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Measuring Visual Acuity in Awake Mice Using Visually Evoked Potentials (VEPs)

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Measuring Visual Acuity in Awake Mice Using Visually Evoked Potentials (VEPs)

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Measuring Visual Acuity in Awake Mice Using Visually Evoked Potentials (VEPs)

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Abstract:

We investigated a means to measure visual acuity in awake mice using visually evoked potentials (VEPs). Using counter-phasing sinusoidal gratings as stimuli, we compared the effectiveness of vertical and horizontal orientations in generating VEPs. Using stereotaxic implanted electrodes in the animal's primary visual cortex, the evoked VEPs were recorded and analyzed. At the lowest spatial frequency, vertical stimuli evoked the largest VEP amplitude. However, at higher spatial frequencies, a reversal occurs where horizontal gratings evoked larger VEPs. These data suggest vertical and horizontal stimuli have different effects on generating VEPs although further investigation is required to determine which stimulus is most suitable for measuring visual acuity in mice. Uncovering this relationship will also help us to understand the link between physiological activity of the brain and behavioral function.

Introduction:

The visual system creates a spatial representation of the surrounding environment and thus, the visual system is integral for the survival of the animal. One way to assess the function of the visual system is by measuring visual acuity. Visual acuity is defined as the as the ability to recognize and resolve high contrast visual stimuli (King et al., 2015). Measuring visual acuity can be a direct form in assessing the health of the animal's visual system (Kang et al., 2013). Therefore, if the health of the visual system wants to be evaluated, developing techniques in measuring visual acuity is essential.

Historically, visual acuity has been tested in multiple ways (Guire et al., 1999; Prusky et al., 2000; Ringach et al., 2016). One way to evaluate visual acuity is through electrophysiological recordings. The physiological recording technique gives a direct measure of neuronal activity through chronically implanted electrodes. These low impendence electrodes are sensitive to neural population activity and represent regional activity in the visual cortex. The visual acuity of the animal is dependent on the function of the primary visual cortex. (Baroncelli et al., 2011). By recording visual cortex population responses, we can estimate the visual acuity of the animal. This thesis investigated a means to measure visual acuity in awake mice using visually evoked potentials (VEPs) and compared the efficacy of different orientations of sinusoidal gratings in evoking VEPs.

We evaluated visual acuity by measuring Visually Evoked Potentials (VEPs) in the primary visual cortex of mice. Visually Evoked Potentials (VEPs) are a sum neural potential evoked by a visual stimulus (Ridder and Nusinowitz, 2006). Generated in the primary visual cortex, VEP's can be used to estimate the animal's visual acuity (Odom et

al., 2016). Through chronically implanted electrode embedded in the primary visual cortex, the VEP amplitude reflects the strength of the visual stimuli. For example, a broad salient stimulus evokes a large VEP amplitude. By incrementally increasing the spatial frequency of the stimulus, a threshold response can be reached, which is used to estimate visual acuity (Ridder and Nusinowitz, 2006).

Most typically, counter-phasing sinusoidal gratings are used in evoking VEPs. Traditionally, the stimuli is delivered in vertical orientation. Since previous studies have shown that primary visual cortex neurons respond best to that orientation (Tobimatsu et al., 1993; Venkataraman et al., 2016). It is speculated that the neural pathways traveling through the retina into the lateral geniculate nucleus and into primary visual cortex is best tuned in to a specific stimulus (Vreysen et al., 2012.) Thus, counter-phasing sinusoidal wave gratings are adequate in stimulating a means of measuring visual acuity. The range of saliency between the differing black and white lines of the sinusoidal wave grating is known as spatial frequency. At a low spatial frequency, large VEP amplitudes are evoked from the animal as it is easily distinguishable (figure 1). As the spatial frequency is increased, the VEP amplitude decreases as it becomes more difficult to distinguish. When the spatial frequency incrementally increases to a point where a VEP is no longer evoked, it can be interpreted as the specific animal's visual acuity. Likewise, the data can be compared with correlative techniques such as behavioral acuity literature (Prusky et al., 2000) to identify if two separate measures of visual function give the same results. If a disparity exists between two separate measurements of visual function, it reveals that we do not fully understand the relationship between neural activity in the brain and behavioral function.

