Binocular stereopsis in V2, V3, and V3A of the macaque monkey

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## Table of contents

Abstract........................................................................................................................................1

List of figures................................................................................................................................2

Introduction..................................................................................................................................3

Materials and methods................................................................................................................14

Results.........................................................................................................................................24

Discussion....................................................................................................................................47

References....................................................................................................................................56
Abstract

Stereopsis is a binocular cue to depth, calculated using the disparity between images projected on the retina of each eye. In the 1960s, Hubel and Wiesel identified neurons selective for this disparity in macaque monkeys, but a full account of the work was never published: the cells lay in “area 18,” a region whose organization was then poorly understood. Using functional magnetic resonance imaging (fMRI), we generated an atlas of macaque visual areas that we aligned with histological sections from Hubel and Wiesel’s experiments on stereopsis. Collaborating with Hubel and Wiesel and guided by their laboratory notes, we located most of their original recording sites, allowing us to place their findings in new context with contemporary results. Disparity-tuned cells resided not only in V2, the area now synonymous with area 18, but also in V3 and V3A. Hubel and Wiesel’s recordings show that the disparity-tuned cells were biased for near disparities, tended to prefer vertical orientations, clustered by disparity preference, and often required stimulation of both eyes to elicit responses, features strongly suggesting a role in stereoscopic depth perception.
List of figures

Figure 1. Near, zero, and far absolute disparities. .................................................................4
Figure 2. Response of binocular depth cell to variations in binocular disparity.................6
Figure 3. Relative disparity....................................................................................................11
Figure 4. Hubel-Wiesel electrophysiology setup. .................................................................19
Figure 5. Disparity tuning of cells in area 18.......................................................................27
Figure 6. Receptive field maps for a single penetration in area 18.......................................28
Figure 7. Response to varying binocular disparity of a binocular “tag” cell in V1...............29
Figure 8. Responses of two extrastriate cells to variations in vertical and horizontal disparity.................................................................................................................................30
Figure 9. Retinotopic mapping of visual areas in the alert macaque. ..............................34
Figure 10. Localization of Hubel-Wiesel cells using electrode path reconstructions........35
Figure 11. Six registrations of Hubel-Wiesel electrode path reconstructions to MRI atlas..36
Figure 12. Disparity tuning by visual area: Hubel-Wiesel electrophysiology and fMRI data. .....................................................................................................................................40
Figure 13. Receptive field size v. eccentricity in V2, V3, and V3A. ......................................41
Figure 14. Orientation tuning of disparity-tuned cells underrepresents the horizontal......44
Figure 15. Spatial analysis of Hubel-Wiesel cells reveals clustering by preferred disparity.................................................................................................................................45
Figure 16. Obligate binocularity: single-cell and fMRI results..........................................46
Stereopsis is the perceptual faculty that enables us to see the world in three-dimensional depth by computing the difference between the images projected on each retina, a difference termed binocular disparity. Stereopsis is a particularly important cue to depth in tasks requiring fine depth discrimination, such as threading a needle. Only within the past fifty years have neuroscientists begun to identify the regions of the brain that contribute to this remarkable ability.

Charles Wheatstone was the first to demonstrate, through his invention of the stereogram, the effectiveness of disparity in producing the impression of three-dimensional depth. The stereogram produced the illusion of three-dimensionality by presenting the viewer’s right and left eyes simultaneously with photographs taken from slightly different points along the horizontal axis (Wheatstone, 1838). That disparity was sufficient to generate perception of stereoscopic depth was later confirmed by the random-dot stereograms of Bela Julesz (1960). In these, pairs of dots in two colors were displaced horizontally from each other. With anaglyph 3D glasses, each dot color could be isolated for viewing by one eye, producing a percept in three dimensions and confirming that a point disparity alone was enough to yield an impression of stereoscopic depth.

In 1965, Jack Pettigrew hypothesized, on the basis of Julesz’s work, that neurons whose response properties were sensitive to horizontal binocular disparity would be involved in calculations of stereopsis (Pettigrew, 1965). He proposed that this response property would derive from a relative offset of receptive fields in each eye within binocular cells. Cells preferring zero disparity stimuli would possess receptive fields that overlapped when the foveae of the two eyes are directed at the same point in the visual field (Figure 1).
Cells preferring non-zero disparities would possess receptive fields in each eye that were horizontally offset, such that their overlap would occur only with a stimulus before or behind the plane of fixation. For instance, in a cell preferring “near” stimuli (in front of the plane of fixation) the right eye’s receptive field would be offset leftward of the left eye’s when both eyes are in register. Rightward displacement of the segment of visual field viewed by the right eye, as would occur for a stimulus present in front of the plane of fixation, would align the receptive fields of the individual eyes and increase the cell’s firing rate.

![Figure 1. Near, zero, and far absolute disparities.](image)

To test for the presence of such cells in the cat, Peter Bishop, Pettigrew's supervisor, developed a method to present disparity stimuli to animal subjects by deviating one eye's position in the visual field with respect to the visual field of the other eye (Pettigrew et al., 1968). This deviation was accomplished using a Risley prism, which consists of two prisms rotated in opposite directions, displacing the light passing through the prism and allowing
a researcher to alter the section of visual field seen by one eye. Using Risley prisms to present disparity stimuli, the research groups of H.B. Barlow – and, concurrently, P.O. Bishop – were able to identify disparity-sensitive cells in the primary visual cortex of the cat (Barlow et al., 1967; Nikara et al., 1968). As Pettigrew had posited, the receptive fields of these cells were horizontally offset with eyes in register, but overlapped with eyes positioned at the cell’s preferred binocular disparity (Barlow et al., 1967).

