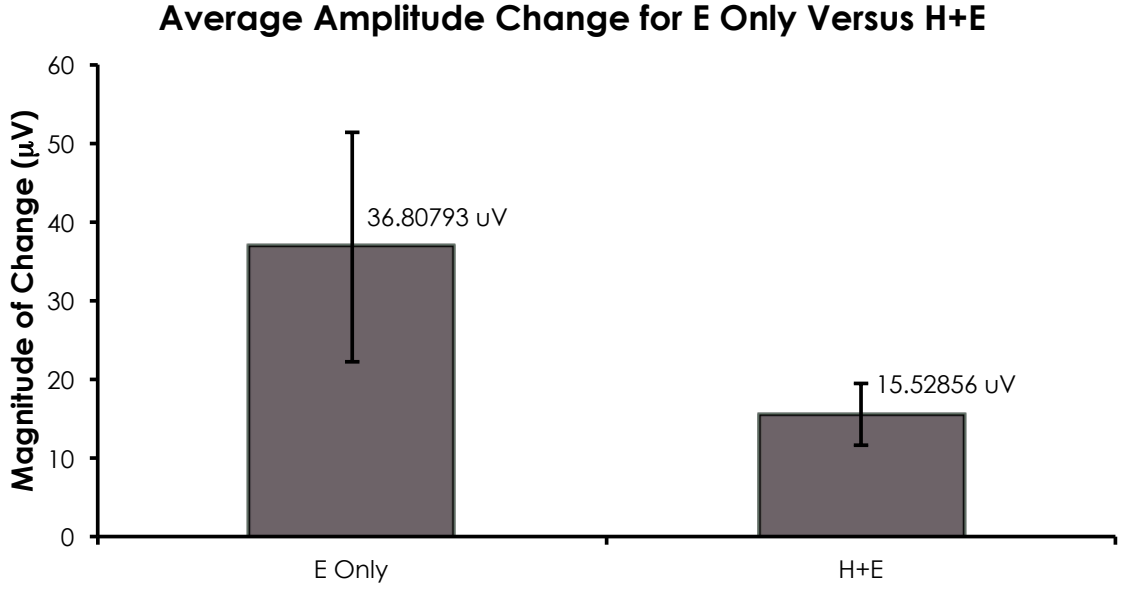
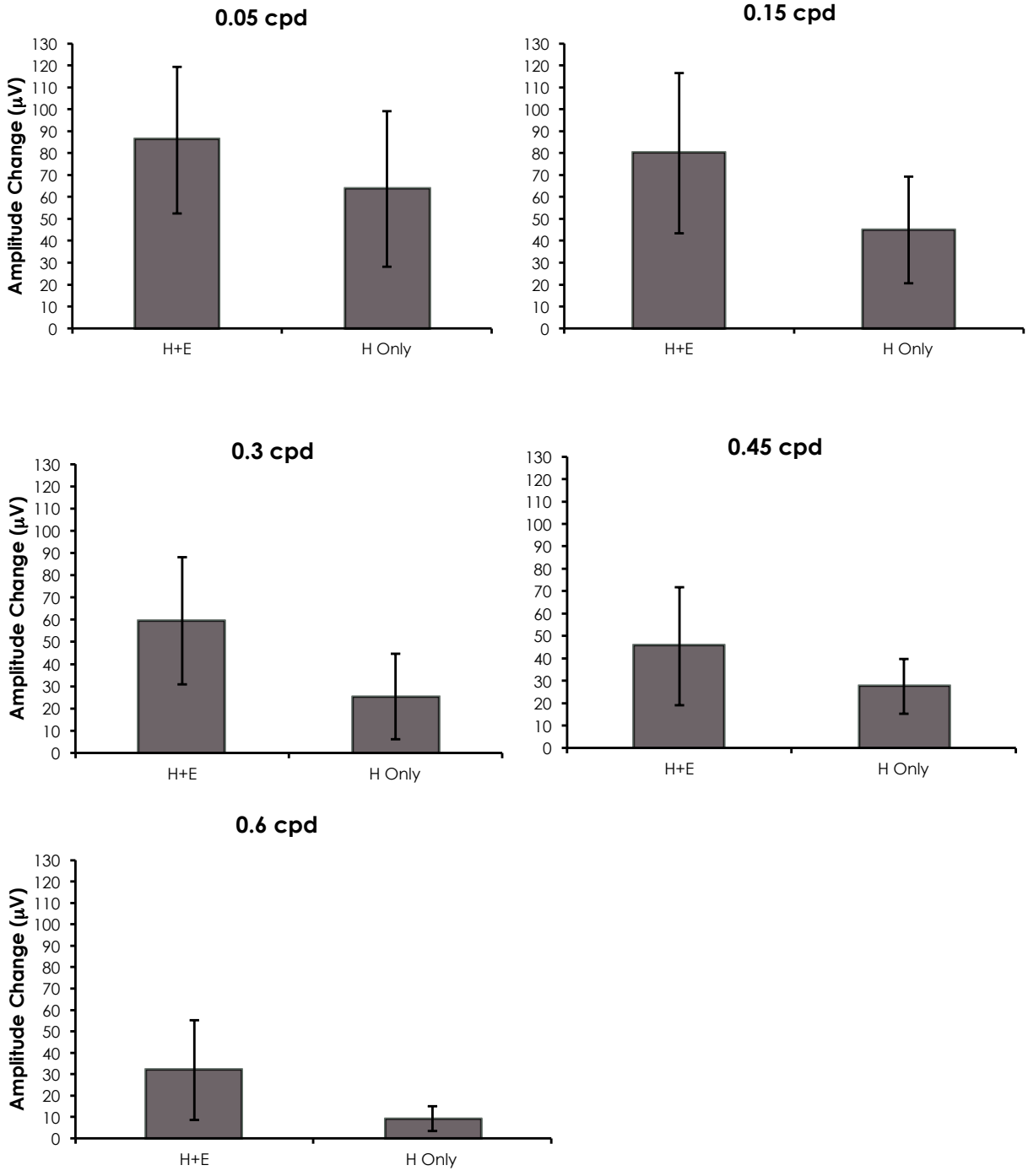