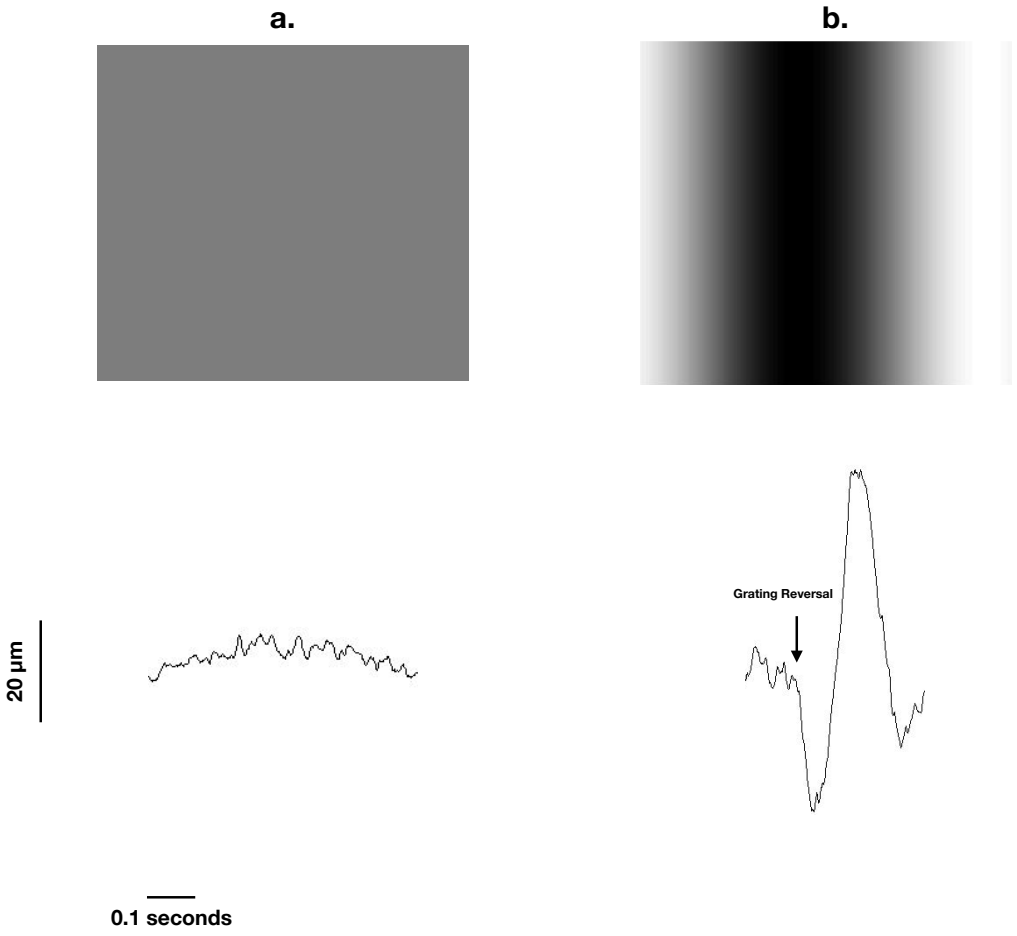

Figure 1: Averaged neural activity in response to gray screen + gratings.
Figure a. Gray screen alone does not evoke VEPs.
Figure b. Low spatial frequencies evoke a large VEP.

However, the question exists if vertical orientation the best way to evoke a VEP? If the spatial frequency is shifted to an horizontal orientation, will it evoke a comparable VEP? Previous research has been published evaluating the effectiveness of differing visual stimuli patterns. In general, contrasting stimuli are effective at in evoking VEPs, however, it is thought vertical sinusoidal gratings best evokes a VEP response (Venkataraman et al., 2016). There has however been no direct comparison between

orientations. Even though this gap exists in the literature, previous research has identified that the cells of primary visual cortex are tuned to best respond to a specific orientation and spatial frequency (Everson et al., 1998). Different populations of neurons have a receptive field best tuned to a specific orientation and spatial frequency independent from the other neuron populations (Everson et al., 1998). However, in general, a low spatial frequency with high contrast evokes the largest VEP amplitude in mice (King et al., 2015). Yet, to our knowledge, visual acuity in mice has not been tested using sinusoidal wave gratings at different orientations to evoke VEPs. This is the focus of our investigation (figure 2).

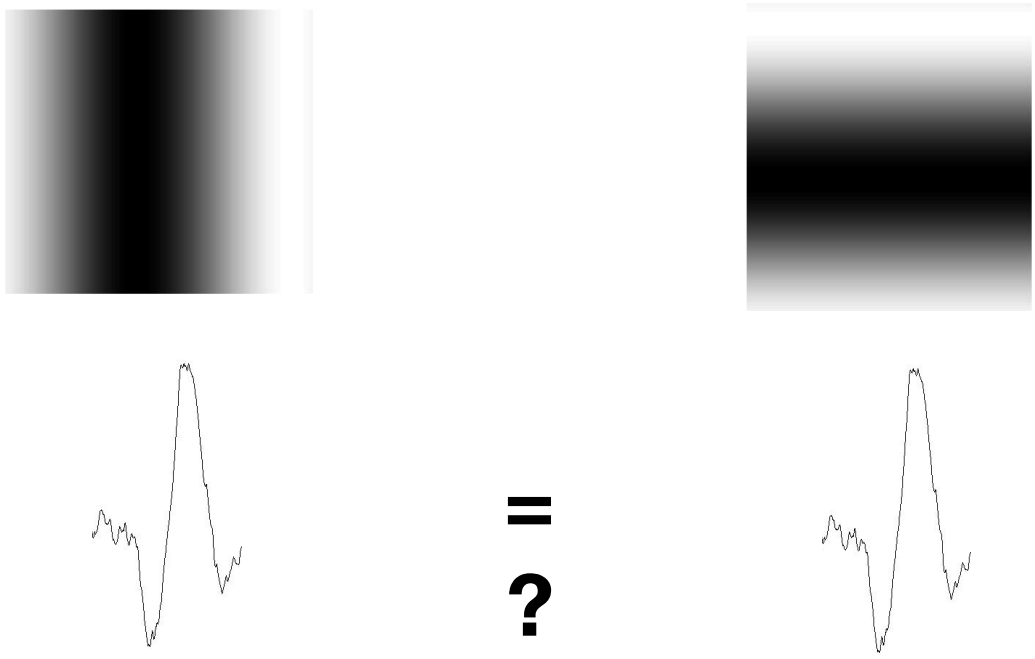

Figure 2:
Will VEPs evoked by a horizontal stimuli be equivalent to VEPs evoked by a vertical stimuli.

Materials and Methods:

Animals:

Mice handling was conducted in accordance with Concordia University's Institutional Animal Care and Use Committee (IACUC) guidelines. Three week old males C57BL6 mice from The Jackson Laboratory were used and housed in standard housing conditions. Mice were housed in groups of three in an 12 hour light/dark cycle held at a 21°C, 40% humidity, and where food and water were available in abundance.

