A Comparison of Koniocellular (K), Magnocellular (M), and Parvocellular (P) Receptive Field Properties in the Lateral Geniculate Nucleus (LGN) of the Owl Monkey (*Aotus trivirgatus*)

Xiangmin Xu [1], Jennifer M. Ichida[1], John D. Allison[4], A.B. Bonds[4] and

Vivien. A. Casagrande [1,2,3,*]

Departments of Psychology[1], Cell Biology[2], Ophthalmology and Visual Sciences[3], and Electrical Engineering[4] Vanderbilt University Nashville, TN 37232-2175

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[*]Address all correspondence and reprint requests to:

V. A. Casagrande

Department of Cell Biology Phone: (615) 343-4538

Vanderbilt Medical School Fax: (615) 343-4539

Medical Center North RM C2310 Email: vivien.casagrande@mcmail.vanderbilt.edu

Nashville, TN 37232-2175

Summary

1. We examined the receptive properties of the Koniocellular (K), Magnocellular (M), and Parvocellular (P) cells in the lateral geniculate nucleus (LGN) of the New world nocturnal simian owl monkey, aiming to characterize receptive field properties of K LGN cells by comparing the K cell properties with those of M and P LGN cells. The key objective of the present study was to proceed by analogy to previous physiological work on magnocellular (M) and Parvocellular (P) lateral geniculate nucleus (LGN) cells and put together a physiological profile of koniocellular (K) LGN cells that might be linked to particular visual perceptual attributes.

2. Conventional extracellular recording techniques were employed to study the receptive field properties of neurons in the LGN or their axons in silenced V1 cortex in nine anesthetized paralyzed owl monkeys. Receptive field center and surround properties were mapped using flashing spots and field center sizes were measured. Spatial and temporal tuning, and contrast response characteristics of cells were examined by using drifting sine-wave gratings. Cells were tested with counterphased sine-wave gratings to examine linearity of spatial summation. Locations of recorded cells were determined based upon electrode tracts marked with lesions as well as changes in eye dominance, and structural boundaries determined by histological (Nissl), histochemical (cytochrome oxidase), or immuncytochemical (K cells contain calbindin-D 28K) criteria.

3. The receptive field properties of a total of 133 LGN cells and 10 LGN afferent axons were analyzed at eccentricities ranging from 2.8 to 31.3 degrees. From this population 38 K, 45 P, and 34 M units could be assigned with confidence to specific LGN layers.

4. The K cells as a population are quite heterogenous in response properties and receptive field organization, relative to the P and M cells. A significant proportion (34%) of K cells responded poorly or not at all to drifting gratings whereas only 9% of P and 6% of M cells responded poorly to drifting gratings. Cells in all 3 classes exhibited increases in receptive field center size with eccentricity but the K population showed more scatter.

5. All K, P and M cells except one M cell, tested with counterphased gratings, showed linearity in spatial summation of their receptive fields.

6. K, P, and M cell populations showed significant overlap in spatial and temporal resolution and in contrast response. On average, at matched eccentricities, K cells exhibited lower spatial resolution than the P and M cells, and showed temporal resolution values that fell intermediate between those of the P and M cells. The contrast thresholds and contrast gains of K cells were more similar to those of M cells than those of P cells. The differences in achromatic properties between P and M cells in owl monkeys resembled those found in other primates; M cells tended to exhibit lower spatial and higher temporal resolution and contrast gain than P cells.

7. K populations in different K LGN layers differed in their average spatial, temporal and contrast characteristics, with K3 cells having higher spatial resolution and lower temporal resolution than K1/K2 cells.

8. Taken together with previous results these findings suggest that the K cell population is made up of several classes some of which could contribute to conventional aspects of spatial and temporal resolution.

Introduction

In the primate lateral geniculate nucleus (LGN), three principal types of relay cells have been identified: the koniocellular (K), the parvocellular (P), and the magnocellular (M) cells. These relay cell classes can be distinguished based upon a number of criteria including laminar location, morphology, connections, and neurochemistry in several primate species including bush babies, owl monkeys, marmosets, and macaque monkeys (See Casagrande & Kaas, 1994 and Casagrande, 1999 for review). K LGN cells are, on average, the smallest relay cells. They form thin layers that lie below the M and P layers, they contain the calcium binding protein calbindin-D28k, and they send their axons to the cytochrome oxidase (CO) blobs in cortical layer III and to cortical layer I of primary visual cortex (V1) (Lachica & Casagrande, 1992; Hendry & Yoshika, 1994; Johnson & Casagande, 1995; Ding & Casagrande, 1997). In contrast, M and P LGN cells are large and medium in size, are located in the ventral and dorsal layers of the LGN, contain the calcium binding protein parvalbumin and send their axons principally to the upper and lower tiers of cortical layer IVC of Brodmann (1909), respectively (Jones & Hendry, 1989; Lachica & Casagrande, 1992; Hendry & Yoshika, 1994; Johnson & Casagande, 1995; Ding and Casagrande, 1997; Goodchild & Martin, 1998).

The physiology of only two of these three LGN cell classes, the M and P cells, has been studied in detail across primate species (e.g., bush baby: Norton & Casagrande, 1982; Irvin et al., 1986; Norton et al., 1988; Irvin et al., 1993; macaque monkey: Kaplan & Shapley, 1982; Derrington & Lennie, 1984; Hubel & Livingstone, 1990; Reid & Shapley, 1992; Spear et al., 1994; owl monkey:

Sherman et al., 1976; O'Keefe et al., 1998; Usrey & Reid, 2000; marmoset: Kremers et al., 1997; White et al.,1998). M and P cells have been hypothesized to support distinct extrastriate visual pathways, based upon differences in physiological signatures and upon their separate projection pathways to V1 and within V1 (See DeYoe & Van Essen, 1988; Livingstone & Hubel, 1988; Merigan & Maunsell, 1993, Casagrande and Kaas, 1994 for review). For example, the greater sensitivity of P cells to chromatic contrast (in macaque monkeys and marmosets) and to higher spatial frequencies (in all primate species examined) has been linked to the appreciation of detail and color while the greater sensitivity of M cells to higher temporal frequencies has been linked to motion perception (DeYoe & Van Essen, 1988; Livingstone & Hubel, 1988).

Few studies, however, have examined the response properties of K LGN cells, especially in simian primates. K LGN cells have been studied in the most detail in the nocturnal prosimian bush baby (*Galago crassicaudatus*) (Norton & Casagrande, 1982; Irvin et al., 1986; Norton et al., 1988; Irvin et al., 1993). In contrast to M and P cells, K cells in bush babies were found, on average, to have larger receptive fields and slower orthodromic and antidromic conduction velocities. Like cat W cells, bush baby K cells (referred to earlier as W-like) also appeared to be heterogenous as a group; some could not be driven well by grating stimuli or were only poorly driven by such stimuli. Other bush baby K cells, however, were unlike cat W-cells, and responded briskly to gratings exhibiting contrast sensitivity functions whose resolution levels lay intermediate between those of the average M or P cell (Norton et al., 1988).

