MODULATION OF FREE VIEWING AND SACCADES ON VISUAL CORTEX

By

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Dedicated to my extraordinarily wise father, Cao Xinxing

and

my amazingly brave and supportive mother, Wan Huarui

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V1FO: V1 region corresponding to the retina fovea (<2°)

V1EF: V1 region corresponding to the retina extra-fovea (>2°)

V2FO: V2 region corresponding to the retina fovea (<2°)

V2EF: V2 region corresponding to the retina extra-fovea (>2°)

V4FO: V4 region corresponding to the retina fovea (<2°)

V4PF: V4 region corresponding to the extra-retina fovea (2~6°)

V4EF: V4 region corresponding to the retina extra-fovea (>6°)

RG: Iso-luminant red-green grating stimuli

BW: 100% contrast achromatic grating stimuli

Fix1: behavioral mode under which the monkey keeps fixation

Fix2: behavioral mode under which the monkey freely views the monitor

Fix3: behavioral mode under which the monkey makes saccades guided by the stimuli

INTRODUCTION

"Vision science would be wonderfully simplified if the spatial relationship between the world and the retinal receptor sheet never changed" (Connor, 2003). Following this appealing idea, a lot of studies have been done using anesthetized animal or awake fixating animals. Eye movement was restricted, and stimuli were presented repeatedly to activate the same retinal area and probably the same cortical regions and same group of neurons in a certain region. There are neither many parameters to handle, nor any eye movement feedback to affect visual stimulus-generated response. Maybe the only disadvantage is, it is not the case in the real world.

In real life, however, the center of gaze continually jumps to position the fovea over important and meaningful regions of the visual images. Under these conditions, neuronal responses in visual cortex become obviously different. Gallant and colleagues (1998) found the activity and modulation in V1, V2, and V4 were all generally suppressed when monkeys freely viewed natural scenes, compared to viewing gratings and natural image patches under fixation mode. Moreover, the sparseness of V1 neuronal activity was found to increase during free viewing, and also when activating non-classical receptive field (Vinje & Gallant, 2000). The results are different from but congruent with earlier finding from Livingstone et al. (1996), who also trained monkeys to free view images. They found unless a filter was applied to only count the burst but not scattered spikes, could analysis of V1 neuronal responses reflect the receptive fields and what the animals had been viewing. This indicated great reliance on response 'bursts' in V1. These studies together suggested visual system might use a different coding mode under natural, free

viewing conditions; or say it in another way, the results obtained under eye movement restricted conditions probably couldn't reflect the neural coding principles in the real world.

Besides a lot of confounds, for example, the complicated mindset difference, the most important reason is the occurrence of saccades. As Ross et al. (2001) summarized, saccades suppress visual sensitivity and lead to errors in perceiving visual space during the process of gaze reallocation. While most neural correlates of erroneous position perception are found in dorsal pathway and parietal cortex (Burr & Morrone, 2001), saccadic suppression exist overspread the visual system, including LGN (Ramcharan et al., 2001; Reppas et al., 2002; Royal et al., 2006), MT and MST (Thiele et al., 2002), V1 (Vallines & Greenlee, 2006), V4 and V7 (Kleiser et al., 2004). Thus, saccadic suppression would be our focus since our project focuses on V1, V2 and V4.

During saccades, the low spatial frequencies that would normally be so conspicuous are suppressed (Burr et al., 1982, 1994; Volkmann et al., 1978). Saccades also suppress motion perception significantly (Burr et al., 1982). Furthermore, research shows that in forced choice tasks, people's sensitivity to luminance stimuli decreases while sensitivity to color stimuli remains unchanged or even increases (Burr et al., 1994). Neural evidence was reported for some of these psychophysics results: very meaningfully, Kleiser and his colleagues (2004) observed that BOLD signal in human cortical areas V4, V7 and MT decreased during a saccade by luminant stimuli but not isoluminant stimuli. These findings together suggest that magnocellular system is suppressed during saccades while parvocellular system is not. Contradictorily, Royal et al. (2006) reported both M and P cells in macaque LGN and Lee & Malpeli (1998) reported both X and Y cells in cat LGN

showed perisaccadic suppression. Which side is correct? We are also going to study this interesting issue.

We use optical imaging to study the effect of free viewing and saccades in V1, V2, V4. Optical imaging records hemodynamic activity of the cortex, which can reflect the intracellular activities of neuronal populations, including both dendritic and axonal; it is also great at studying domains and can record from a large region within the same cortical area or across different areas simultaneously. With optical imaging, we aim to confirm others' results about saccadic suppression and the different effect of isoluminant color and luminant stimuli, we are also interested in the reaction of different domains and how the functional map would change: our lab have already done a lot of work to make clear functional domain distribution of macaque V1 and V2 (e.g. Lu & Roe, 2008), and is going to make V4 clear (Tanigawa et al., 2007). Another reason we are interested in the response of V1, V2 and especially V4 is the effect of attention. In free viewing, the allocation of attention and the arousal state must be different from fixation. V4 is not only a crucial area in ventral pathways, processing form, color and contour information, but is also a very important area in attentional effect, for example, attention to color and luminance (Motter, 1994). V1 and V2 are also modulated by attention a lot (Motter, 1993; Luck et al., 1997).

In this project, we designed a third behavioral mode besides fixation and free viewing: guided saccades. Many of the studies applied various cues to induce saccades, while in natural free viewing conditions, eye movements are sometimes more internally controlled rather than externally guided, the underlying mechanisms including attention might be different. With the comparison of free viewing and guided saccades, we seek to define

the effects of saccades in visual perception under free viewing, and build connection between previous free viewing and saccades studies.

METHODS AND EXPERIMENTS

1. Animal:

We obtained results from one female macaque monkey in the awake condition. This monkey was first implanted with a head post, and then trained on a visual fixation task. The animal was rewarded with a drop of juice for holding fixation within a 1.2 deg window for 4.5 s during training (off the window, then no reward). If the animal blinked or looked out of the fixation window for longer than 200 ms, the trial was aborted. There were different fixation conditions, as will be explained later. The monkey was also reinforced with juice reward to sit still in their primate chair during training and experiments. At the time we started imaging, the monkey had already learned to sit still and fixated very well.

A craniotomy and durotomy were performed to expose visual areas V1, V2 and V4 (near the lunate sulcus) in the left hemisphere (see Figure 1). A chronic optical chamber was implanted, and a transparent artificial dura was used to cover the brain, to allow repeated imaging over months (Chen et al. 2002). All surgical and experimental procedures conformed to the guidelines of the National Institute of Health (NIH) and were approved by the Vanderbilt Animal Care and Use Committees.

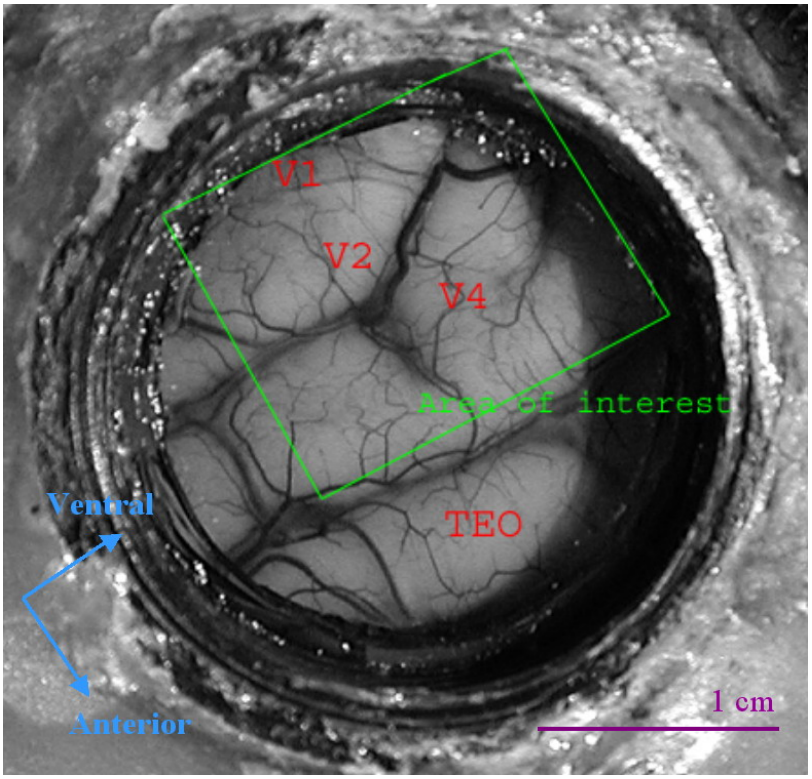

Figure 1: Chamber for imaging. Left hemisphere. The upper portion is ventral and the lower portion is dorsal.

2. Optical Imaging:

The brain was stabilized with agar and images were obtained through a glass coverslip. Images of reflectance changes corresponding to local cortical activity were acquired using a CCD camera (DALSA) and Imager 3001 (Optical Imaging Inc., Germantown, NY) with 630-nm illumination (for details, see Roe and Ts'o 1995; Ramsden et al. 2001). A two lens combination was used: a tandem of 50/85 lenses providing an 8 mm field of view (cf. Ratzlaff and Grinvald 1991). The size of acquired images was always 504*504 pixels. Signal-to-noise ratio was enhanced by trial averaging; experiments were conducted on six different days (year: 2008, date: 0104, 0111, 0215, 0220, 0331, 0401) with 48-68 trials per stimulus each day. All stimuli were presented in blocks of randomly interleaved conditions. Each stimulus was presented for 4 s, and imaging also lasted for 4

s in each trial, during which 16 consecutive image frames were taken (0.25 s per frame). Stimulus presentation stated after the first two frames (500ms) of image recording on five out of the six dates, and stimulus presentation and the recording started simultaneously on 0215.

3. Visual Stimulus:

Full-screen drifting square-wave gratings were created using Matlab and VSG software and presented on a CRT monitor (800*600 pixels). There were four basic grating stimuli: isoluminant red-green grating, horizontal orientation (H_RG); isoluminant red-green grating, vertical orientation (V_RG); achromatic high contrast (100%) grating, horizontal orientation (H_Lum); achromatic high contrast (100%) grating, vertical orientation (V_Lum). A gray screen of equal mean luminance was used as blank condition. The mean luminance for all stimuli was equal.

The screen extent was 18.5*13.9 degrees or 42.0*32.1 degrees, depending on whether the distance was 1181 mm or 500 mm between the monitor and monkeys' eyes (date 0104, 0111: 18.5*13.9 deg, 1181mm; date 0215, 0220, 0331, 0401: 42.0*32.1 deg, 500mm). The spatial frequency of the gratings was automatically adjusted by VSG software to make sure retinal projections of gratings have the same spatial frequency (1.5 cycle/deg) and the same moving temporal frequency (1 cycle/s). The gratings moved randomly in two directions perpendicular to the grating orientations.

Three behavioral modes were studied in these experiments: fixation (fix1), free viewing (fix2), guided saccades (fix3) (see Figure 2).

(1) In ‘fixation’ mode, a small light blue fixation dot was presented at or near the center of the screen (in different days, see table1) for 4 s, and the monkey was required to fixate at the fixation dot during the entire period.

(2) In ‘guided saccade’ mode, the fixation dot appeared at four locations, whose horizontal and vertical axis values relative to center fixation point were: (-3, -3), (3, -3), (3, 3), (-3, 3) for date 0215, 0220, 0331 and 0401, (0, -2), (2, 0), (0, 2), (-2, 0) for 0104 (unit: degree). The fixation dot was presented for 1 s at each location, the four one-second periods were not related to the phase of the gratings. The monkey was trained to saccade to each new position of the fixation dot. Being well trained, the monkey performed fixation and saccades precisely (see eye movement results).

(3) In ‘free viewing’ mode, no fixation dot was presented, and the monkey could view freely and make self-initiated saccades within a large fixation window inside the screen for 4 s.

The size of fixation dot was 0.2 deg * 0.2 deg on 0104 and 0111, and 0.45 deg * 0.45 deg on the other four dates.

Fifteen stimuli (five basic stimuli of four gratings and one blank, under three behavioral modes) were presented in randomly interleaved fashion in each block. So there were roughly equal numbers of trials for each of the fifteen stimulus conditions across days before we did analysis abandoning some failure trials (some trials were eliminated due to noise or behavioral error). Failure trials, in which the monkey made very long blinks or looked outside the big fixation window, were abandoned before analysis. The failure percentage was less than 30%, see Result Section, Part 1.

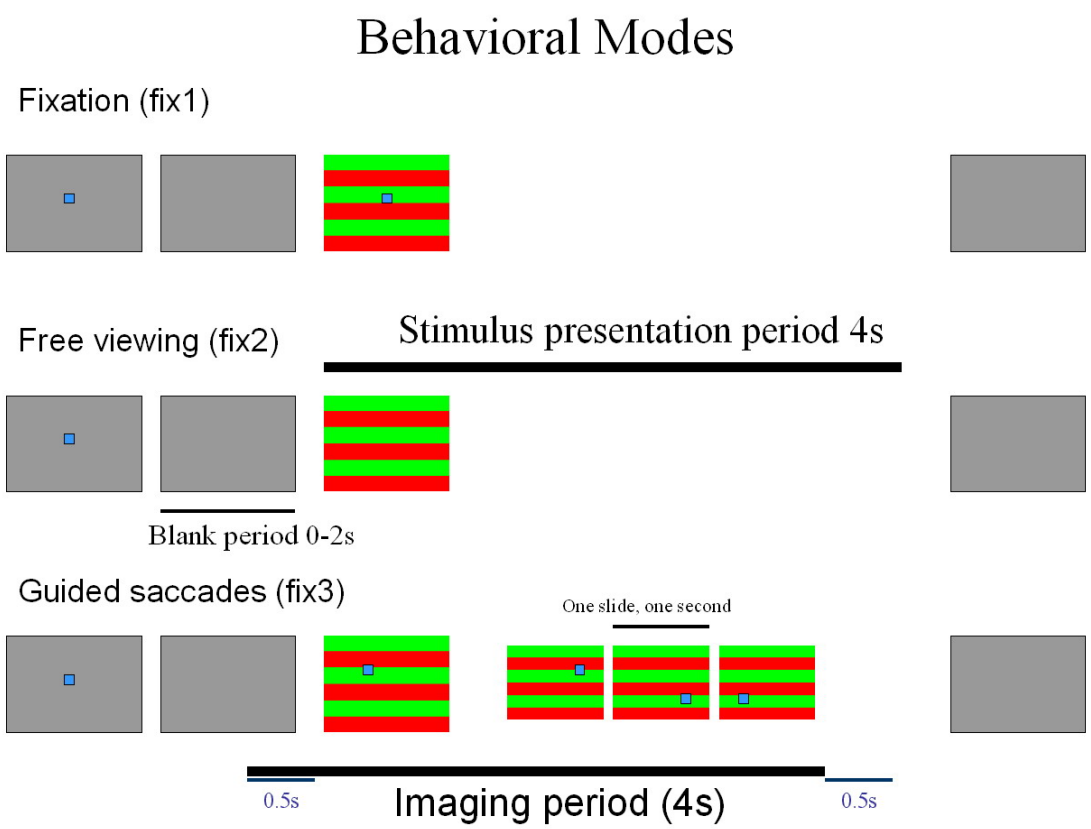

Figure 2: Illustration of the three behavioral modes. A trial started with a brief (300-500 msec) presentation of a fixation dot at (or near) the center of the screen followed by a gray screen (0-2 sec). This is then followed by 4 seconds of stimulus presentation (either a full-screen grating or blank screen). During stimulus presentation, there was a single stationary fixation dot (fix1), or no fixation dot (fix2), or a series of fixation dots presented at 4 locations (fix3). Imaging period started 0.5 s before stimulus presentation began in all the experiment days except 0215. In 0215, the imaging began at stimulus onset, and continued through the entire stimulus presentation process until the reward was given.

The same fifteen stimulus conditions were presented on five of the experiment days (0104, 0215, 0220, 0331, 0401). But note on 0111, there was no guided saccades mode. Instead we used a second 'fixation' mode in which the light blue fixation dot was replaced with a white fixation dot. The purpose and meaning would be explained later, in Part "Defining Sample Regions".

4. Experiment Procedure:

A trial began with a light blue fixation dot and a gray screen background. This dot serves as a cue to the monkey for starting the trial and lasted for a very short period (300-500 ms). Then the dot disappeared, leaving the gray screen for 0-2000 ms. Afterwards stimulus presentation began and lasted for 4 s, during which the monkey was required to fixate in fixation mode, or follow the shifting dot in saccades mode, or freely view within the screen in free viewing mode. The stimulus was randomly given and there was no way to predict that. Although we did not use small fixation window to strictly control the monkey's eye movement in fixation and saccade modes, the monkey performed fixations very reliably (See Result Section, Part 2). Soon after the stimulus presentation ended, the monkey was rewarded with a drop of juice. The reward period was always 500 ms. Inter-trial interval was 1.5-2 s.

Testing Different Baselines. On five of six experiment days (except 0215), optical recording started 0.5 s (2 frames) before stimulus presentation started, lasted 4 s, and ended 0.5 before the end of stimulus presentation. We used the first two frames, frames prior to stimulus onset, as our baseline. To test possible effects of using different baselines, we introduced some differences in the image acquisition on different days. On 0104 and 0111, the first frame was taken during intertrial interval right before onset of the cue while the second frame was taken when the cue was shown. On 0220, 0331, and 0401, the first two frames were taken during the gray screen period after the cue and before stimulus presentation. On 0215, to test the change from a stimulated baseline, image acquisition began at the start of stimulus presentation. The first frames on 0104, 0111, 0220, 0331, and 0401 are likely to be more comparable because the screen was

always blank. The first frames on 0215 may be significantly different, as measuring the signal from stimulus onset may result in significant differences in magnitude and variance of the initial baseline value. Differences in stim and no-stim baseline will be evaluated. For all experiment parameters, see Table 1.

Elimination of Trials. We removed blocks with high behavioral error rates or large artifacts due to, for example, excessive translational brain movement or camera position drift. Therefore, the number blocks in the final analysis less than all the blocks collected (see Table 2).

Monitoring of Eye Movement. Eye movement was monitored by SMI (www.smivision.com) and the Cortex system (NIH) or, on 0331 and 0401, a behavioral system written in Labview (Rob Friedman). The SMI scan rate was 500/sec, and eye position was recorded precisely for every trial.

Table 1: Experimental Parameters for the Six Experiment Days. The following parameters were consistent across days and are not shown in this table: SF: 1.5 cycle/deg; TF: 1 cycle/sec; Stimulus presentation period: 4 s; Imaging period: 4 s, 16 frames, 250 ms/frame; Reward period: 500 ms.

Date	0104	0111	0215
Number of used blocks	50	48	67
Distance (from eyes to screen) (mm)	1181	1181	500
Screen size (deg)	18.5*13.9	18.5*13.9	42.0*32.1
Fixation window size (deg)	10*10	10*10	20*20
Fixation dot size (deg)	0.2*0.2	0.2*0.2	0.45*0.45
Center fixation dot position (relative to screen center) (deg)	(0, 0)	(0, 0)	(0, -4)
Fix3 dot positions (relative to center fixation dot)	(0, -2), (2, 0), (0, 2), (-2, 0)	No fix3	(-3, -3), (3, -3), (3, 3), (-3, 3)
Trigger duration (ms)	300	300	300
Blank screen period (ms)	0	0	500
Start of Imaging prior to stimulus presentation start (ms)	500	500	0
Intertrial interval (s)	2000	2000	2000
Anything special		no fix3	imaging period not perfect

Part2

Date	0220	0331	0401
Block number	62	68	60
Distance (from eyes to screen) (mm)	500	500	500
Screen size (deg)	42.0*32.1	42.0*32.1	42.0*32.1
Fixation window size (deg)	20*20	20*20	20*20
Fixation dot size (deg)	0.45*0.45	0.45*0.45	0.45*0.45
Center fixation dot position (relative to screen center) (deg)	(0, -4)	(0, -4)	(0, -4)
Fix3 dot positions (relative to center fixation dot)	(-3, -3), (3, -3), (3, 3), (-3, 3)	(-3, -3), (3, -3), (3, 3), (-3, 3)	(-3, -3), (3, -3), (3, 3), (-3, 3)
Trigger duration (s)	300	500	500
Blank screen period (s)	2000	1500	1500
Interval Imaging prior to stimulus presentation (s)	500	500	500
Interstimuli interval (s)	2000	1500	1500
Anything special			

ANALYSIS

1. Single-Condition Maps:

A ‘single-condition map’ is constructed for each stimulus condition. The gray value of each pixel in the ‘single-condition map’ represents the percentage change of the light reflectance signal after the stimulus was presented. Specifically, the gray value of each pixel was calculated using this equation:

$$\Delta R = F_{5\text{-}16} - F_{1\text{-}2}$$

ΔR represents changes of reflectance, and it was obtained by subtracting the average raw reflectance value of frames 1-2 ($F_{1\text{-}2}$) from that of frames 5-16 ($F_{5\text{-}16}$). The first two frames were taken before stimulus onset and thus represented the baseline activity. For stimulus-induced intrinsic signal, reflectance change magnitude changes from 0.01% to 0.2%, and the peak occurs about 2-3 s after stimulus onset. In single-condition maps, darker pixels represent highly activated regions, while lighter pixels represent less-activated regions. Single-condition maps are often also used for calculation of other maps such as subtraction maps or for other further quantification.