Prompted by these findings, David Hubel and Torsten Wiesel sought out similarly disparity-tuned neurons in the primary visual cortex of the macaque monkey, whose brain bears greater homology to humans’. At the time, they could find none. Nearly a decade later, Poggio and Fischer documented disparity-tuned cells in the striate cortex of behaving macaques (1977). In retrospect, Hubel and Wiesel’s early inability to identify disparity-tuned cells in primary visual cortex was likely a result of the small size of V1 receptive fields, and the even smaller size of the corresponding disparities between them when measured in the two eyes separately (Poggio and Fischer, 1977). Identification of disparity tuning in such cells would be especially challenging in anesthetized animals, as used by Hubel and Wiesel, since the position of the eyes drift under anesthesia (Hubel and Wiesel, personal communication).

Compelled by the findings of Barlow et al. and Bishop et al. in the cat, Hubel and Wiesel persevered in their search for disparity-selective neurons in monkeys, taking their exploration into extrastriate cortex. It was here that Hubel and Wiesel identified the first disparity-tuned cells in primates (macaque monkey), in the late 1960s. They published a preliminary paper on this finding in *Nature* in 1970, reporting the discovery of cells in
macaque extrastriate cortex with similar properties to those previously described in V1 of the cat by Barlow et al. and Nikara et al. (Figure 2).

Figure 2. Response of binocular depth cell to variations in binocular disparity. (A-E), Oscilloscope traces for a cell in area 18 in response to disparities ranging from 0° to 1° in steps of 15’. This cell responds optimally to a displacement of the right eye’s (RE) receptive field 30’ to the left of the left eye’s (LE), or to a stimulus .5m in front of the plane of fixation (Adapted from Hubel and Wiesel, 1970). Arrows indicate direction of stimulus motion.

Hubel and Wiesel’s publication, like many in Nature at the time, was a brief description of their findings that would have been expected to be followed by a much longer paper, containing a fuller representation of their data (Hubel and Wiesel, 2004). Although the 1970 paper contained only one figure (see Figure 2), Hubel and Wiesel had “recorded from over 600 cells and reconstructed 23 penetrations with the results tabulated
and figures drawn, in [preparation for] what would have been a long paper even for us” (Hubel and Wiesel, 2004). However, they never published a complete description of this research: their disparity-tuned cells lay in a region they described as “area 18,” whose functional subdivisions were not well characterized at the time of their experiments.

The posterior border of area 18 is marked by the anterior edge of striate cortex or area 17, whose border with extrastriate cortex is clearly visible in histological slides due to the characteristic stripes for which striate cortex is named (Paxinos, 2000). However, the anterior border of area 18 is not clearly defined by anatomical or cytoarchitectonic boundaries that were apparent with the technology then available (Hubel and Wiesel, personal communication). As a result, it was difficult for Hubel and Wiesel to assign their depth cells to a visual area with confidence. In large part due to this uncertainty, they delayed indefinitely the publication of a report that would describe their findings in full (Hubel and Wiesel, 2004).

Today, however, advances in the field of neurophysiology afford neuroscientists a far more detailed map of the visual brain than was available to Hubel and Wiesel when they performed their experiments on stereopsis. Such technology permits us to address for the first time the question of which contemporary visual areas comprised the “area 18” referred to by Hubel and Wiesel and their contemporaries. While in more recent publications, “area 18” has become synonymous with visual area V2 (Hubel and Livingstone, 1987a; Livingstone and Hubel, 1987; Hubel and Wiesel, 2004; Rockland, 2004), there is little evidence to support the assumption that the anterior boundaries of modern-day V2 correspond with the anterior boundaries of area 18 as defined by Hubel and Wiesel during their study of disparity tuning. Rather, it is very likely that their
electrode penetrations encompassed other regions of extrastriate cortex as well (Zeki, 1978; Tsao et al., 2003; Hubel and Wiesel, 2004).

Hubel and Wiesel’s protocols for these experiments refer frequently to penetrations within the annectant gyrus, a gyrus buried beneath the lunate sulcus in the dorsal occipital lobe. Later study has confirmed that the annectant gyrus contains not only dorsal V2, but also a segment of dorsal V3 and V3A, and potentially dorsal V4 (Zeki, 1978; Felleman and Van Essen, 1987; Gegenfurtner et al., 1996). Decades after this paper’s publication, Hubel and Wiesel themselves have posited that some proportion of the depth-sensitive cells they described may have been in “what is known as the V-3/V-3A complex, and not 18 or visual area 2 as we thought at the time” (Hubel and Wiesel, 2004). Although the precise boundaries of these areas have proven challenging to map, this evidence suggests that the historical understanding of Hubel and Wiesel’s 1970 paper in Nature as a study of V2 alone is an incomplete representation of the research’s anatomical scope.

