Retinal Correspondence of Monocular Receptive Fields in Disparity-Sensitive Complex Cells from Area V1 in the Awake Monkey

Rogelio Perez,1,2 Adrian F. Castro,1 Maria S. Justo,1 Maria A. Bermudez,1 and Francisco Gonzalez1,3

PURPOSE. To explore the neural mechanisms underlying disparity sensitivity in complex cells of the macaque visual cortex, the relationship between interocular receptive field (RF) positional shift and disparity sensitivity was studied in area V1.

METHODS. Single-unit recordings were made from area V1 of awake Macaca mulatta. Monocular RFs were mapped by means of a reverse cross-correlation technique, and their centers were determined after performing a bidimensional Gaussian function fitting. Interocular RF shifts were calculated for both bright and dark stimuli. Similarly, Gabor adjustments were obtained from disparity profiles to bright and dark dynamic random-dot stereograms (RDSs).

RESULTS. Twenty-five complex cells were studied. The response profiles to disparity were similar for bright and dark RDSs. Interocular RF positional shift correlated significantly with both the peaks of Gabor fittings of disparity-sensitivity profiles and the peaks of the Gaussian envelopes of these Gabor fittings. Correlation between interocular RF positional shift and the peaks of the Gaussian envelopes was stronger than correlation between interocular RF positional shift and peaks of Gabor fittings.

CONCLUSIONS. Interocular shift of monocular RFs is more related to the center of the range of disparities to which the cell is sensitive, than to the preferred disparity of the cell. (Invest Ophthalmol Vis Sci. 2005;46:1533–1539) DOI:10.1167/iovs.04-1061

A remarkable feature of the visual system is its ability to construct depth from the two-dimensional images projected on the left and right retinas. Because both eyes are horizontally separated, the image of an object, either in front of or behind the fixation plane projects on both retinas with slight horizontal and vertical differences. The positional variation that causes these differences is known as retinal disparity. Although there are other means of estimating depth, stereopsis, the process of recovering depth information from retinal disparity, is usually the most robust and accurate at near distances. Moreover, horizontal disparity is by itself a sufficient cue to produce a vivid perception of depth.

It is believed that the neural processing of visual information for stereopsis begins in the striate cortex, because this is the first stage along the visual pathway where neurons may be activated from either eye. Because of the discovery that neurons in the striate cortex of cats and monkeys are selective for retinal disparity, describing how these neurons encode it has become an important question for understanding the neural mechanisms of binocular fusion and stereopsis.

Two hypotheses for how this encoding occurs are currently available. The first is that cells have left and right RFSs with the same spatial structure but with noncorresponding retinal positions, known as positional shift. The second postulates that cells have left and right RFSs at retinal corresponding positions, but each RF has differences in its structure, creating what is known as phase offset. Cortical simple cells have RFs with separate regions that respond to bright or dark stimuli, whereas complex cells have RFSs with these areas overlapped. It is believed that simple cells combine RF positional shift and RF phase offset to achieve disparity sensitivity and that the positional shift hypothesis is based on the assumption that the RF is well modeled by Gabor functions. Complex cells may display sensitivity to disparity by means of a positional shift or because they inherit it from other cells.

The purpose of the present study was to elucidate whether RF positional shift determines disparity sensitivity in complex cells from area V1 of the macaque. For this, we used both a reverse cross-correlation technique to determine the RF position and RDSs to assess sensitivity to disparity in alert, fixating monkeys.

MATERIALS AND METHODS

Animal Preparation

The experimental setup and physiological recording were similar to that described previously. Two monkeys (Macaca mulatta) were trained to maintain a steady visual fixation on a small target for which they earned a daily fluid ration. The fixation target was a small, bright bar (0.3°×0.2°), and the allowed reaction times were short enough to force an attentive and steady visual fixation during each trial. The behavioral task consisted of a series of trials separated by an intertrial interval (~1 second). Each trial began with the presentation of the target and was continued if the animal pressed a key with its right hand. Then, a visual stimulation period of 1 to 2 seconds started, during which the animal stared at the target. Next the fixation target turned into a cross, and the animal had to release the key to receive a drop of water as a reward. Loss of fixation or inadequate releasing of the key aborted the trial. The training sessions were finished when the animals achieved a level of 90% of correct trials.