Only recently has there been an effort to examine the response properties of K cells in any simian primate (Martin, et al., 1997; White, et al., 1998; Solomon et al., 1999). Studies of K cells in the

diurnal New World marmoset monkey revealed one population of K cells that appeared to receive input from blue-ON ganglion cells suggesting involvement of this pathway in the processing of chromatic information (Martin, et al., 1997; White, et al., 1998). Since K cells send axons to the CO blobs of V1, the latter result is consistent with the proposed role of CO blob cells in chromatic processing (Livingstone & Hubel, 1988), although recent studies in primate V1 conflict with earlier reports and suggest that CO blob cells are not unique in their selectivity for chromatic stimuli (Lennie et al. 1990; DeBruyn et al., 1993; Edwards et al., 1995; Leventhal et al. 1995). Because bush babies have only a single cone type, lack blue cones and also have well defined CO blobs, K cells and their CO blob targets likely perform some more universal visual function than the processing of chromatic signals or perform more than one role across species (Winkler & Rakic, 1990, Casagrande & Kaas, 1994). Regardless, given the paucity of information available on K cell physiology a key objective of the present study was to proceed by analogy to previous work on M and P cells and put together a physiological profile of this class of cells that might be linked to particular perceptual attributes.

Our aim for the present study was to fully characterize receptive field properties of K LGN cells in the nocturnal simian owl monkey and compare K cell properties to those of M and P cells. Owl monkeys, like prosimian bush babies, have only one cone type and lack blue cones entirely, and like marmosets, are New World simian primates (Winkler & Rakic, 1990; Jacobs et al, 1993). Owl monkeys offered us several other advantages. First, with the exception of macaque monkeys, the visual systems of owl monkeys have been studied in the most detail (For review see Casagrande & Kaas, 1994). Second, owl monkeys have well-developed LGNs with simple laminar patterns consisting of two P layers, two M layers, and at least three well-defined K layers. Finally, the axon structure and

cortical target cells of K LGN cells in owl monkeys have been studied in detail (Ding & Casagrande, 1997; 1998). Some of the results reported here were presented previously in abstract form (Xu et al., 1999).

Method and Materials

General preparation

Conventional extracellular recording techniques were employed to examine the receptive field properties of LGN neurons in nine adult owl monkeys (*Aotus trivirgatus*). In seven owl monkeys single units were recorded directly from neurons in the LGN. In the remaining two monkeys recordings were made from LGN cell afferent axons in V1 where intrinsic neuronal activity was inhibited by the $GABA_A$ agonist muscimol (Chapman et al., 1991; Boyd et al., 1998). All monkeys were handled according to the National Institutes of Health Guide for the Care and Use of Animals under an approved protocol from the Vanderbilt University Animal Care Committee. Animals were pre-medicated with injectable atropine (0.1 mg/kg), Acepromazine (0.5-1 mg/kg) and dexamethasone (2 mg/kg). Anesthesia was induced with an intramuscular injection of ketamine HCL (8-12 mg/kg) and mask inhalation of Isoflurane. Animals were maintained with these anesthetics while a cannula was inserted into the femoral vein of one hind-limb for subsequent delivery of anesthetic and paralytic agents. Anesthesia then was maintained with injections of ketamine for the remainder of the surgical manipulations. After a cannula was placed in the trachea, the animals were mounted in a stereotaxic apparatus, the scalp was reflected on the midline and stainless steel screws were inserted in the skull over the frontal lobe for recording EEGs to monitor general levels of arousal. Animals were then paralyzed with an intravenous injection of 1-1.5 mg/kg vecuronium bromide (Norcuron) and artificially

ventilated with a mixture of 75% N_2O, 23.5% O_2 and 1.5% CO_2 delivered at a rate of 28-35 strokes/min with a volume of about 15 cc to maintain the peak end tidal CO_2 level at 4%. Paralysis and anesthesia were maintained by intravenous infusion of vecuronium bromide (0.2 mg $\cdot$ kg^{-1} $\cdot$ h^{-1}) and sufentanil citrate (Sufenta: 12-15 µg $\cdot$ kg^{-1} $\cdot$ h^{-1}) mixed in 5% dextrose lactated Ringer's delivered at a rate of about 2.7 ml/h.

For LGN recording, a small craniotomy (4×5 mm) was made over the location of the LGN according to stereotaxic coordinates established previously and the dura reflected. The brain was protected with a layer of agar. After the electrode was inserted into the brain, the opening in the skull was covered with an additional layer of paraffin wax to ensure recording stability. For recordings made from silenced V1, holes were drilled directly over the *area centralis* representation in V1 in the posterior lateral cortex; the dura in the hole was incised, and 25-50 µl of 50 mM muscimol were applied to the exposed cortex for 5-10 minutes to silence cortical cellular activity. The cortical surface was then rinsed with saline before sealing the craniotomy (Chapman et al., 1991; Boyd et al., 1998). Muscimol was reapplied to the cortical surface as soon as there were signs of recovery of neural activity. Generally cortical neural activity did not recover for at least 8 hours.

Pupils were dilated with atropine eye-drops (1% ophthalmic atropine sulfate). Individually fitted clear gas permeable contact lenses were used to render the retina conjugate with the viewing screen 57 cm distant. In some animals lenses with 3mm artificial pupils were used. Retinal landmarks (optic disk and area centralis) were projected onto the plotting screen with a reversible ophthalmoscope. The electrode was then lowered into the brain using a microdrive. Responses to visual stimuli were monitored as the electrode was lowered until characteristic LGN responses were found. During physiological recording, EEG, EKG, end tidal CO_2 level and rectal temperature were monitored and

maintained at appropriate levels.

Recording, stimulation and data acquisition

Commercially made parylene-coated tungsten electrodes (FHC) with an impendence of 5 -10 MS were used to record from LGN cells. Well-isolated units were used to trigger standard pulses, which could be played over the audio monitor and counted by a computer that also controlled the presentation of stimuli.

The receptive fields of each unit was initially plotted by manually controlled stimuli displayed on the tangent screen, with eye dominance determined and the receptive-field boundaries drawn. The receptive field centers and surrounds were identified as either ON, OFF or ON-OFF. In addition, any strong suppressive effect of the surround on the center was noted. The horizontal and vertical extent of the receptive field center was measured and the average of these two values was taken as the diameter of the receptive field center. We also qualitatively differentiated cells into sustained and transient categories according to their response to stationary contrast stimuli presented for 5 to 10 seconds within the center of the receptive field. Units that exhibited above spontaneous maintained discharge during this period were categorized as sustained.

Stimuli consisted of drifting sine-wave and counterphase gratings presented at different spatial and temporal frequencies, contrasts and orientations, and phases in the case of counterphase gratings. Stimuli were generated by an image-processing board (Pepper PRO 1280) with a capacity of 1,024x1,280 pixels by 8 bits of modulation and presented on a CRT screen that subtended an angle of 10 degrees with a background illuminance of 110 cd/cm^2. For the cells having strong suppressive surrounds stimuli were presented within a 2 degree window instead of the full 10 degree screen. Cells

were tested with spatial frequencies ranging from 0.1 – 9.6 c/deg, temporal frequencies ranging from 1 to 32 Hz at the optimal spatial frequencies, and contrasts ranging from 3% -56% at optimal spatial and temporal frequencies. Linearity was tested using different phase angles of the counterphase sine-wave gratings stepped through the receptive field such that the cell's responses were sampled at all positions within the center and surround at least once. The initial linearity test was run with spatial, temporal and orientation parameters optimized for the cell at moderate contrast (28%). Next the cell was tested again at double its preferred spatial frequency. If the cell still responded adequately it was retested at 3 times the optimal spatial frequency, and so forth, until the cell no longer responded. By increasing the spatial frequencies we could ensure that non-linearities in spatial summation would be reliably detected if present (Hochstein & Shapely, 1976; Derrington & Lennie, 1984).