Average Amplitude Change for E Only Versus H+E
Magnitude of Change (μV)
60
50
40
30
20
10
0
36.80793 uV
15.52856 uV
E Only
H+E

Figure 8. Comparison of Amplitude Change Between H + E and H Only Shows Amplitude Increase is Experience-Dependent

VEP amplitude increase is dependent on visual experience, or daily stimulus exposure. The magnitude of average VEP amplitude change from day 1 to day 10 is shown for all animals in H + E (n=4) and all animals in H Only (n=3) at 0.05, 0.15, 0.3, 0.45, and 0.6 cpd. The average amplitude change is larger for H + E, that received regular stimulus exposure, when compared to H Only that did not receive regular stimulus exposure.


0.05 cpd
0.15 cpd
0.3 cpd
0.45 cpd
0.6 cpd
Amplitude Change (µV)
130
120
110
100
90
80
70
60
50
40
30
20
10
0
H+E
H Only

Discussion

Habituation is Necessary to Produce a Reliable VEP Signal

We found that habituation is necessary to produce a reliable VEP signal. The animals in E Only, that did not receive habituation, produced highly variable VEPs, while the animals in H + E, that received a habituation period of 10 days, produced more reliable VEPs. Just as in H + E, we would expect to see a SRP effect in E Only as well considering the animals are receiving regular stimulus exposure. However, there is no observable SRP effect for E Only. Why is this? Does habituation play a role in SRP?

Amplitude Increase is Experience-Dependent

We also showed that VEP amplitude increase is experience-dependent as the amplitude increase for those animals in H + E, that received stimulus exposure, was greater than the animals in H Only, that were habituated and did not receive daily exposure. Habituation alone may play a small role in VEP amplitude increase since there was a slight upward trend after the habituation period for H Only. However, it is clear that visual experience plays a crucial role in the increase of VEP amplitude as expected based on previously published literature (Cooke & Bear, 2010; Cooke & Bear, 2014; Fischer, Aleem, Zhou, & Pham, 2007; Frenkel, et. al., 2006; Guo, et. al, 2017; Prusky, et. al., 2000b)

The Role of Stimulus-Selective Response Potentiation (SRP)