With the monkeys under general anesthesia and in aseptic conditions, a head post was implanted on the anterior part of the skull by means of metal screws and dental cement to fix the monkey to a head-holder system. For this, the monkey was anesthetized with intra-
muscular ketamine (10 mg/kg) followed by intravenous pentobarbital sodium (27 mg/kg). Supplementary pentobarbital doses were given when necessary during the surgical procedure, and an analgesic (intramuscular naropinormidipine 150 mg/kg) and antibiotic (intramuscular penicillin 50.000 IU/kg) were given at the end of surgery. The implant was cleaned to make it aseptic whenever necessary. All animal procedures were performed in accordance with the guidelines of the Bioethics Committee of our Institution and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Ocular movements were monitored by means of a video camera under infrared illumination. A frame grabber (Imagination PXC200; CyberOptics Semiconductor, Beaverton, OR) and a conventional computer were used to detect the corneal reflex on the left eye and abort the trials when eye movements exceeded a fixation window of 1° × 1°. The resolution of the system was 20 minutes of arc at a sampling rate of 60 Hz.

Cell Recording

Single-cell activity was recorded by means of metal microelectrodes inserted into the brain with an impedance of 5 MΩ (AM Systems Inc., Carlsborg, WA). For this, after the same surgical procedure as just described, a stainless steel cylindrical chamber was attached to the skull covering the occipital cortex, first on the left hemisphere and then on the right hemisphere. To access area V1, small craniotomies 5 mm in diameter were made in the exposed skull within the chamber. Microelectrode penetrations were made nearly perpendicular to the surface of the dura in both hemispheres with an electrohydraulic microdriver mounted on the chamber. The contact and penetration of the electrode through the dura was visualized by means of a surgical microscope. When the dura thickened and microelectrode penetrations were not possible, a new craniotomy was made. When the penetrations in the left hemisphere were completed, 25-gauge stainless steel needles were inserted into the brain centered in the craniotomies and parallel to the electrode penetrations. Then, the cylinder was removed under general anesthesia as described earlier and a similar cylinder was implanted on the right hemisphere after the same procedure. During the recordings neural voltage signals were amplified and filtered using conventional electronic equipment. For this a time-amplitude window discriminator (Bak Electronics, Rockville, MD) was used to convert the amplified, filtered neural signal to TTL pulses, which were used to construct an online raster. Qualitative judgments about reliability of response and RF characteristics were made while the experiment was in process by directly inspecting the rasters. Quantitative single-cell response analysis was made offline. For this, the multunit analog signal recorded by the microelectrode was digitized by an A/D converter (sampling rate 32 kHz) and stored together with the parameters and the event markers. Single-cell responses were extracted from this recording by using a software digital discriminator that performed a spike discrimination similar to that performed by a conventional time-amplitude window discriminator like the one just mentioned. Up to three single cells were isolated from each multunit recording.

Visual Stimulation

The presentation of stimuli and data collection were performed by conventional computers running software developed in our own laboratory. For statistical purposes and to construct the final RF maps, we used commercially available software. For binocular stimulation the animal sat in a primate chair with its head fixed in front of a two-mirror system that allowed simultaneous and separate viewing of two monitors (Model CPD-520GST; Sony, Tokyo, Japan) placed laterally 57.7 cm away from the monkey’s eyes (Fig. 1A). Cold mirrors that transmit light at an angle produce a stereo effect. Two conventional CRT monitors (Model CPD-520GST; Sony, Tokyo, Japan) were set up with a 0.14° pixel size. The dot jumped in a pseudorandom sequence from position to position on a 16 × 16 grid every one-seventieth of a second. To construct the RF-map each spike was correlated with the recent history of the stimulus sequence resulting in a two-dimensional histogram of stimulus occurrences.

FIGURE 1. (A) Schematic representation of the experimental setup. The animal viewed two images on two separate screens (left and right) by means of two cold mirrors. The monitor screen had a constant background luminance. For bright dynamic RDSs each dot was brighter than the background, whereas for dark stereograms each dot was darker than the background. For the sake of clarity, the illustration shows a bright dynamic RDS in which dots have been exaggerated, and the inset square represents the area of dots with horizontal disparity. (B) Representation of the stimulus used to perform the reverse cross-correlation. The mapping of the RF was made by using a jumping dot (bright or dark relative to the background) that covered a surface of 2° × 2° centered on the RF of the cell. The dot jumped in a pseudorandom sequence from position to position on a 16 × 16 grid every one-seventieth of a second. To construct the RF-map each spike was correlated with the recent history of the stimulus sequence resulting in a two-dimensional histogram of stimulus occurrences.