A fuller understanding of the locations of Hubel and Wiesel’s binocular depth cells paper will not only alter the neuroscience community’s understanding of Hubel and Wiesel’s classic paper – it will also reveal important information regarding the areas of extrastriate cortex involved in stereoscopic depth perception. Hubel and Wiesel’s data represents a critical contribution to the field in that their work involved study of a very large number of research animals. Minimal restrictions on the use of non-human primates in medical research at the time made it simple to use many more animal subjects than would be used today. Hubel and Wiesel’s standard technique for these experiments was to perform a craniotomy in the morning, record until late into the night, and then perfuse the animal afterward. Rarely was an animal used in more than one experiment. This allowed
Hubel and Wiesel to record from 21 separate monkeys in their study of binocular depth cells, a number of research animals which would not be feasible today due to prohibitive expense. Their data confirm the finding of cells sensitive to binocular disparity in a large sample of subjects, and provide valuable information on individual variations in the processing of disparity stimuli.

In addition, because the Hubel-Wiesel data were recorded via progressively advanced electrodes, they supply information on the laminar organization of cortical disparity computation that is not available with the fixed electrodes used in contemporary chronic recordings. Different layers of visual cortex each contain distinct inputs and projections, both local and distant. These connections have been well documented for striate cortex (Lund, 1988), but their organization in extrastriate cortex is not as thoroughly understood. Detailed analysis of the laminar organization of the Hubel-Wiesel data on disparity sensitivity in extrastriate cortex may yield important insight on the cortical circuitry that facilitates disparity calculations.

Localization of the Hubel-Wiesel depth cells will also provide a critical contribution to an ongoing debate on the relative specialization of different extrastriate areas to cortical calculations of stereopsis. fMRI studies have demonstrated that non-zero disparity stimuli activate V3A alone in humans (Backus et al., 2001), and both V3A and the caudal intraparietal sulcus (CIPS) in humans and macaques (Tsao et al., 2001). However, very few electrophysiology studies have been performed to study disparity tuning in areas V2, V3 and V3A.

The lack of electrophysiology data on disparity tuning in these regions is problematic: while fMRI results demonstrate concerted activity in a broad range of
spatially clustered neurons, single-unit electrophysiology provides information about the activity in individual cells, making it a critically important tool in understanding calculations of stereopsis at the cellular level. The few electrophysiology studies performed in V2, V3, and V3A have implicated a broader range of regions in computations of stereopsis than have fMRI studies of these regions (Tsao et al., 2003). These electrophysiology studies have also yielded variable reports of the relative disparity tuning of each of these visual areas (Tsao et al., 2003; Anzai et al., 2011). Felleman and Van Essen, using anesthetized monkeys, reported that only 45% of the cells in area V3 were tuned to binocular disparity (1987). A later study from the Poggio group, using behaving animals, found that 80% of the cells in V3/V3A were disparity-tuned (1988). This proportion was greater than that identified in V2 or V1, suggesting a relative specialization for stereopsis in V3/V3A (Poggio et al., 1988). However, the criteria for disparity-tuning classifications of the Poggio group has not been published, an omission which has been commented upon by Tsao et al. (2003) and Anzai et al. (2011). Compounding this debate, more recent electrophysiology work with awake macaques indicates that V3 and V3A as a whole are no more specialized for disparity processing than V1, V4, or MT (Anzai et al., 2011).

Different visual areas may also specialize in computation of different types of binocular disparity. Contemporary research on stereopsis makes a distinction between so-called “absolute disparity” and “relative disparity” (Anzai et al., 2011). The former refers to what has been classically termed “binocular disparity,” the horizontal distance in the visual field between images projected on each retina. Relative disparity is the difference between two absolute disparities (see Figure 3), and is important for fine calculations of depth (Westheimer, 1979; Kumar and Glaser, 1992). fMRI study of extrastriate cortex has showed
that in monkeys and humans, areas V3 and V3A respond strongly to stimuli containing both absolute and relative disparity (Backus et al., 2001; Tsao et al., 2003). However, recent single-unit electrophysiology recording in extrastriate cortex indicates that V3 and V3A may, in fact, be specialized for absolute over relative disparities (Anzai et al., 2011). The Hubel-Wiesel disparity data represents absolute disparity stimuli, and will provide useful information regarding the specialization of extrastriate regions to this disparity type.

**Figure 3. Relative disparity.** Relative disparity is the difference between two absolute disparities. This difference can be quantified as the difference between the change in angle of the line of gaze of each eye (α, β) for fixation on each of two points (P, Q). Figure adapted from Parker (2007).

This wide variation in estimates for regional disparity tuning between studies is likely related to variation in criteria for determining disparity sensitivity, as well as the small sample size of most studies on disparity tuning in these regions (2 monkeys or fewer). It may also result from the columnar organization of disparity-tuned cells identified in V2 (Hubel and Livingstone, 1987a), V3 (Adams and Zeki, 2001; Poggio et al., 1988) and V3A (Poggio et al., 1988). Further study is required before definitive conclusions can be drawn regarding the specialization of cells in extrastriate regions for disparity computation. Identifying the precise location of Hubel and Wiesel’s disparity-tuned cells
will allow us to interpret their results in context with these more recent findings, clarifying the relative contribution of V2, V3, and V3A to calculations of stereopsis within a large experimental sample.

