Vivien Casagrande, PhD

Visual Neuroscience Lab

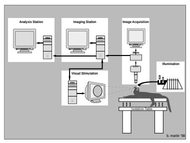
Departments of Cell & Developmental Biology, Psychology, and Ophthalmology & Visual Science

http://www.psy.vanderhttp://www.psy.vanderbilt.edu/faculty/Casagrande/Casagrandelab/index.htm

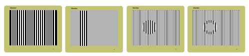
VANDERBILT Vision Research Center

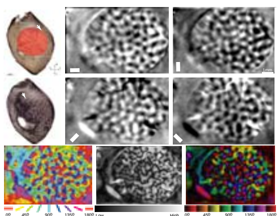
Optical Imaging

Our goal in this project is to determine functional organization of visual cortex. The visual system of primates has been the most intensely studied sensory system given its major importance to humans and other primates. Of special importance are studies that compare the anatomical and functional organization of visual cortex across various primate species, since persistent similarities across species can add to our understanding of common underlying principles of the visual system. We used a new technique, optical imaging of intrinsic signals, to examine the functional organization of primary visual cortex (V1) in distant primate relatives of humans, the bush babies (*Otolemur garnetti*) and owl monkeys (*Aotus trivirgatus*). Until recently, the most commonly used technique to study the functional organization of the primate brain was recording from individual neurons (by means of electrophysiology). While this method is a powerful study tool, allowing one to record the responses of individual cells with very fine temporal resolution, it is not very effective in creating overall functional maps of entire cortical areas, such as V1. By contrast optical imaging can be used to map the functional organization of large regions of visual cortex simultaneously at fairly high resolution

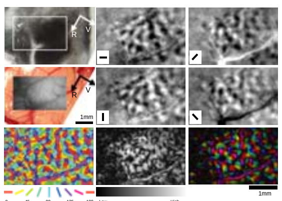


Side panel depicts our experimental set up. Animal is are placed on the isolation table to eliminate vibration artifacts. Animals observe stimuli on the monitor, while the CCD camera acquires images of the brain illuminated with red light. Active regions of the brain consume oxygen, which changes the ratio of oxy- to deoxy-hemoglobin resulting in more light being absorbed. Acquired data is transmitted to a computer where preliminary analysis is done. Further analysis is done off-line on the analysis computer. Bottom panels depict the stimuli that were used in this study. Most cells in the primary visual cortex, and several higher visual area are very sensitive to moving gratings of different orientations. Confining such gratings to an area allows us to probe functional properties of confined parts of visual cotex, while full screen stimuli allow us to study either a big part of a given visual area or even several visual areas simultaneously..

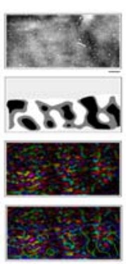




Functional maps of are V1 in bush baby. Top left panel depicts alignment of the digital image with the vasculature obtained during the experiment on the first histological section. The white outline (white arrow) depicts the imaged area with the area of exposed brain shown as a circular outline in the center.
Center left image depicts the correspondence between the imaged area and the cytochrome oxygenase (CO) stained sections. These two panels were used to confirm that the data was acquired from area V1 which in bush babies has intense CO blob staining.
Four images shown at the right depict differential images of V1 responses (left to right, top to bottom) to 0, 90, 45, and 135 degree oriented full screen gratings. These images were used to create the color coded orientation map seen in the bottom left panel, the magnitude map (bottom center) and the combined map (bottom right)



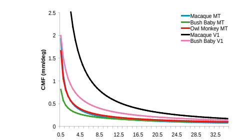
Functional maps of area MT in owl monkey. Top left. Myelin stained section showing heavy myelination in and around the middle temproal visual area (MT.) White rectangle represents the studied area. R-rostral; V-ventral. Middle left. Green light reference image of the cortical surface showing vascular pattern used to align optical images with histological sections. Reference image is aligned with color photo of the cortical surface. Differential images of orientation preference (four top right), with the orientation of the darker patches indicated at the lower left corner of each panel. Bottom Left. Orientation preference map obtained by vector summation of differntial maps with color key shown below. Bottom middle. Magnitude map showing strength of activation across MT, with key shown below. Bottom right Polar map showing both orientation preference and magnitude of activation.