2. Subtraction Maps (or Difference Maps):

All maps for each single condition were summed to increase signal to noise ratio. Then subtractions of pairs of summed maps were obtained to show response preference for different stimuli. For example, subtracting activation maps of vertical gratings from those of horizontal gratings can reveal distribution of orientation preference, and subtracting maps of achromatic gratings from those of isoluminant red-green gratings can reveal distribution of color vs. luminance preference. Subtraction maps were clipped at 1

to 1.5 standard deviations from the mean of the pixel distribution and then scaled to gray values of 0-255. In subtraction maps, pixel values represent activation preference for each of the two stimuli (or two groups of stimuli): 0 represents strong activation induced by one, and 255 by the other, while 127 represents no strong preference for either condition. Main blood vessels were masked during clipping to avoid contamination from large artifactual vessel-related reflectance changes.

3. Time Course Analysis and Statistics:

ANOVA and Multi-Comparison Procedure: The time course of reflectance change, during the period of image acquisition (4 s, 250 ms per frame), was obtained over nine regions (see Table2, Figure 10, 11). These nine regions would be introduced later, in Result Section, Part 4, since a lot of results are presented and used to define these regions. Time course analysis can reveal the temporal change, and we can select the most appropriate time period for our statistical analysis.

Absolute raw reflectance values vary significantly depending on lighting conditions and specifics of the preparation. Therefore, we used the percentage of changes in reflectance signal as a way of comparing across images and cortical regions. The percentage change from baseline was calculated by first-frame subtraction, which means subtracting activation in the first 250 ms from activation in each of the subsequent 250 ms frame. The subtraction value was later divided by absolute value of the first frame:

$$dR/R = (F_{any} - F_1)/F_1$$

The mean, standard deviation, and standard error of the mean (SEM) were calculated for each frame and each sample region. Not only the peak value, but also the overall trend of

the time course curves was taken into consideration. To compare activations of several stimuli (or several groups of stimuli) during the overall period, typical frames were first selected for statistical analysis. Data from these frames under one behavioral mode (the same frames for three modes) were combined into one set, and then there were three sets of data representing three different behavioral modes, one-way ANOVA procedures and appropriate multiple comparisons (Fisher LSD n=3; Bonferroni, n=4; Scheffe, n>=5) were then conducted. These frames were selected based on three criteria:

(1) Select distinct period for each case (date*region), the period that is most prone to significant difference. If the dR/R curves don't intersect (usually they don't), make the period as long as possible, because the longer the period it is, the more frames there are, and the bigger the sample size is, and then the greater the signal-to-noise ratio is, the more possible to show significant difference.

(2) These periods must contain the peak frame.

(3) Avoid the first four or five frames because the values of these frames are not decided by stimulus condition (see Result section, Part 8).

Figure 3 presents two examples for our frame selection. For 0220 V2EF (extra-fovea), the main difference among the three modes is between sum_fix3 and the other two, the difference can be clearly seen as early as the fourth or fifth frame, and is always very obvious across the later period. Usually, the more frames with obvious difference we select, the more possible ANOVA analysis would show significant difference. Therefore, we can select frame 5-16, and to avoid the beginning period, we can change it to frame 6-16. It also fits the criteria that the peak must be contained. For another case, 0401 V1FO (fovea), the difference is not as obvious as in last case, but can still be observed from

frame 9 or 10 to frame 16. Considering the peak, it is best to choose frame 9-16. It also fits the criteria that the beginning must be avoided. Although the judgment is somewhat arbitrary, by obeying these three criteria, we can still make systematic selections and later reliable analysis. For each case, the same frames were used in later modulation index analysis (Result Section, Part 6, 7).

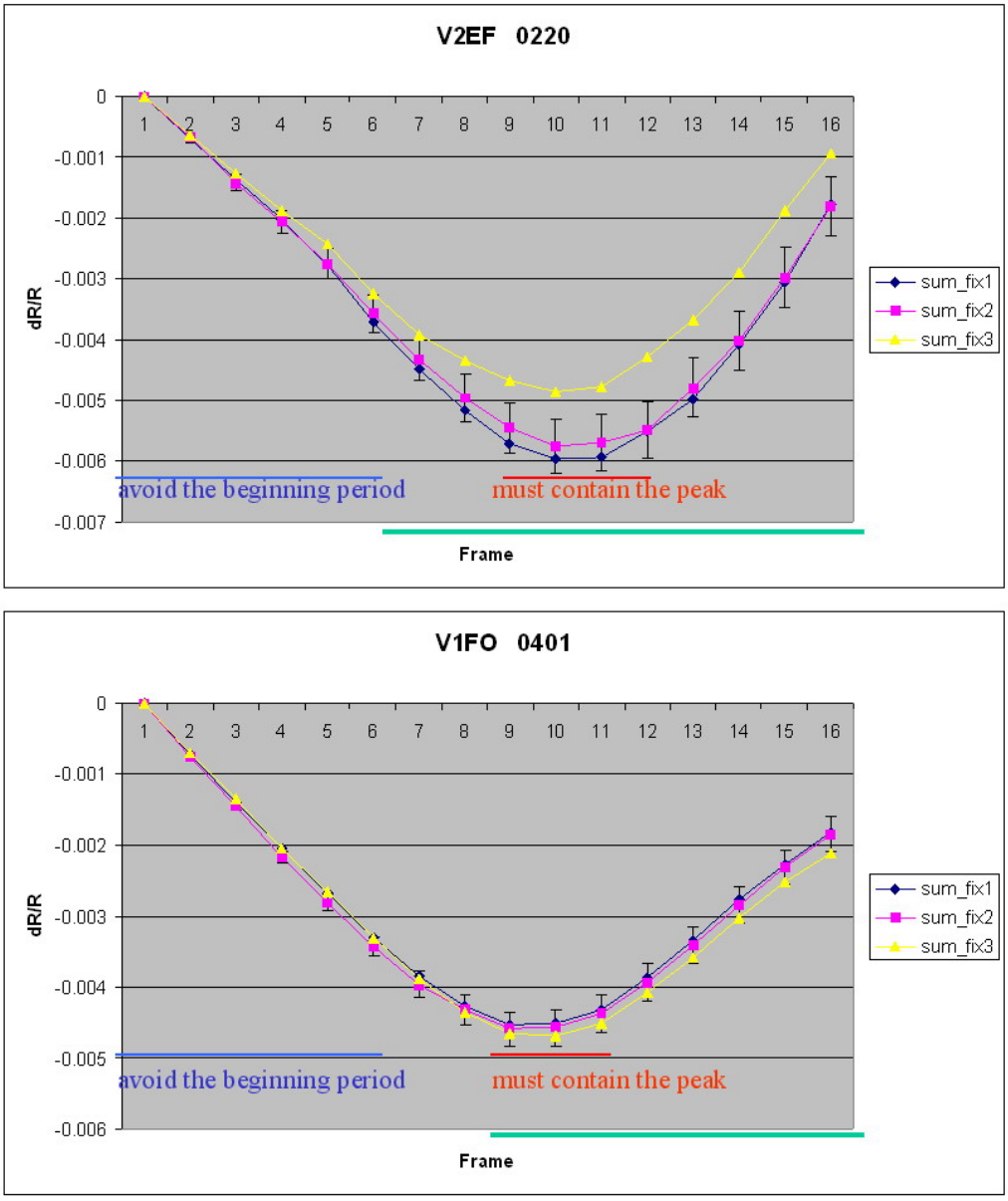

Figure 3. Two examples of frame selection. The green bars denote frames selected for ANOVA analysis. To be sensitive to differences in time course, for each case, the periods with most significant difference are used to evaluate differences between stimulus and behavioral conditions.

Saccade Modulation Indices: To examine activation under the three fixation conditions, we calculated modulation indices as a ratio of saccade/fixation activation (MI1) or saccade/free viewing activation (MI2), as defined below:

(1) MI1 = mean of activation of stim_saccade / mean of activation of stim_fixation.

in which 'stim' means a specific stimulus condition. Standard deviation of MI was calculated following this formula:

$$sd(x/y)/mean(x/y) = sqrt(sd(x)^2/mean(x)^2 + sd(y)^2/mean(y)^2). \quad \ldots\ldots \textbf{(a)}$$

This is called 'delta method' in statistics. We took dR/R values as the activation results.

(2) MI2 = mean of activation of stim_saccade / mean of activation of stim_free viewing.

There are data from 5 days, a maximum of 9 sample regions from one day, and a total of 38 cases (date*sample region) available for MI analysis (all dates except 0111). In each case, there are five stimulus conditions: Blank, H_RG, H_Lum, V_RG, V_Lum, and two stimulus combinations: RG (H_RG + V_RG), Lum (H_Lum + V_Lum). We obtained two modulation indices (MI1 and MI2) and their standard deviations for each frame of all these stimulus (or stimulus combination) condition in all the cases. To compare modulation effect under two stimulus (or stimulus combination) conditions, we combined several frames into one set, calculated the average MI and the corresponding standard deviation for both conditions, and finally conducted a z-test (normal distribution). Calculation followed the formula below:

$$average\ mean = (mean1 + mean2 + \ldots + mean(n))/n \quad \textbf{……(b)}$$

$$average\ sd^2 = (sd1^2 + sd2^2 + \ldots + sd(n)^2)/n^2 \quad \textbf{……(c)}$$

Mean1 and sd1 denote mean and standard deviation of the first selected frame, mean(n) and sd(n) denote mean and standard deviation of the nth selected frame, and n denotes the number of selected frames. These selected frames were exactly the same ones used in previous one-way ANOVA.

Z test was done by:

z = (mean(x) – mean(y)) / sqrt(sd(x)^2 + sd(y)^2). ***……(d)***

To be clearer, let's take an example. We intend to compare modulation effect of saccades in V1 fovea under color (RG gratings) and luminance (Lum gratings) stimulus conditions on 0401 (in this example, just consider one single case: 0401, V1 fovea). So we need to calculate modulation index and its standard deviation under these two stimulus conditions, and then do a z-test. Now let's see what we have:

1. dR/R mean and SEM value (SEM: standard error of the mean) of all sixteen frames in two conditions;

2. The typical period: the selected frames are exactly the same ones used in previous one-way ANOVA, because the period composed of these frames is most sensitive to saccadic suppression. In case 0401, V1 fovea, the period is frame 9-16.

Then the calculation includes 5 steps, and we only consider MI1 (fix3/fix1) in this example:

1. Calculate Modulation Index of each frame for two conditions:

RG condition MI1(frame n) = dR/R mean(RG, fix3, frame n) / dR/R mean(RG, fix1, frame n).

The same for Lum condition.

2. Calculate standard deviation of Modulation Index of each frame for two conditions, using formula (a):

sd(x/y)/mean(x/y) = sqrt(sd(x)^2/mean(x)^2 + sd(y)^2/mean(y)^2).

Here, for each frame, mean(x/y) is the MI1 value obtained in step 1; sd(x/y) is the standard deviation of MI1 which we want to get; mean(x) and mean(y) are dR/R mean values of fix3 and fix1, respectively; and sd(x) and sd(y) are dR/R SEM values of fix3 and fix1.

3. Calculate Average MI1 for selected frames, using formula (b):

Avg MI1 = [MI1(frame9) + MI1(frame10) + … + MI1(frame16)] / 8 .

4. Calculate standard deviation of the average MI1 for selected frames, using formula (c):

Avg SD = sqrt{[SD(frame9)^2 + SD(frame10)^2 +…+ SD(frame16)^2]/8}.

Step 1, 2, 3, 4 can be performed respectively for RG and Lum stimulus conditions, and finally get four values: Avg MI1 (RG), Avg SD (RG), Avg MI1 (Lum), Avg SD (Lum).

5. Do a z-test, using formula (d):

z = [Avg MI1 (RG) – Avg MI1 (Lum)] / sqrt [Avg SD (RG)^2 + Avg SD (Lum)^2].

Then we can get the p value and judge the significance.

Similarly, we can also compare two regions under the same stimulus condition, to test the effect of regions on saccadic modulation.

4. Eye Movement:

Eye positions were recorded for stimulus presentation period on two days: 0331 and 0401. Eye position data was averaged for each stimulus condition in fixation and saccade modes on each day, in order to show the trajectory of eye movement. We didn't average

eye movement data for free viewing mode, because eye position trajectories have no pattern and are mostly scrambled, averaging would make no sense. The movement was recorded for both eyes, and as they are equivalent, we show right eye data only in the examples.

Linear transformation was carried out to transform original eye position data to data of gaze position on the screen. From session to session, the gain and offset values would change, so in each session we estimated average gain and offset values. The average offsets were estimated following this procedure: 1) check average eye position result for Blank_fix1, 2) if eye position values remain the same over most of the stimulus presentation period (true, see Result Section, Part 2), take the average of x and y eye position values during those periods as the offsets. The average gains were estimated following this: 1) check average eye position result for Blank_fix3, 2) check that gaze follows four fixation points (true for both 0331&0401), then 3) assign the four positions (-3, -3), (3, -3), (3, 3), (-3, 3) respectively, and calibrate vertical and horizontal gains from these locations. Linear transformation was conducted using this algorithm: gaze position = (eye position – offset)*gain. Reminder: blank_fix1 is a isoluminant gray screen with a single fixation dot, and blank_fix3 is the same isoluminant gray screen with a fixation dot appearing sequentially at four locations.

Error trials were not included in this calculation.

5. Retinotopic Mapping:

We aimed to examine the effects of free viewing and saccades in different regions (for example, foveal and extra-foveal regions); therefore it was necessary to obtain at least

coarse retinotopic organization of V1, V2 and V4. We were able to define the foveal region of V1 and V2 and relation between the two regions. The foveal region of V4 in this chamber had been previously determined by retinotopic mapping (Tanigawa et al. Soc Neurosci Abstract 2007).

Tanigawa et al. used three paradigms to define V4 retinotopy (manuscript in prep) (Figure 4). One was ring and line paradigm. In the ring paradigm, a thin ring (width: 0.1 or 1 degree) was presented with the fixation dot as the center. The ring was composed of four types of drifting gratings with the same average luminance: red-green, horizontal; red-green, vertical; black-white, horizontal; black-white, vertical. In the line paradigm, a thin radially extending line (width: 0.1 or 1 degree) was presented with the fixation dot as the center. The line was composed of four types of drifting gratings with the same average luminance: red-green, horizontal; red-green, vertical; black-white, horizontal; black-white, vertical. The third was a patch paradigm. The patch had three possible sizes: 0.25*0.25, 0.5*0.5, and 1*1 degrees, and was composed of the same drifting gratings. The temporal frequency and spatial frequency were 1 cycle/s and 1.5-2 cycle/deg for all paradigms. During retinotopic mapping, the monkey was always required to keep fixation.

V4 was imaged following similar procedures as described above. Retinotopy could be obtained by calculating difference maps, subtracting achromatic grating from red-green grating with the same eccentricity.

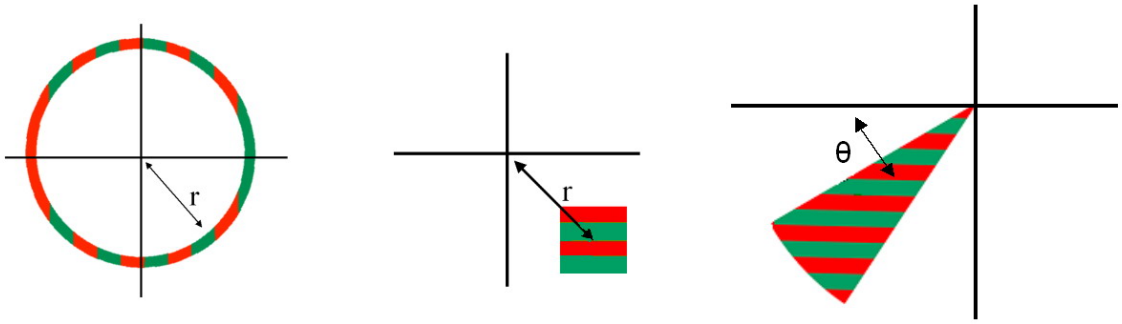

*Figure 4: Ring, patch and line paradigms. In ring paradigm, ring width was 0.1 or 1 degree; In patch paradigm, the size of the patch ranged from 0.25*0.25 to 1*1 degrees, and eccentricity was calculated from the fixation dot to the center of the patch; In line paradigm, the line was very thin (1 degree, not to scale in figure). Ring, patch and line were all composed of red-green or black-white grating, and the orientation was horizontal or vertical. Temporal frequency and spatial frequency were 1 cycle/s and 1.5-2 cycle/deg. The monkey was required to keep center fixation in both paradigms. The horizontal and vertical lines here are drawn to make the graph clear, but are not shown during experiments.*

RESULTS

1. Behavioral data:

Overall, the monkey performed very well in the tasks (see Table 2). The monkey did more than 48 blocks of trials (48*15=720 trials) every day, with a success rate higher than 80% on five of the six days. The failure trails were caused by either monkey looking out of the window or long blinks. The success rate in blocks for analysis is over 70% on all days, after removing some blocks with too many errors or with a big shift of camera position. The overall high successful rate is because the monkey was well trained and the task was not hard: the monkey was just required to follow the fixation dot within a big

fixation window. Reminder: no matter what behavioral mode is, if the monkey kept gaze within the fixation window during the entire trial, it was recorded as a successful trial; and if the monkey looked outside the fixation window or made a very long (>200ms) eye blink, it was considered a failure trial.

The success rate for the free viewing condition was lower than the overall because the paradigm without any fixation dot was still new to that monkey. Since there were only two times of free viewing training before recording, the monkey was obviously still learning in the experiment process. The success rate under free viewing first decreased and then increased to a perfect level, partly because the fixation window became larger on later days, and partly because the monkey finally acquired this task and adapted to the task structure.

Successful rate are very close for fixation and saccades, both very high (Supplemental Table 1).

Table 2. Behavioral Data in Six Days of Experiments. The overall successful rate was high, successful rate under free viewing conditions was lower but reached a high level at last.

Experiment day	Overall successful rate	Number of used blocks	Successful rate in used blocks	Successful rate under free viewing
0104	0.83	50	0.835	0.808
0111	0.86	48	0.855	0.721
0215	0.87	67	0.879	0.701
0220	0.60	62	0.703	0.619
0331	0.96	68	0.963	0.941
0401	0.93	60	0.989	0.983

2. Eye Movement:

Eye movement data were averaged for every stimulus condition in fixation and saccade modes. Figure 5 clearly shows that in fixation mode, following a saccade to the center fixation dot at the beginning of each trial, the monkey almost always maintains fixation within a small area during most of the stimulus presentation period. This figure only shows some examples of the results, but under other stimulus conditions (of fixation mode) or on other days, the gaze trajectories were very similar. Therefore, though the fixation window we applied was very big, the monkey still performed the fixation very well.

Figure 6 shows two examples of gaze trajectory under the guided saccade mode. A and B were averaged results for Blank_fix3 and V_Lum_fix3, obtained from data of 0401. Other stimulus conditions in saccade mode and other days (0331) showed similar results: the monkey followed the shifting fixation dot very well, at least at the first three locations. In some conditions, the monkey did not saccade fully to the last location, but stopped at the third location or the saccade fell short between the third and the fourth ones. The likely reason is that, knowing it was the end of the trial and that juice reward was impending, the monkey was less motivated to perform accurately. Overall, the monkey at least made three guided saccades in a trial: from somewhere to the first location, from the first to the second location, from the second to the third.

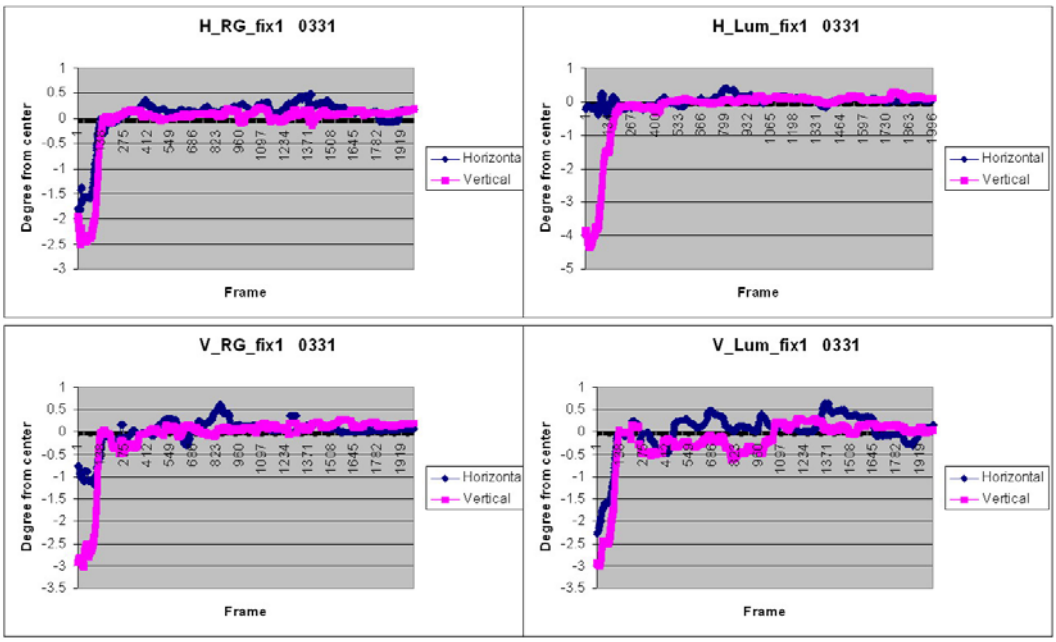

Figure 5. Gaze trajectories under fixation mode (fix1), obtained by averaging all trials (n=62~68) for the same stimulus condition. These four graphs show trajectory under four different stimulus condition, all under fixation mode. The entire period was 4 s, composed of 2000 frames, and the scanning rate was 500/sec. Horizontal axis is the frame number, and vertical axis represents degree value from the center fixation dot position. This figure shows that the gaze almost always remained within a 1x1 degree-size window (±0.5 x ±0.5) after a saccade to the fixation dot at the beginning of the trials. On horizontal axis: positive means rightward; on vertical axis: positive means upward. Terms: Blank: iso-luminant gray screen; H: horizontal; V: vertical; Lum: blank-white grating; RG: red-green grating; fix1: fixation mode; 0331: date of experiment.