To resolve the historical uncertainty regarding the locations of Hubel and Wiesel’s stereo cells, we localized their recording sites using contemporary atlases of macaque visual brain, an endeavor made possible by the preservation of Hubel and Wiesel’s experimental notes, protocols, and histological slides for virtually all of their experiments on stereopsis, including much unpublished data. We generated a functional atlas of macaque visual regions using functional magnetic resonance imaging (fMRI) in awake behaving macaques. This was accomplished with standard retinotopic mapping, which exploits the retinotopic organization of visual cortex to mark the boundaries of each early visual area. As each early visual area contains a discrete representation of the contralateral visual field (Logothetis et al., 1999), it is possible to map these representations on the cortex by stimulating the visual field along horizontal and vertical meridians during fMRI. Activation in response to meridian stimuli can then be projected on a computationally inflated cortical surface to delineate the boundaries of early visual regions (Logothetis et al., 1999). This technique can be used to map even visual areas such as V3A, which represents not one but both quadrants of the contralateral visual field (Van Essen and Zeki, 1978).

This atlas was compared directly with reconstructions of the Hubel-Wiesel electrode penetrations, as the majority of electrode penetrations in these experiments lay within the sagittal plane and could therefore be matched to sagittal MRI slices. Alignments were made using Hubel and Wiesel’s record of the site of each craniotomy relative to the
midline and the nuchal ridge of the skull, features which vary little between animals. In combination with a perceptual match of gyri and sulci between each histological slide and MRI slice, this information enabled the assignment of each of Hubel and Wiesel’s original electrode penetrations to an MRI atlas slice of the same location in the sagittal plane.

These alignments could then be used to assign each cell to a visual area. Hubel and Wiesel had generated for all experiments an electrode-path reconstruction using electrolytic lesions, clearly visible in histological slides, made during experiments to track electrode progress. These reconstructions also had single-cell resolution, as Hubel and Wiesel had recorded the depth at which each recording had been made. The fidelity and detail of these reconstructions enabled the assignment of each of their disparity cells to a corresponding point in our functional atlas.

In close collaboration with Hubel and Wiesel, and guided by their detailed laboratory notes, we assigned each cell in their experiments on disparity tuning in area 18 to a contemporary anatomical region. This analysis facilitates comparisons between the Hubel-Wiesel data and more recent findings, allowing us to place their results in better context with contemporary data on the role of extrastriate cortex in stereo processing. Our localizations confirm that the Hubel-Wiesel disparity cells lay not only in V2, but in V3 and V3A. In fact, V3 and V3A contained a higher proportion of disparity-tuned cells than V2, supporting prior findings of a specialization for stereopsis in V3 and V3A (Adams and Zeki, 2001; Tsao et al., 2003).
Materials and methods

Electrophysiology

From April 9, 1968 to March 17, 1970, Hubel and Wiesel recorded from 627 cells in “area 18,” involving over 31 penetrations in 21 animals. (These numbers include only cells recorded after the initial identification of cells tuned to binocular disparity.) We have been able to recover detailed information about 626 of these cells. Of these, it is possible to reconstruct 28 penetrations in 18 animals. (Three penetrations could not be reconstructed, as neither histology nor electrode path reconstructions were available.)

In addition, Hubel and Wiesel also recorded from 535 cells in primary visual cortex over the same period; however, these cells were excluded from analysis in this paper as their location in V1 was certain and Hubel and Wiesel did not identify sensitivity to disparity in V1 cells.

The majority – approximately 90% - of penetrations – were in the sagittal plane. The electrode was usually angled forward to enter the cortex at a 90° angle to the surface.

Surgery

Animals were anesthetized with intraperitoneal sodium pentothal (35 mg/kg), and additional doses of the drug were given at half-hour intervals. Eyes were paralyzed with curare and gallamine triethiodide (2-3mg/kg), administered via intramuscular injection every half hour. Body temperature was carefully monitored, as fall in temperature forced early termination of an experiment due to an increase in background firing rate and decreased stimulus specificity of responses, effects which were more pronounced in extrastriate cortex than in V1. No experiments lasted longer than 19 hours.
Visual stimuli for electrophysiology experiments

Visual stimuli were generated using standard Hubel-Wiesel protocols (Hubel and Wiesel, 1959; Hubel and Wiesel, 1962). The animal’s head was stabilized using a Horsley-Clarke stereotactic apparatus, and eyes were held open and protected with contact lenses. Lenses were fluid-filled so that eyes focused on the screen, which was 1.5 m away (Figure 4). A streak retinoscope (Copeland) was used to monitor eye focus. The location of the foveae and optic discs on the screen was determined according to previously documented methods (Hubel and Wiesel, 1959).

To vary binocular disparity, the left eye’s line of gaze was altered using a Risley prism (two wedge prisms that counter-rotate to vary the angle at which light is deflected) (Barlow et al., 1967; Nikara et al., 1968). With this setup the line of gaze for the left eye could be altered by up to 30° in any direction. Displacement of the left eye line of gaze was monitored by projecting a small laser onto a small mirror at a 45° mirror next to the left eye. Light from this was projected through the prism onto the screen, generating a spot of approximately 1 minute φ that could be used to quickly check left-eye deviation from the Risley prism. Since at a distance of 1.5 m, 1 inch is equal to 1°, this displacement could be measured in degrees directly from the screen.