Monocular RF Maps

After a disparity-sensitive cell was isolated, its two-dimensional structure of left and right RFs were mapped by using a reverse cross-correlation technique. For this, a small bright or dark dot of the same size and luminance as a single dot of the stereogram was flashed (flash duration one-seventieth of a second, one frame of the monitor) on a grid with the same background luminance as that used for the RDS and with 16 × 16 spatial locations covering a visual area of 2.2° × 2.2° (Fig. 1B). The stimulus was presented in rapid succession on the grid (7°1-142 presentations per trial) with its presentation order randomized in such a way that a particular spatiotemporal sequence of presentation was never repeated. When the RF of one eye was being mapped, the fellow eye viewed the fixation target on the background. Both the left-right eye dots and dark-bright dots were interleaved from trial to trial. We obtained online RF maps to keep good control of the collected data while performing the experiment.
A more detailed series of the RF maps was constructed off-line. To construct the RF maps, cell discharges were collected together with the stimulus coordinates on the grid. Spike occurrences were correlated with the stimulus position at various prespike times (–20 to –100 ms in 20-ms steps). Series of RF maps were constructed for both the left and the right eyes. To avoid the effect of a nonuniform distribution of the stimulus on the grid we divided the value we had obtained after the reverse cross-correlation was made by the actual number of dot presentations on each particular grid position. Thus the result was a surface distribution of relative stimulus occurrences on a given grid position for a given prespike time. A coupling between the stimulus and response at a particular prespike time will cause a pattern to emerge on this particular RF map (Fig. 2). Otherwise, the RF maps will show no structure. These RF maps were used to classify the cells as either simple or complex and to determine the RF center. To produce reliable maps, each sequence of stimulation normally required at least 20 presentations of the stimulus on each location of the stimulus grid for each eye.

**Simple and Complex Cells**

To discriminate simple from complex cells, first the maps were filtered with a Gaussian filter (σ = 0.14°) to reject high-frequency components. Then they were filtered again with a band-pass filter to reject low-frequency components and minimize the Gaussian component. These smoothed maps were finally fitted to bidimensional sinusoidal functions. A cell was considered complex when the maps for bright and dark stimuli were 90° out of phase in at least one axis for at least one eye.55

**RF Center and RF Positional Shift**

Because all the cells we included in the study were complex, to determine the center of the RF, we fitted the RF maps to a Gaussian function. For this, each RF map was first smoothed with a Gaussian filter (σ = 0.14°) and then fitted to a bidimensional Gaussian function (R > = 0.80). The maximum of the Gaussian function was considered to be the center of the RF. Determination of the RF center was made for each eye (left and right) and for each type of stimulus (bright and dark). The interocular RF positional shift was calculated by subtracting the horizontal component of the RF center position for the left eye from the horizontal component of the RF center position for the right eye (Fig. 2), for both the bright and dark stimuli.

**Disparity Sensitivity**

A detailed off-line analysis of responses to disparity was performed to assess disparity sensitivity. For this, disparity-sensitive cells were identified by ANOVA (factor disparity, significance level P < 0.05) and the response profiles were fitted to Gabor functions (R = 0.80). For statistical purposes the average cell response rate (spikes per second) was calculated for the set of presentations with the same disparity. According to the phase shift of the Gabor function, disparity-sensitive cells were grouped into four categories as reported by Poggio and Fischer: tuned excitatory (TE; phase shift between –45° and 45°), near excitatory (NE; phase shift between 45° and 135°), tuned inhibitory (TI; phase shift between –135° and 135°) and far (FA; phase shift between –45° and –135°).

Fittings of the RF maps and disparity-sensitivity profiles to Gabor, Gaussian, and sinusoidal functions were made by using the Levenberg–Marquardt algorithm as implemented on computer (MatLab ver. 6.5r12; The MathWorks, Inc., Natick, MA).

**Histology and Electrode Track Identification**

Visual areas V1 and V2 of the rhesus monkey are folded in such a way that an electrode penetration perpendicular to the surface would first reach area V1 and then area V2. We were able to identify the transition between V1 and V2 because of the changes in the recording as the electrode entered the white matter. At the end of the experiments one of the animals was deeply anesthetized with pentobarbital. The brain was perfused with 10% formalin, removed, and placed in the same solution for several days. The region where the penetrations were made was then blocked and sliced in the horizontal plane. Sections were cut at 50 μm, mounted on microscope slides, and stained with toluidine blue for cell bodies. Electrode tracks were reconstructed using the marks left by the needles together with the readings in the microdriver counter and the relative position of the penetration to the center of the craniotomy.