Data were collected by a generic PC-386. The primary data analysis tool was construction of 2-s, 128 bin/s post-stimulus time histograms. The interleaved histogram technique of Henry et al. (1973) with randomization was adopted to reduce artifact from the inherent non-stationarity of the visual system. A stimulus set was specified and comprised each measuring condition as well as a null condition (a blank screen at the mean luminance of the gratings) to assess the maintained discharge. Each element in the stimulus set was presented once in a random order with a 1 s interval of blank screen between each presentation. Presentation of the set was then repeated in a random order until each stimulus condition had been tested completely (5-10 times). With 4-s presentation periods, data are based on 20-40 s of averaging for each condition. The PST histograms for each cell were Fourier transformed, and the fundamental (F1) and second harmonic (F2) components of the response were analyzed. Data were plotted with IGOR 3.1 (WaveMetrics, Inc.) and Matlab 5.0 (MathWorks, Inc.) programs. The following receptive field properties were measured for each cell: peak spatial frequency

and cut-off, peak temporal frequency and cut-off, response to stimulus contrast, and linearity of spatial summation. In addition, we determined orientation selectivity for each cell tested and all remaining tests were done with the grating stimuli set to the preferred orientation if one existed. Data on the orientation selectivity of LGN cells in owl monkey will be submitted for publication separately. If the cell exhibited no orientation selectivity than all tests involved vertically oriented drifting gratings.

We examined linearity of spatial summation with counterphased gratings in all the cells that responded well to drifting gratings. The ratio of the mean amplitude of the second harmonic (averaged across all spatial phases) to the amplitude of the fundamental at the best phase was used as an index of non-linearity (Hochstein & Shapley, 1976; Derrington & Lennie, 1984), with a value greater than 1.0 indicating a substantial non-linearity. In the present study, cells with 2nd harmonic(F2)/1st harmonic(F1) ratios of <1.0 at all spatial frequencies tested were classified as linear cells. Cells with F2/F1 ratios >1.0 at any one of the spatial frequencies tested were classified as non-linear cells.

Spatial and temporal frequency peaks were defined as the frequencies at which cells exhibited the highest first harmonic response. Cutoffs were determined by extrapolating the high-frequency limb of the curves (vs. log frequency) to control levels determined from responses to the blank control screen (baseline).

Several measures of contrast sensitivity were compared. Threshold contrast was determined by extrapolating to baseline (vs. log contrast). Contrast gain was determined based upon the slope of the linear portion of the contrast response curve (vs. log contrast) where responses were well above threshold. In addition, we attempted to fit contrast response curves with a hyperbolic function in the form of $Response(C) = R_{max} \cdot C^n / C^n + C_{50}^n)$, where R_{max} = maximum response rate, C_{50} = contrast required for half maximal response, and the exponent n = rate of change (Albrecht &

Hamilton, 1982). Such hyperbolic fits were useful only in those cells that exhibited response saturation. In the latter group we also compared C_{50} values obtained from these curve fits.

Statistical Analysis

Statistical comparisons of receptive field properties across the K, P and M groups was done by one-way ANOVA with post hoc mean difference tests (Tukey and LSD tests), provided that the data did not violate the prerequisite of variance homogeneity across groups. For groups with both unequal variances and unequal samples, we used instead the Kruskal-Wallis test, which is a non-parametric procedure commonly advocated as an alternative of the ANOVA (Kruskal & Wallis, 1952). In the latter case, a Mann Whitney U test was used for between group comparisons. Alpha levels of $p\#0.05$ were considered significant.

Histological procedures

The position of each recorded cell was noted by the depth indicated on the microdrive. Electrolytic lesions (5 µA × 5 sec) were made to mark the location of electrode tracks (see Fig.1). At least two electrolytic lesions were made on each electrode penetration to aid in reconstruction of the track and to calculate tissue shrinkage. At the termination of each experiment, the animal was deeply anaesthetized with an overdose of Nembutal (sodium pentobarbital) and perfused transcardially with a saline rinse followed by fixation with 0.1 % glutaraldehyde and 3 % paraformaldehyde in O.1 M phosphate buffer. The brain was removed and cryoprotected overnight in a solution containing 30% sucrose in 0.1 M phosphate buffer. For LGN afferent axons in V1, K axons can be distinguished anatomically since they terminate within cortical layers 3 and 1, while M and P axons terminate within

the upper and lower halves of cortical layer 4, respectively (see Ding and Casagrande, 1997). To identify cortical layers alternate V1 sections were stained for Nissl and cytochrome oxidase (CO), respectively. LGN axon types were classified based upon the laminar position established via reconstructions of the electrode tracts. To locate the LGN cells recorded, alternate sections of the LGN were stained for Nissl bodies, CO and immunostained for calbindin-d28K. CO staining was performed using the method described by Boyd and Matsubara (1996).

Calbindin immunostaining was used to identify the K layers, which are numbered K1-K4 beginning with K1 located between the optic tract and the first M layer (see Fig.1). Only those axons or cells that could be located histologically with confidence were included for further data analysis.

Figure 1 about here

Calbindin immunostaining was performed using a rabbit polyclonal antibody (Swant CB-38). Before being placed in the primary antibody, the sections were placed in 0.3% H_2O_2 for 30 minutes followed by three rinses in 0.1M Tris-buffered saline (TBS; pH 7.4). Sections were then incubated for one hour in blocking buffer consisting of 10% normal donkey serum, 1% bovine serum albumin and 0.1% Triton X-100 in TBS. Then the sections were placed in primary antibody diluted 1:5000 in antibody buffer consisting of 10% normal horse serum, 0.2% bovine serum albumin, and 0.2% Triton X-100 in TBS. Sections were incubated overnight in the primary antibody and then rinsed three times in TBS before being placed in the secondary antibody. The secondary antibody (biotinylated donkey anti-rabbit [Chemicon]) was diluted at 1:200 in antibody buffer. Sections were incubated in secondary antibody for two hours, rinsed three times in TBS and placed in tertiary antibody (Elite ABC kit,

Vector) prepared in TBS. After two hours the sections were rinsed three times with TBS and the calbindin immunoreactivity was visualized by placing the sections into a solution containing 50mM imidazole, 25mM nickel ammonium sulfate, 0.01-0.02% 3,3'-diaminobenzadine and 0.0003% H_2O_2 in 0.05M TBS. Sections remained in this solution until labeled cells were visible and the reaction product was quite dark (usually 15-20 minutes).

Results

We recorded from 133 LGN cells and 10 LGN afferent axons in silenced V1 with eccentricities ranging from 2.8° to 31.3°. All LGN axons recorded from silenced V1 had eccentricities of less than 10°. From this total population 38 K units (36 cells & 2 axons), 45 P cells, and 34 M units (29 cells & 5 axons) could be assigned with confidence to specific layers based upon histological reconstructions.