SRP is a type of experience-dependent plasticity in which VEP amplitude increase is positively correlated with the number of exposures to the visual stimulus (Cooke & Bear, 2010; Cooke & Bear, 2014; Cooke, Komorowski, Kaplan, Gavornik, & Bear, 2015; Frenkel et. al., 2006; Hosang, Yusifov, & Löwel, 2017). The SRP effect is

clearly demonstrated in the H + E group as the amplitude increases over time. The H only group also experienced an increase in amplitude, although the increase in amplitude in the H + E group over 10 days was much larger than amplitude increase for the H only group, demonstrating that experience plays a larger role in amplitude increase than habituation alone. Plasticity in general has been shown to be highly experience-dependent within the visual system (Cooke & Bear, 2010; Cooke & Bear, 2014; Cooke, et. al., 2015; Fischer, et. al., 2007; Frenkel, et. al., 2006; Guo, et. al., 2017; Hosang et. al., 2017; Kalogeraki, Pielecka-Fortuna, Hüppe, & Löwel, 2016; Prusky, et. al., 2000b; Young, et. al., 2018). However, it is unclear whether the increase in the H Only amplitudes could be due to SRP. Would we expect to see an SRP effect after only two days of stimulus exposure followed by 7 days of habituation? The effects of habituation alone, while controlling for SRP needs to be further studied.

Other Factors Influencing the VEP Signal

Electrodes

We have found that habituation is necessary to produce a reliable VEP signal, however there are several other factors that may affect the signal that we did not investigate. Placement and diameter of recording and reference electrodes may have an effect on the VEP signal. For example, we placed 0.0005-inch diameter electrodes at a depth of 450 um, corresponding to layer four of the visual cortex. Placement, including depth, of the recording electrode was determined using a mouse brain atlas. Different sizes and placements of the recording electrodes will record from different populations of neurons and may result in better VEPs. Marenna, Castoldi, d'Isla, Marco, Comi, & Leocani conducted a study in 2019 investigating and comparing different semi-invasive

and non-invasive recording methods. They found that "amplitude from invasive screws was lower than the ones from semi- and non-invasive electrodes" (p. 6). Given that screws are considered the "gold standard" for VEP testing, this raises questions about the reliability of different electrode placements: Which is more accurate? Why are certain placements producing higher or lower amplitudes? Can we safely assume that the "gold standard" is most accurate?

The material of which the electrodes are made may also have an effect, as conductivity differs between materials. We used silver reference electrodes and platinum recording electrodes since this material is inert and highly conductive, however different materials may enhance or take away from the VEP signal. For example, stainless steel screws are commonly used to record VEPs and have been shown to produce a reliable VEP signal (Makoweiecki, Garrett, Clark, Graham, & Rodger, 2015). Santangelo et. al. conducted a study in 2018 examining the reliability of epidermal cup electrodes and found that they are comparable to epidural screws. However, a direct, widespread comparison between electrode types and nuances has not been examined, to our knowledge.

Presentation of Spatial Frequencies

Another factor influencing the VEP signal could be the sequential versus random presentation of spatial frequencies. We consistently presented the animals with spatial frequencies in ascending order. It is unknown whether sequential presentation has any differing effect on the signal than presenting spatial frequencies to the animal in a random order. For example, there may be experience-dependent effects on VEPs when visual stimuli are always presented the same way, similar to SRP.

Repeated Measures

The effects of repeated measures at the same spatial frequency within one day should be explored since it may be useful in testing the reliability of VEP signals within one day versus over several days. It is also unknown whether a 24-hour period is required to see VEP potentiation (Cooke & Bear, 2010; Cooke & Bear, 2014; Cooke, et. al,. Frenkel, et. al, 2006; Guo, et. al, 2007; Hosang, et. al., 2017) or if it can be induced within one day. Makowiecki et. al. showed that VEPs were reliable within the same session, but were not reliable when comparing sessions on day 1 to sessions on day 7. The reliability of VEPs between days and between sessions should be further investigated. It is also unknown whether a brief "warm-up" presentation period, during which each spatial frequency would be presented to the animal for a short window of time prior to VEP testing, would be beneficial to producing a reliable signal.