Anesthetic + Surgery:

Mice were anesthetized in a chamber through inhaled isoflurane (induction at 4.0-4.5%). Once anesthetized, the animal was transferred and mounted into a stereotaxic frame with its head fixed in place by two ear bars and was jaw fixed into a mouth bar and continuously breathing isoflurane (maintained at 2.0-2.5%). Eyes were coated with a layer of ophthalmic ointment to prevent drying and injury during surgery. A feedback controlled heat pad used to maintain body temperature at 37°C. The scalp hairs were trimmed using scissors and a fur trimmer and Lidocaine and prilocaine cream (2.5%/2.5%) was applied to the shaved scalp. Ethanol and providone-iodine (Betadine surgical scrub) was applied three times to sterilize the exposed scalp. A small incision was made horizontally on midline and then vertically posterior to anterior exposing the skull. Acetone was applied to clean and dry the skull. Minor holes (1 mm diameter) were drilled into the skull using a microdrill and a platinum electrode (0.005-inch diameter; impedance, ~0.4-MΩ) was implanted into layer 4 of primary visual cortex in one

hemisphere. (Atlas coordinates 0.0 mm lambda anteroposterior, 3.00 mm mediolateral, 0.45 mm dorsoventral from midline). A silver reference electrode was placed lateral in the frontal cortex. A head post was fixed to the skull at the anterior of the animals head. The electrodes and head post were adhered in place using cyanoacrylate glue.

Post-Surgery Care:

Following the surgery, the animal was giving a subcutaneous injection of Carpofin (0.1mL of 0.1M) to alleviate any discomfort. The animal was placed in a recovery environment by itself and supplied with ample amounts of food and water. Its behavior was monitored for 24 hours. Once recovered, the animal was transferred into their standard housing with case mates.

Visual Stimulus:

Visual Stimulus was projected using flat screen monitor (Dell, 17 inches, 144Hz refresh rate) and generated through a custom script in Matlab. The program delivered the orientated stimuli at various spatial frequency (cycles per degree c/d) and counter phased at every 1 second (1080 resolution, 45 screen height, 20 distance). Control stimulus was a gray screen. The screen was at a viewing distance of 20 centimeters from the mouse. The mouse was placed 10 cm above to establish a midline viewing of the screen. The mouse was held by positioning the head post into a holding platform and harnessed by placing the mouse in an open body plastic fitting to establish perpendicular viewing of stimulus. Trials lasted for a 300 seconds.

VEP Data Analysis:

The electrical signal traveling from the electrodes is sent to an amplifier (Warner Instruments, DP-311 Differential Amplifier). Once from there, the data is sent to an data acquisition unit (Cambridge electronics, Micro3, 1401) where it is processed and translated into the computer. The data acquired is processed by the software (Cambridge Electronic Design, Spike2, 8.12). and aligns the recorded neural activity to the triggers that correspond to grating reversals in order to generate an averaged waveform. The VEP amplitude is measured. If necessary, further processing can be done through the use of additional filters to remove extraneous noise.

Baseline neuronal activity is recorded using a control gray screen to evoke a standard neuronal reading. The baseline neuronal activity is used as a primary visual threshold against the experimental evoked VEPs. When an animal is stimulated to evoke a VEP, the recorded VEP wave contains both the evoked VEP wave and also the baseline neuronal activity. The baseline visual threshold is set to filter out the evoked VEP wave. Once the visual threshold is set, the certain VEP amplitude that is evoked and crosses below the visual threshold can be interpreted as the specific animal's visual acuity.