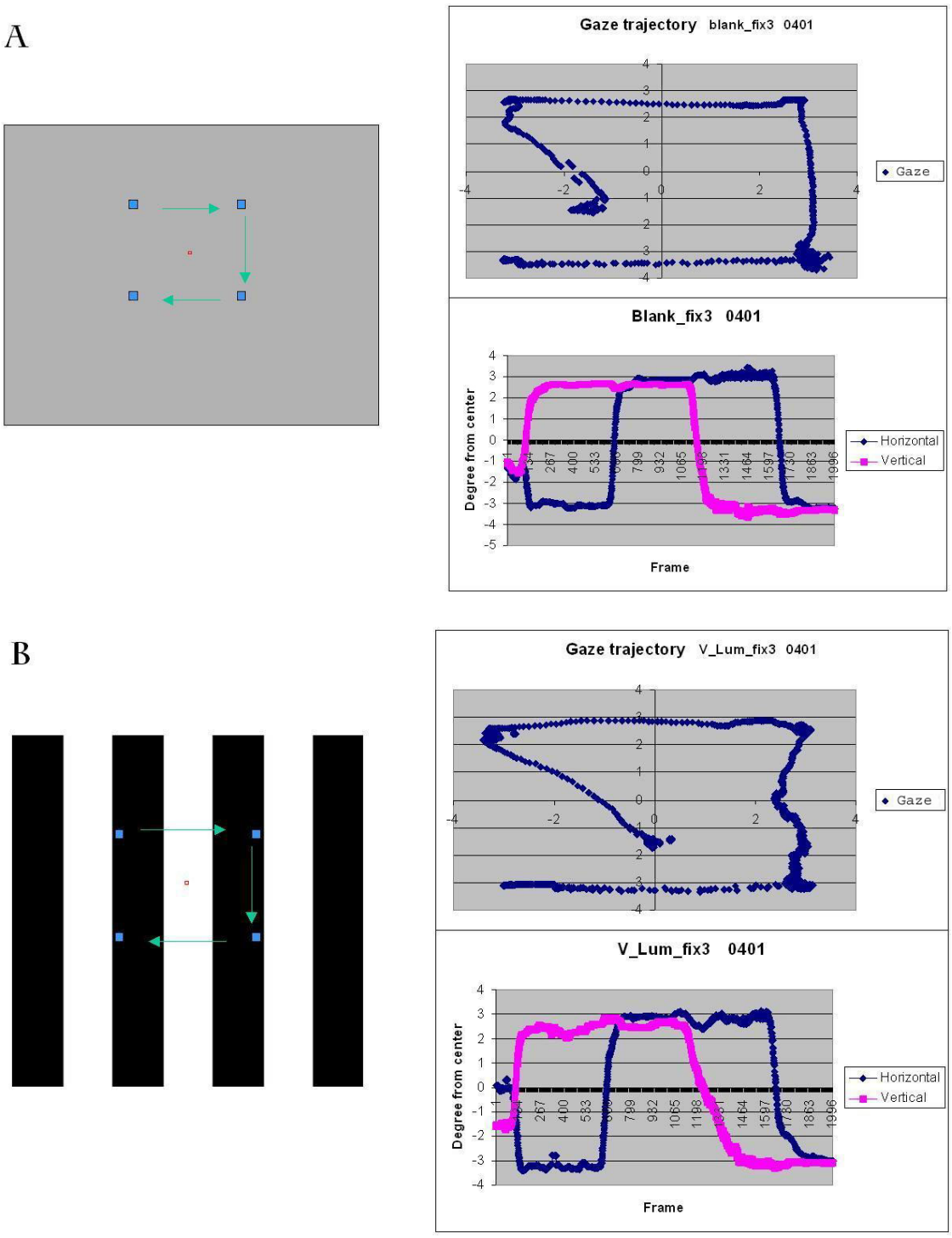

Figure 6. Gaze trajectories under saccade mode (fix3). A: Blank screen (iso-luminant gray), saccade mode, and the gaze trajectory; B: Vertical, black-white grating, saccade mode, and the gaze trajectory. Both are obtained by averaging all trials (n=58~60) for the same stimulus condition. For both A&B, Left: stimulus, the background (either blank

or grating) remained the same, while the fixation dot shifted following the directions of the arrows, and was presented at each location for 1 sec. The red dot at the center refers to the center fixation dot position, which is at or near the screen center. The four locations of fixation dots were (-3, -3), (3, -3), (3, 3), (-3, 3) (unit: degree), relative to the center fixation dot. Note, in real experiments, this center fixation dot was not shown under saccade mode. Right Upper Panel: gaze trajectory projected on the screen, (0,0) represents the location of center fixation dot. Right Bottom Panel: Degree from the center at horizontal and vertical directions.

Averaging across trials does make sense for fixation mode and saccade mode, but not free viewing mode. Under free viewing condition, the monkey was making random eye movements without any fixed pattern, averaging could only provide us some scrambled images. Figure 7 shows two randomly selected examples of free viewing trials; the monkey was making big random saccades during the processes.

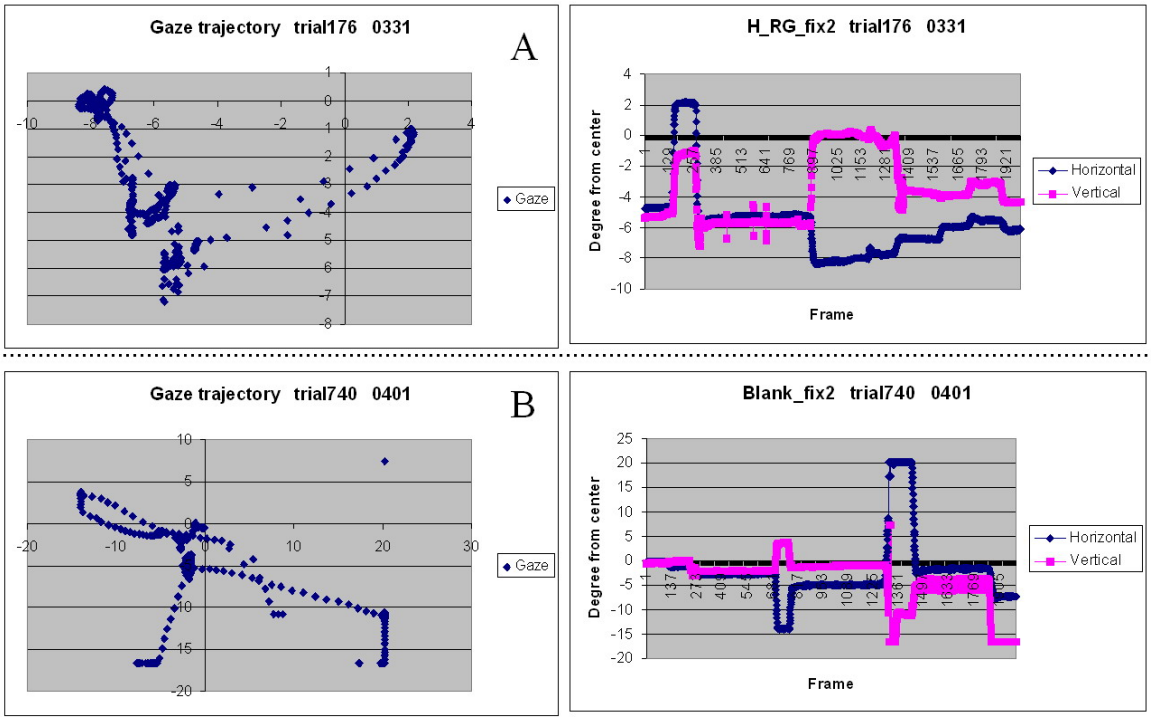

Figure 7. Gaze trajectories of two examples under free viewing mode. Left: gaze trajectories projected on the screen. Right: changes of distance from the center fixation location (not present under free viewing), horizontal and vertical location.

3. Functional maps:

Many optical imaging works used difference maps to show their results. For instance, *Roe and Ts'o 1999; Chen et al. 2003;Roe et al. 2005; Xu et al. 2004; Lu and Roe 2008* all used difference maps as a main analysis method, which is great to show functional architecture, such as ocular dominance maps in V1 and the 'stripes' in V2 (Ts'o et al., 1990), S1 topography (Chen et al. 2003), color domain in V1 and V2 (Lu & Roe, 2008) etc. Though we mainly use time course, which can avoid clipping and keep the original data, for statistical analysis in this project, we still need to present some functional map results for three purposes:

(1) To check whether functional architecture is affected by free viewing or saccades.

(2) To at least obtain a rough map of functional architecture and use it to define domains in time course analysis.

(3) To show that time course changes here are mainly real signal induced by corresponding stimuli, but not noise or random effect.

Functional Map and Nature of Time Course Changes: Difference maps in figure 8 shows clear orientation domains and color domains. The upper three are obtained by subtracting sum cortical activation induced by all vertical gratings from sum by all horizontal gratings, including both isoluminant red-green and black-white (100% contrast achromatic). The dark regions prefer horizontal orientation while the bright regions prefer vertical orientation. The bottom three are obtained by subtracting sum cortical activation induced by all red-green gratings from sum by all black-white gratings, including both horizontal and vertical. The dark regions prefer color stimuli, while the bright regions prefer luminance stimuli. Difference maps obtained under all three behavioral modes are able to show orientation and color domains, although certain regions are not very clear in some maps. Note some special parts of the images, for example, V1 and V2 areas of 'H-V_RGLum_Fix3' image are very bright. This kind of widespread darkness or brightness doesn't mean that the entire area has the same preference. Their preference are probably different, because clipping assigns all pixels brighter than median+1.0*SD (or 1.5*SD) pure white, and pixels darker than median-1.0*SD (or 1.5*SD) pure black.

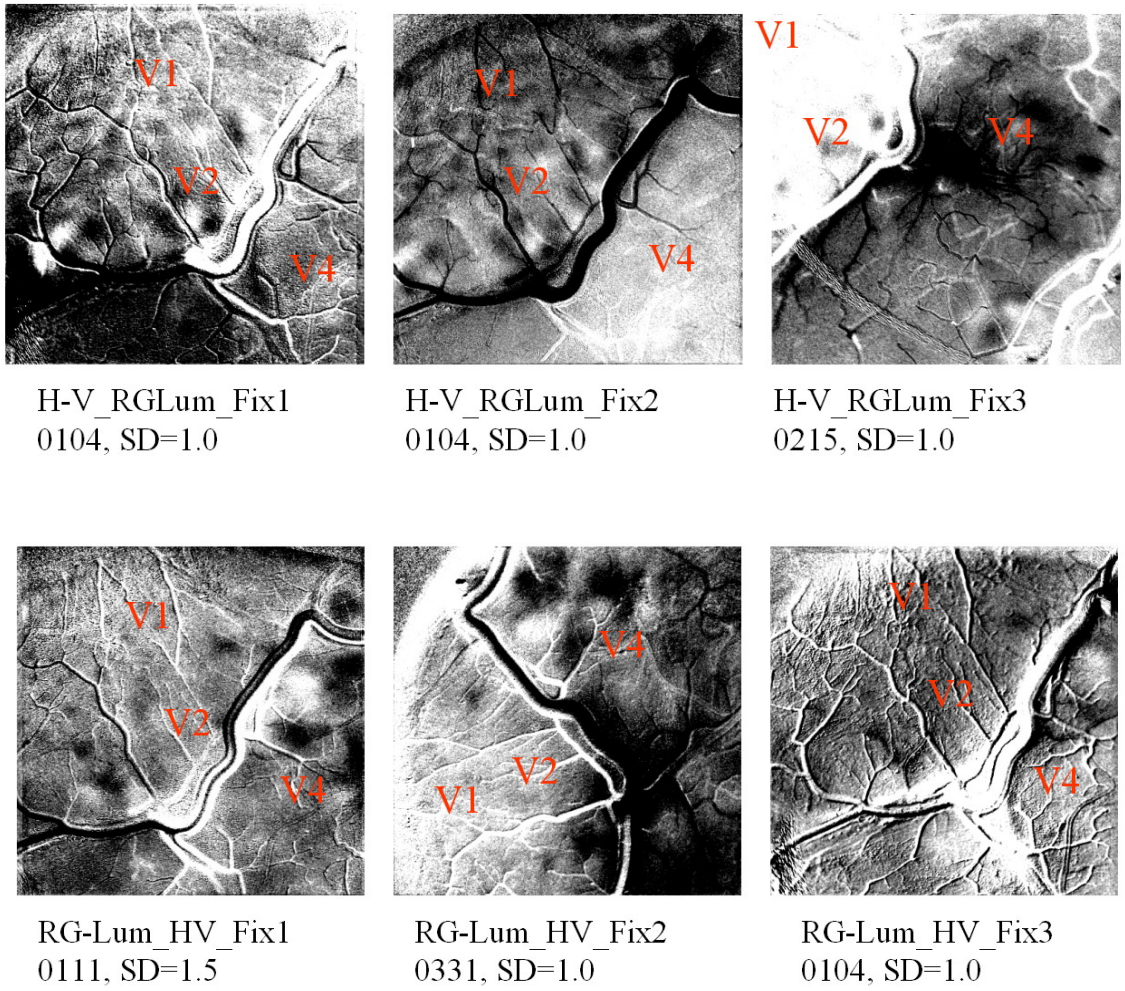

Figure 8. Difference maps that show orientation domains (upper three images) and color domains (bottom three images). Imaging areas were different across days, which caused vessels and visual areas to be at different locations across maps. No filtering was applied to obtain these maps. ***Note all the difference maps have a size of 8 mm*8 mm, including maps in this figure and all the following figures.*** *Terms: H-V_RGLum: sum (of cortical activation) of all horizontal gratings including both red-green and black-white subtracts sum of all vertical gratings. RG-Lum_HV: sum of all red-green gratings including both horizontal and vertical subtracts sum of all black-white gratings. Fix1: fixation mode; fix2: free viewing mode; fix3: saccade mode. SD=1.0 (or 1.5): clip to +-1.0 (or 1.5)*SD; 0104 (etc.): date of experiment.*

These maps indicate that stimuli can activate the cortex and their corresponding domains under any of the three behavioral modes. Therefore we can trust the time course data as stimulus-induced.

Effect of Behavior on Functional Architecture: But does the functional architecture remain unchanged by the monkey's behavior? Or would free viewing or saccades make domain distribution different?

The answer is no. We found the domain distribution is consistent across the three behavioral modes, although the maps are clearer in some cases than in others.

Figure 9 shows difference maps obtained from 0104: the upper three reflect orientation preference while the lower three reflect color-luminance preference. In the upper row, yellow arrows denote dark patches that prefer horizontal stimuli, these dark patches are at the same locations across the three maps, and so are the light patches right beside, which prefer vertical stimuli. In the lower row, the dark and light patches are areas that prefer color and luminance stimuli respectively, and they also appear at the same locations across the three maps. Indeed, the patches are clearer and more easily seen in some cases than in others, but the reason is very complicated: clipping, vessel artifact, system noise, etc. However, no matter how the salience of the patches changes, most importantly, the locations never change. Another example, figure 10, data from 0220, presents the same results. Data from the other four days also show consistent results (no figure presented). These indicate that the domain distribution is unaffected by the behavioral state. In other words, the stimulus preference of the neural populations is not affected much by either free viewing or saccades.

Based on this result, it is obvious that the following sample region defining procedure is reliable: no matter the region was defined using data under any one behavioral mode, it can be applied to analyze the other two modes.

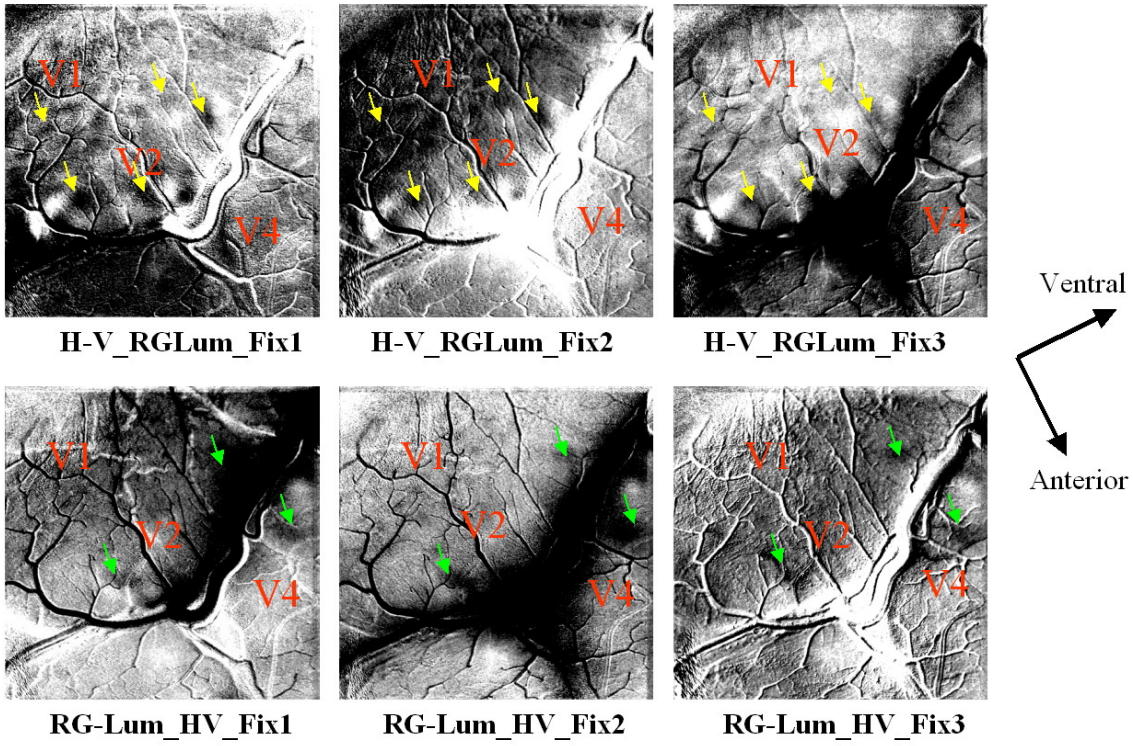

Figure 9. Difference maps obtained from 0104. Averaged from 50 blocks, clipping to 1.0 SD. The upper three are calculated by this formula: (H_RG + H_Lum) – (V_RG + V_Lum), the dark regions are horizontal orientation preferred, while the light regions prefer vertical orientation. The lower three are calculated by this formula: (H_RG + V_RG) – (H_Lum + V_Lum), the dark and light regions are color and luminance preferred, respectively. Yellow and Green arrows both point to dark patches. Terms: RG: red-green grating; Lum: achromatic grating, 100% contrast; H: horizontal; V: vertical; Fix1: fixation; Fix2: free viewing; Fix3: guided saccades.

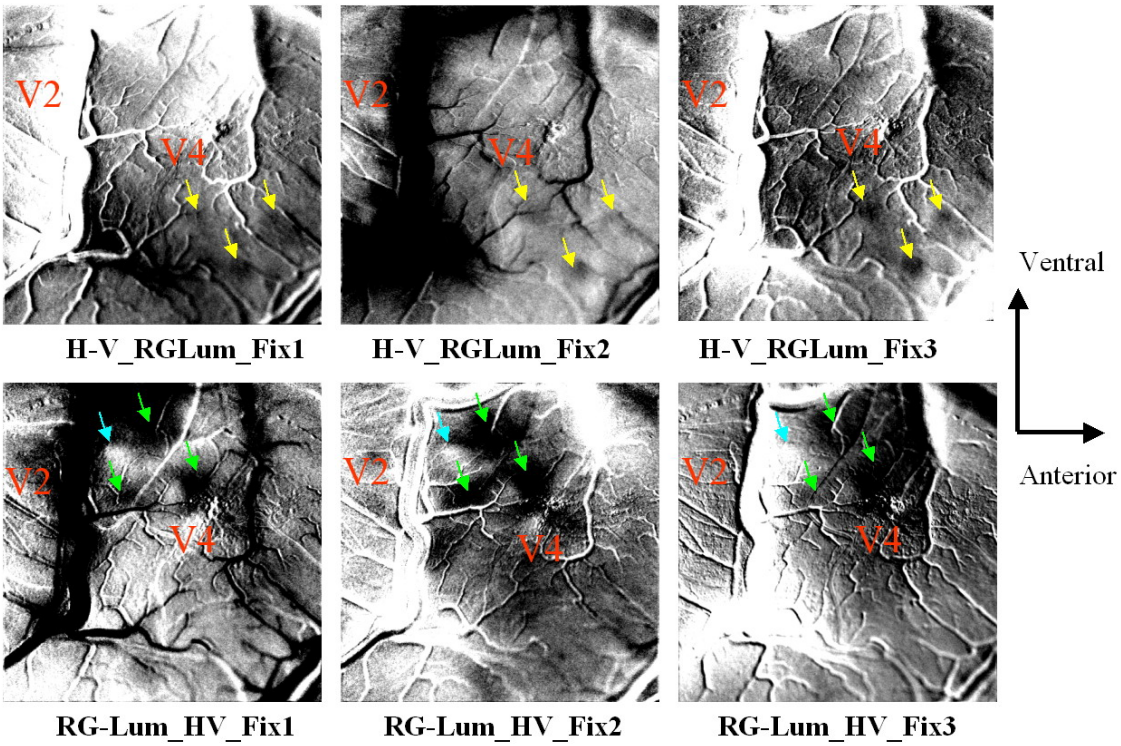

Figure 10. Difference maps obtained from 0220. Averaged from 62 blocks, clipping to 1.0 SD. The meaning of the terms and labels are the same as in Figure 16. Blue arrows in the bottom row refer to bright areas that prefer luminance stimuli.