Receptive Field Structure and Size

Twenty of the 38 K units analyzed (53%) gave a sustained responses to a stationary stimulus held in the receptive field center for at least 5 seconds. The remaining 18 (47%) responded transiently. Twenty-three K units (66% or 23/35) exhibited typical center/surround receptive fields either ON center with an OFF surround or the converse. Thirty-four percent of the K units (12/35) had either strong suppressive surrounds, ON-OFF surrounds or no clear surrounds. Thirteen K units (34% or 13/38) were poorly driven by grating stimuli, but responded well to flashing spots or single light bar stimuli moved manually. Of the 13 K cells that did not respond to gratings, 2 appeared to respond only to changes in luminance, and although this impression was not tested quantitatively these cells seemed to

respond very much like the "luminance units" described in the cat retina by Barlow and Levick (1969). In addition, two other K cells seemed to have unusually long onset latencies to flashing spots of light.

Thirteen of the 34 M units analyzed (38%) responded in a sustained manner and the remainder responded transiently to a stationary stimulus of appropriate contrast. In contrast to the K and M populations the majority, (71% or 32/45) of P cells responded in a sustained fashion. Ninety percent of the P and M units showed standard center/surround receptive fields; the remainder showed either weak or unclear surrounds or suppressive surrounds. Also, in contrast to the K population, only 4 P cells (9%) and 2 M cells (6%) responded poorly to grating stimuli although all of these cells responded briskly to flashing spots or moving bars of light.

In all three LGN cell classes receptive field center size tended to increase with eccentricity, but this relationship was least clear for the K population where there was a large degree of scatter at all eccentricities. Figure 2 shows the relationship between receptive field center size and eccentricity for the subset of the K (14), P (19) and M (18) cells where center boundaries were unambiguous. For this population average receptive field center diameter was 1.05 ± 0.25 deg for K cell cells, 0.87 ± 0.11 deg for P cells, and 0.92 ± 0.13 for M cells.

Figure 2 about here

Linearity of spatial summation

In cats the major feature that is used to distinguish X and Y retinal ganglion and LGN cells is linearity or nonlinearity of spatial summation (Enroth-Cugell and Robson, 1966; Hochstein and Shapley, 1976). According the Hochstein and Shapley (1976) the identification of a cell as an X cell on the basis

of linear summation requires not only a strong dependence on spatial phase and response at the fundamental modulation frequency, but also that spatial phase dependence be demonstrated at higher than the cell's preferred spatial frequency. This is because Y cells can exhibit a strong spatial phase dependence and respond quite well at the fundamental modulation frequency if the grating is presented at a low spatial frequency. Therefore, we also examined for spatial phase dependence both at the preferred spatial frequency of the cell and at least 2x the preferred frequency. Cells were considered to respond linearly if they showed a clear null F1 response. Also, if the F2 response became dominant over the F1 response we classified the cell as non-linear (Hochstein and Shapley, 1976). In Y cells the F2 component was found to be phase insensitive. We calculated the F2/F1 response ratio as an index of nonlinearity. In cats, X cells were always found to have a nonlinearity index of less than 1.0 while for Y cells this index was found to be greater than 1.0 (Hochstein and Shapley, 1976).

Figure 3 show examples of spatial phase dependance in the responses of typical K, P and M cells, respectively. We found that all K (N=17), P (N=32), and all but one M (N=27) unit could be classified as linear according the above described criteria.

Figure 3 about here

The K cell shown in Figure 3A was tested at its optimal spatial frequency of 0.8 c/deg. The peaks of the F1 responses are much higher than average F2 responses at all phases outside of the null points. This cell exhibited a non-linearity index of 0.21. Nulls are clearly evident at phase positions -120º and 60º. Figure 3B shows the responses of the same cell tested at 2× the optimal spatial frequency. As would be expected the F1 responses decrease as the spatial frequency is increased

beyond the cell's preferred frequency but at no point is the average F2 response higher than the F1 response. Also even at 2x the preferred spatial frequency this cell still exhibits clear evidence of a null.

The P cell shown in Figure 3C also shows clear evidence of null positions in the F1 response curve at - 60º and 120º phase angles when tested at its preferred spatial frequency. As shown in Figure 3D when this same cell is tested at 2× its preferred spatial frequency, it still exhibits clear null responses. The peaks of the F1 curve are always higher than those of the F2 at all phases outside of the null positions.

The spatial phase responses of a typical M cell shown in Figure 3E also exhibit clear evidence of spatial linearity. Figure 3E shows the cell's responses when tested at 2x its preferred spatial frequency. The F1 response curve exhibits nulls at -90º and 90º. At the peak responses of this cell the F1 curve is always higher than the F2 curve. Figure 3F show the same cell tested at 4× its optimal spatial frequency and nulls are still evident. Only one M cell showed any indication of nonlinearity (Figure 4). In this cell the peak F2 responses are higher than the F1 response. However, unlike cat Y cells this cell shows evidence of phase dependence.

Figures 4 and 5 about here

Figure 5 shows a summary distribution of the non-linearity indices for all cells in the population tested at their optimal or close to optimal spatial frequencies. Only one M cell (also shown in Figure 4) exhibited an index of greater than 1.0 suggesting spatial non-linearity. The distributions of the non-linearity indices for the other cells show no clear trends that correlate with cell class. The majority of K, M, and P cells show non-linearity indices of <0.4 with a peak for each of the populations at 0.2.

Spatial and temporal resolution

Spatial and temporal frequency tuning curves and responses to contrast are shown for representative K, P and M cells in Figures 6, 7, and 8, respectively. The K cell shown in Figure 6A exhibited a peak spatial frequency at about 0.8 c/deg and a high spatial frequency cutoff at around 6 c/deg. Typical of all K cells tested this K cell showed a broad band-pass tuning curve with a sharper drop off in response to higher than lower frequencies. As shown in Figure 6B this K cell responded to temporal frequencies over a broad range from 1.0 Hz (the lowest temporal frequency tested) to 6.0 Hz with a clear peak at 2Hz. The contrast response function for this same K cell is shown in Figure 6C. The extrapolated contrast threshold for this cell is about 2.5 %, and its contrast gain (the slope for the linear segment of rising phase) is 29 spikes $\cdot s^{-1} \cdot$ log contrast %.

Figure 6 about here

Spatial and temporal tuning curves for a representative P cell are shown in Figure 7. Unlike K and M cells most P cells exhibited symmetrical band pass spatial frequency tuning curves. This cell showed a peak spatial frequency response at 0.8 c/deg and a cutoff around 6.4 c/deg (Fig.7A). Like the K cell shown in Figure 6 this P cell had a peak temporal frequency at 2.0 Hz but a higher cut-off at 10 Hz. The contrast response curve for this P cell is shown in Figure 7C. It shows a sigmoid shape and an extrapolated threshold of around 5% contrast and a contrast gain of 29.2 spikes $\cdot s^{-1} \cdot$ log contrast %. Most P cells had contrast gains of less than 15 spikes $\cdot s^{-1} \cdot$ log contrast % , which was lower than that found in most M and K cells.