**RESULTS**

The identification of the recording site was made on the basis of the functional properties of the activity recorded as the cortex was traversed by the electrode and the histologic sections made in one animal. The histology showed that V1 was the first area traversed by the electrodes and that all the cells...
were recorded from this area. The identification of the white matter immediately underneath V1 was clearly observed while recording because of the drop in neural activity. In addition, in most penetrations, we continued the advance of the electrode until we reached layer 6 of area V2. At this point, a sudden shift in the RF position was observed and confirmed the transition from V1 to V2. The cellular RF positions matched the known representation of the visual field in area V1. We recorded 75 cells sensitive to disparity (ANOVA test, \( P < 0.05 \)), as tested with dynamic RDSs. However, we were able to obtain reliable RF maps for each eye and for bright and dark stimuli in 27 of these disparity-sensitive cells (27/75, 36%), which formed the data set of this study.

**Simple and Complex Cells**

The bidimensional sinusoidal fitting of the RF maps of two cells (2/27, 7%) showed phase shifts to bright and dark stimuli larger than 90° in both eyes and therefore were considered as simple cells. The remaining 25 cells (25/27, 93%) were considered to be complex cells, because the fittings showed phase shifts <90° for at least one eye. Of these 25 complex cells, 21 (21/25, 84%) showed phase shifts of <90° in both eyes, whereas the remaining 4 (4/25, 16%) cells showed phase shifts larger than 90° in the right eye and <90° in the left eye.

**Disparity Sensitivity**

According to the phase shifts found in the Gabor fittings of disparity-sensitivity profiles, cells \((n = 25)\) were grouped in four categories. For bright stereograms 4 (16%) cells were classified as TE, 5 (20%) as TI, 13 (52%) as NE, and 3 (12%) as FA. Gabor phase shifts for the bright RDS ranged from \(-141°\) to \(172°\) (Fig. 3A). For dark RDSs, 2 (8%) neurons were classified as TE, 7 (28%) as TI, 13 (52%) as NE, and 3 (12%) as FA cells. Phase shifts for dark RDSs ranged from \(-180°\) to \(176°\) (Fig. 3A).

**FIGURE 3.** (A) Phase shift distribution of disparity-sensitivity profiles. Histograms representing the phase shift of the Gabor adjustment of the disparity-sensitivity profiles of all cells included in this study for bright and dark RDSs (bin size = 10°). (B) Disparity sensitivity to bright and dark RDSs. Phase shifts of Gabor adjustments of disparity-sensitivity profiles obtained with bright and dark RDSs are represented on the vertical and horizontal axes, respectively. Phase shifts for dark RDSs were correlated with those for bright RDSs \((P < 0.0001; R = 0.96)\).

**FIGURE 4.** Interocular RF positional shift and disparity sensitivity. (A) Relationship between disparity tuning and interocular RF positional shift in response to bright stimuli. Disparity tuning was measured in two forms: as the peak of the Gabor adjustment of the disparity profile obtained with bright stereograms and as the peak of its corresponding Gaussian envelope. Interocular RF positional shift was obtained by subtracting the center of the bidimensional Gaussian envelope of the RF map for the left eye from the center of the bidimensional Gaussian envelope of the RF map for the right eye, both obtained with a bright stimulus. In the graphs, interocular RF positional shift data are represented on the horizontal axis, whereas peaks of Gabor adjustments and corresponding Gaussian envelopes of disparity profiles are represented on the vertical axis. Interocular RF positional shifts correlated with Gabor \((P < 0.05; R = 0.58)\) and Gaussian \((P < 0.0001; R = 0.75)\) peaks of disparity profiles. (B) Relationship between disparity tuning and interocular RF positional shift for dark stimuli. Disparity tuning was measured in two forms, as the peak of the Gabor adjustment of the disparity profile obtained with dark RDSs and as the peak of its corresponding Gaussian envelope. Interocular RF positional shift was obtained by subtracting the center of the bidimensional Gaussian envelope of the RF map for the left eye from the center of the bidimensional Gaussian envelope of the RF map for the right eye. Interocular RF positional shifts correlated with Gabor \((P < 0.005; R = 0.58)\) and Gaussian \((P < 0.0001; R = 0.75)\) peaks of disparity profiles.
Two neural mechanisms are currently considered to subserve disparity detection. The first mechanism, known as RF positional disparity, is based on the view that the left and right RFs of a cell have retinal positions at noncorresponding retinal points, creating an interocular RF positional shift. The rational part of this view is that if a binocular neuron is to serve as a depth detector, then the left and right RFs would have an identical organization to ensure that the cell would respond to the same object features in both eyes, but with a positional disparity that would match their optimal disparity sensitivity. The second mechanism, known as the RF phase offset, assumes that internal structural differences between left and right RFs are the cause of disparity sensitivity.