Our analysis here is mostly qualitative, to do further quantitative analysis, we can either do a threshold analysis or a time course statistical analysis. In this paper we emphasize time course analysis, threshold analysis will be done in later projects.

Functional Architecture Map: By further analyzing the functional maps, comparing orientation preference map (H-V) and color-luminance preference map (RG-Lum), we got functional architecture maps to show V1\V2 border (Figure 11), V1 blobs (Figure 12), V2 strips (Figure 13), and V4 domains (Figure 14). These areas and domains were identified by the following criteria:

(1) V1 blobs are color-preferred blob-shaped regions, which are the blob-shaped dark regions in RG-Lum_HV difference maps.

(2) In RG-Lum_HV difference maps, V1 is characterized by color-preferred blobs, and V2 includes larger stripes but not blobs. These features are important in defining V1/V2 border, V1 and V2 are both posterior to lunate sulcus. The dorsal border between V1 and V2 runs roughly parallel to the lunate sulcus and is located a few millimeters posterior to it.

(3) V2 is a narrow bank posterior to lunate sulcus, and V4 is anterior to lunate sulcus.

(4) V2 thin stripes include both dark and bright regions in V2 in RG-Lum_HV difference maps, because thin stripes are involved in the overall processing of the surface properties of objects and contain both representation of color and achromatic luminance change (Wang et al., 2007).

(5) V2 pale and thick stripes are areas with orientation preferences, and are identified as both bright and dark regions in H-V_RGLum maps.

(6) In V4, we identified color and luminance processing regions as the dark and bright regions in RG-Lum_HV difference maps, and orientation processing regions as the dark and bright regions in H-V_RGLum maps.

Data from all three behavioral modes were added together for this analysis. RG-Lum_HV difference map was calculated by formula (H_RG + V_RG) – (H_Lum + V_Lum); and H-V_RGLum map was calculated using formula (H_RG + H_Lum) – (V_RG + V_Lum).

Note in V4, color-luminance processing regions and orientation processing regions are separate, this suggests contour and surface information are processing separately in V4. It has been discussed in Tanigawa et al., 2007.

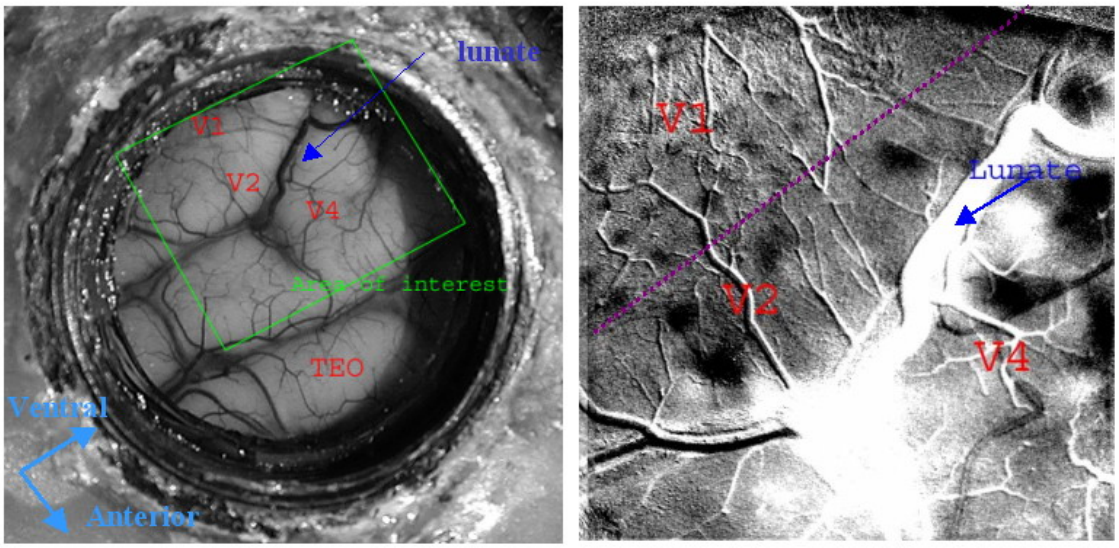

Figure 11. Identify V1, V2 and V4 in the chamber. Left: the optical chamber over visual cortex. The lunate sulcus is buried under the big vessel and constitutes the border between V2 and V4. Right: The difference map obtained by imaging for isoluminant red-green vs. achromatic luminance grating preference (average from 48 blocks). V1 is characterized by color-preferring blobs and V2 is characterized by larger color-preferring domains within thin stripes. A dashed purple line denotes the border between V1 and V2. The upper is ventral and the lower is dorsal. Clipping: 1.5 SD.

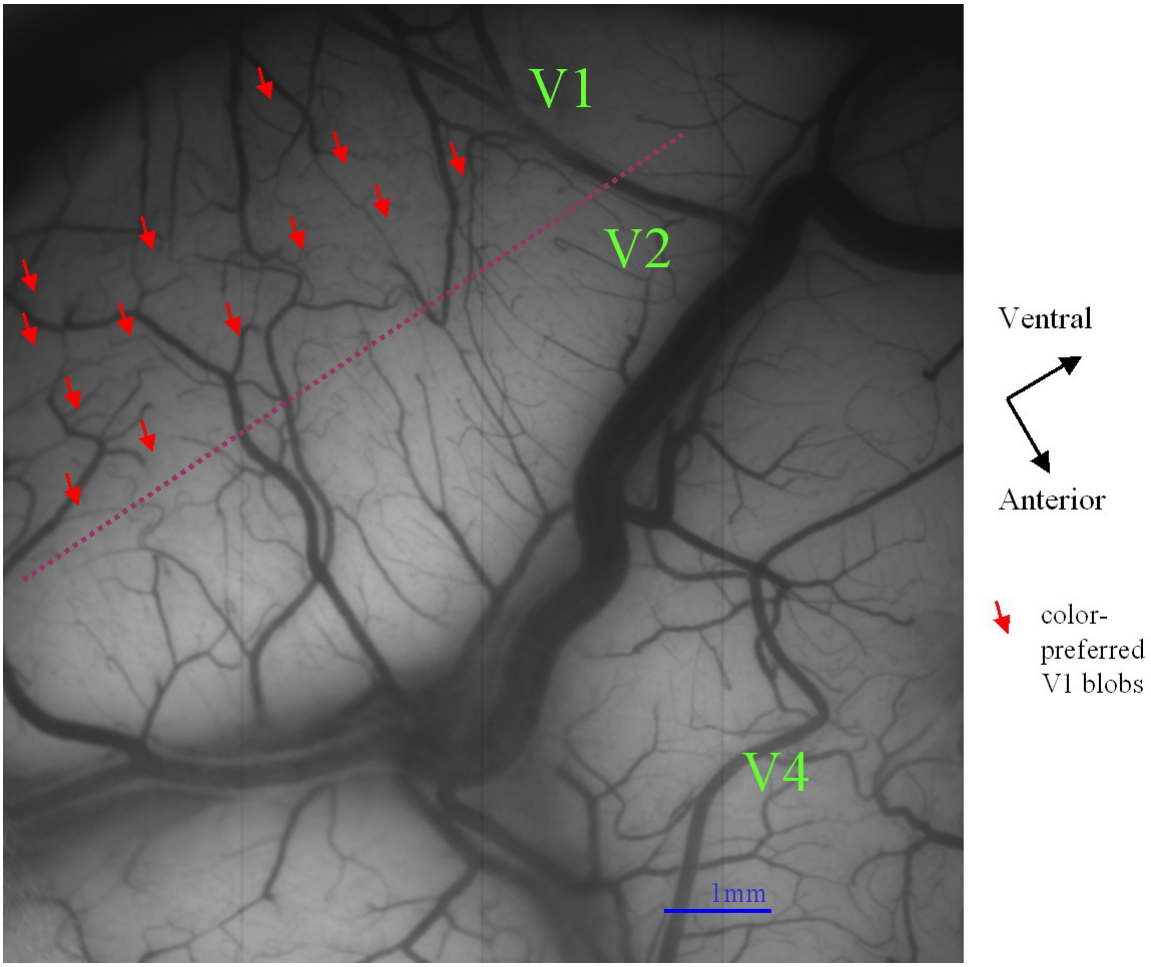

Figure 12. Map presenting V1 blobs. V1 blobs are dark regions in RG-Lum_HV maps. Results are obtained together from data of 0104 and 0111, average of 50 and 48 blocks. We use arrows but not boundaries to label because the boundaries among domains are obscure and hard to define.

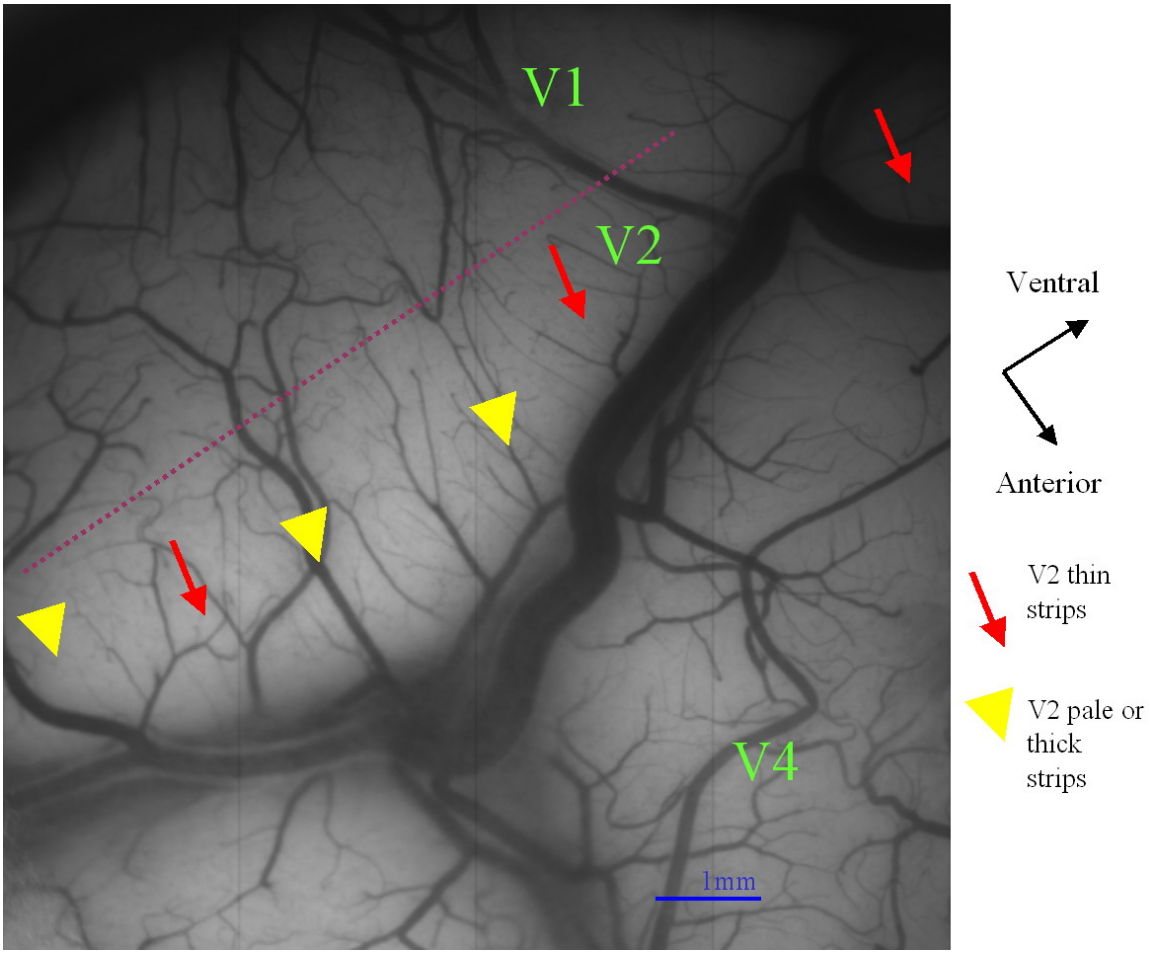

Figure 13. Map presenting V2 thin stripes and pale or thick stripes. V2 thin stripes are dark and bright regions in RG-Lum_HV maps, and V2 pale and thick stripes are dark and bright regions in H-V_RGLum maps. Results are obtained together from data of 0104 and 0111, average of 50 and 48 blocks. Arrows point to estimated centers of the stripes, and the areas near the arrows are still the corresponding stripes.

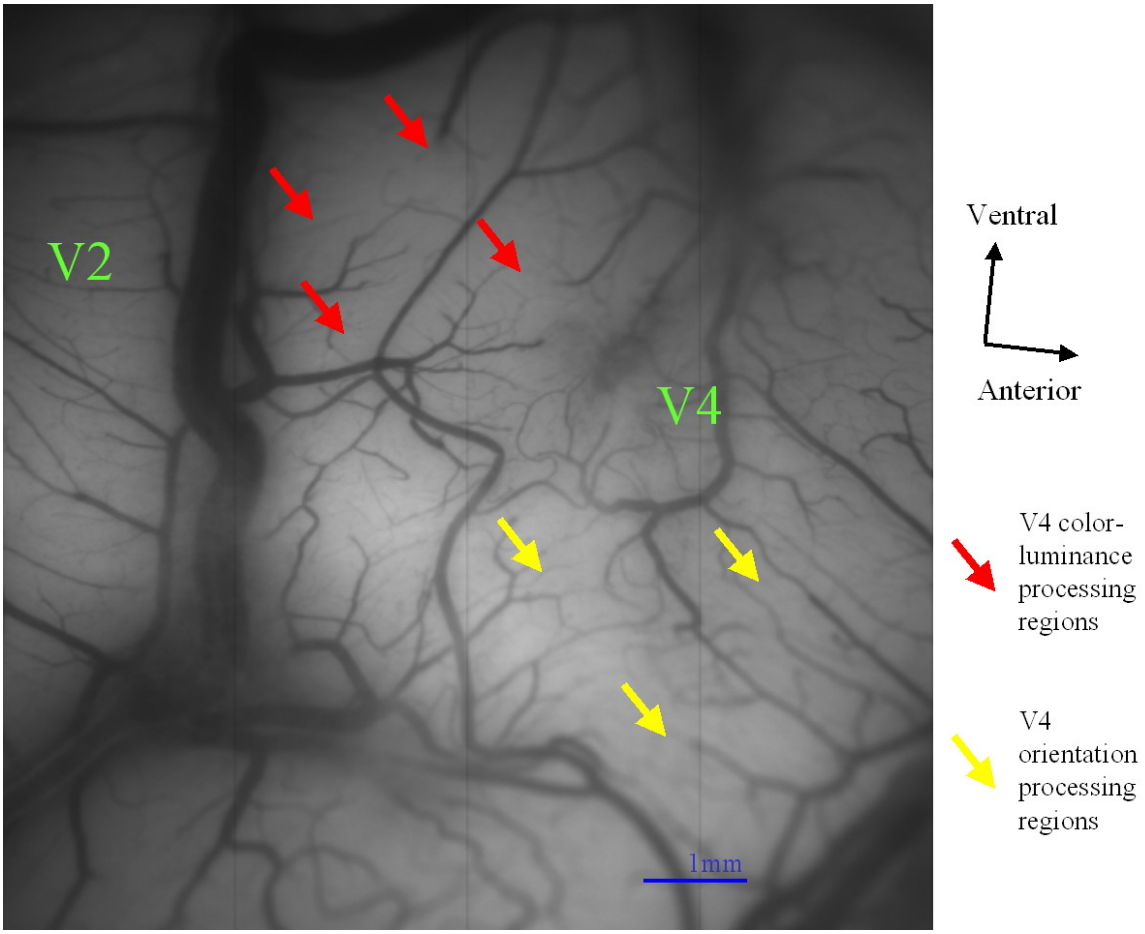

Figure 14. Map presenting V4 surface information (color-luminance) processing and contour information (orientation) processing regions. Results are obtained from data of 0220, average of 62 blocks. Data of 0215 (67 blocks), 0331 (68 blocks), and 0401 (60 blocks) are also taken into consideration.

4. Defining Sample Regions:

General Introduction of Nine Regions: We defined sample regions in V1, V2, and V4 for analysis. V1, V2 and V4 are localized by blob and stripe distribution, and the spatial relation to lunate sulcus (Already explained in Result Section, Part 3 and Figure 11). Since cortical activation under free viewing and saccade paradigms may have different effects on foveal and extra-foveal regions, we divided V1, V2, V4 into sub-

regions of different eccentricity. Nine sample regions were selected: V1 fovea (<2°), V1 extra-fovea (>2°), V2 fovea (<2°), V2 extra-fovea (>2°), V4 fovea (<2°), V4 para-fovea (2°-6°), V4 extra-fovea (>6°). In addition, to examine whether free viewing or saccades have different effects on specific functional domains, we also selected regions overlying color domains and luminance domains in the foveal region of V4.

To define these regions, we relied on our retinotopic mapping results (Tanigawa et al 2007) and functional mapping results. Table 3 below shows the methods for defining sample regions, and it also shows the dates when each region was imaged. Note that all the sample regions were as big as possible to minimize the noise, in prerequisite of avoiding major blood vessels. Maps obtained on different days were aligned to ensure the same sample regions were selected.

Table 3. Sample Regions for Time Course Analysis. 'Fovea' means regions representing areas 0º-2º from the center, V1 and V2 extra-fovea: >2º, V4 para-fovea: 2º-5º, V4 extra-fovea: >6º. Three methods were used to define sample regions: functional domain mapping; 'retinotopic mapping (Tanigawa et al., 2007); 'other' means not only using results from this project and related retinotopic mapping, but also others' publications.

Sample Region	Experiment days that have results of this region	Methods to define this region
V1 fovea	0104, 0111, 0331, 0401	other
V1 extra-fovea	0104, 0111, 0331, 0401	other
V2 fovea	0104, 0111, 0215, 0220, 0331, 0401	subtraction map
V2 extra-fovea	0104, 0111, 0215, 0220, 0331, 0401	other
V4 fovea	0104, 0111, 0220, 0331, 0401	retinotopy mapping
V4 para-fovea	0104, 0111, 0215, 0220, 0331, 0401	retinotopy mapping
V4 extra-fovea	0104, 0111, 0215, 0220, 0331, 0401	retinotopy mapping
V4 fovea color domain	0104, 0111, 0220, 0331, 0401	subtraction map
V4 fovea luminance domain	0104, 0111, 0220, 0331, 0401	subtraction map

V1 and V2 Fovea: We defined V2 fovea by retinotopic mapping and inferred V1 fovea by reflection across the V1/V2 border (Newsome et al., 1986; Yang et al., 2007).

On date 0111, instead of saccade mode, we used a second 'fixation' mode replacing the light blue fixation dot with a white fixation dot. With this mode, we first intended to investigate the effect of fixation dot, but didn't find any interesting effect. However, comparison of these two fixation modes with the same background can help us identify visual cortical region corresponding fovea region ('fovea area' for short in the following paragraphs). When the monkey keeps fixation, a fixation dot on a gray background (blank condition) activates the fovea, therefore, difference map by comparing two blank conditions with these two different fixation dots would show fovea regions. The two fixation dots have different color and luminance. Be clearer, difference map calculating "blank screen_white fixation dot – blank screen_blue fixation dot" could show fovea regions. This difference map is shown on the right part of Figure 15. For some unknown reason there was no clear difference in V1, but a dark region in V2 certainly denoted V2 region corresponding to fovea. This fovea region was confirmed by Tanigawa et al.'s retinotopy results (Figure 16). Note, though these two methods both used color stimuli, it doesn't mean the dark region is only thin stripe, but it also contains pale or thick stripe (see Figure 21).

As described above, V1 fovea should be the region adjacent to V2 fovea right across V1/V2 border, which is the golden box area in Figure 6.

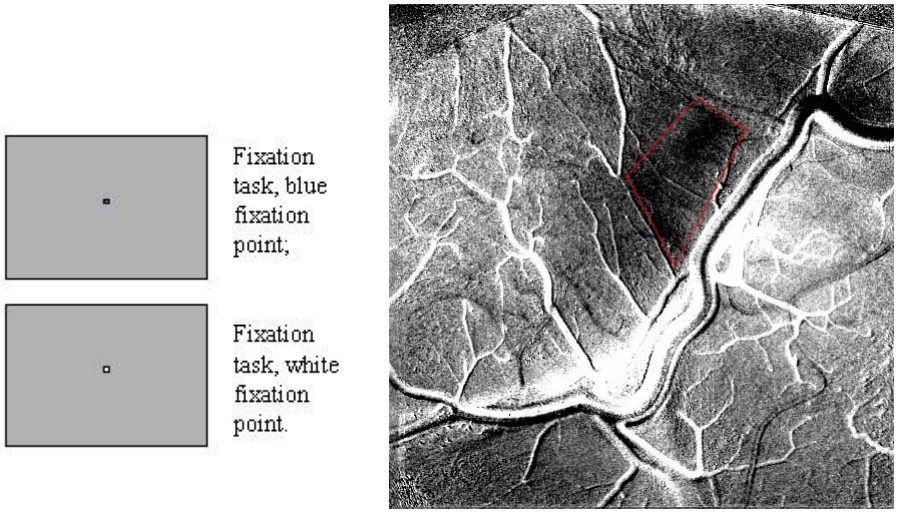

Figure 15. Fixation dot activation and V2 fovea region. Left: two fixation tasks with different fixation dots, one fixation dot is light blue, and the other is white; the background is a gray screen. Right: the difference map resulting from subtracting blue fixation point condition from white fixation point condition. The dark region (within the red box) in V2 represents visual fovea. Clipping to 1.5 SD was applied for the map on the right and no filtering was applied.