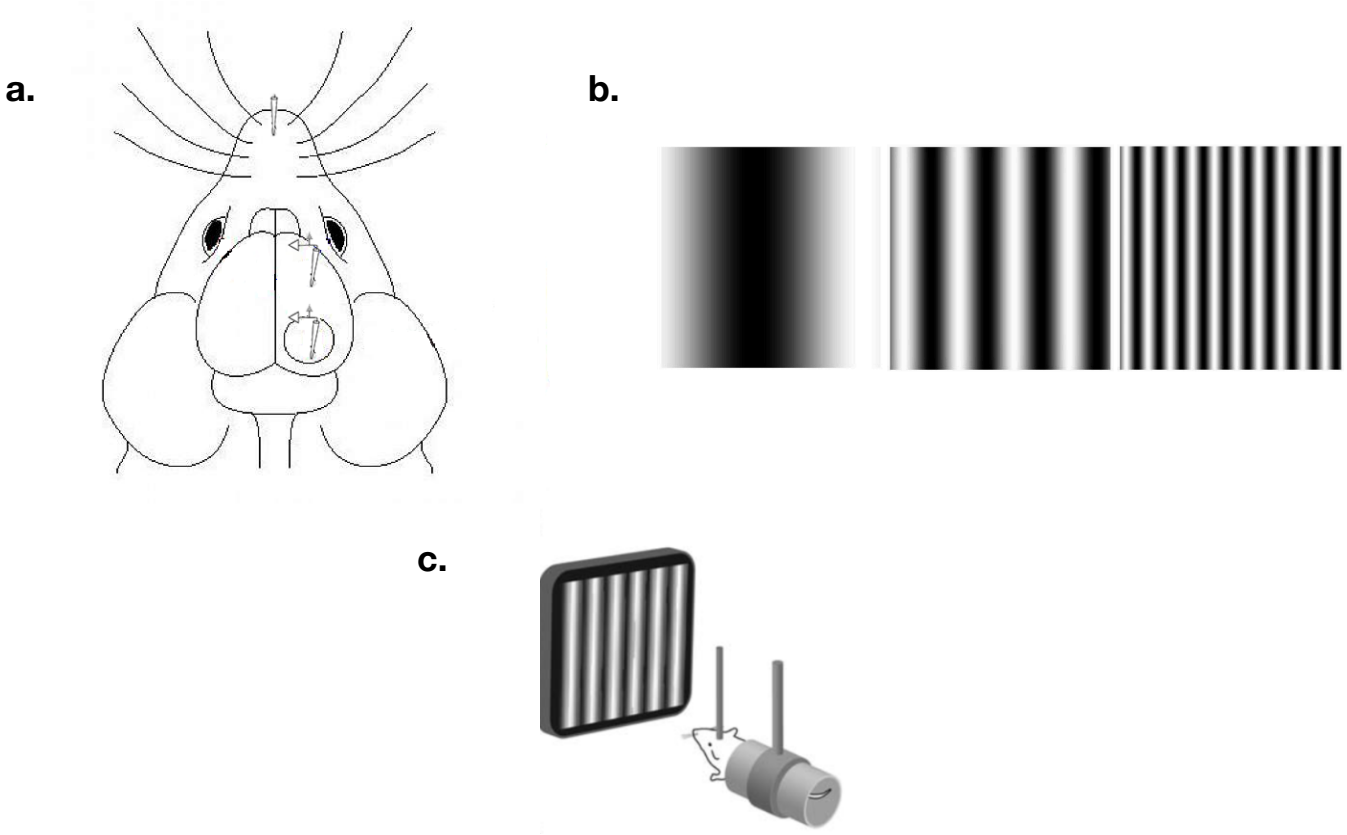

Figure 3: Experimental Setup

Figure a: Chronically implanted electrode in primary visual cortex, with reference electrode at anterior coordinates (see methods).

Figure b: The progression from the low spatial frequency (left panel) to mid (middle panel) to high spatial frequency (right panel) become more difficult to discern.

Figure c: Overview illustration of visual stimulus apparatus. Head-fixed mouse viewing VEP generating stimulus.

Results:

	Reggie	Barry	Thelonius	Charlie	Astro	Tim	Sam	Averages (μV)	± Standard Error
				Vertical					
0.05	133.374	111.151	23.4545	172.326	27.1089	39.465	96.9445	86.2605	21.82
0.15	110.438	80.2873	21.4853	131.429	51.835	42.5736	59.8602	71.1297	14.67
0.3	62.8657	38.6456	8.72209	100.666	28.5104	29.4807	51.0197	45.7014	11.25
0.45	57.753	25.7971	10.6209	99.0938	30.0379	30.9876	53.2603	43.9358	11.04
0.6	96.9302	19.4401	19.1113	63.8324	29.2866	35.4751	45.8578	44.2762	10.59
0.75	85.1727	29.3158	27.921	70.7915	20.0371	33.1484	41.105	43.9273	9.24
1	51.5558	40.4087	34.1991	39.6913	21.626	14.8134	52.4421	36.3909	5.35
				Horizontal					
0.05	74.544	87.9961	37.5207	112.945	36.3205	55.5542	41.1395	63.7171	11.07
0.15	88.4974	94.9163	24.2446	74.9421	29.5597	60.4294	34.3986	58.1411	11.01
0.3	101.977	52.3243	15.0929	78.7277	25.6844	49.0664	33.0968	50.8527	11.57
0.45	94.2496	44.866	13.564	87.7533	28.0638	69.7919	53.9442	56.0332	11.31
0.6	104.861	33.447	21.0066	58.632	29.0127	71.2361	43.402	51.6567	11.04
0.75	117.78	38.1265	26.5401	26.5518	19.6193	55.9516	73.7617	51.1901	13.22
1	90.9009	30.3604	32.6753	40.4098	27.7232	46.6332	76.3674	49.2957	9.32
Gray	14.9012	18.2892	18.8736	27.0935	13.6227	12.8098	20.4839	18.0105	1.86

Figure 4: Data Table displaying the VEP averages of every mouse (n=7) and the total collective averages at every spatial frequency and differing orientations

The data reveals the basic trends of high VEP amplitudes evoked from lower spatial frequencies. When graphed, it is clearly seen that lower spatial frequencies evoke the highest VEP amplitude while higher spatial frequencies evoke a lower VEP amplitude.

Variable	Observations	Obs. with missing data	Obs. without missing data	Minimum	Maximum	Mean	Std. deviation
Vertical	7	0	7	36.391	86.261	53.089	18.278
Horizontal	7	0	7	49.296	63.717	54.412	5.163

t-test for two independent samples / Two-tailed test: 95% confidence interval on the difference between the means:	[-18.323 ;	15.676 [

Difference	-1.324
t (Observed value)	-0.184
|t| (Critical value)	2.368
DF	7
p-value (Two-tailed)	0.859
alpha	0.050

Figure 5: Data Table running statistical analysis between averages of vertical and horizontal VEPs.

From a statistical analysis, the independent t-test on the two averages presents that the null hypothesis cannot be rejected. The averages for vertical and horizontal VEPs generated a p-value higher than 0.05 resulting in us not being able to reject the null hypothesis. While this can imply that the data is random, it also reflects that further investigation is needed. A greater sample size and more VEP recordings is needed in order to produce a better statistical mean.