Recovery Time After Surgery

Recovery times after electrode implant surgery may play a role in the reliability of VEP signals. The electrode implant surgery is invasive, causing inflammation and possible bleeding within the brain. Allowing for adequate recovery time following surgery, during which the brain has time to properly heal, may play a significant role. Campbell and Wu (2018) describe the tissue reaction and electrical changes that take place upon electrode implantation within the brain. They describe that the electrode implant triggers the "foreign body reaction and sustained inflammation" (2018, p. 6). These bodily responses and their ongoing interaction with the electrode implant may interfere with the neural signal during the post-operative period. When the electrode is inserted, it ruptures, severs, and pulls capillaries and arteries which leads to "bleeding,

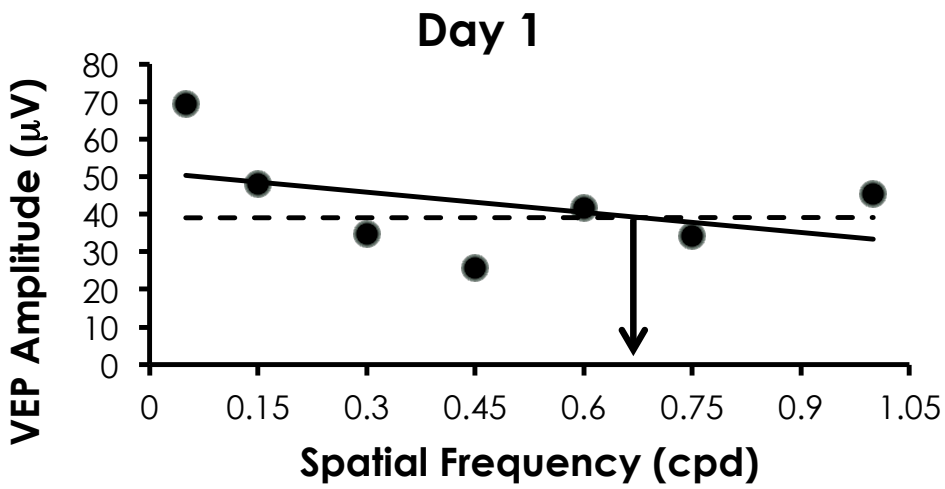
serum protein leakage, and infiltration of neutrophils, blood-bone macrophages, and T-lymphocytes." The implant also tears the extracellular matrix, "ruptures neuronal and glial cell bodies, and causes tissue displacement" (Campbell & Wu, 2018, p. 11). This tissue damage may interfere with the reliability of the VEP signal.