Figure 7 about here

Spatial and temporal resolution curves for a representative M cell are shown in Figure 8. Unlike K and P cells, some M cells did not exhibit a low spatial frequency roll off as shown in Figure 8A. This cell had a peak spatial frequency of 0.2 c/deg and spatial frequency cutoff of 6 c/deg. This cell also responded well to all temporal frequencies from 1.0 Hz (the lowest tested) to 8 Hz but still responded above background at 20Hz. This cell's peak temporal frequency is the same as that for the K and P cells shown earlier, 2 Hz (Fig. 8B). The contrast response curve for this M cell is shown in Figure 8C. It shows an extrapolated threshold of 2.5 % contrast and a contrast gain of 18.9 spikes $\cdot s^{-1} \cdot$ log contrast %.

Figure 8 about here

Table 1 provides the average ± SE spatial and temporal resolution values for each population of cells which included a total of 25 K, 41 P and 32 M units. Statistical comparisons were confined only to the 15 K, 27 P and 16 M cells (Table 2) at roughly matched eccentricities (>10E). The histograms in Figures 9-11 compare the eccentricity matched populations for each parameter measured. As can be seen in table 2 and Figure 9 K, P, and M cells show broad overlapping ranges of peak and cut-off spatial frequencies. A one way ANOVA comparison of the populations at matched eccentricities revealed significant differences in peak spatial frequencies and cutoffs (p = 0.008 and 0.05, respectively). A post hoc mean test showed that the P cells had significantly higher peak spatial

frequencies than K or M cells (p = 0.02 and 0.03, respectively); the mean peak spatial frequencies for K and M cells did not differ ($p > 0.90$). P cells differed significantly from M cells in their spatial frequency cutoffs (p = 0.05). Although K cells tended to have lower average spatial cut-offs than P cells, this trend did not reach significance (P vs K p = 0.26 / M vs K p = 0.73).

Interestingly, as shown in Figure 9A the K cells peak spatial frequency distribution appeared to have two modes suggesting that K cells have subclasses.

Figure 9 and tables 1 & 2 about here

Figure 10 compares the peak and cut-off temporal frequencies for K, P and M cells. Mean values are given in Table 2. As can be seen in Figure 10A, K, P and M cells have very similar temporal frequency peak distributions with most cells preferring 2.0 Hz. Figure 10B shows that K cells have temporal frequency cut-offs that lie between those of P and M cells although here again the populations show broad areas of overlap. Temporal frequency cut offs were found to differ significantly between the 3 groups (Kruskal-Wallis test, $p = 0.003$). This difference can be accounted for by differences between P and M cells ($p = 0.001$) since Mann Whitney U tests showed that the K cell population did not differ from either the M (p = 0.22) or P (p = 0.07) populations. As can be seen, the temporal resolution values of K cells seem to lie between those of P and M cells.

It is also noteworthy that as demonstrated for K cells in the peak spatial frequency domain, M cells exhibit double peaks indicative of two modes in both spatial and temporal frequency cutoffs (Figures 9B and 10 B). The double peaks seen in the M distributions also may hint at the existence of subclasses.

Figure 11 compares contrast threshold values (A) and the contrast gain values (B) for each of the populations; mean values are shown in table 2. Contrast thresholds of M cells were significantly lower than those of P cells (Mann Whitney U test, p = 0.05). K cells did not differ significantly from M cells or P cells (p = 0.26/0.45). M cells exhibited significantly higher average contrast gains than P cells (Mann Whitney U test, p = 0.05), although K cells did not differ significantly from either P or M cells (p = 0.48/0.53). Thus, as with temporal resolution, the contrast characteristics of K cells seem to lie intermediate between those of M and P cells.

Figures 10 & 11 about here

We also attempted to fit contrast response curves for all the cells tested with a hyperbolic function in the form of $Response(C) = R_{max} * C^n / C^n + C_{50}{}^n)$, where R_{max} = maximum response rate, C_{50} = contrast required for half maximal response, and the exponent n = rate of change or contrast gain index (Albrecht & Hamilton, 1982). The contrast response curves of the majority of M (24/32) and K cells (15/23) were well fit by a hyperbolic function, however, the curves of most P cells (25/37) showed little response saturation and thus could not be adequately fit by this function. For those K, P and M cells where the fit was good, the average C_{50} differed between populations, for K cells it was 25.9 ± 4.0, for P cells 39.0 ± 5.3, and for M cells 20.7 ± 2.6. The average C_{50} was differed significantly between the 3 groups (ANOVA, $p = 0.005$). The average C_{50} of P cells was significantly different from either that of M or K cells (P vs M p = 0.001; P vs K p = 0.029). However, the average C_{50} of K cells did not differ significantly from that of M cells (p = 0.29).

Properties of K cells in different layers

There are 4 K layers in the owl monkey based upon the distribution of calbindin labeled cells. Of these K layers, three are well developed, with K1 and K3 exhibiting the largest numbers of K cells (see Figure 1C and 1D). Of our sample of K units, reconstructions indicated that 2 K axons in V1 were in layer 3B suggesting that they probably arose from LGN layer K3; 4 were in LGN layer K1, 11 were in LGN layer K2, 20 were in LGN layer K3, and 1 was in LGN layer K4. However, of the total sample of 38 K units, only 3 K1, 6 K2, 14 K3 cells plus the 2 K, responded well enough to grating stimuli for quantitative measures to be made. Since our previous anatomical studies in owl monkeys showed that the different K cell layers (K1 & K2 verses K3) have distinct axonal termination patterns in V1 (Ding and Casagrande, 1997), we asked whether these anatomical distinctions correlated with any differences in K cell receptive field properties. As shown in Figure 12 differences were found between the properties of cells in the different K layers. Cells in K1/K2 tended to be selective for lower spatial frequencies and higher temporal frequencies than cells in layer K3 (Figure 12A & 12B). These trends were significant, however, only for the differences between temporal frequency cut-offs (Mann-Whitney U, $p = 0.05$) perhaps due to the small N. In addition, K1/K2 cells exhibited a trend toward higher contrast thresholds and lower contrast gains than K3 cells (Figures 12C, D), although this trend did not reach statistical significance. Overall, these K layer differences suggest that the ventral most K layers, K1/K2 resemble M cells more than P cells in their spatial and temporal resolution characteristics whereas the resolution values of K3 cells tends to lie intermediate between those of P and M cells. Clearly, a larger N will be required to confirm the trends seen.

Figure 12 about here

Discussion

Our key objective was to examine the receptive field properties of K LGN cells and, by comparing the characteristics of this population with those of M and P LGN cells, provide some insights into the function(s) of this cell class. A main finding was that the majority of K cells in the owl monkey overlap extensively in terms of spatial and temporal resolution with M and P cells. Nevertheless, unlike M and P cells, a significant minority of K cells could not be driven by standard drifting gratings leading to our second main finding namely that the K population is more heterogeneous in terms of its responses to visual stimuli than either the M or P class. Finally, our data provide evidence that some of the heterogeneity within the K population can be accounted for by position in the LGN since cells in different K layers can be distinguished based upon spatial and temporal resolution. Below we consider each of these conclusions in light of studies conducted by others.