Relations of high or low orientation selectivity bands and cytochrome oxygenase (CO) dark stripes in V2 of owl monkeys. Top. CO stained section. Interestingly, originally CO dark thin stripes were proposed to process color. Their presence in monochromatic species questions that assumption. Second from the top. CO thresholded image, showing in black and gray areas of most intence and less intence stainging for CO, respectively. Third from the top. Polar map showing both orientation preference and responce strength. Bottom. Polar map with contour plots transferred from the CO thresholded image of the same region of V2 as shown in second image. Green contours encircle CO dark stripes, blue contours encompass the CO light stripes: border areas lie between the two colors.

Quuantitative comparisons between CO dark and light stripes and stripes of high and low orientation selectivity suggest that 4 separate functional compartments exist in visual area V2 of owl monkeys.

Some relationship appears to exist between alternating areas of high and low orientation selectivity in V2 and alternating CO dark stripes, i.e, every other CO dark stripe appears to correspond to a zone of low orientation selectivity.



Plot of linear cortical maginfication factor (CMF, a coefficient showing how many millimiters of cortex analyze one degree of visual space), as a function of eccentricity (E) for the following species:

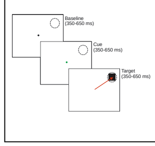
owl monkey	(red)	$CMF = 1.051*E^{-0.663}$	(Present study)
macaque MT	(blue)	$CMF = 1.14*E^{-0.76}$	(Albright & Desimone, 1987)
bush baby MT	(green)	$CMF = 0.573*E^{-0.504}$	(Xu, et al., 2004)
macaque V1	(black)	$CMF = 10.1*(E+0.82)^{-1.14}$	(Van Essen et al., 1984)
bush baby V1	(pink)	$CMF = 2.36*(E+0.73)^{-0.8}$	(Rosa et al., 1997)

In central vision, the CMF of owl monkeys is very close to one obtained for macaque monkeys and is larger then the CMF obtained for bush babies. Note that, due to the anisotropic representation of the horizontal compared to the vertical meridian (see Figure 6), the CMF obtained in this study represents the average CMF at any given eccentricity.

Awake Behaving Monkeys

Our primary research goal is to understand more fully the functional role of the visual thalamus in human vision. We combine the precision of single unit electrophysiology with the power and freedom of an awake behaving animal preparation. We work with various species of macaque monkeys (indigenous to Asia and Northern Africa) because they have visual systems designed very similar to humans, and equally important, they are intelligent enough to learn very complicated visuomotor tasks.

Behavioral Training

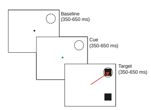


Before recording from single neurons, monkeys are trained to perform a series of tasks like the one shown at left. Here the monkey was required to fixate the fixation spot (◦) until a colored stimulus was presented on screen (■). In this task, shifting gaze to the stimulus elicited reward (a drop of juice).

Tasks vary in complexity. The visually guided saccade task shown at left represents one of the first and most basic tasks the monkeys learn.

Another topic of great interest is the role attention plays in filtering our visual experience. We know that the eyes see much more than we are aware of at any given moment. This process of filtering the visual stream could occur at a number of sites within the visual pathway, including the LGN. We hypothesize that visual filtering occurs at the LGN and that the combination of awake behaving monkeys , complex, attention-demanding tasks, and electrophysiology will help reveal this process.

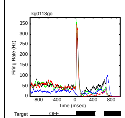
Behavioral Training



The task outlined at left is what we call a Two Stimulus Visually Guided Saccade Task. The monkey's task here is to shift gaze to one of two targets (one inside our single unit's RF (dashed circle) and the other outside the RF). If attention impacts LGN cells, and if we implement very demanding tasks, we may see differential activation of LGN cells depending on the locus of attention.

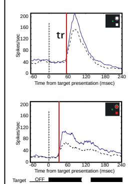
Electrophysiological Recordings

After behavioral training, recording from cells can begin. The first step is to locate our thalamic nucleus of interest, the lateral geniculate nucleus (LGN). We accomplish this via MRI and a sterotaxic atlas of the monkey brain. At left is a coronal MRI image showing the location of both LGN (circled in black). The vertical black line represents our recording electrode with its tip positioned within the left LGN.



Many LGN cells are color selective. This figure is a peri-stimulus time histogram (PSTH) showing the average response of a single LGN cell to presentations of colored stimuli inside its receptive field (RF) center. This cell increased its firing rate when red, green, and white stimuli were flashed in its RF (red trace, green trace, and black trace, respectively) and decreased its firing rate when a blue stimulus was flashed in its RF (blue trace). This cell would be classified as a Blue OFF/Yellow ON color opponent cell.