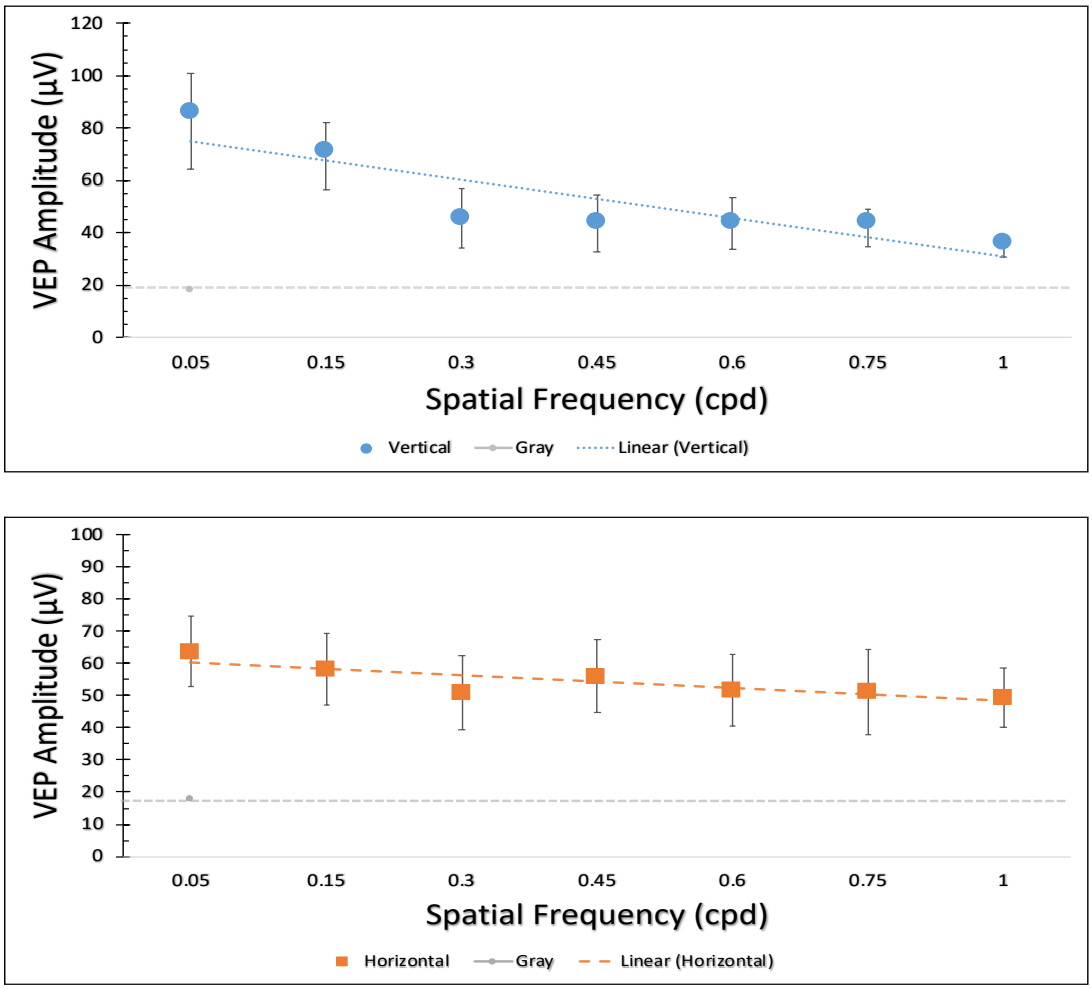

Figure 6: Average VEP Amplitudes at ranging spatial frequencies (n=7)**. TOP**: Plot of average VEP amplitudes generated from vertical orientation stimuli**. BOTTOM:** Plot of average VEP amplitudes generated from horizontal orientation stimuli

At all spatial frequencies, a VEP was evoked. As seen, the trend can be observed that increasing spatial frequencies evoke lower VEP amplitudes. For vertical orientations at a low spatial frequency (0.05 cpd) the averaged VEP amplitude is 86.3 µV (±21.82). At a high spatial frequency (0.75 cpd) the averaged VEP Amplitude was 43.9 µV (±9.24). For horizontal orientations, at a low spatial frequency (0.05 cpd), the averaged

VEP amplitude wave was 63.7 µV (±11.07). At a high spatial frequency (0.75 cpd) the averaged VEP amplitude of 51.2 µV (±13.22).