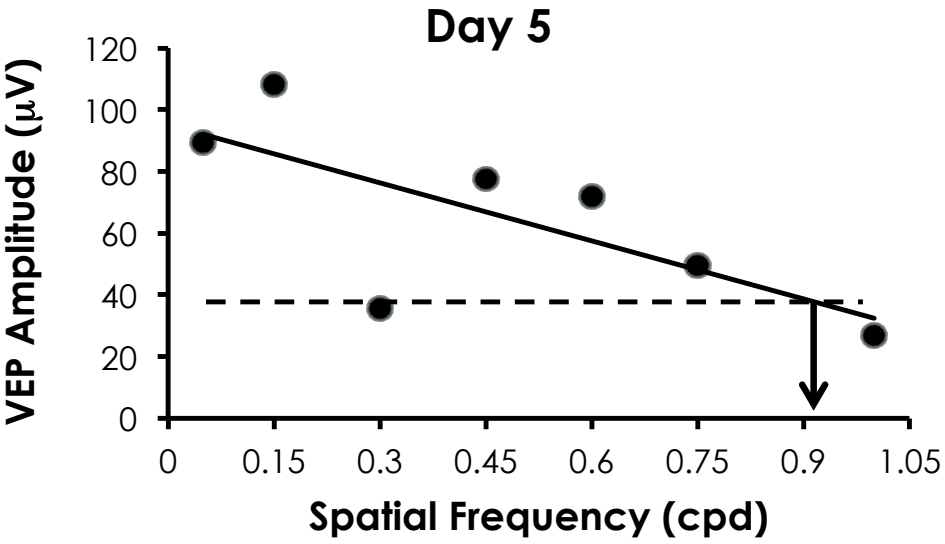
Visual Acuity

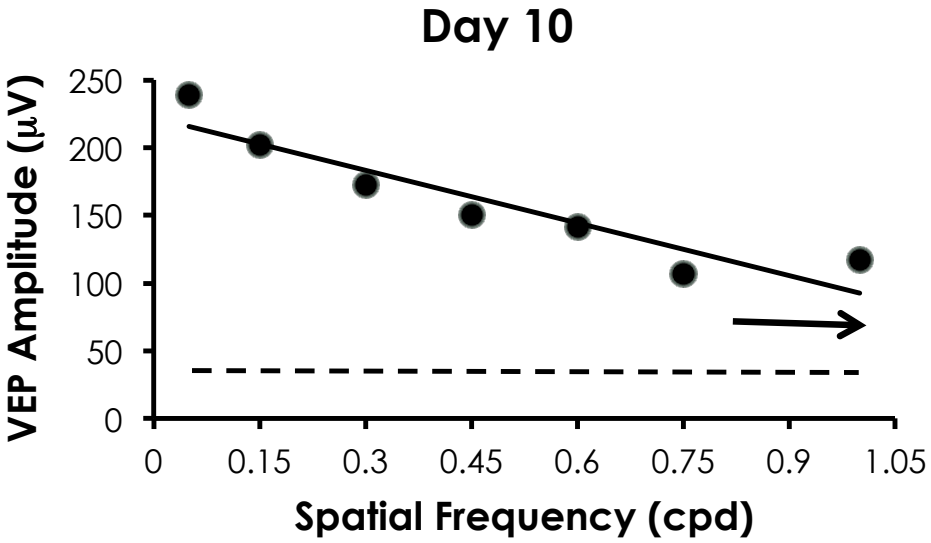
VEPs are used to quantify ocular dominance, stimulus-selective plasticity, and visual acuity in mice (Cooke & Bear, 2010; Cooke & Bear, 2014; Cooke, et. al, 2015; Fischer, et. al., 2007; Frenkel, et. al., 2006; Hosang, et. al., 2017; Porciatti, et. al. 1998; Prusky, et. al., 2000b; Ridder III, et. al., 2006; Tokashiki, et. al., 2018; Young, et. al., 2018). For example, in ocular dominance studies, researchers have used VEPs to investigate experience-dependent plasticity of neural circuits (Frenkel, et. al, 2006; Hosang, et. al., 2017; Prusky, et. al., 2000b). Importantly, visual acuity can be found by comparing the VEP amplitude, evoked by a visual stimulus, to the inherent noise level in the brain. A visual acuity threshold corresponding to the animal's visual acuity can then be determined (Cooke & Bear, 2010; Heimel, et. al., 2007; Porciatti, et. al., 1998; Prusky, et. al., 2000a; Prusky, et. al., 2000b; Tokashiki, et. al., 2018). Figure 9 shows visual acuity measures for an individual animal from H + E on days 1, 5, and 10 of VEP testing. This is to show that our testing apparatus can carry out the function for which it is designed. There is also an observable SRP effect as the VEP amplitudes continue to increase at each spatial frequency over time and number of exposures. Disentangling SRP from visual acuity measures should be further explored.

Figure 9. Visual Acuity Measures Are Subject to SRP

The amplitude at each spatial frequency as well as the visual acuity threshold is seen to increase over time. VEP amplitude is plotted over spatial frequency for an individual animal from H + E on days 1, 5, and 10 of VEP testing. The dashed line represents the average noise amplitude for that animal. The highest spatial frequency where the VEP amplitude evoked is higher than the average noise amplitude is the visual acuity threshold. On the graph, where the trend line for the VEP amplitudes intersects the noise line (where the arrows are pointing) is deemed the animal's visual acuity.


Day 1
VEP Amplitude (μV)
80
70
60
50
40
30
20
10
0
0
0.15
0.3
0.45
0.6
0.75
0.9
1.05
Spatial Frequency (cpd)


Day 5
VEP Amplitude (μV)
120
100
80
60
40
20
0
0
0.15
0.3
0.45
0.6
0.75
0.9
1.05
Spatial Frequency (cpd)


Day 10
VEP Amplitude (μV)
250
200
150
100
50
0
0
0.15
0.3
0.45
0.6
0.75
0.9
1.05
Spatial Frequency (cpd)

Sources of Error

Possible sources of error within this study include small sample sizes as each experimental group consisted of 3 or 4 animals. We did not do a post-mortem analysis of the brain or electrode. This means we did not check the electrode implant location to ensure that each electrode was recording from the correct layer of V1. We also did not assess the condition of the electrode meaning there was no way to tell if an electrode had degraded or worn out other than comparing incoming data to previous data within the same animal.

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