Non-Standard receptive field properties

In owl monkeys a third of the K cells responded poorly or not at all to drifting gratings whereas less than 10% of P and M cells fell in this category. Of those that did not respond to gratings a larger proportion of K cells than M and P cells also were difficult to characterize with other stimuli that were tried including manually moved light bars and flashing spots of various sizes. This description of K cells fits well with earlier reports of K cells in the prosimian bush baby and the New World simian marmoset. In the bush baby more than three quarters of the K population responded poorly to gratings; in marmosets approximately 13% were found to be unresponsive to such stimuli (Norton and Casagrande, 1982; Irvin et al.,1986; Solomon et al., 1999; White et al.,submitted). Although the

percentages of K cells that were unresponsive to gratings were clearly less in the two new world simians (owl monkey, present study) and marmoset (White et al., submitted) than in bush babies, a larger proportion of P cells in marmosets (32%) were found to be unresponsive than in owl monkeys. Although K cells have not been studied in detail in old world simians, earlier studies of P and M cells in macaque monkeys suggest that, as we find in the owl monkey, only a small percentage of M and P cells (19 out of 389) would not respond to drifting sine wave grating stimuli (Spear et al., 1994).

The existence of a relatively large number of K cells in owl monkeys and bush babies that can not be driven by standard stimuli supports the idea that this population of cells, at least in these two primate species, is quite distinct from M and P cells. Whether these difficult to categorize cells are similar in other ways across primate species is difficult to say since attempts to categorize these cells have not been systematic. It is noteworthy, however, that some of the non-standard characteristics that have been reported for both simian and prosimian K cells such as very large difficult to plot fields, cells with non-standard center/surround organization, cells that respond sluggishly or variably to a variety of visual stimuli have also been reported for cat W cells suggesting that some K cells may be analogous to cat W cells (Sur and Sherman, 1982; see also Norton and Casagrande, 1982). Whether some K cells and W cells are utilized functionally in the same way in primates and in cats, respectively, will require more detailed study but the number of other characteristics that are shared between K cells and W cells such as larger average receptive field sizes, thinner axons with slower conduction velocities, and projection patterns to layer 3 of striate cortex all hint at some functional relationship between these cell classes (see Casagrande, 1994 for review).

Receptive field structure and size

Consistent with an earlier study of LGN receptive field properties in owl monkeys (Sherman et al.,1976) we found that M cells on average were more transient in their responses to center appropriate standing contrast stimuli than were P cells. Approximately half the K cells we tested responded in a transient manner. Although not examined in detail, we did not find a correlation with degree of response transience and other properties of K cells. Besides owl monkeys, sustained/transient responses have only been examined for all three cell classes in the bush baby (Norton and Casagrande,1982). Examination of sustained verses transient responses in bush baby K, M and P cells showed similar differences to those described here for owl monkeys with most M cells exhibiting transient responses, most P cells exhibiting sustained responses, and K cells falling in between.

Receptive field center sizes in owl monkey, as in the LGNs of other primates, tend to increase with increasing eccentricity (Present study; for review see Casagrande and Norton, 1991). K cells in the current study in owl monkey were found to differ from this pattern only in the overall degree of variation in center size seen. Some K cells had significantly larger receptive field center sizes than P or M cells at the same eccentricities while other K cells had receptive field center sizes that fell in the range of the smallest we encountered. In bush babies and marmosets, it has been reported that K cells have relatively larger receptive field centers than those of P and M cells at same eccentricities with the variability in size also reported to be higher in K cells than in P and M cells (Norton and Casagrande, 1982; Irvin et al, 1993; White et al., submitted). In marmosets, however, M cells also showed high variability in size (White et al., submitted). Receptive field center sizes for P cells have consistently been reported to be smaller than those of M cells at any eccentricity (Sherman et al., 1976; Norton & Casagradne, 1982; Derrington & Lennie, 1984; Norton et al., 1993; Kremers & Weiss, 1995; O'keefe et al., 1998; Usrey & Reid, 2000). In owl monkeys two reports (O'Keefe et al., 1998; Usrey

and Reid, 2000) have shown that receptive field sizes of P were smaller than those of M cells at all eccentricities. P and M cells in owl monkeys were also found to be larger than those of macaque monkeys by a factor of about 2 and smaller than those of the bush baby by a factor of 2 which fits with the differences in visual acuity of these three primate species (O'Keefe et al., 1998). Although our data are in general consistent with these results we find more overlap between the sizes of P and M receptive fields than was reported previously in owl monkeys. It is noteworthy, that in macaque monkeys, although P cells have been found to have smaller centers than M cells at matched eccentricities significant overlap also has been reported (Derrington & Lennie, 1984; Spear et al., 1994; see Merigan & Mausell, 1993 for review).

Spatial summation

Although linearity of spatial summation has been useful in distinguishing cell classes in cat LGN its usefulness in primate LGN cell classification remains controversial (see Casagrande and Norton 1991 for review). In this study we found that all K, P and M cells with the exception of a single M cell were linear. Since we examined spatial linearity at the highest spatial frequency to which each cell would respond we believe it is unlikely we failed to drive non-linear subunits adequately and so missed a population of non-linear LGN cells. Consistent with our results, Usrey and Reid (2000) report that all the M and P LGN cells that they tested in owl monkey and squirrel monkey LGN (K cells were not examined) were linear as measured by a null test. Additionally, very few spatially non-linear LGN cells (3M and 1 K out of 36 cells) were found in bush babies that resembled Y-cells in cats, using counterphased gratings (Norton & Casagrande, 1982). In other primates, however, higher percentages of non-linear LGN cells have been reported (Kaplan & Shapley, 1982; Derrington &

Lennie, 1984; Blakemore & Vital-Durand, 1986; White et al., submitted). For example, in a recent study in marmosets 11% of M cells, 6% of P cells and 13% of K cells were found to be spatially non-linear and the majority of those in M and P cells were located very close to K layer borders (White et al., submitted). White et al.,(submitted) have suggested that the non-linear cells (around 4.5%) reported to be P or M cells in macaque monkey (Kaplan & Shapley, 1982; Derrington & Lennie, 1984; Blakemore & Vital-Durand, 1986) may actually have been mis-classified K cells given their finding almost all non-linear LGN cells were in or very near the borders of K layers in marmosets. Regardless, it would appear that non-linearity of spatial summation is not a consistent feature of LGN cells in primates. At present it is unclear why some species have some spatially non-linear LGN cells while others have virtually none.

Spatial and temporal resolution

Examination of the differences between the spatial and temporal resolution of populations of K, P, and M cells in our study in owl monkeys suggests that although statistically demonstrable differences can be found between K, M, and P classes the overlap between them is substantial. This finding suggests that cells in all 3 classes could contribute jointly to different aspects of conventional vision depending upon the demands of the task, or at least those that respond well to gratings. In other words, the K cells that respond to gratings, which constitute the majority, do not stand out by deviating substantially in terms of spatial and temporal resolution from M and P cells. On average, however, at matched eccentricities, K cells exhibited lower spatial frequency cut offs than the P and M cells, and showed temporal resolution values that fell intermediate between those of the P and M cells. The

contrast thresholds and contrast gains of K cells were more similar to those of M cells than those of P cells.

As reported by others in owl monkeys and other simian and prosimian primates we found that M cells tended to exhibit lower spatial resolution and higher temporal resolution and contrast gain than P cells (O'Keefe et al., 1998; Usrey and Reid, 2000; Norton et al., 1988; Kaplan & Shapley, 1982; Derrington & Lennie, 1984; Blakemore & Vital-Durand, 1986; White et al.,submitted). In owl monkeys, O'Keefe et al. (1998) reported that P cells in owl monkey LGN had higher high spatial frequency cut-offs, lower optimal temporal frequencies and cut-offs, and lower levels of responsivity than did M cells. Our results are consistent with this previous study (O'Keefe et al., 1998), except that the P and M cells in our study preferred similar peak temporal frequencies. Both our work and that of O'Keefe et al., 1998 found that the differences between owl monkey M and P cells in contrast sensitivity and gain are markedly lower than those reported for macaque M and P cells (Kaplan & Shapley, 1982; Spear et al., 1994).