Stimuli were spots or lines (slit, edge, or dark bar) projected on the screen with a hand-held slide projector (Figure 4). The light-dark ratio for such stimuli was 1-1.5 log units. The maximum luminance intensity of dark areas onscreen was about 1 log cd/m². As previously described, interference filters were used to create colored stimuli (Hubel and Wiesel 1966). To assess tuning curves and quantify cell properties, stimuli were generated with a computer whose monitor, a large TV screen, was 1.5 in front of the animal. Using this
setup, it was possible to continuously alter the size, location, orientation, and speed and range of motion for slits, dark bars, edges, and tongues.

A video recorder (Sony EV 210) was used to record both stimuli and oscilloscope traces. Hand-projected stimuli were recorded with a camera; electronic stimuli were recorded via the computer used for stimulus generation. Stimuli, responses, and relevant experimental notes were recorded during experiments using a typewriter.

Recordings

Electrophysiology recordings were made according to previously described procedures (Hubel and Wiesel, 1962; Wiesel and Hubel, 1966). Vinyl-insulated tungsten wire microelectrodes (Hubel, 1957) were inserted hydraulically through a 2 mm diameter craniotomy and durotomy. These electrodes, and the cortical surface, were separated from the surrounding atmosphere.

For each unit for which receptive fields were mapped, the relative positions of the two eyes were confirmed using a novel electrophysiology technique: while recording from extrastriate cortex in the right hemisphere, Hubel and Wiesel would maintain a second electrode inserted in primary visual cortex of the left hemisphere in an area where receptive fields were as similar as possible to the fields of cells being studied in extrastriate cortex (Figure 4). This “tag” electrode recorded from a binocular cell insensitive to binocular disparity (see Figure 6 for an example of such a cell). This cell could then be used to confirm alignment of the two eyes; if the experimenters suspected any drift in eye position, the lines of gaze could be brought into alignment via the Risley prism and this response could be confirmed via evaluation of the tag cell’s response to stimulation of each eye alone. If an eye had drifted, its monocular response to stimulation of its receptive field
would decline, indicating that its position needed to be re-adjusted to bring the eyes into alignment again. In this way the tag cell was used to check eye alignment after recording from each individual unit in extrastriate cortex. Each tag cell was recorded from for as long as possible, usually for several hours and sometimes for the duration of an entire experiment.

This technique was accurate to within about 4’ of arc of disparity between each eye’s receptive field, more precise than monitoring disparity through foveal position and often both faster and simpler for the experimenters to implement.

To record the location of the microelectrode, small electrolytic lesions were made within each penetration by passing current through the electrode tip, typically 2 μA for 2 seconds (electrode negative). At the end of each penetration, a lesion was generated to mark the furthest depth of the electrode.

Histology

Brains were fixed with formalin and gluteraldehyde and sectioned in the sagittal plane to facilitate tracking of the electrode path through the lunate sulcus. Brain sections were Nissl stained.

Functional Magnetic Resonance Imaging

Overview

Two adult male rhesus macaque monkeys (7-8 kg) were scanned at Massachusetts General Hospital Martinos Imaging Center (MGH) in a 3T Allegra with an AC88 gradient insert (Siemens, New York, New York) scanner using a custom-made four-channel send/receive surface coil (Martinos Center, MGH, Charlestown, Massachusetts) and standard echo planar imaging [repetition time (TR) = 2 s, 98 × 63 × 98 matrix, 1 mm³]
voxels]. Animals were trained with liquid reward to jump into a chair and sit in the “sphinx” position while fixating on a dot in the center of the screen. Animals’ heads were stabilized using custom surgically-implanted plastic head posts (see Conway et al., 2007 and Lafer-Sousa et al., 2012 for detailed methods). Eye position was tracked with an infrared eyetracker (ISCAN, Burlington, MA). Animals were rewarded for maintaining fixation within 1 degree of the onscreen fixation point. To improve fMRI signal detection, animals were injected intravenously with monocrystalline iron oxide nanoparticle (MION) contrast agent, Feraheme (AMAG Pharmaceuticals, Cambridge, Massachusetts). Feraheme was injected into the femoral vein below the knee (8–10 mg/kg, diluted in saline) before MR scanning. As an increase in the MION signal corresponds to a decrease in the blood-oxygen-level-dependent (BOLD) signal (Vanduffel et al., 2001), MION time courses are here shown inverted to facilitate comparison with BOLD responses. MR imaging and surgical procedures conformed to the local and National Institutes of Health guidelines and were approved by the Harvard Medical School Institutional Animal Care and Use Committee.

Visual stimuli for fMRI experiments

Stimuli were projected to a screen (41° × 31°) 49 cm in front the animal with a JVC DLA projector (1024 × 768 pixels). Stimuli covered the whole screen and included a small cross at the center of the screen as a fixation point. All stimuli were presented using a block paradigm. Three sets of experiments were performed to assess I. Extent of early visual areas via retinotopic mapping, II. Response to non-zero vs. zero binocular disparity, III. Sensitivity to monocular v. binocular stimuli.
Figure 4. Hubel-Wiesel electrophysiology setup. Extracellular recordings were taken from the right hemisphere along the lunate sulcus via a “business” electrode, while drift in eye position was monitored with a “tag” electrode in left hemisphere V1. Stimuli were projected to a screen in front of the monkey using a slide projector. Binocular disparity was controlled with a Risley prism, and checked by projecting a spot of light (via the beam projector) through the prism to the screen.
I. Retinotopic mapping

Visual cortex was mapped retinotopically by presenting the animal with black-white checkerboard wedges along the vertical and horizontal meridians. Checks flickered once per second, in phase. Maps comparing significance of response to each type of meridian stimulus were projected on a computationally-inflated cortical surface of each animal (Figure 4A). Centers of each meridian representation were used to draw boundaries for visual areas V1, V2, V3, V3A, V4, V5/MT, and CIPS. These boundaries were confirmed by comparison to a standard atlas of the macaque brain (Paxinos 2000).