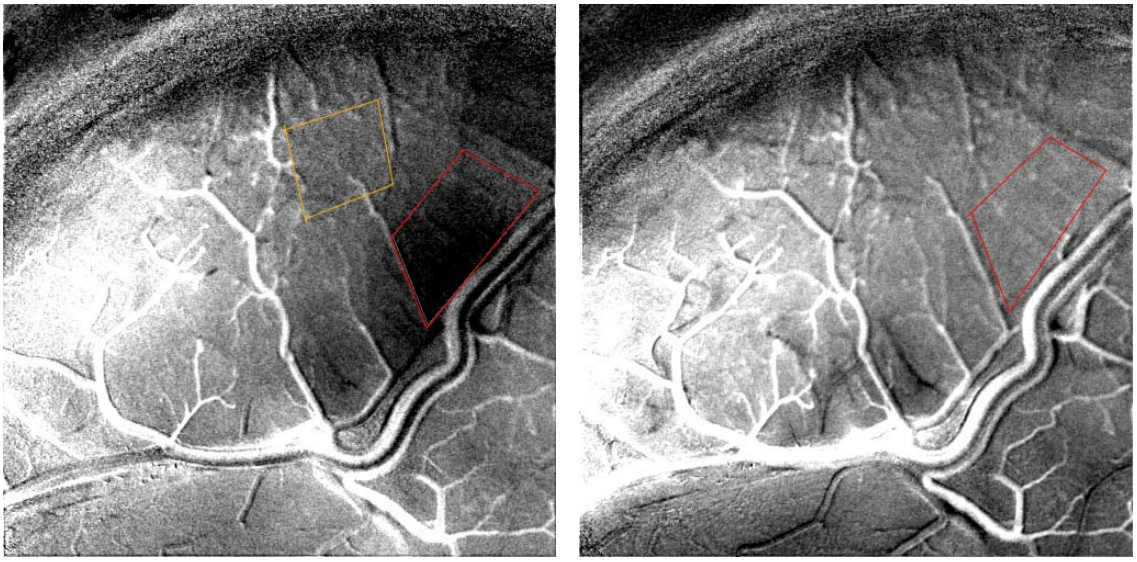

Figure 16. Retinotopic results and V1, V2 fovea regions. Left: Difference map obtained from subtracting entire screen black-white grating activation from activation result of a black-white grating ring with 0.25° diameter. The orientations of the gratings are the same: both random orientations. The dark region (red box) is V2 fovea, and it matches the result in Fig2. The golden box should be V1 fovea, which is adjacent to V2 foveal region across V1/V2 borderline. Right: Difference map obtained from subtracting entire

screen black-white grating activation from activation result of a black-white grating ring with 0.50° diameter. No clear activation is seen in the red box region, which indicates this region represents center fovea of very small eccentricities. Clipping to 1.5 SD was applied for these maps but no filtering was applied.

V1 and V2 Extra-Fovea: The coarse retinotopy in V1 and V2 of the macaque V1 and V2 is well established (e.g., Daniel & Witteridge 1961; Tootell et al., 1988; Van Essen et al., 1984). As one moves outward from the fovea toward the periphery along the vertical meridian, the cortical representation shifts from lateral to medial along the V1/V2 border. So here if we found an area a few millimeter dorsal to V1 or V2 fovea right along the V1/V2 border, it must represent lower peripheral visual field. With this method, we defined V1 and V2 extra-fovea regions in Figure 17. The areas were made as large as possible in prerequisite of avoiding big blood vessels.

V4 Fovea: Different from V1&V2, V4 retinotopy was described clearly by Tanigawa et al.'s results. This V4 area seen in our chamber is V4d. Figure 18 includes seven RG-Lum (Ring) difference maps (the meaning of 'RG-Lum' has already be explained above). In these maps, dark and bright regions are color-preferred and luminance-preferred regions respectively, and they both correspond to the eccentricity of the rings. The active regions induced by ring from 0.25 deg to 1.5 deg eccentricities, both dark and bright, were all located within the red box, so this region was defined V4 fovea.

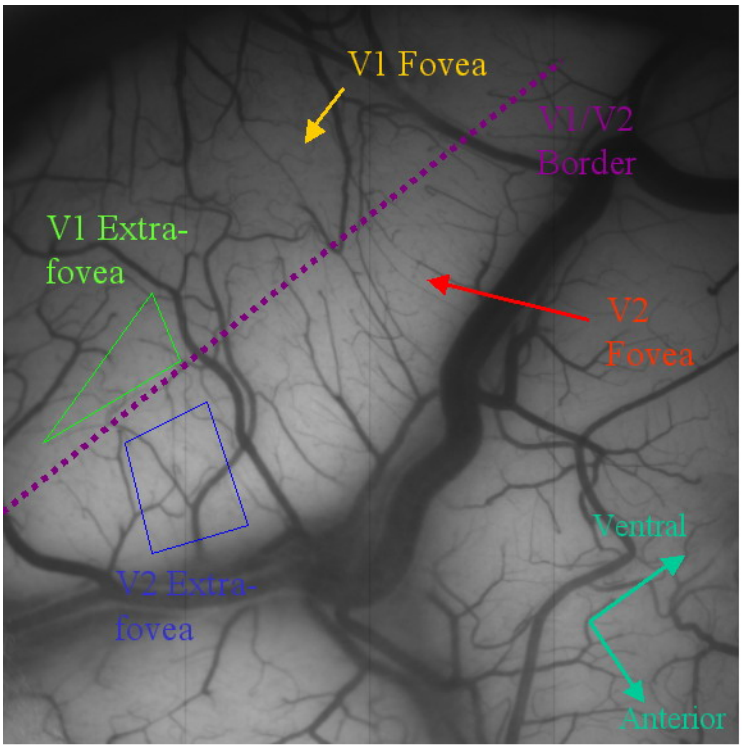

*Figure 17. V1 and V2 extra-fovea sample regions, which are a few millimeters dorsal to V1 and V2 fovea along the V1/V2 border. The scale of this entire map is 8 mm*8 mm.*

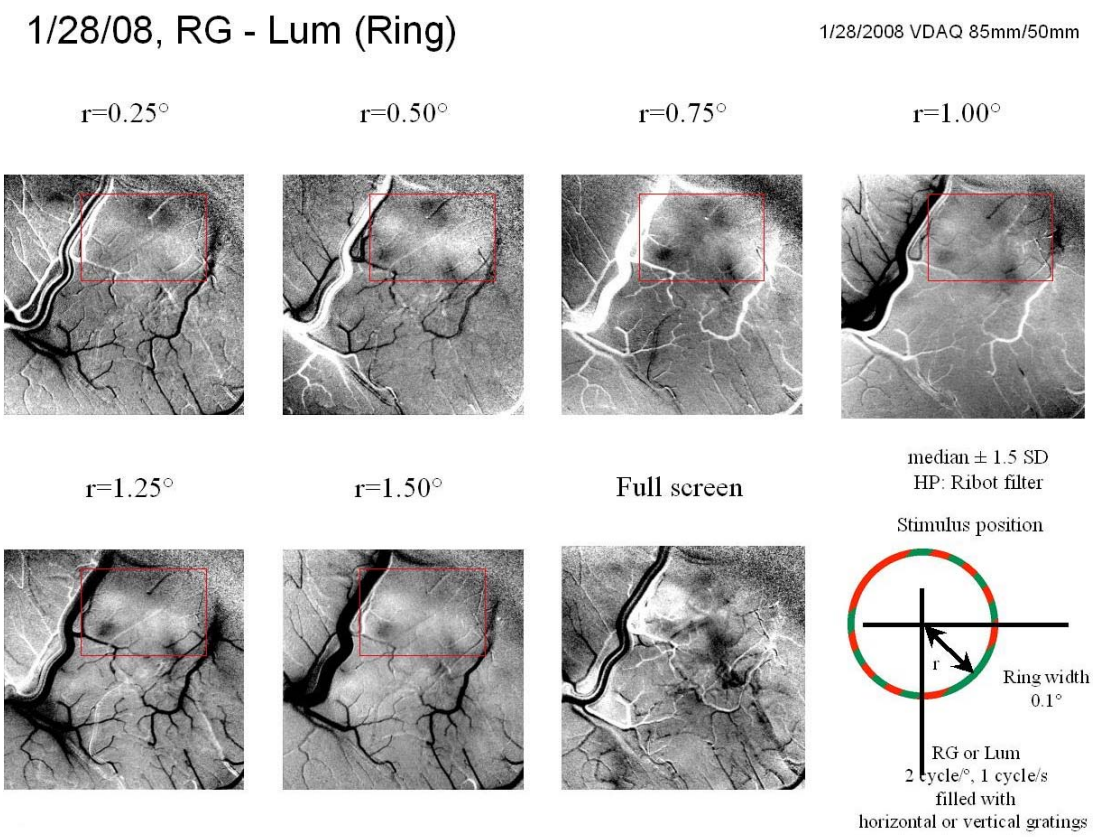

*Figure 18. RG-Lum difference maps obtained from ring paradigm (eccentricity: 0.25-1.50 degree). The red boxes denote the active regions responding to the ring stimulus. Each map was calculated using a ring pattern of a certain eccentricity. Ring width was 0.1 degree. The ring consisted of gratings, either red-green or black-white, and either horizontal or vertical orientated. Temporal frequency of the drifting was 1 cycle/s, and spatial frequency of the grating was 2 cycle/degree. All the maps were clipped to median+-1.5*SD and a high pass Ribot filtering was applied.*

Para- and Extra-Fovea V4: To define extra-foveal V4, we used mapping results from Tanigawa et al. 2007. In Figure 19, the red boxes denote areas responsible for stimuli with eccentricity larger than 6 degree, which was defined as extra-foveal V4. The area within the blue boxes (r=2° & 4°) was activated by the ring of 2-degree eccentricity and slightly activated by 4-degree, so this area was defined para-foveal V4. These retinotopy results are consistent with and extend Gattass et al.'s results (1988) that V4d contained a

coarse but systematic retinotopic map, with fovea in ventral region and extra-fovea in dorsal region.

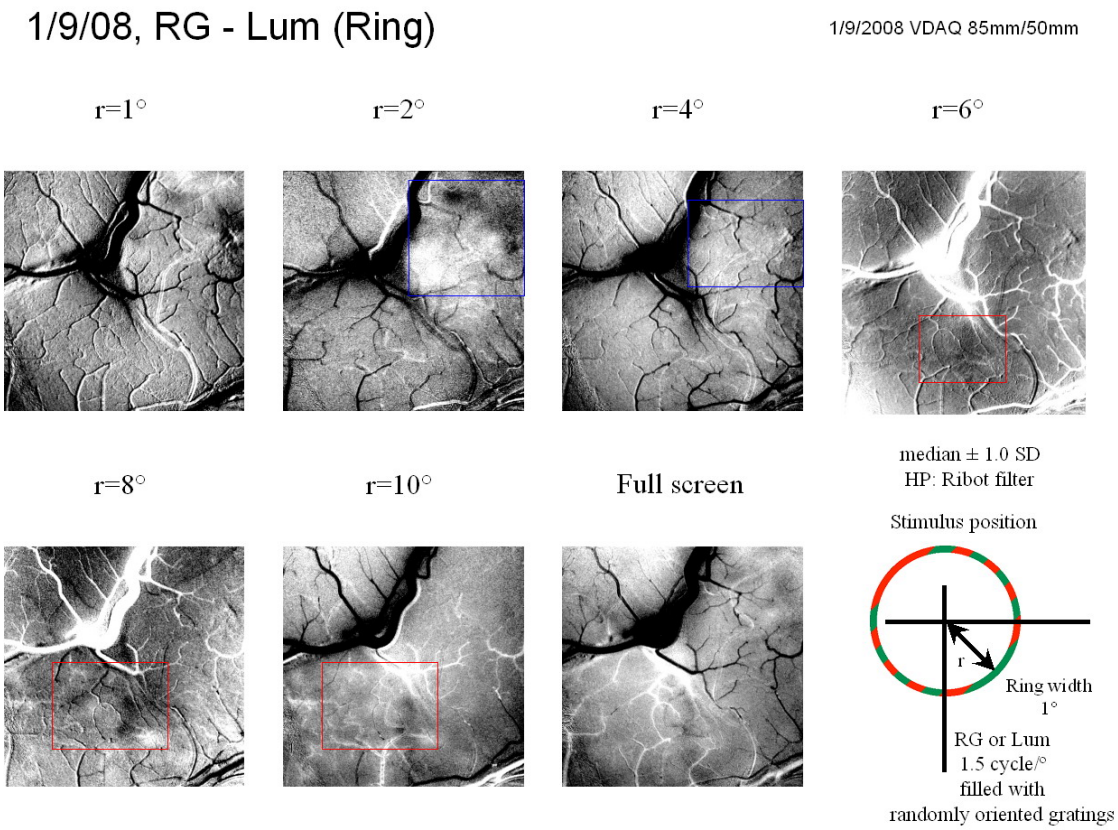

*Figure 19. RG-Lum difference maps obtained from ring paradigm (eccentricity: 1-10 degree). Blue boxes correspond to V4 para-fovea region responsible for 2-4 degree eccentricity and red boxes correspond to V4 extra-fovea region responsible for eccentricity no smaller than 6 degrees. Each map was calculated using a ring pattern of a certain eccentricity. Ring width was 1 degree. The ring consisted of gratings, either red-green or black-white, and either horizontal or vertical orientated. Temporal frequency of the drifting was 1 cycle/s, and spatial frequency of the grating was 1.5 cycle/degree. All the maps were clipped to median+-1.0*SD and a high pass Ribot filtering was applied.*

Summary of Seven Regions: Based on all the analysis above, we selected seven sample regions denoted in Figure 20. Every green or red map has a size of 8mm*8mm.

Each region also excluded large blood vessels, and the size was sufficiently large to ensure a high signal-to-noise ratio, and was included in the field of view on almost all of the six imaging sessions days (on some days, not all these seven regions were imaged).

Figure 21 shows the spatial relation between V1, V2, V4 domains and the seven sample regions. V1 fovea and extra-fovea both contain blobs and interblobs, and V2 fovea and extra-fovea both contain thin stripes and pale or thick stripes. V4 fovea sample region is color-luminance selective, and V4 para-fovea region contains some orientation selective domains, but they two also include some regions among the arrows, and the functional selectivity of these "inter-arrow regions" is not clear. The functional architecture of V4 extra-fovea is also unclear. We made all these seven regions large enough so they all include more than one functional specific domain because we didn't intend to emphasize the effect of functional architecture in time course analysis in these seven regions. The purpose was to simplify the analysis and first focus on the general effect of free viewing and saccades. We mainly investigated the effect of domains in analysis of the other two sample regions: V4 fovea color domain and luminance domain.

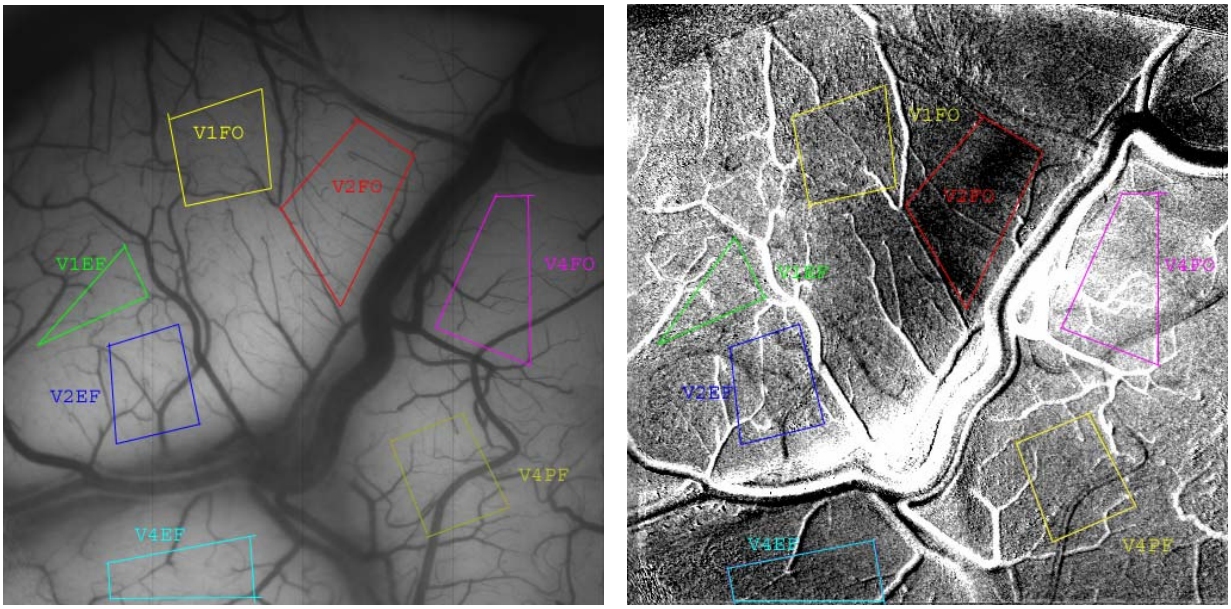

Figure 20. Sample regions for time course analysis, shown in blood vessel map (left) and difference map (right). Blood vessels with in V2EF, V4FO and V4PF won't affect the accuracy and reliability of the analysis because most of them are arteries, located deeply inside the cortex. V1FO: V1 fovea; V1EF: V1 extra-fovea; V2FO: V2 fovea; V2EF: V2 extra-fovea; V4FO: V4 fovea; V4PF: V4 para-fovea; V4EF: V4 extra-fovea.

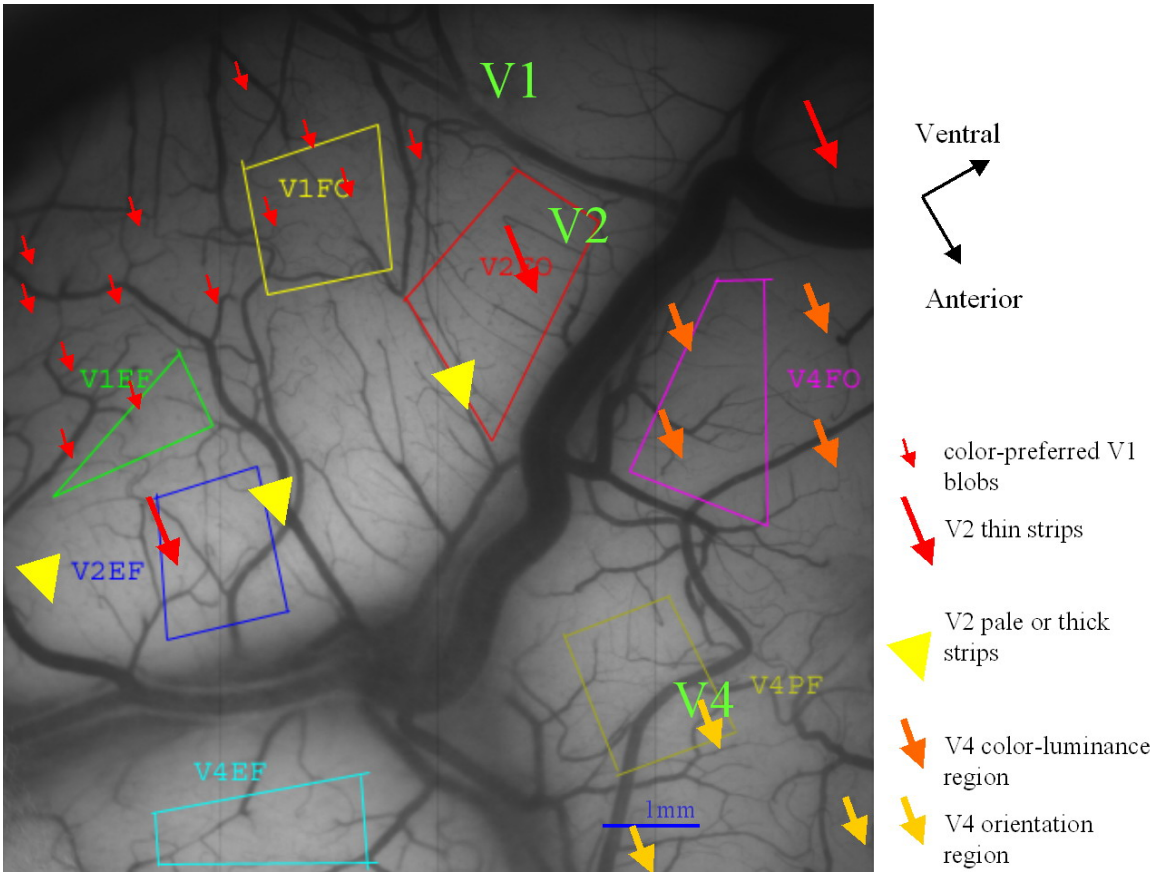

Figure 21. V1 blobs, V2 stripes, V4 domains, and seven sample regions. Terms: V1FO: V1 fovea; V1EF: V1 extra-fovea; V2FO: V2 fovea; V2EF: V2 extra-fovea; V4FO: V4 fovea; V4PF: V4 para-fovea; V4EF: V4 extra-fovea.