Physiological subclasses of K cells

Previous anatomical studies in owl monkeys done in our lab showed that the different K layers (K1 & K2 vs K3) have distinct axonal termination patterns in V1, with axons from K3 cells mainly terminating within the cytochrome oxidase (CO) blobs in layer 3 of V1 and axons from cells in K1& K2 mainly terminating in cortical layer I (Ding & Casagrande, 1997). These anatomical differences suggested that some physiological differences may exist between these populations, a suggestion that is supported by our data. Our results show that K cells in K1/K2 are more selective for lower spatial frequencies and higher temporal frequencies than cells in layer K3. In other words the K cells that lie

between or below the M layers tend to resemble these layers in resolution whereas the K3 cells have resolution values that lie intermediate between the average values for M and P cells (at least in terms of temporal resolution) matching their anatomical position between the LGN M and P layers.

The distribution of the peak spatial frequencies of K cells also exhibited two modes suggesting subclasses. Interestingly, M cells also showed double peaks within their distributions of spatial and temporal frequency cutoffs indicating the existence of M subclasses. The presence of M subclasses is supported by anatomical work in owl monkeys (Boyd et al., 2000) and anatomical and modeling data in macaque monkeys (Lund *et al.*, 1995; Bauer *et al.*, 1999).

Additional support for K subclasses comes from preliminary immunocytochemical work (Song et al., 2000) which shows that K cells in macaque monkeys are neurochemically diverse. Two marker proteins used to identify K cells in macaque monkeys calbindin-28 D (CB) and the alpha form of calcium/calmodulin dependent kinase II ("CaMK II) did not completely overlap in their distributions within the different LGN K layers. In fact, based upon double-label immunocytochemistry three subclasses of K cells could be identified, classes that contained either CB only or "CamK II only and a class that was double-labeled. The proportion of each type was found to vary within the different K layers suggesting that even within K layers different subclasses of K cells may co-exist. At present we do not know if the same markers will identify subclasses of K cells within the K layers of the owl monkey but it may be useful in the future to combine physiology with the immunocytochemistry for these markers to investigate this issue.

A role for K cells in vision

Our physiological results in owl monkeys can not directly address the most interesting question, namely, what role do K cells play in the vision of primates. These data, however, do provide descriptions of K cell response characteristics from which we can draw a few conclusions. The first is that K cells in owl monkeys are a heterogeneous population given that a significant fraction do not respond to grating stimuli and that differences were found between the K cells in the different K layers. The idea that K cells contain subclasses is also supported by older work in the prosimian bush baby where many more K cells than P or M cells were reported to exhibit non-standard properties (Irvin et al.,1986). Among simian primates detailed analysis of K cell physiology is only available for marmosets (Martin et al.,1987; Solomon et al.,1999; White et al.,1998; White et al.,submitted). Here again K cells were reported to be heterogenous with some responding to color (blue-ON), others responding only to achromatic gratings, and still others being unresponsive to gratings. Our recent study showing neurochemical diversity among macaque monkey K cells described above and data showing that projections of different K layers differ (see Casagrande, 1994 for review) also support the hypothesis that the K pathway contains different subclasses.

Our physiological results in owl monkey and comparable data in bush babies suggest that many K cells exhibit spatial and temporal resolution values in the range that would allow these cells to contribute to conventional aspects of spatial and temporal vision. If this is true than the question arises why is the anatomy of the K pathway so different from that of the P and M pathways. Why do K cells send their axons either to cortical layer I or to the CO blobs within cortical layer 3 and not to a subdivision of layer 4 as is the case with M and P LGN cells? Casagrande (1994) suggested that clues about the K pathway might be gained by examining the role of the target cells of the K pathway as well as any extraretinal inputs that are unique to K cells. Concerning cortical targets what comes to mind

first about the CO blobs is color vision, based upon the now famous papers by Livingstone and Hubel (1994). The latter suggested that cells in the CO blobs are tuned specifically to chromatic stimuli. The finding of blue-ON K cells in marmosets fits with this hypothesis but does not fit with the facts that CO blobs are ubiquitously well-developed in all primates, and that K cells project to CO blobs in all primates examined, but blue cones are absent in the nocturnal owl monkey and in the bush baby (Winkler & Rakic, 1990; Jacobs et al, 1993). Clearly K cells, CO blobs, and the anatomy of this pathway are highly conserved features across primates. Perhaps some K cells contribute uniquely to brightness contrast information but also color contrast in species that have color vision. Alternatively, K cells could contribute to a variety of other aspects of vision that might only be tested adequately in the awake behaving preparation. The subgroup of the K cells that do not respond well to gratings could, for example, contribute to eye movement related signals given the fact that K LGN cells in all primates receive a direct input from the superficial layers of the superior colliculus (input that P and M cells lack), and that a significant number of K cells project directly to the dorsal medial visual area (DM), an area concerned more with motion than with object vision (Harting et al., 1981; 1991; Beck and Kaas,1998.

Finally, the neurochemistry and projection patterns of some K cells indicate that they are part of a neuromodulatory pathway. LGN K cells and cells within the adjacent inferior pulvinar nucleus are similar in that they both contain calbindin (Jones & Hendry, 1989). Both K cells and the inferior pulvinar project to the most superficial layer of V1, layer I (Robinson & Petersen, 1992; Casagrande & Kaas, 1994). Projections to layer I in all cortical regions are in a position to modulate signals within all cortical layers given the fact the apical dendrites of the majority of cortical neurons extend into layer I (Vogt, 1991). Moreover, a number of investigators have proposed that the pulvinar is involved in visual attention (Robinson & Petersen, 1992; Sakai & Miyashita, 1994). Perhaps, some of the K cells

that respond poorly in the anesthetized animal project to layer I and are active along with the pulvinar in regulating visual attention. Future work in which K cells can be queried with an electrode in awake behaving monkeys may be able to more directly address these hypotheses.

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Figure Legends

Figure 1. Histological reconstruction of recording sites. Photomicrographs of adjacent parasagittal LGN sections showing lesions in sections stained for Nissl bodies (**A**), CO (**B**), and calbindin-D 28K (**C, D**). Calbindin-D 28K labels cells mainly in layers K1, K2, and K3. Calbindin labeled cells in K4 are sparse. The arrow and arrowhead in **B** indicate a pair of lesions located in the contralateral M layer (arrow) and in layer K2 (arrowhead) that mark a single electrode penetration. The arrows in **A** and **B** point to the same lesion, arrowheads in B and C point to the same lesion. **D**, shows a higher power photomicrograph of the distribution of K cells immunostained for calbindin in K layers K1-K3 in another case. Scale bar in **A**-**C** = 200 μm, **D**=100 μm .

Figure 2. Receptive field center diameter vs eccentricity. K, P and M cells, represented by open squares, solid diamonds, and open triangles, respectively, show increases in receptive field center size with eccentricity. K cells, however, show more scatter.