Stimuli for retinotopic mapping consisted of 32-second presentations of wedges along the horizontal meridian (99% luminance contrast, stimulus breadth covering 30°) and along the vertical meridian (covering 60°). Vertical and horizontal meridian stimuli were each presented 4 times, with each presentation of a meridian stimulus preceded and followed by 32 seconds of neutral gray.

Experiments performed separately in the same animals identified the representation of the center 3° of visual angle. The paradigm for stimulus presentation was identical to that in retinotopic mapping experiments, but stimuli altered between a flickering checkerboard at the central 3° and a checkerboard spanning the peripheral visual field outside this central region.

High-resolution anatomical scans (0.35 mm³ voxels) were also collected separately for each animal. For these experiments, animals were lightly anesthetized. Retinotopically-defined visual areas for each animal were projected onto these scans; a different color was assigned to each region (Figure 4B). Retinotopy was determined from K (K2) functional volumes in M1 (M2) obtained during L (L2) scanning sessions.
II. Sensitivity to zero- and non-zero disparity stimuli

We measured response to disparity stimuli using 32-second presentations of random-dot stereograms, guided by the earlier work of Tsao et al. (2003). Binocular disparity was generated through red-cyan colored dots, paired with red-cyan anaglyph glasses. The diameter of dots was roughly .1°, and dot density was 5%. Luminance of red dots was 20.36 cd/m² through the red filter, and 1.38 cd/m² through the cyan filter. Luminance of cyan dots was 23.98 cd/m² through the cyan filter, and 3.64 cd/m² through the red filter. Sensitivity to disparity stimuli was determined from K (K2) functional volumes in M1 (M2) obtained during L (L2) sessions.

III. Sensitivity to binocular versus monocular stimuli

We also measured response to binocular v. monocular visual stimuli. In the binocular condition, light was allowed into both eyes; in the monocular condition, a mechanical shutter shielded one eye at a time from external light. This shutter was controlled with a pulley from a room separate from the MR scanner. Sensitivity to binocular v. monocular stimuli was determined from K (K2) functional volumes in M1 (M2) obtained during L (L2) sessions.

fMRI Data Analysis

Data analysis was performed with FreeSurfer software (http://surfer.nmr.mgh.harvard.edu/). Anatomical volume surfaces were reconstructed and inflated, and each animal’s functional data was registered to its anatomical volume. To correct for any motion of the head in the scanner, we applied the “AFNI” motion correction algorithm (Cox and Hyde, 1997). To account for changes in the intensity of the MR signal, data were also normalized for signal intensity. Data mapped on inflated cortical surfaces
were spatially smoothed (full width at half maximum = 1.5 mm). Detailed methods for these processes are described in Conway and Tsao, 2006; Conway et al., 2007; Lafer-Sousa et al., 2012. Time courses were generated after “detrending” the fMRI response, which corrects for the drift in signal intensity over time that frequently occurs in fMRI signals. Temporal drift was modeled by the following second-order polynomial:

\[ x(t) = s(t) + at^2 + bt + c, \]

where \( x(t) \) was the raw fMRI signal and \( s(t) \) was the detrended signal. The coefficients \( a, b, \) and \( c \) were calculated using the Matlab function \textit{polyfit}. The percentage deviation from the mean of the fMRI signal, \( s'(t) \), represented in time courses as the y-axis values, was calculated as:

\[ s'(t) = 100 \times (s(t) - \bar{s} / \bar{s}), \]

where \( s(t), t=1,2...N, \bar{s} \) is the mean of \( s(t) \) and \( N \) is the number of TRs in the experiment.

To generate bar plots of the fMRI signal, the response from each voxel in each time block was calculated as the average response from the prior 10 TRs in that block, minus the average from TRs of immediately preceding and following blocks of gray. As each block contained 16 TRs, we calculated the average from the 6th to 16th TR to minimize potential confounding effects from hemodynamic delay. The response of the kth non-gray stimulus block in a run, denoted as \( R_k \), would then be modeled as

\[ RS \_ 100 \times R_k - R_{k-1} \_ Rk_12 \_ / Rk_1 \_ Rk_12 \_ : \]

where \( R_{k-1} \) denotes response to the gray block before this stimulus block and \( R_{k-1} \) denotes response to the gray block after it.

Our analysis was further constrained to voxels demonstrating the highest ratio of signal to noise (calculated by dividing mean response by standard deviation). This corrects
for potential spatial gaps in the fMRI signal (Winawer et al., 2010), excludes voxels of white matter that may have been erroneously included in the manual creation of ROIs, and finally, accounts for the possibility of error in motion correction and registration of the data. Final analysis included only voxels demonstrating a signal-to-noise ratio in the top 90% in response to presentation of blocks of achromatic gratings, stimuli which should induce a strong response in V1.