V4 Color and Luminance Domains: Color and luminance-preference sample regions were selected in V4 fovea based on our difference maps. Though we had intended to select similar regions in para-foveal and extra-foveal V4, we failed to confidently define color and luminance domains there. Sample regions of two V4 foveal functional domains are shown below. These regions are consistent with Tanigawa et al. 2008.

The reason to focus on V4 fovea is that (1) fovea is very important in attention and saccades: fovea is the locus of overt attention, and saccades bring objects under covert attention into fovea. (2) V4 is a crucial area in ventral pathway and affected by attention. (3) Color and Luminance Stimuli have already been reported to show difference in effect

of saccades (e.g. Burr et al., 1994), and color and luminance domain in V4 fovea has been clearly identified.

Here our nine sample regions have all been introduced.

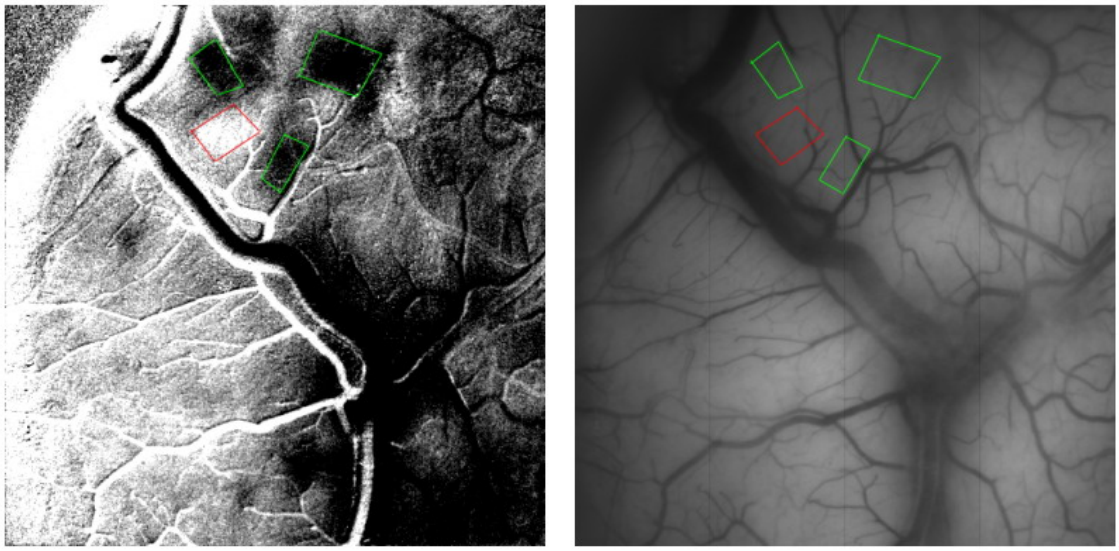

Figure 22. V4 fovea color and luminance domains. Left: subtraction map obtained from subtracting sum of activation by horizontal and vertical RG gratings from sum of activation by horizontal and vertical BW gratings, under free viewing circumstance. Clear results are seen in V4 fovea region, dark regions are responsible for processing color information while light regions for luminance information. Right: Color domain and luminance domain in V4 fovea region, shown in vessel map.

5. Overall Effects of the Three Behavioral Modes:

With time course analysis and statistics, the first question we want to ask is: would free viewing generally enhance or suppress the visual cortex? If there were any effect, would this differ among V1, V2 and V4? How about saccades?

In order to investigate effects of free viewing and guided saccades, activation results (dR/R) of the four gratings were summed under each of the three behavioral modes. The

four grating types are: isoluminant red-green grating, horizontal orientation; isoluminant red-green grating, vertical orientation; black-white (100% contrast achromatic) grating, horizontal orientation; black-white grating, vertical orientation. Four examples of time course curves are shown in Figure 23. In each case (region*date), there are at least 33, and as many as 68 successful trials under any single grating condition. After summing the four gratings, at least 151 successful trials were analyzed under any behavioral mode in each case.

Typical frames were selected individually for each case to conduct ANOVA and multiple comparisons. The criteria of frame selection are explained in Method section, Part 3. Following these criteria, the selection was somewhat arbitrary but still systematic. All comparisons with significant difference are listed in Table 4. Note a special case (V4EF on 0215) deserves special attention. In this case, fix2 activation was first greater and later smaller than fix1 & fix3, and with this data ANOVA would show no significant difference when averaging across the entire imaging period. The best description should be that activation under free viewing arrived faster but lasted shorter.

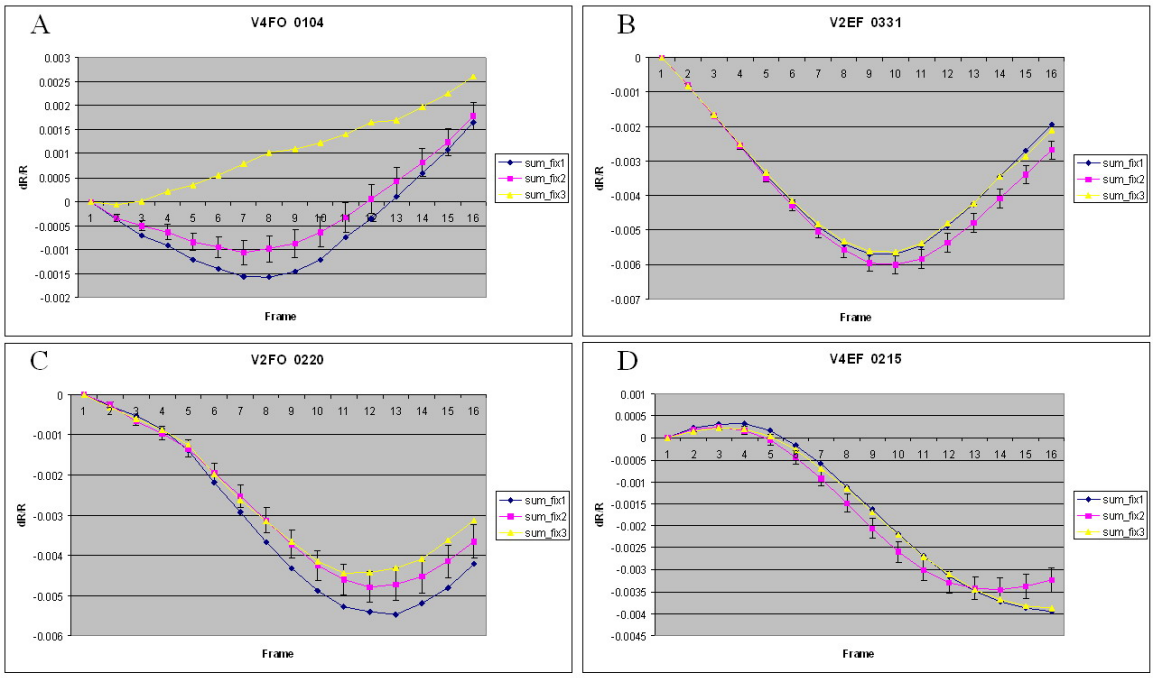

Figure 23. Examples of time courses, three behavioral modes. An initial dip is generally considered to indicate activation and is the focus of optical imaging study. Shapes in A, B and C are more common while D is an example that comparison between fix2 and fix1&fix3 shift after the 13th frame. Terms: fix1: fixation mode; fix2: free viewing mode; fix3: saccade mode; FO: fovea; EF: extra-fovea. Every point has a sample size of at least 151.

Table 4. Comparisons of sum of activation by all grating stimuli under three behavioral modes. Typical frames were selected, and ANOVA and LSD multiple comparisons were conducted. Post-hoc multi-comparisons were done under the prerequisite of significant ANOVA effect. Multi-comparison results are listed for all cases, those inequations in italic style approach significance (0.05<p<0.1), others present significant difference (p<0.05). "-" means no data: this region was recorded on that specific day. "=" means no significant difference. There were only fix1&fix2 on 0111, so a t-test was conducted. "0215 V4PF" in Table A is complicated and would be discussed in the paragraphs. Terms: fix1: fixation mode; fix2: free viewing mode; fix3: saccade mode.

Table A. fix1 vs. fix2						
Region\Date	0104	0111	0215	0220	0331	0401
V1 fovea	fix1>fix2	fix1>fix2	-	-	=	=
V1 extra-fovea	fix1>fix2	*fix1>fix2*	-	-	fix1<fix2	=
V2 fovea	fix1>fix2	fix1>fix2	=	fix1>fix2	=	=
V2 extra-fovea	fix1>fix2	*fix1>fix2*	fix1<fix2	fix1>fix2	fix1<fix2	=
V4 fovea	fix1>fix2	*fix1>fix2*	-	fix1>fix2	fix1<fix2	fix1<fix2
V4 para-fovea	fix1>fix2	fix1>fix2	fix1<fix2	=	fix1<fix2	fix1<fix2
V4 extra-fovea	fix1>fix2	=	complicated	=	*fix1<fix2*	fix1<fix2

Table B. fix2 vs. fix3					
Region\Date	0104	0215	0220	0331	0401
V1 fovea	fix2>fix3	-	-	=	=
V1 extra-fovea	fix2>fix3	-	-	fix2>fix3	=
V2 fovea	fix2>fix3	=	=	fix2>fix3	=
V2 extra-fovea	fix2>fix3	fix2>fix3	=	fix2>fix3	=
V4 fovea	fix2>fix3	-	=	=	=
V4 para-fovea	=	fix2>fix3	=	fix2>fix3	fix2>fix3
V4 extra-fovea	=	complicated	fix2>fix3	*fix2>fix3*	fix2>fix3

Table C. fix1 vs. fix3					
Region\Date	0104	0215	0220	0331	0401
V1 fovea	fix1>fix3	-	-	=	=
V1 extra-fovea	fix1>fix3	-	-	=	=
V2 fovea	fix1>fix3	=	fix1>fix3	=	=
V2 extra-fovea	fix1>fix3	=	fix1>fix3	=	=
V4 fovea	fix1>fix3	-	fix1>fix3	=	=
V4 para-fovea	=	=	=	=	=
V4 extra-fovea	fix1>fix3	=	fix1>fix3	=	=

From Table 4, we make three observations.

(1) Although relations among cortical activity magnitude under the three behavioral modes varied across different dates, they were consistent or at least not contradictory (no opposite result) on each date no matter which region was investigated. It doesn't differ much among V1, V2 and V4, and among fovea and extra-fovea regions. For example, on 0104, cortical activity was significantly stronger in fixation than in free viewing in all seven regions, significantly stronger in fixation than in saccades in six out of seven regions, and significantly stronger in free viewing than in saccades in six out of seven regions. This suggests that free viewing or saccades caused widespread effect over a large part of visual cortex.

(2) Activation under saccade mode (fix3) was the smallest. It was never greater than activation under fixation (fix1) and free viewing (fix2). Comparing fix2 and fix3, in 14 out of 30 cases ('case' means date*region), the cortical activity was significantly weaker in fix3 than in fix2; in 1 out of 30 cases, activity was weaker in fix3 than fix2, and it was approach significance; in other 14 out of 30 cases, activity was not significantly different. While comparing fix1 and fix3, in 10 out of 30 cases, activity was significantly weaker in fix3 than in fix1, and in other 20 out of 30 cases, they were not significantly different. This suggests that saccades have a general suppression effect on visual cortex, which has already been revealed by a lot of studies (Kleiser et al. 2004; Thiele et al, 2002; Vallines & Greenlee, 2006). This result is also consistent with psychophysics result that saccades decrease visual sensitivity (Burr et al, 1982, 1994; Campbell & Wurtz, 1978).

(3) The effect of free viewing was not consistent across days. Cortical activity was greater under free viewing compared with fixation on three days (10 out of 18 cases

showed significance or approach significance), but weaker on the other three days (16 out of 19 cases showed significance or approach significance), so it becomes very hard to make a clear conclusion about free viewing effect.

6. Modulation Effects of Saccades under Various Stimulus Conditions:

Many studies, both psychophysical and neurophysiological, indicate that saccadic suppression exhibits selectivity. It tends to suppress motion perception more (Thiele et al., 2004; Burr et al, 1982; Ilg & Hoffman, 1993; Shiori & Cavanagh, 1989) and to suppress the magnocellular pathway more so than the parvocellular pathway (Kleiser et al. 2004; Burr et al., 1994). We wondered whether optical imaging results show any similar selectivity with respect to saccadic suppression, especially between color and luminance signals, which were red-green (isoluminant) and black-white (100% achromatic) gratings in our experiments. To further study how stimulus condition and areas or domains affect these effects, two modulation indexes were calculated. Modulation index 1 (MI1) is the ratio of activation under saccade mode to that under fixation (fix3/fix1), and modulation index 2 (MI2) is the ratio of activation under saccade to that under free viewing (fix3/fix2). Modulation indices of less than 1 would be consistent with the presence of saccade suppression. The smaller MI is, the greater saccadic suppression is.

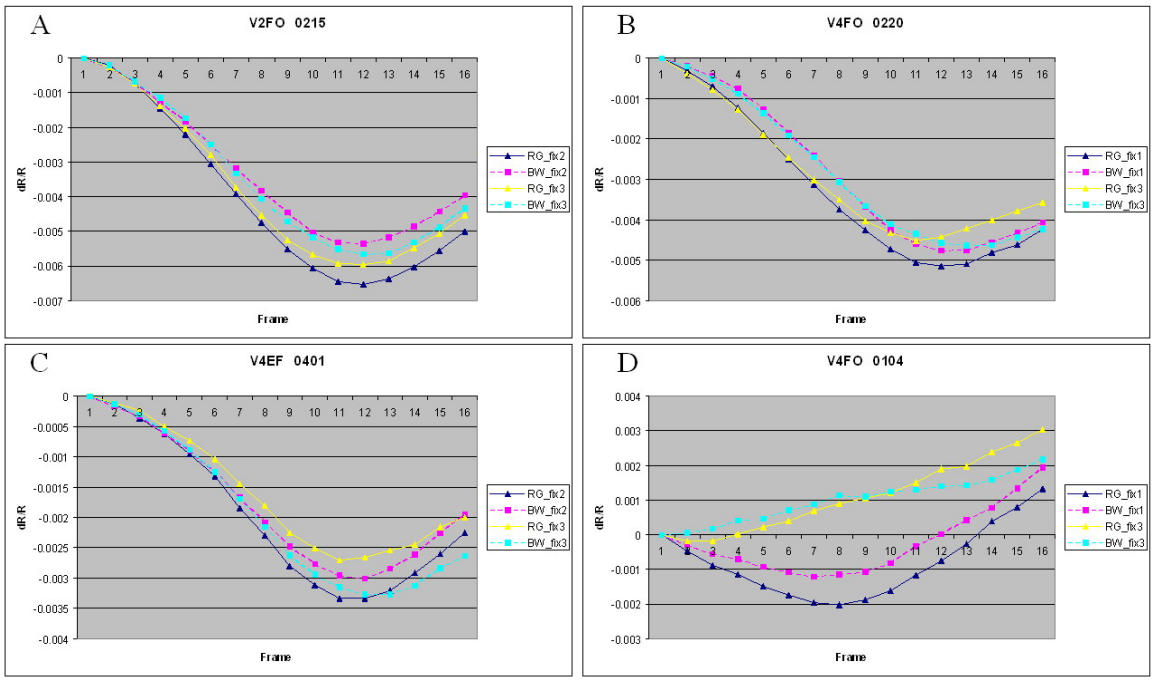

Figure 24. Examples of activation by RG and BW stimuli under three behavioral modes. RG stimuli tended to activate the cortex less under saccade condition compared with fixation or free viewing conditions, while BW stimuli showed the opposite effect. In A&C, fix2 and fix3 are showed, and in B&D, fix1 and fix3 are showed. D is an example from 0104, in which fix3 values were mostly positive, causing MI to be negative and very hard to interpret. However, saccades still showed greater suppression under RG stimulation versus BW stimulation. Every point has a sample size of more than 80.

We intended to investigate whether saccadic suppression has any selectivity between color and achromatic stimuli. Figure 24 presents four examples of time course data of activation by red-green and black-white gratings. MI1 and MI2 for red-green stimuli and black-white stimuli were then calculated and compared to study how color and luminance processing relate to the effects of saccades. Analysis of both MI1 and MI2 were done for each case no matter whether previous ANOVA showed significant effect or not. We intended to dig out something ANOVA might conceal. Comparisons were done only for 0215, 0220, 0331 and 0401, but not 0104 and 0111: on 0111, there was no saccade mode

(fix3); on 0104, dR/R values for fix3 were mostly positive, which caused both MI1 and MI2 to be negative and therefore hard to interpret.

Figure 25 presents four examples of modulation index time course. At many time frames, modulation indices are not significantly different for red-green gratings and black-white gratings, however after combining several frame into one set, the two indices can probably be significantly different. B of Figure 23 is a good example. To do statistical test, MI values of these frames should be averaged and the new corresponding standard deviation should be calculated, then a z-test can be performed. Take V2FO 0215 (Figure 25) as an example, this 'combining' method is quite reliable because MI(BW) is consistently larger than MI(RG) across frame 6-16, though not significant. There is no contradictory result. By combining several frames together, we didn't conceal any important information but simply increased statistical sample size and so increased signal-to-noise ratio. Therefore, the significant difference could be revealed. The selected frames for each case are exactly the same frames used in previous ANOVA analysis, in Result Section, Part 5.

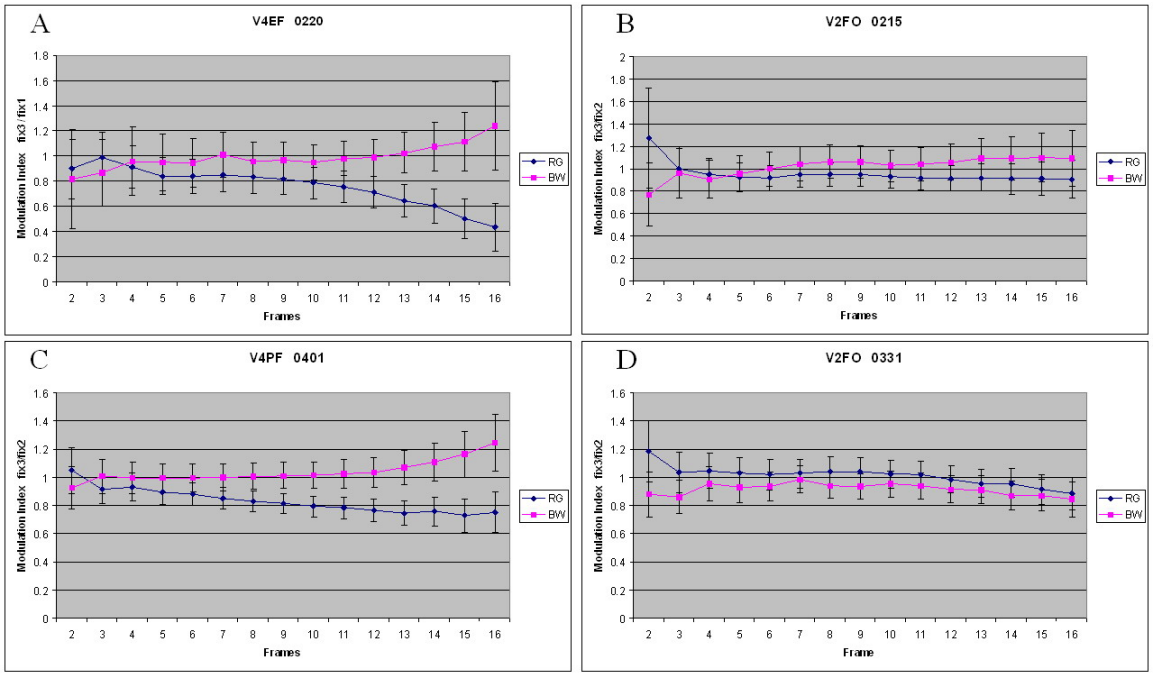

Figure 25. Four examples of modulation index time course. A presents modulation index 1 (fix3/fix1) while B, C, D present modulation index 2 (fix3/fix2). A, B, C are example with significant difference. Though the error bars overlap at all time points in B, significant difference still exist after averaging MI across several selected frames. D is an example without significant difference.