Electrophysiological Recordings



PSTHs from two LGN cells that were recorded during the Two Stimuli task described at left. The blue trace refers to trials where the monkey shifted gaze to the RF and the black dashed trace refers to trials where the monkey shifted gaze to the nonRF location. The red line indicates the cell's response latency to target onset as determined via the Poisson analysis. It should be noted that the response latencies to target onset were not significantly different between the two tasks for these cells.

The cells' mean firing rate from the time of target onset to the time of saccade initiation was significantly higher when the monkeys made saccades to the RF location.

How Many Retinogeniculocortical Pathways Are There?

Given that all visual signals for conscious visual perception are transmitted to cortex via the dorsal lateral geniculate nucleus (LGN), it is important to understand how different aspects of visual stimulus reach the cortex in parallel. Since the initial proposal of the existence of parallel visual streams by Livingstone and Hubel (1988), the question still remains: How many retinogeniculocortical pathways are there? In an attempt to investigate this question, dLGN layers were identified by extracellularly recording visually evoked potentials. Either small (0.25ul) or large (1.5ul) injections of dextran-tetramethylrhodamine were injected into specific LGN layers to retrogradely label ganglion cells. After a 3-5 day survival the animals were deeply anesthetized, living retinas were then dissected, and ganglion cell morphology was revealed through photofilling (Dacey, 2003). Cellular structure was analyzed by confocal microscopy, digital photography and calibrated Photoshop™ measurement. At least six different RGC classes were found to project to the LGN in bush babies based upon each cell's: 1) dendritic field diameter 2) aspect ratio of dendritic arbor 3) organization and complexity of dendritic array.
In addition to verifying that midget and parasol ganglion cells project to the LGN parvocellular and magnocellular layers, respectively (Yamada et. al., 1998), we found theta-like cells (as seen in cat retina; O'Brien et. al., 2002), large sparse cells (as seen in macaque monkey retina; Dacey & Packer, 2003), and a 'fan' cell type not characterized previously. Given the fact that all but one of these classes of cells has been identified in several species supports that idea that there are at least 5 classes of ganglion cells, and thus at least 5 distinct functional pathways, that send parallel messages to the brain important for conscious visual perception. Currently we are continuing this project by doing much smaller injections (using iontophoresis) of modified live Rabies virus (which produces Green Flourescent Protein) into single layers in the LGN in order to be able to better localize which types of ganglion cells projection of specific functional layers of the LGN.

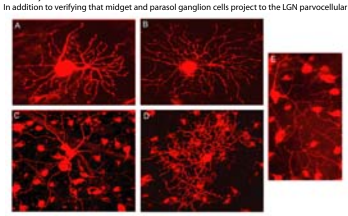


Figure 1
Photographs of bush baby retinal ganglion cells taken from 25º-30º eccentric from the center of area centralis. All cells were retrogradely labeled with dextran, tetramethylrhodamine and filled by photofilling. **A** is a midget cell digitally photographed at 63X. **B** is a parasol cell digitally photographed at 40X. **C** is a confocal image of a large sparse cell taken at 40X. **D** is a confocal image of a theta-like cell taken at 40X. **E** is a digital image of a fan cell type taken at 40X.

Dynamics of spatial frequency tuning and its generation in primate primary visual cortex (V1)

The response selectivities of visual system neurons are dynamically regulated by feedforward and intracortical pathways. Prior studies of V1 in Macaque monkeys showed that the preferred spatial frequency (SF) of some neurons shifts over time toward higher SFs (Bredfeldt and Ringach, 2002; Mazer et al. 2002). This shift could either reflect: 1) the relative SF preferences and latencies of lateral geniculate nucleus (LGN) magnocellular (M), parvocellular (P) and koniocellular (K) input pathways, 2) a removal of selectivity to lower SFs through cortical inhibition, or 3) some combination. In this study we used the Utah one-hundred electrode Utah array (Cyberkinetics) and reverse correlation analysis to examine the response dynamics of V1 neurons in the prosimian primate bush baby (Otolemur garnetti). Results showed that the shifts of preferred SF could be divided into four groups. Not only increases but also decreases of preferred SF over time were found. The latency of activity increased with the higher SF of stimulus, no matter in one neuron or between neurons. We also found that the temporal dynamics of SF preference do not differ between cells located in CO blobs and interblobs. These result suggest that the dynamics of SF tuning could be accounted for by both feedforward and intracortical pathways.