**Alignment of Hubel-Wiesel histology to MR atlas**

I matched Hubel and Wiesel’s electrode penetration reconstructions to sagittal functional atlas slices, with matches guided by anatomical landmarks visible in histology and the MR atlas and restricted to slices near the coordinates (lateral from the midline) of each craniotomy recorded in the original Hubel-Wiesel protocols. On average, match slices were within +/-1.5 mm lateral of the original Hubel-Wiesel penetration. Assignments were independently confirmed by a research technician naïve to the purpose of the task. These assignments were very often identical: when they did not match, they typically differed by no more than one slice (slice thickness = .35 mm). In the rare event that assignments were separated by more than one slice, a slice was selected from slices intermediate between the two initial assignments.

Match slices were then overlaid on reconstructions of Hubel-Wiesel penetrations. For a small minority of overlays, an affine transform was applied to the reconstruction of the Hubel-Wiesel penetration, in order to improve the registration to anatomical landmarks in the MR atlas. Using these overlays, we determined the probable location of each Hubel-Wiesel cell within our MR atlas and assigned it either to an extrastriate cortical region (V2, V3, or V3A) or to unlabeled cortex.
Results

Hubel and Wiesel recorded from a total of 627 cells in extrastriate cortex from April 9, 1968 to March 17, 1970. Their initial paper on these data, the first to document disparity-sensitive cells in primates, reported that 43% of these 627 cells modulated their response in response to varying binocular disparity (Hubel and Wiesel, 1970). They classified such disparity-tuned neurons as binocular depth cells. As disparity is required for and is sufficient to induce stereoscopic depth perception, these cells were presumed to contribute to computations for stereoscopic depth.

Disparity tuning of sample Hubel-Wiesel binocular depth cells

Figure 5 illustrates tuning curves for two sample binocular depth cells. Binocular depth cells were distinguished by their narrow tuning curves in response to variations in binocular disparity. This definition included cells that exhibited a sharp peak with the receptive fields of both eyes in alignment (i.e., zero binocular disparity). Binocular cells generally peak with the receptive fields of both eyes aligned, but in order for cells to be determined to be stereo-tuned the peak in response to alignment of the two eyes had to be particularly sharp. (Very frequently, these cells were insensitive to stimulation of either eye alone, a tuning property that, at the time these recordings were made, had not previously been documented in relation to disparity tuning.)

Figure 5A shows paper taped to the screen during a Hubel-Wiesel disparity experiment, illustrating both a mapped receptive field and Hubel and Wiesel’s method of measuring disparity during an experiment. The receptive field for the right eye in this cell is mapped at top; arrows indicate the direction of motion preferred by the cell. “31” indicates that this cell was the 31st unit recorded with the “business” (extrastriate)
electrode within this experiment. The point of alignment of the two eyes is marked with vertical lines; displacement of the left eye to 30’ is indicated in 10’ increments by dots along the horizontal axis. The disparity generating the cell’s peak response is marked with pencil at approximately 30’, or 12 mm on the screen (Figure 5A). This disparity corresponds to an object presented approximately 15-20 inches in front of the screen. For a demonstration of this cell’s disparity tuning, see supplemental video.

We generated a tuning curve for this cell, using an archival film documenting the cell’s response to variation in binocular disparity while the cell was presented with its preferred stimulus, a bar of light moving orthogonal to its orientation (3°). The video’s sound was bandpass filtered to isolate the sound from the oscilloscope; a MATLAB script counted the number of spikes in response to each stimulus condition taped, yielding a tuning curve quantifying the cell’s preference for approximately ½ degree of near disparity (Figure 5B). With the eyes positioned at the cell’s preferred disparity, single eye responses were also assessed by masking each eye with a hand. Responses to the single-eye condition were highly suppressed compared to the binocular condition (Figure 5B; filled square: response to right eye only; open square: left eye only). Hubel and Wiesel identified cells demonstrating this strong suppression of response to monocular viewing as “obligate binocular.”

Figure 5C-E describe the tuning properties of a binocular depth cell tuned to zero disparity, recorded from the same electrode penetration as the cell in 5A-B. (Figure 6 illustrates a receptive field schematic for all cells recorded in this penetration.) Panel 5C illustrates this cell’s response to varying binocular disparities: disparity between the two eyes is illustrated at left: the right eye (solid line) remains fixed, while the left eye (dotted
line) is displaced increasingly leftward. The cell’s response peaks with eyes in register, distinguishing it from a binocular cell not tuned to disparity (see Figure 7 for an example). This sharp peak at zero disparity is quantified in a tuning curve (5D). Each point in the curve represents the average response from ten trials of the cell’s preferred stimulus at that disparity. Figure 5E shows oscilloscope traces illustrating this cell’s obligate binocular tuning, evident in its highly suppressed response to stimulation of single eyes.