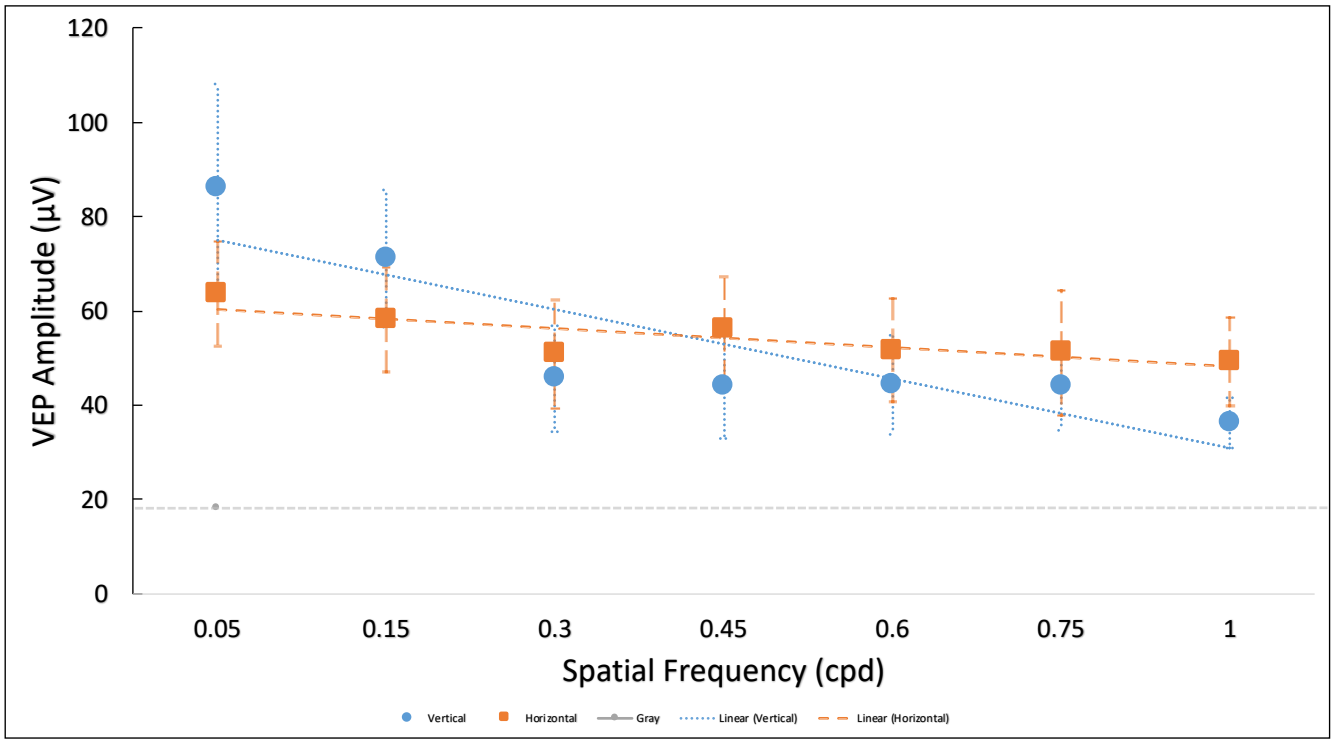

Figure 7: Overlay and comparison of averaged VEP amplitudes evoked from vertical stimuli (blue circle) and horizontal stimuli (orange square).

An interesting trend emerges when vertical and horizontal stimuli are compared (figure 7). At the lowest spatial frequency (0.05 cpd), the vertical stimuli evoked an average VEP amplitude greater than the horizontal stimuli average VEP amplitude (Vertical 86.3 µV ±21.82 compared to Horizontal 63.7 µV ±11.07). When the stimuli is increased to a medium spatial frequency (0.3 cpd), the average VEP amplitude evoked from a horizontal orientation is greater than the average VEP amplitude evoked from a vertical orientation (Horizontal 50.9 µV ±11.57 compared to Vertical 45.7 µV ±11.25). For the remaining increasing spatial frequencies (0.75 cpd; 1.0 cpd), the same result of

horizontally oriented VEPs is higher in amplitude than vertically orientated VEPs is observed (at 0.75 cpd, Horizontal 51.2 μV ±13.22 compared to Vertical 43.9 μV ±9.24). However, the trend line generated from the vertical orientated VEP amplitude averages regressed far greater than the horizontally oriented VEP amplitude averages. If both trend lines are extended, the vertical trendline will cross the baseline threshold first. This reveals that at a vertical orientation, the animal will reflect a lower visual acuity than at a horizontal orientation.

However, from a visual standpoint, a vertical orientation evokes a cleaner looking VEP visual image. In Figure 8, the VEP evoked from a vertical stimulus contains less baseline neuronal noise compared to a VEP evoked from a horizontal stimulus at the same spatial frequency (0.45 cpd). However, even though the vertically orientated evoked VEP contains less neuronal noise, it averaged a lower VEP amplitude (0.45 cpd Vertical average 43.9 μV ±11.04 compared to Horizontal average 56.0 μV ±11.31). While the horizontal oriented evoked VEPs had an higher amplitude average, they also had a more noisy baseline neuronal activity.