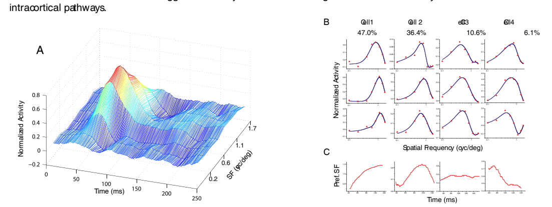


Figure: A, Example of the response dynamics of neurons to the presentation of different SFs. X axis, time; Y axis, SF; Z axis, normalized activity for one neuron. **B.** Four examples of the shift of preferred SF over time. The first row shows the SF tuning curve at T-dev. The second row shows the SF tuning between T-dev and T-dec. The third row shows the SF tuning curve at T-dec. Four types of shifts over time in preferred SF were observed in 66 cells in two cases: 1) the preferred SF increased monotonically (31 cells, 47.0%); 2) the preferred SF first increased over time to a maximum and then decreased (24 cells, 36.4%); 3) The preferred SF stayed the same (7 cells, 10.6%); 4) the preferred SF decreased monotonically from T-dev to T-dec (4 cells, 6.1%). The four cells presented are examples from each of these four groups. **C.** Evolution of preferred SF in time

We also study the role of neuronal synchrony in the coding of visual information. Synchrony has been considered an alternate to changes in firing rate as a method of neural coding. It is attractive in light of the fact that many perceptual phenomena (e.g. hyperacuities) cannot be explained in terms of changes in firing rate, and also the fact that a code based on synchrony has a great dynamic range is easily generates downstream responses.

We have had great success studying synchrony in the bush baby. Using the Cyberkinetics multielectrode array (See Fig. 1), we have generated strong and reliable recordings from over 75 neurons over a 30 hour period. Our data have shown a few possible ways that a code based on spike synchrony can be better than a code based on firing rate. For example, our data have shown us that spike synchrony is more sensitive to fine changes in orientation, spatial frequency and temporal frequency. In addition, our data have shown that synchronous population responses are propagated through the visual hierarchy very quickly and efficiently. Our goals are to investigate the feed-forward, local and feed-back circuits that are involved in the generation of spike and oscillatory synchrony. In addition, we will examine the role of neuronal synchrony in coding for contours embedded in illusory images. These studies will shed light on coding of visual information in the visual cortex in the primate brain.

Figure 1: The Cyberkinetics Multielectrode Array. It is 4X4 mm array with 100 electrodes.

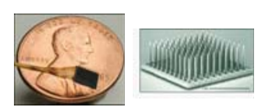
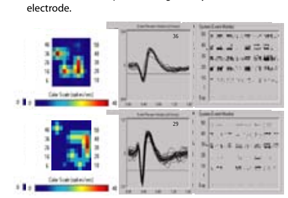


Figure 2: Data from the Bionics Acquisition Program. A, Firing rates of each electrode integrated over 100 ms. B, Graphs of the last 25 spikes for each electrode. C, Raster plot showing activity of each electrode.



Other past and future projects in our lab.

Histological studies of connections between different layers of visual cortex and LGN. It is known that LGN sends visual information to visual cortex via at least three different streams. Also, visual cortex is subdivided into six major layers. One of the areas of research in our lab is to obtain detailed anatomical information on subcortical and cortical connections of parallel visual pathways.

Neurons communicate with each other via synapses. There are many types of synapses in the brain, with different morphologies, neurotransmitters and receptors used. The types of synapses and receptors determine the range of functions performed. Our lab has conducted a variety of ultrastructural studies to determine the microcircuitry of visual cortex.

Molecular mechanisms of axonal guidance. During development, billions of nerve cells make presise connections with each other. How is such specificity achieved? One of the studies in our lab concentrated on L1, a molecule playing an important role in pathfinding.

Studies of multisensory perception. It is not immediately apparent, but our different senses, such as vision and hearing or touch can interact with each other. For instance, the ventriloquist effect is a perception of a voice comming from the dummy, due to visual perception of the dummy's mouth moving in accordance with sound. Our lab is planning to conduct a series of electrophysilogical experiments to determine mechanisms of interaction between vision and audition.