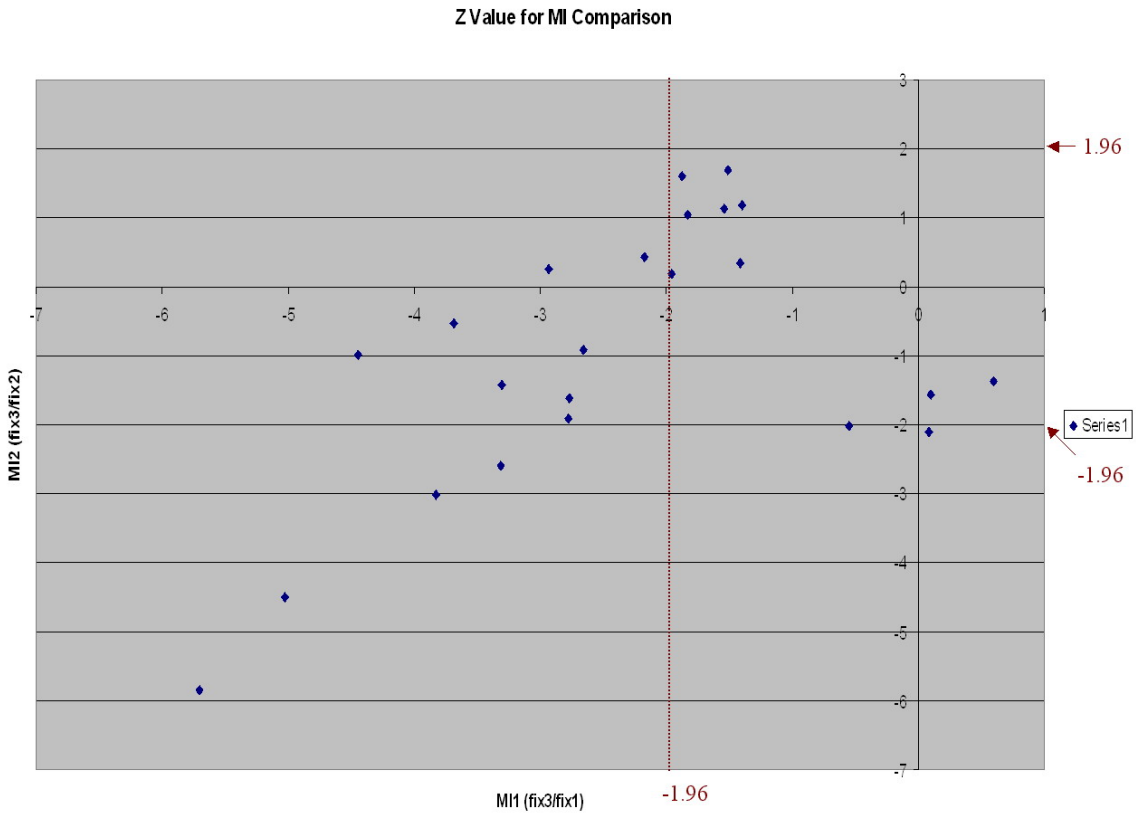

Figure 26. Distribution of z values for MI comparisons (data from 0215, 0220, 0331, 0401). Horizontal axis represents z values obtained from comparison between MI1 (fix3/fix1) of RG and BW gratings and Vertical axis represents z values of MI2 (fix3/fix2) comparisons. z=(mean of MI(RG) – mean of MI(BW))/sqrt(SD of MI(RG).^2 + SD of MI(BW).^2). When z=+-1.96, p=0.05 for two-tailed test.

Figure 26 shows the results of all MI comparisons (RG – BW). Z test was applied for statistical analysis:

z = [Mean MI(RG) – Mean MI(BW)]/sqrt[SD MI(RG)^2 + SD MI(BW)^2].

z>0 means MI(RG)>MI(BW) while z<0 means the opposite. If z<-1.96 or z>1.96, then the difference is significant.

In half of the cases (12/23), MI1 for RG and BW are significantly different, while in one fourth of the cases (6/23), MI2 for the two are significantly different. In some cases, there is no significant saccade suppression for sum of all gratings, but indeed significant

for RG gratings. Note that though MI comparisons are not significant for many cases, all the cases with significant difference show MI(RG)<MI(BW). This indicates that suppression effect of saccade mode is stronger for color stimuli (RG) than for luminance stimuli (BW), this effect is overspread because significant difference appears in V1, V2 and V4. For more details, see Supplemental Table 2.

One question is raised: is this difference more due to difference between RG and BW activation under saccade mode or under other modes? The answer is both. Table 5 lists results of all comparisons that compared cortical activation elicited by color gratings versus by luminance gratings, under the three behavioral modes. Comparisons were only done for cases with significant MI difference between color and luminance stimuli, plus cases from 0104.

Though the answer is both, we see greater contribution by fix1 and fix2 than by fix3. **(1)** Under fixation and free viewing, color gratings activated visual cortex more than luminance gratings did. This effect was significant in almost all cases, across V1, V2 and V4: under fixation, 18 out of 19 analyzed cases showed significance, and the other one was approach significance; under free viewing, 13/13 cases showed significance.

Table 5. Comparison (F test) of activation elicited by RG and BW gratings (sum of horizontal and vertical). Part A lists all cases with significant difference in modulation index 1 comparison between RG and BW stimulus conditions, and Part B modulation index 2. Data from 0104 is also analyzed here. Inequations with "(a)" means approach significant difference (0.05<p<0.1), while other inequations denote comparisons with significant difference (p<0.05). "=" means no and not approach significant difference (p>0.1).

A. Modulation Index 1 (fix3/fix1)		
Case\Comparison	Saccade Mode (fix3)	Fixation Mode (fix1)
0220 V2EF	RG < BW	RG > BW (a)
0220 V4PF	RG < BW (a)	RG > BW
0220 V4EF	RG < BW	RG > BW
0331 V2EF	=	RG > BW
0331 V4PF	=	RG > BW
0331 V4EF	=	RG > BW
0401 V1EF	=	RG > BW
0401 V2FO	=	RG > BW
0401 V2EF	RG < BW (a)	RG > BW
0401 V4FO	RG < BW	RG > BW
0401 V4PF	RG < BW	RG > BW
0401 V4EF	RG < BW	RG > BW
0104 V1FO	=	RG > BW
0104 V1EF	RG < BW (a)	RG > BW
0104 V2FO	=	RG > BW
0104 V2EF	=	RG > BW
0104 V4FO	RG < BW (a)	RG > BW
0104 V4PF	RG < BW	RG > BW
0104 V4EF	RG < BW	RG > BW

B. Modulation Index 2 (fix3/fix2)		
Case\Comparison	Saccade Mode (fix3)	Free Viewing Mode (fix2)
0215 V2FO	=	RG > BW
0215 V2EF	=	RG > BW
0401 V2EF	RG < BW (a)	RG > BW
0401 V4FO	RG < BW	RG > BW
0401 V4PF	RG < BW	RG > BW
0401 V4EF	RG < BW	RG > BW
0104 V1FO	=	RG > BW
0104 V1EF	RG < BW (a)	RG > BW
0104 V2FO	=	RG > BW
0104 V2EF	=	RG > BW
0104 V4FO	RG < BW (a)	RG > BW
0104 V4PF	RG < BW	RG > BW
0104 V4EF	RG < BW	RG > BW

(2) Under saccades, color gratings induced smaller activation than luminance gratings, but significant effect was no so widespread as in fixation or free viewing: 7/21 cases showed significance, 6/21 cases were approach significance, 8/21 showed no significance, and there was no opposite result. Moreover, significant effect only existed in V2EF and V4. These two effects might probably be due to different mechanisms.

Here another question is raised: does this difference also exist between color and luminance domains? Is saccade suppression stronger in color domains than in luminance domains?

7. Modulation Effects of Saccades under Different Domains:

We mainly investigated the effect of domains in analysis of two sample regions: V4 fovea color domain and luminance domain. The reason to focus on V4 fovea is that (1) fovea is very important in attention and saccades: fovea is the locus of overt attention, and saccades bring objects under covert attention into fovea. (2) V4 is a crucial area in ventral pathway and affected by attention.

The answer to the last question in Part 6 is: 'not likely'. We still used modulation index for analysis. We compared modulation indices of V4 color domain and V4 luminance domain. There are four comparisons:

(MI1, V4 color domain, RG gratings) vs. (MI1, V4 luminance domain, RG gratings);

(MI2, V4 color domain, RG gratings) vs. (MI2, V4 luminance domain, RG gratings);

(MI1, V4 color domain, BW gratings) vs. (MI1, V4 luminance domain, BW gratings);

(MI2, V4 color domain, BW gratings) vs. (MI1, V4 luminance domain, BW gratings).

The comparison procedure was the same as in Part 6: combine selected frames into one set, calculate average modulation index and the corresponding standard deviation, do z-test. For each day, the selected frames for both domains are exactly the same ones used in ANOVA analysis of V4 fovea.

Table 6 lists p value of these four comparisons, and modulation index mean values of all these eight conditions listed above. Out of twelve comparisons in total, only one shows significant difference. On 0220, being activated by BW gratings, saccadic suppression (MI2=fix3/fix2) is stronger in V4 color domain than in V4 luminance domain. This one-out-of-twelve possibility is not convincing enough, and may just be caused by some random effect, e.g. noise. Therefore, the selective saccadic suppression, which is greater for color stimuli than for luminance stimuli, is not specialized in any functional domain, but rather overspread across the entire V4 fovea area.

Table 6. Results of modulation index analysis. Analysis compared modulation indices of the two functional domains. Three days' data were analyzed, and for each day, four comparisons were done: (MI1, V4 color domain, RG gratings) vs. (MI1, V4 luminance domain, RG gratings); (MI2, V4 color domain, RG gratings) vs. (MI2, V4 luminance domain, RG gratings); (MI1, V4 color domain, BW gratings) vs. (MI1, V4 luminance domain, BW gratings); (MI2, V4 color domain, BW gratings) vs. (MI1, V4 luminance domain, BW gratings). Only one out of twelve comparisons shows significant difference. P values for z-test, and the MI mean values are listed.

	RG gratings, MI comparison			BW gratings, MI comparison		
		Color Domain	Luminance Domain	P value	Color Domain	Luminance Domain
	P value	MI Mean value	MI Mean value	P value	MI Mean value	MI Mean value
0220 MI1	0.7663	0.8639	0.8453	0.5789	0.9465	0.9914
0331 MI1	0.6677	0.9983	0.9777	0.8975	1.0758	1.0668
0401 MI1	0.9774	0.9596	0.9607	0.9435	1.1306	1.1268
0220 MI2	0.1522	0.9553	0.8530	**0.0099**	**0.7177**	**0.8763**
0331 MI2	0.9790	0.9840	0.9827	0.4356	0.9027	0.9444
0401 MI2	0.8431	0.9272	0.9344	0.9102	1.0486	1.0434

8. Task Structure Related Activity

In the process of analyzing effect of free viewing and saccades, we accidentally found an interesting phenomenon that during the beginning one to two seconds after the stimulus onset, the cortical activity increased but was virtually unaffected by stimulus presentation, and not much affected by saccades.

Unaffected by Stimulus Presence: Figure 27 shows four examples of time course comparison between blank_fix2 (blank screen) and sum_fix2 (sum of all conditions). In every case, blank_fix2 has a sample size of no less than 16, which is small but still enough, while sum_fix2 has always more than 151. Blank_fix2 was a condition in which no stimulus even not a fixation dot was presented: the monkey was given an isoluminant gray screen to freely view. We previously thought it would be a good control and expected the dR/R curves to be flat (e.g. Figure 5 in Chen et al. 2005). However, to our surprise, dR/R curves for blank_fix2 were not flat, and, in fact, the magnitude was comparable to that under stimulation (sum_fix2). This means the cortex was very active even when there was no visual stimulation.

Statistical analysis was applied for comparison between blank_fix2 and sum_fix2. For each case (date & sample region), an overlapping period was selected during which the two dR/R curves were very close, and an one-way ANOVA was applied to test the effect of stimuli. The 'overlapping period' always began from the first frame and typically lasted for at least 1.5 seconds, which was 1 second after stimulus onset (Figure 28) (except 0215, 1.5 sec for 0215). Specialty of experiment procedure on 0215 has been illustrated in Figure 2 and Table 1. Note, since this is a group comparison with two-levels,

one-way ANOVA here has exactly the same effect as t-test (conclusion from SAS Institute).

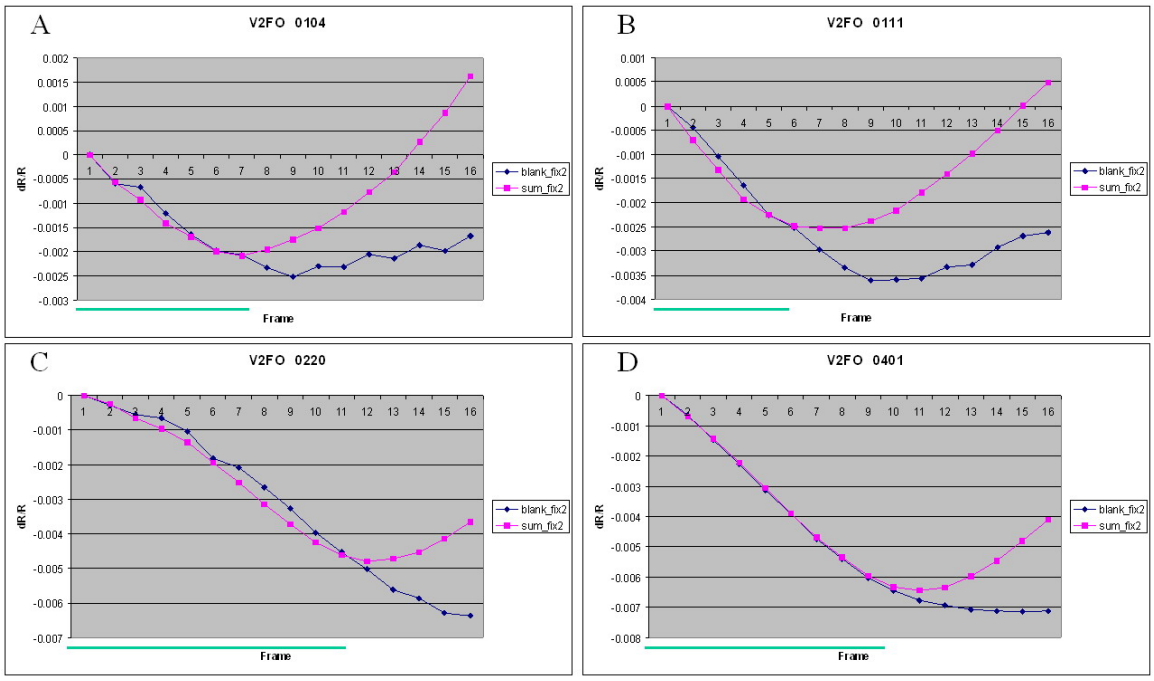

Figure 27. Four examples: time courses of activation by blank_fix2 and sum of gratings under fix2. The beginning parts (indicated by green bars) of blank_fix2 and sum_fix2 overlap, indicating this part of change is not caused by stimuli. Terms: V2FO: V2 foveal region; fix2: free viewing; blank: isoluminant gray screen; sum: summation of activation by all the four gratings.

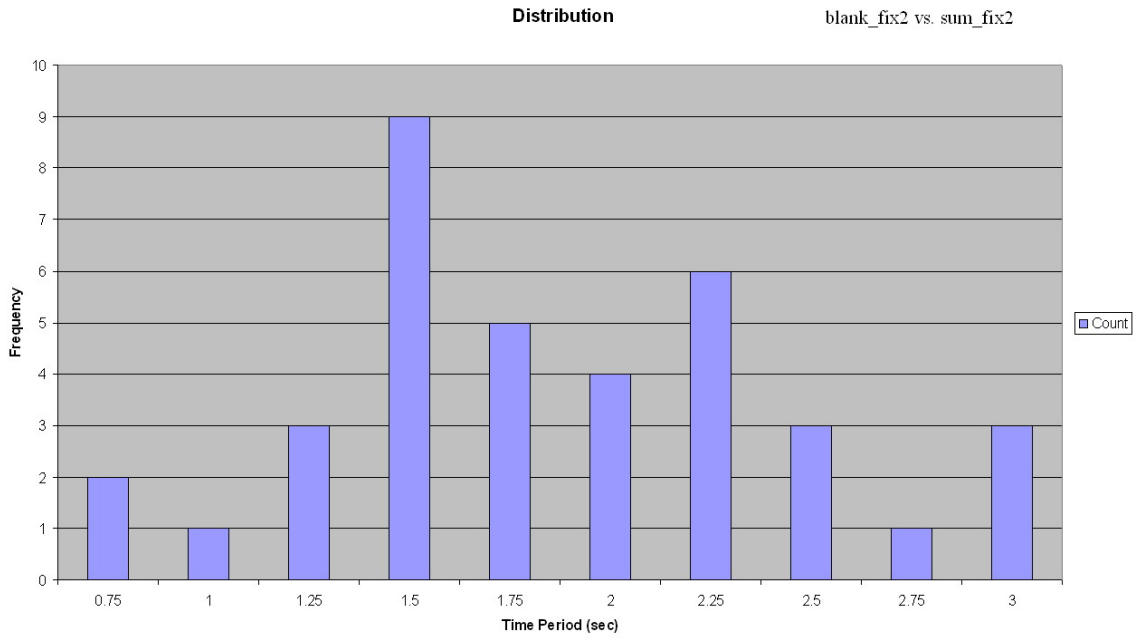

Figure 28. Distribution of the length of 'overlapping periods' selected for comparisons between blank_fix2 and sum_fix2. The mean is 1.87s, and 31/37 of the cases are no shorter than 1.5s.

For each case, a similar ANOVAs were done for a later period, usually the last one to two seconds, and this 'later period' included the frames most possible to have significant difference. Almost all the overlapping periods failed to show significant difference between blank_fix2 and sum_fix2, and most of the later periods show significant effect. Moreover, p values for later periods were always smaller than for beginning overlapping periods except one case (0104, V4EF) (Figure 29). This indicated that the difference between conditions didn't appear until one to two seconds later after stimulus onset. Similar phenomena were found in comparisons between blank_fix1and sum_fix1, and between blank_fix3 and sum_fix3, though not so typical as between blank_fix2 and sum_fix2. With the same behavioral mode and the same task procedure, the main difference was the stimulus presence or absence. Therefore, during the beginning one to

two seconds or more after stimulus onset, the activity in V1, V2 and V4 were not affected by stimulus presence.

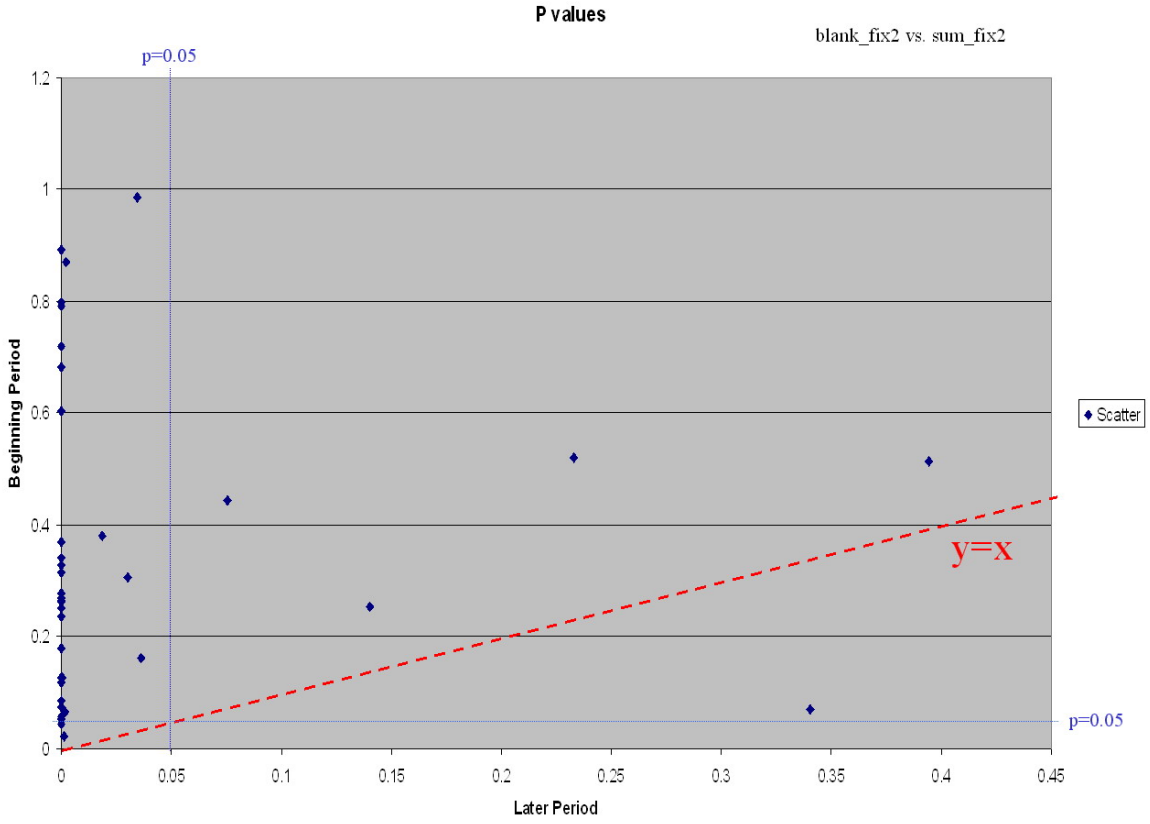

Figure 29. P values of ANOVA results, comparisons between blank_fix2 and sum_fix2. ANOVA were applied to examine the difference between blank_fix2 and sum_fix2 during selected periods: beginning overlapping period and later period. One single dot indicates the p values for the overlapping period and the later period of one case (date & region).

Unaffected by Presence of Saccades: Another series of comparisons, between blank_fix1 and blank_fix3, were carried out under similar procedure to examine the effect of saccades. Sample size of these two conditions are always larger than 40. Blank_fix1 was an isoluminant background gray screen with a fixation dot at the center, and blank_fix3 was an isoluminant gray screen with a fixation dot appearing sequentially at four locations. The stimulus was only a small fixation dot under these two conditions,

and the main difference was the distinct behaviors. Four examples are shown in Figure 30. In this series of comparisons, the overlapping periods were shorter than in late series: the length ranged from 0.75s to 2s, and a lot of them were shorter than 1.5s (Figure 31). Most dots in Figure 32 were still above the y=x line, but there were six cases which showed significant difference in the beginning period. This means, actually there were no 'overlapping period' in these six cases that were all from 0401, across V1, V2 and V4 in both fovea and extra-fovea. The reason why date 0401 showed distinct result might be very complicated, but it indeed weakened the final conclusion. Generally, during a beginning period after stimulus onset, cortical activity in V1, V2 and V4 were not much affected by behavior modes. However, the unaffected period was relatively shorter, probably 0.5 to 1.5 seconds, and the behavior effect was small but somehow still existed.