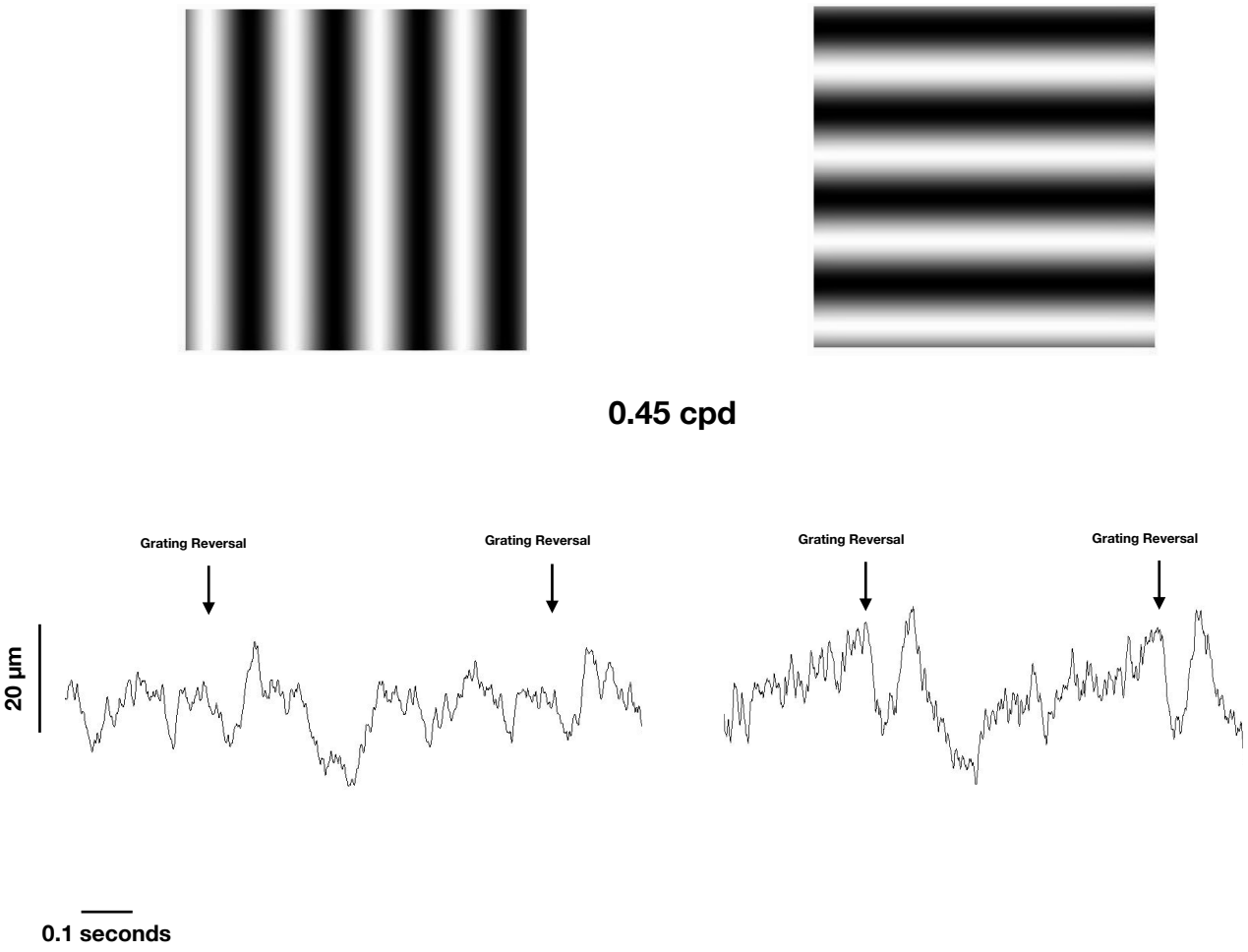

Figure 8: Comparison of Raw VEP data evoked from the same spatial frequency at different orientations.

Discussion:

Vertical Versus Horizontal Stimuli

This study begins to show that different visual stimuli have an influence on VEPs and possibly visual acuity estimates. The dependent variables of spatial frequency and orientation all directly impact the ability to generate a neuronal response. Unsurprisingly, the lowest spatial frequency evokes the Largest VEP amplitude. However, even at the same low spatial frequency (0.05 cpd), vertical orientation evoked an average VEP amplitude of 86.3 μV ±21.82 while horizontal orientation evoked an average VEP amplitude of 63.7 μV ±11.07, This suggests that the visual system is in more tuned to vertical orientations at low spatial frequencies (0.05-0.15 cpd).

Orientation Tuned Cells in Primary Visual Cortex

We showed that both vertical and horizontal orientations of the spatial stimuli evoke a VEP response. This is in agreement with single cell recordings in visual cortex, that show that receptive field in mouse's visual cortex is tuned to specific vertical or horizontal stimuli (Vaiceliunaite et al., 2013). Receptive field can be defined as a boundary in which a single cell can detect changes in the spatial environment (Marshel et al., 2011; Vreysen et al., 2012). In the visual cortex, the mouse's receptive field is organized and tuned to best recognize a specific region of visual space (Iacaruso et al., 2017; Zmarz and Keller, 2016) The receptive field of neurons in the primary visual cortex are more in tune to sinusoidal wave grating stimuli in vertical form (Marshel et al., 2011; Ringach et al., 2016; Vaiceliunaite et al., 2013; Vreysen et al., 2012; Wang et al., 2015). This explains the significant difference in average VEP amplitude at 0.05 cpd

spatial frequency (Vertical 86.3 μV ±21.82 compared to Horizontal 63.7 μV ±11.07). If all factors held constant, a vertical low spatial frequency stimuli will evoke a higher VEP amplitude than a horizontal low spatial frequency stimuli. However, the discrepancy occurs when the stimuli's spatial frequency is increased. The increasing spatial frequency soon becomes ambiguous to the receptive field resulting in an inconclusive data set. The data for horizontally evoked VEP's is only slightly different between the average VEP amplitude at a low spatial frequency (0.05 cpd = 63.7μV ±11.07) compared to a high spatial frequency (1.0 cpd = 49.3 μV ±9.32). This difference in only 14.4 μV between the lowest spatial frequency and the highest spatial frequency can conclude the horizontally evoked average VEP amplitudes as inconclusive. This is also seen in the statistical analysis. Because the t-test statistical null hypothesis cannot be rejected, a higher sample size is needed in order to have more conclusive results.

Effect of Repeated Exposure on VEP Amplitude in Mouse's Primary Visual Cortex

During our research investigation, some mice underwent more trials than others. We observed that the mice who had more repeated exposure to the stimuli had an small increase in VEP amplitude over trials. Each of the three animal cases represented differing responses to repeated exposure (figure 9). One mouse showed a positive increase in VEP amplitude (mouse 3), while one mouse reflected a negative decrease (mouse 1) and the other mouse did not change (mouse 2).