The study of stereopsis in alert monkeys has the advantage that the actual position of both monocular RFs can be measured because the animal can be trained to fixate a steady target. However, even in a very well-trained monkey small eye movements remain under binocular fixation. To minimize the influence of these movements on the assessment of the RF position, we used a dot jumping in a random fashion over a given area of the visual field and mapped the RF by using a reverse cross-correlation technique. It has been shown that the spatiotemporal structure of visual RFs can be obtained by using techniques of white noise analysis because we also used a bidimensional Gaussian fitting, we were able to obtain the center of the RF with reasonable accuracy, although the small eye movements during fixation could have blurred the RF limits.

We recorded 27 disparity-sensitive cells from V1 whose RF structure could be mapped from each eye separately. The majority of disparity-sensitive cells were of the complex type. Congruent with these data, in our sample, 93% of disparity-sensitive neurons were considered complex, which is an amount similar to that reported by other investigators in V1 of macaques. It is assumed that visual cells from V1 have monocular RFs of the same type in both eyes. However, we found four cells that showed simple RF in one eye and complex RF in the other.

Most disparity-sensitive cells we found belonged to the same disparity group, regardless of whether a bright or dark RDS was used. In fact, phase shifts of Gabor fittings of disparity profiles for the dark RDS correlated significantly with those to bright RDS (P < 0.0001; R = 0.96). This finding indicates that disparity sensitivity to RDS in neurons included in our sample is contrast independent, which is in agreement with the known properties of complex cells.

For simple cells of cats, a model has been proposed in which disparity sensitivity would be defined by the phase offset of their RF subregions. Data supporting this model in disparity-sensitive simple cells of macaque have also been reported. According to this model, disparity-sensitive cells would be sensitive to the position and contrast of the stimulus over the RF. However, because RFs of complex cells present overlapping regions where bright and dark stimuli elicit re-
corresponding Gaussian envelope were reported in an example. Note that whereas peaks of Gabor adjustment and correlation between interocular RF positional shifts and pre-spike occurrences were position and contrast invariant.

For complex cells, a model of disparity sensitivity has also been proposed in cats. This model needs the presence of a large number of simple cells in the visual cortex to generate disparity sensitivity in complex cells. However, in the present study we detected only 7% of simple cells among those with disparity sensitivity. This reinforces the reported finding that complex cells are clearly dominant among those with disparity sensitivity in macaque V1.

The other presumed mechanism to achieve disparity sensitivity in complex cells is interocular RF positional shift. If this were the case, one should expect a strong correlation between interocular RF positional shift and disparity sensitivity. However, in agreement with previous data, we found a weak correlation between interocular RF positional shifts and prefered disparity, as determined by the peak of Gabor fitting of disparity profiles. We found a stronger correlation between the interocular RF positional shift and the peak of the Gaussian envelope in the Gabor adjustment of the disparity profile. This occurred for both bright (P < 0.0001; R = 0.73) and dark (P < 0.0001; R = 0.75) RDS. This observation may indicate that the interocular RF positional shift determines the center of the range of disparities to which the cell is sensitive, instead of the tuning of the cell for a given disparity. Figure 5 shows the case of a disparity-sensitive unit in which peaks of both Gabor fitting of the disparity profile and the corresponding Gaussian envelope and interocular RF positional shift were calculated as an example. Note that whereas peaks of Gabor adjustment and corresponding Gaussian envelope were −0.87° and −0.19°, respectively; interocular RF positional shift was −0.15°.

Other models try to explain disparity selectivity more on the basis of functional properties of the monocular inputs to the cell than on the basis of the RF structure. Facilitatory and inhibitory interactions would occur at the dendritic tree level to compute the input from each eye and trigger disparity sensitivity. In fact, disparity sensitivity has been described in units with a strong ocular dominance45 and in cells with a strong inhibitory monocular input,45 in which none of these models could be applied. Figure 6 shows one such cell, which displays a clear disparity sensitivity, whereas no left RF can be elicited. Therefore, in this case only the functional characteristics of the left input could explain disparity sensitivity, whereas nothing can be inferred from the RF structure.

In addition, most disparity-sensitive complex cells recorded in this study showed phase shifts of their disparity profiles larger than 90° and smaller than −90°.35 This suggests that in complex cells, suppressive interocular interactions may play a more relevant role than excitatory interactions for disparity sensitivity and favors the idea that simple and complex cells could have different mechanisms to achieve disparity sensitivity.

**References**


**Acknowledgments**

The authors thank Yanai Duran for valuable help with the animals and the laboratory, Carmen Cadarso for performing statistical analyses, and Dolores Fernandez for performing the histology.