Figure 3. K, P and M cell linearity test. The first harmonic component (F1) is shown in solid circles and the second harmonic component (F2) in open squares. **A**, An example of a K cell tested with a phase angle range (from -180º to 180º) in 12 steps at its optimal spatial frequency (SF 0.8 c/deg), optimal temporal frequency (TF 2Hz) and moderate contrast (28%). The F1 curve had null positions around -120º and 60º. **B**, The same K cell tested at 2× the optimal SF. Its F1 responses decreased, but the peaks of the F1 curve were still higher than the average F2 curve across all phase angles. **C**, Phase tuning curve for a P cell at its optimal spatial frequency (SF 1.6 c/deg), optimal

temporal frequency (TF 2Hz) and moderate contrast (28%). The F1 curve had null positions around -60º and 120º. **D**, The same cell tested at 2× the optimal SF. Its F1 responses decreased, but the peaks of the F1 curve were still higher than the average F2 curve across all phase angles. **E**, Phase tuning curve for an M cell at 2x its optimal spatial frequency (SF 0.8 c/deg), optimal temporal frequency (TF 2Hz) and moderate contrast (28%). The peaks of the F1 curve were higher than the average F2 curve at all phase angles. The F1 curve had null positions around -90º and 90º. **F**, The same cell tested at 4× the optimal SF. Its F1 responses decreased, but the peaks of the F1 curve were still higher than the average F2 curve across all phase angles. See text for details.

Figure 4. Spatial non-linearity in one M cell. Only one M cell exhibited any evidence of spatial non-linearity. This cell was tested with a global phase angle range (from -180º to 180º) in 12 steps at its optimal spatial frequency (0.6 c/deg), optimal temporal frequency (2Hz) and moderate contrast (28%). Average F2 responses across all phase angles except at the center of the field are higher than F1 responses with a ratio of F2/F1 greater than 1.0 at the peaks. Hochstein and Shapley (1976) have argued that such ratios are indicative of spatial non-linearity.

Figure 5. Non-linearity index (F2/F1) histograms of K, P, and M cells. Most K, P and M cells have a non-linearity index of less than 0.4. Out of 76 cells (K=17, P =32, M=27), one M cell had an index greater than 1.0 suggestive of spatial non-linearity.

Figure 6. K cell spatial frequency, temporal frequency and contrast tuning curves. The F1 response curves for one K cell are shown for different spatial frequencies (SF) in c/deg in **A** and

different temporal frequencies (TF) in Hz in **B**. This cell had a SF peak at 0.8 c/deg and a cutoff around 6 c/deg. The peak TF was 2 Hz and the cutoff was around 6 Hz. The contrast response curve is shown in **C.**

Figure 7. P cell spatial frequency, temporal frequency and contrast tuning curves. The F1 response curves for one P cell are shown for different spatial frequencies (SF) in c/deg in **A** and different temporal frequencies (TF) in Hz in **B**. This cell had a SF peak at 0.8 c/deg and a cutoff above 6.4 c/deg. The peak TF was 2 Hz and the cutoff was 10 Hz. The contrast response curve is shown in **C.**

Figure 8. M cell spatial frequency, temporal frequency and contrast tuning curves. The F1 response curves for one M cell are shown for different spatial frequencies (SF) in c/deg in **A** and different temporal frequencies (TF) in Hz in **B**. This cell had a SF peak at 0.2 c/deg and a cutoff around 6 c/deg. The peak TF was 2 Hz and the cutoff was above 20 Hz. The contrast response curve is shown in **C.**

Figure 9. Histograms of peak spatial frequencies and cut-offs for K, P and M cells with matched eccentricities of >10°. A, the peak spatial frequencies (SF) of K, P and M cells are represented with solid, open and cross hatched bars, respectively. The average peak SF was 0.43 ± 0.07 c/deg for K cells (n=15), 0.69 ± 0.06 c/deg for P cells (n=27), and 0.43 ± 0.06 c/deg for M cells (n=16). P cells had a significantly higher peak SF than K and M cells (ANOVA and post hoc mean tests; p #0.05), but K and M cells were not significantly different. **B**, the average SF cut-off was 2.7 ± 0.4

c/deg for K cells, 4.0 ± 0.4 c/deg for P cells, and 3.1 ± 0.5 c/deg for M cells. SF cut-off differed significantly between P and M cells ($p \# 0.05$), although the cut-off for K cells did not differ significantly from P or M cells.

Figure 10. Histograms of peak temporal frequencies and cut-offs for K, P and M cells with matched eccentricities of >10°. **A**, the peak temporal frequencies (TF) of K, P and M cells are represented with solid, open and cross hatched bars, respectively. The average peak TF was 2.25 ± 0.25 Hz for K cells, 1.96 ± 0.11 Hz for P cells, and 2.06 ± 0.17 Hz for M cells. Peak TF did not differ significantly between K, M and P cells. **B**, the average TF cut-off was 11.2 ± 1.2 Hz for K cells, 9.1 ± 0.8 Hz for P cells, and 14.9 ± 2.0 Hz for M cells. K cells were not significantly different from P and M cells, but P cells had a significantly lower cut-off than M cells (Mann Whitney U, $p \# 0.05$).

Figure 11. Histograms of contrast threshold and contrast gain for K, P and M cells at matched eccentricities of >10°. **A**, the average contrast threshold was 3.8 ± 0.6 for K cells, 5.0 ± 0.8 for P cells, and 2.9 ± 0.3 for M cells. K cells were not significantly different from P and M cells, but M cells had a significantly lower threshold than P cells (Mann Whitney U, $p \# 0.05$). **B**, the average contrast gains were 17.6 ± 2.8 for K cells, 14.5 ± 1.3 for P cells and 18.2 ± 1.4 for M cells. K cells were not significantly different from P and M cells, but M cells had a significantly higher gain than P cells (Mann Whitney U, $p \# 0.05$).

Figure 12. Spatial and temporal characteristics and contrast sensitivity of K cells in different

layers. **A**, the average peak spatial frequency (SF) was 0.42 ± 0.12 c/deg for K1/K2 cells (n=9) and 0.64 ± 0.10 c/deg for K3 cells (n=14). The average SF cut-off was 2.5 ± 0.5 c/deg for K1/K2 cells and 3.6 ± 0.5 c/deg for K3. Peak SF and SF cut-off did not differ significantly between K1/K2 and K3 cells. **B**, the average peak temporal frequency (TF) was 2.44 ± 0.29 Hz for K1/K2 cells and 1.96 ± 0.23 Hz for K3 cells. The average TF cut-off was 12.8 ± 1.7 Hz for K1/K2 cells and 9.5 ± 0.9 Hz for K3 cells. Peak TF did not differ significantly between K1/K2 cells and K3 cells, but K1/K2 cells had a significantly higher TF cut-off than K3 cells (Mann-Whitney U; p #0.05). **C**, the average contrast threshold was 4.7 ± 1.1 for K1/K2 cells and 4.3 ± 0.6 for K3 cells, in which K1/K2 cells did not differ significantly from K3 cells. **D**, the average contrast gain was 14.2 ± 1.5 for K1/K2 cells, and 20.9 ± 3.6 for K3 cells. Contrast gain did not differ significantly between K1/K2 cells and K3 cells.