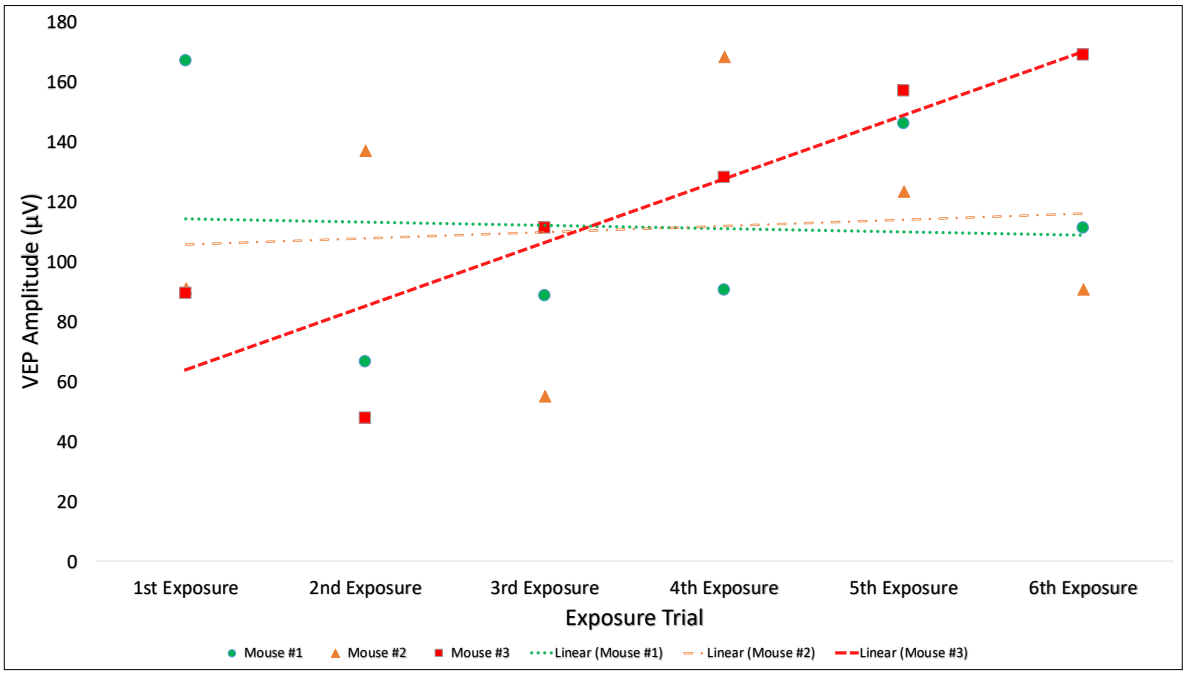

Figure 9: Overlay of trials from Mice (n=3) who reflected SRP effect. Mouse 1 displayed a negative decrease. Mouse 2 displayed a neutral increase. Mouse 3 displayed a great positive increase.

However, the overall average reflects an increasing trend in VEP amplitude (Figure 10). Although a limitation is our sample size, the data overall matches previously published data describing this trend (Frenkel et al., 2006; Shepherd and Bear, 2011). Stimulus-selective response potentiation (SRP) occurs when exposure to repeated stimuli enhances the response to the stimulus which is thought to represent perceptual learning (Cooke and Bear, 2010; Cooke et al., 2015). Further investigation is needed to understand how SRP impacts our measure of visual acuity, if at all.

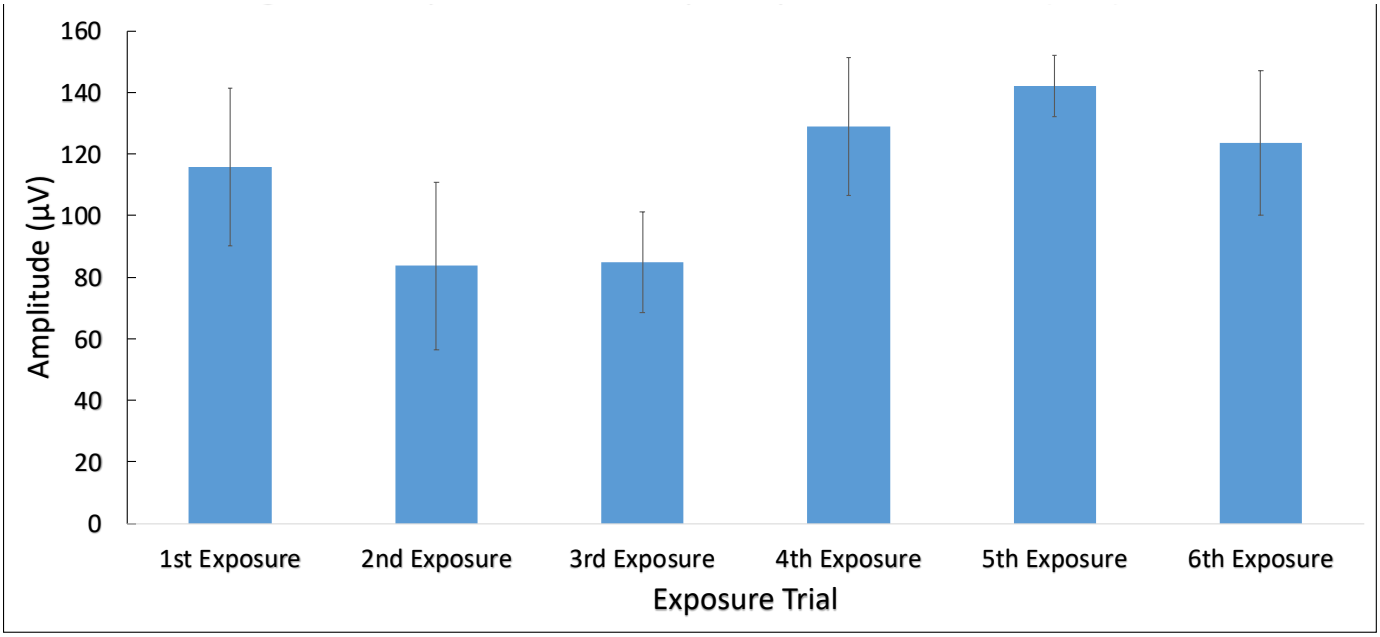

Figure 10: Average VEP Amplitudes from Mice (n=3) who reflected SRP effect.

Relevance of VEP Technique in Comparison to Other Visual Acuity Measurements

A commonly used behavioral technique to measure visual function is the visual water task (Prusky et al., 2000). The visual water task is a two-alternative forced task where, over a set of trials, the animal is trained and visual acuity can be evaluated through the animal's behavioral performance. When the spatial frequency of the visual stimuli is increased, the animal's performance will begin to decline, and their visual acuity can be estimated.

A direction for future research is to investigate the relationship between the visual acuity measured with VEPs and behavioral function. Through the implanted electrodes in the visual cortex, we can compare activity in the same animal. By

measuring evoked VEPs and behavior from the same animals makes it is possible to capture two separate measures of visual function in parallel. These parallel measurements will warrant direct comparisons of cortical physiology and behavioral function of the same visual pathway, in the same animals, and determine whether the two measurements are causally related or separate. This will help create a standard for measuring visual acuity in mice in the laboratory setting.

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