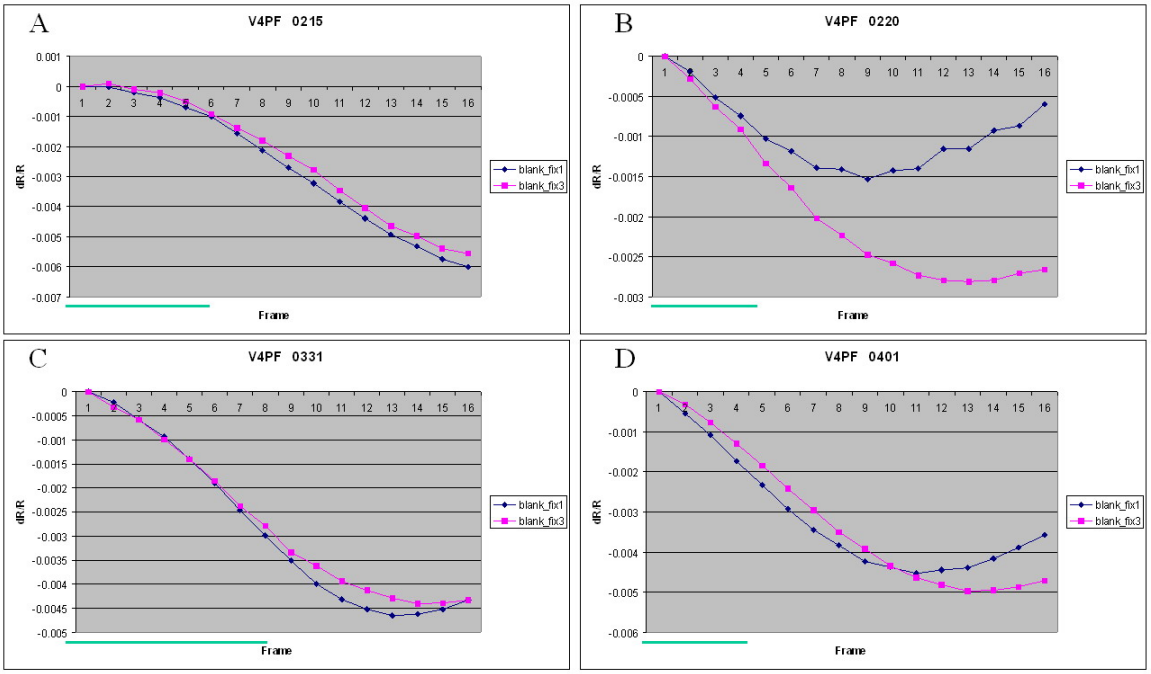

Figure 30. Four examples: time courses of activation by blank screen under fixation and saccade modes. The beginning parts (indicated by green bars) of blank_fix1 and blank_fix3 overlap, indicating this part of change is not caused by behavior. Terms: V4PF: V4 para-foveal region; fix1: fixation; fix3: saccade; blank: isoluminant gray screen.

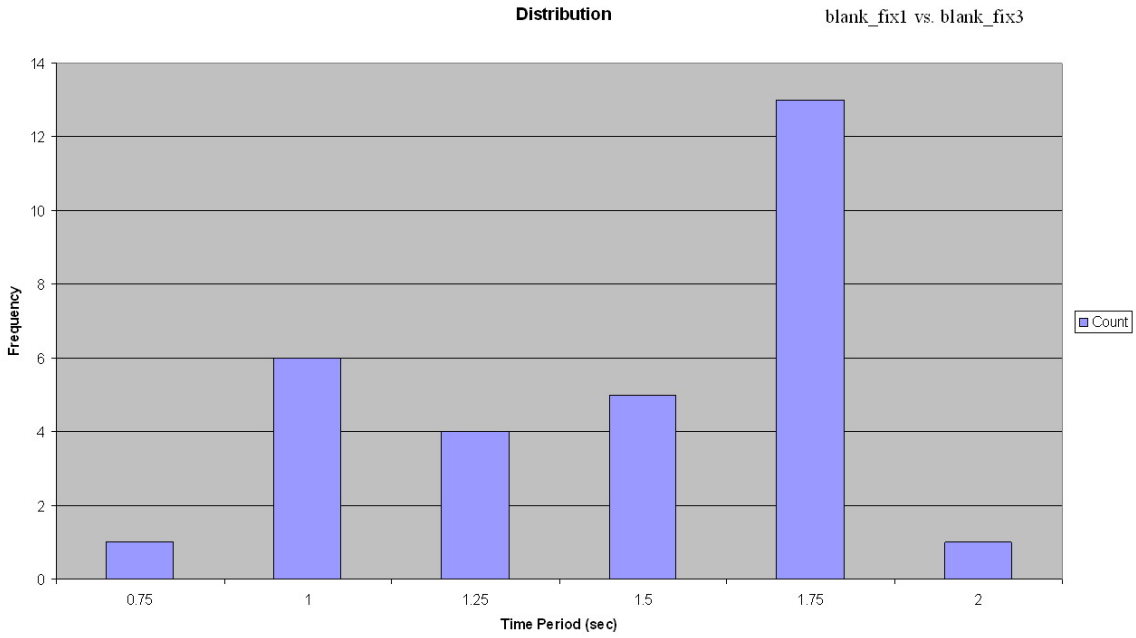

Figure 31. Distribution of the length of 'overlapping periods' selected for comparisons between blank_fix1 and blank_fix3. The mean length is 1.47s, and 29/30 are between 1 and 2 sec.

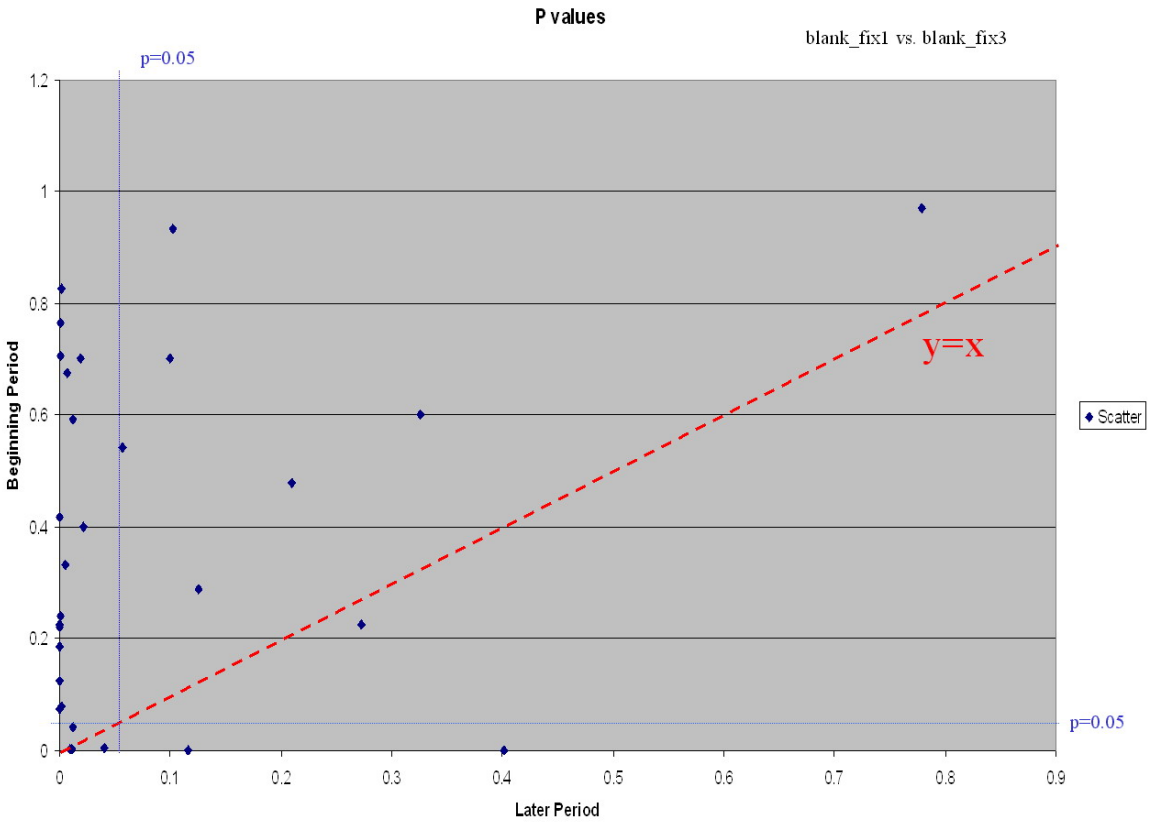

Figure 32. P values of ANOVA results, comparisons between blank_fix1 and blank_fix3. ANOVA were applied to exam the difference between blank_fix1 and blank_fix3 during selected periods: beginning overlapping period and later period. One single dot indicates the p values for the overlapping period and the later period of one case (date & region).

This "unaffected beginning period" effect existed in almost all regions in V1, V2, and V4, with no effect of stimulus presence and very small effect of behavior mode. It was task structure-related and probably due to temporal attention.

DISCUSSION

Real Saccadic Suppression or Not?

Our results indicate that compared with fixation and free viewing, saccades have suppressive effect on neural activity across a large scale of visual cortex. However, this

suppression happens mostly when given isoluminant color stimulus, but little suppression when the stimulus is luminant.

Numbers of previous studies by others show saccadic suppression in the visual system, Zuber and Stark (1966), and Riggs et al. (1974) reported a threshold elevation for detecting spots of light flashed briefly during saccades, even when the stimulus was a phosphene produced under conditions of total darkness (Riggs et al., 1974). The sensitivity for detecting displacement also reduced at the time of a saccade (Bridgeman et al., 1975). Our results are consistent with these studies, but not consistent with later studies that show selective suppression toward luminant stimuli.

In a task that subjects were required to detect briefly presented gratings (Burr et al, 1994), sensitivity to luminant stimuli of low spatial frequency was greatly impaired while there was little suppression of isoluminant stimuli, irrespective of the spatial frequency and in some circumstances stimuli can even be enhanced. Other experiments applying displacement task (Bridgeman & Macknik, 1995) or spectral sensitivity analysis (Uchikawa & Sato, 1995) both show greater saccadic suppression for luminant stimuli than isoluminant stimuli. Moreover, not only psychophysics studies but also neurophysiological works report the same result. Kleiser et al. (2004) observed a saccadic reduction in BOLD signal for luminant gratings, but not for isoluminant gratings, in V4, V7 and MT.

It is still possible that the suppression observed in our experiments is the same "saccadic suppression" reported in other publications though our intrinsic signal result is opposite to BOLD result. Those very few researches studying the relation between intrinsic signal and BOLD (Cannestra et al., 2001; Roe et al., 2006) reported a non-linear

but very complicated relation, no matter in human or animals. Plus, it is also possible that smaller intrinsic signal coexists with better cognitive ability, a good supportive example is that a large amount of information is still kept even when the stimulus is masked and neuronal response is greatly reduced (Rolls et al., 1999). If it is the real "saccadic suppression", the fact it occurs as early as in V1 matches Thilo et al.'s conclusion (2004) that suppression originates between retina and occipital cortex.

However, an alternative explanation seems more convincing. A great distinctness of our project is that we didn't require the animal to perform any detection or recognition task, what the animal had to do was just following the fixation dot. Therefore, the monkey didn't really attend to the background grating stimuli but rather attended to the fixation dot under fixation mode, and was searching for and attended to the dot under saccade mode. The reason that color gratings elicit greater response under fixation and free viewing might be simply that color gratings are greater stimuli for this monkey. While under saccade mode, it was harder for the monkey to search for the small light blue dot on the background of red-green gratings, compared with black-white gratings. Since the harder visual search task occupied more working memory and attentional load, the monkey paid less attention to the background gratings (consider the role of working memory in visual selective attention, see de Fockert et al., 2001). Considering the small dot elicited tiny retinal response compared with the entire-screen stimuli, less attention to the gratings would lead to smaller visual cortical responses. Moreover the significant difference occurred only in V4, between RG and BW stimulation under saccade mode, it was probably because the monkey was attending to blue while the background was other colors, and V4 is more sensitive to color attention (e.g. Motter, 1994).

As described above, this selective suppression may be produced by the oculomotor system, same as "saccadic suppression" described by the many other publications, or it may be an attentional effect. In order to further investigate this in the future, we need a task requiring the monkey to detect or identify a certain stimulus.

Color vs. Luminance Domain in V4

Based on the result the saccades selectively suppress color but not luminant stimuli, we predicted that color domain should show greater suppression than luminance domain, at least for color stimuli. However, the modulation effect of saccade didn't show any significant difference between color and luminance domain in V4 fovea, no matter the stimulus was color or luminant.

The result suggest that color and luminance processing may not be segregated in V4 fovea, this is consistent with Schein and Desimone's (1990) conclusion that large majority of V4 neurons process color. However, because the mechanism of the suppression is still unclear, it can only be a suggestive but not confident conclusion.

Effect of Expectation and Temporal Attention

In this study, we demonstrate an activity increase in a short period after stimulus onset time, which is affected by neither stimulus presence or absence, nor the oculomotor behavior. This period ranges from half a second to more than two second, and the effect exists in V1, V2 and V4, both fovea and extra-fovea cortical region. Then what causes this increase?

Ghose and Maunsell (2002) reported that attention could be dynamically manipulated according to the anticipation of the timing of behaviorally relevant events. Moreover, attentional preparation based on temporal expectation was shown to improve perceptual processing (Correa et al., 2005) and elicit ERP (Correa et al., 2006) components and neural activity in inferior temporal cortex (Anderson & Sheinberg, 2008). Therefore, temporal attention was probably the reason for the increasing cortical activity in visual cortex. Since the monkey was well trained, had performed many similar tasks before this project, and was trained two times with this free viewing and saccade task before recording, the monkey should be very familiar with the task structure even if the task timing somehow changed in the middle. The monkey was probably expecting a stimulus right after the initial cue, and this expectation arouse temporal attention. Attention with (Motter, 1993; Luck et al. 1997) and without (Kastner et al 1999) visual stimulation could both enhance the activity in visual cortex including V1, V2 and V4, and this was exactly the phenomenon found in our results.

Because only one subject was tested, and we lack the control condition that no expectation happens, we can only make a suggestive but not confident conclusion that the cortical activity in the beginning one or two seconds after stimulus onset mostly depends on attentional modulation induced by expectation. Optical imaging focuses on not the later blood flow change but the oxygen consumption which is colocalized with neuronal activity, so although we are analyzing hemodynamic signal, the timing should be about the same for cortical neural activity.

Suggestions for Future Experiments

Every discovery needs a process, same for our project. Though the mechanisms are still unclear, our findings suggest some future research directions: by carefully designing the experiments, we would probably be able to find to what extend attention, arousal state and planning contribute to saccadic suppression, we would also be able to reduce the confounds of the factors, and quantitatively test our above mentioned findings such as the stability of functional domains distribution.

The first suggestion is that a more specific task is needed for the monkey, for example, to respond to the color or the orientation of the gratings, or to search for some specific object with certain color and form features. Because in this project, the monkey could get reward by simply looking inside the fixation window, then it is hard to know what the monkey was attending to and what the monkey was thinking about, especially in free viewing conditions in which even no fixation or following was required. During free viewing, the monkey might be in search for the possible fixation dot, or looking at the background gratings, or looking inside the screen but thinking of something else, etc. Without a task to make the monkey's attentional state similar across three behavioral modes, it would be impossible to know whether the activity difference was caused by saccadic suppression itself, or different attentional state, or others.

Secondly, use as many consistent parameters as possible across days of experiments. Keep during the entire experiment process the same eye-to-screen distance, the same fixation window size, the same fixation dot positions, the same cue period, etc. Then the analysis and interpretation would be greatly simplified if we don't need to consider the effects of so many varying factors. Most importantly, make the baseline period the same

across different days. The reason the parameters were changing in this project was that we were still testing and looking for the best experiment settings.

Thirdly, precisely record eye movements, especially in free viewing conditions. In this project, we found there is no consistent result in comparison between the response magnitudes under fixation and free viewing. The reason is complicated, but at least one important factor should be saccade frequency. Maybe free viewing trials with many saccades would have neural activity more like guided saccade conditions, and those with few saccades would have neural activity more like fixation conditions. With more precise eye movement recording, we can count saccade frequency during every trial, and see how it affects neural activity.

The last suggestion is to randomize the intertrial interval and also have some trials with the trigger cue but no upcoming grating or blank stimulus. The purpose is to eliminate the effect of expectation, which was found significant in this project. If the effect of expectation can be eliminated, the influence of free viewing and saccade can be more easily studied. With the intertrial interval randomized, the monkey won't be able to predict the onset of the trial, and with some incomplete trials, the monkey won't habituate and expect an upcoming stimulus after the trigger every time.

With these four modifications, future research will minimize the mindset confounds, have better designed baseline and controls, and obtain more quantitative and reliable results.

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SUPPLEMENTAL MATERIAL

Supplemental Table 1
Behavioral Data. Successful rates are both very high for fixation and saccades. On 0111, there was no saccade mode, instead there was a second 'fixation' mode with a white fixation dot, and its successful rate was 0.9375.

Experiment day	Number of used blocks	Successful rate in used blocks	Successful rate under fixation	Successful rate under saccades
0104	50	0.835	0.871	0.817
0111	48	0.855	0.908	-
0215	67	0.879	0.958	0.976
0220	62	0.703	0.729	0.761
0331	68	0.963	0.982	0.968
0401	60	0.989	0.993	0.990

Supplemental Table 2.
Results of modulation index analysis. Analysis of modulation index1 (fix3/fix1) and modulation index2 (fix3/fix2) were applied for four days. Mean and SD are listed for each case. Bold digits denote significant difference ($p<0.05$) between RG MI and BW MI, while underlined digits denote approach significant difference ($0.05<p<0.1$). In cases with or approach significance, MI is always smaller for RG than for BW.

Region\Date	0215 MI1 (fix3/fix1)		0215 MI1 (fix3/fix2)	
Mean±SD	RG MI1	BW MI1	RG MI2	BW MI2
V1 fovea	-	-	-	-
V1 extra-fovea	-	-	-	-
V2 fovea	0.9920±0.0411	0.9869±0.0485	**0.9260±0.0372**	**1.0617±0.0528**
V2 extra-fovea	0.9747±0.044	1.0131±0.0547	**0.8539±0.0368**	**0.9853±0.0534**
V4 fovea	-	-	-	-
V4 para-fovea	1.0522±0.0544	1.0051±0.0566	0.7927±0.0359	0.8704±0.0439
V4 extra-fovea	1.1145±0.3765	1.0730±0.1503	0.7833±0.0501	1.1905±0.2568

Region\Date	0220 MI1 (fix3/fix1)		0220 MI1 (fix3/fix2)	
Mean±SD	RG MI1	BW MI1	RG MI2	BW MI2
V1 fovea	-	-	-	-
V1 extra-fovea	-	-	-	-
V2 fovea	0.7295±0.0493	0.8693±0.0560	0.9981±0.0793	0.8441±0.0545
V2 extra-	**0.6161±0.0453**	**0.9198±0.0688**	0.7415±0.0720	0.7865±0.0474

fovea				
V4 fovea	0.8553±0.0455	0.9904±0.0521	0.8751±0.0493	0.8625±0.0443
V4 para-fovea	**0.7959±0.0533**	**1.0597±0.0710**	0.9100±0.0684	0.8876±0.0567
V4 extra-fovea	**0.6564±0.0495**	**1.0419±0.0712**	0.8411±0.0784	0.9387±0.0602

Region\Date	0331 MI1 (fix3/fix1)		0331 MI1 (fix3/fix2)	
Mean±SD	RG MI1	BW MI1	RG MI2	BW MI2
V1 fovea	0.9634±0.0385	1.0592±0.0503	1.0167±0.0404	0.9228±0.0381
V1 extra-fovea	0.9850±0.0520	1.1056±0.0686	0.9651±0.0455	0.8910±0.0431
V2 fovea	0.9113±0.0395	1.0371±0.0563	0.9531±0.0416	0.8915±0.0423
V2 extra-fovea	**0.9289±0.0437**	**1.1005±0.0659**	0.9018±0.0383	0.8781±0.0401
V4 fovea	0.9825±0.0311	1.0645±0.0432	0.9919±0.0321	0.9391±0.0343
V4 para-fovea	**0.9199±0.0336**	**1.1312±0.0543**	0.9009±0.0333	0.9750±0.0402
V4 extra-fovea	**0.9295±0.0355**	**1.1052±0.0523**	0.9069±0.0339	1.0137±0.0445

Region\Date	0401 MI1 (fix3/fix1)		0401 MI1 (fix3/fix2)	
Mean±SD	RG MI1	BW MI1	RG MI2	BW MI2
V1 fovea	1.0142±0.0617	1.1618±0.0841	1.0811±0.0741	1.0468±0.0701
V1 extra-fovea	**0.8979±0.0541**	**1.2472±0.1200**	0.9680±0.0702	1.0576±0.0688
V2 fovea	**0.9380±0.0294**	**1.0770±0.0407**	0.9787±0.0325	1.0611±0.0391
V2 extra-fovea	**0.8437±0.0483**	**1.2533±0.1139**	**0.8624±0.0543**	**1.1003±0.0741**
V4 fovea	**0.9527±0.0270**	**1.1330±0.0387**	**0.9217±0.0261**	**1.0479±0.0326**
V4 para-fovea	**0.8692±0.0309**	**1.1818±0.0452**	**0.7911±0.0277**	**1.0611±0.0370**
V4 extra-fovea	**0.8428±0.0404**	**1.1922±0.0566**	**0.8005±0.0380**	**1.0752±0.0478**