In addition, Hubel and Wiesel examined the relationship between a cell’s preferred orientation and its response to preferred disparity. This was accomplished by evaluating a cell’s response to variations in both vertical and horizontal disparities. For cells preferring non-zero disparity, the disparity generating maximum response was at a right angle to the axis of the cell’s preferred orientation. Figure 8A illustrates this relationship between disparity tuning and orientation for two disparity-tuned cells in extrastriate cortex. Figure 8A shows the responses of one cell to vertical (dashed-line circles at top) and horizontal displacements of the left eye (dashed-line circles at bottom), with the right eye remaining fixed. The cell is broadly tuned along the axis of its receptive field’s orientation, and narrowly tuned along the orthogonal axis, illustrating the dependence of disparity tuning on orientation. Figure 8B shows responses of a second example cell to vertical and horizontal displacements of the left eye. For both cells, the optimal disparity was orthogonal to the cell’s preferred orientation.
Figure 5. Disparity tuning of cells in area 18. (A) Receptive field and preferred disparity mapped for a binocular depth cell in extrastriate cortex. Arrows indicate axis of preferred stimulus motion. Dots below mark disparities up to 30’, with vertical lines marking zero disparity. (B) Tuning curve for cell in (A), reconstructed from audio analysis of archival film. Cell peaks at approximately 30’ (.5°). Response of single eyes indicated with squares: right eye, filled square; left eye, open square. (C) Response of another cell in the same electrode penetration to varying binocular disparities. RE and LE indicate relative positions of right and left eyes. This cell peaks at approximately 0° disparity. (D) Tuning curve for zero-tuned cell in (C). Filled square marks responses from single eye stimulation (roughly equivalent for each eye). (E) Oscilloscope traces of this cell’s response to monocular and binocular stimulation illustrate obligate binocular tuning.
Figure 6. Receptive field maps for a single penetration in area 18. Dashed lines indicate receptive field for left eye; solid lines indicate receptive field for right eye. Horizontal marks indicate the position of the right fovea relative to each receptive field. “S” marks stereo-tuned cells. Cell 20 and 31 are the same cells whose tuning properties are illustrated in Figure 5C-D and A-B, respectively.
Figure 7. Response to varying binocular disparity of a binocular “tag” cell in V1. Dashed lines indicate receptive field for left eye; solid lines indicate receptive field for right eye. (A) Response to stimulation of right eye only – left eye covered. (B) Response to stimulation of left eye only – right eye covered. (C) Response to eyes in register (zero disparity). (D) Response to left eye displaced upward. (E) Response to left eye displaced downward. (F) Response to left eye displaced leftward. (G) Response to left eye displaced rightward. This cell’s response was insensitive to variations in disparity, both horizontal and vertical.
Figure 8. Responses of two extrastriate cells to variations in vertical and horizontal disparity. Insets show oscilloscope traces indicating cell firing in response to a specific disparity. (A) Responses of a cell in extrastriate cortex to varying disparities of the left eye, with the right eye fixed. Dashed circles at top illustrate the cell's response to a shift in horizontal disparity; dashed circles at bottom mark response to a shift in vertical disparity. The cell is broadly tuned along the axis of its preferred orientation, and narrowly tuned along the axis orthogonal to this orientation. (B) Responses of a second extrastriate cell to vertical and horizontal displacements of the left eye.
Localization of Hubel-Wiesel binocular depth cells

To locate Hubel and Wiesel’s binocular depth cells within contemporary anatomical areas, we generated a functional atlas of visual areas in the macaque brain with “meridian mapping,” an fMRI method that exploits the retinotopic organization of visual cortex to draw boundaries for visual areas. Since each early visual area contains a discrete representation of the visual field, stimulation of the visual field along spatially defined axes reveals these area boundaries. Two alert monkeys viewed flickering checkerboards along the vertical and horizontal meridians; the response to these meridian stimuli is here shown projected on an inflated surface of the macaque brain (Figure 9A, left). Boundaries for each area were drawn manually through the center of meridian representations. Localization of meridians and visual areas was confirmed by mapping the response to stimulation of the center 3° of visual field. Retinotopically-defined areas were then labeled on a high-resolution anatomical MR image of each monkey’s brain to generate a functionally-defined atlas of macaque visual areas. Figure 9B illustrates sagittal and horizontal sections of this atlas for one monkey.

In order to locate individual Hubel-Wiesel cells within the area boundaries of this functional atlas, Hubel-Wiesel electrode paths and histology were matched to anatomically corresponding atlas slices. These matches were made in the absence of atlas labels. Matches were primarily guided by the locations of the original craniotomy site, which was recorded for each protocol in these experiments, and were refined using anatomical landmarks (primarily the lunate sulcus). Figure 10 illustrates the application of this method to assign individual cells recorded by Hubel and Wiesel to distinct visual areas. Brains from the original Hubel-Wiesel experiments were fixed, sliced in the sagittal plane,
and Nissl stained. After each experiment, Hubel and Wiesel examined these slices to confirm the location of their electrode within cortex, using the visible landmarks of the electrode track and the electrolytic lesions periodically generated to mark the location of their electrode. With this information, they generated a reconstruction of their probable electrode path.

Figure 10A illustrates Hubel and Wiesel's reconstruction of the electrode penetration in which they recorded from the two cells whose tuning properties are illustrated in Figure 4. This line drawing is overlaid on a Nissl-stained sagittal slice to illustrate the fidelity of Hubel and Wiesel's line drawings to the histology. The electrode enters near the lunate sulcus and passes into the buried annectant gyrus (Figure 10A). Electrolytic lesions generated to mark the electrode's location are marked with filled circles. Arrowheads indicate the boundary between striate and extrastriate cortex (filled) and the point of entry of the electrode (open). Above, a top-down view of the brain shows both the plane of section of the sagittal slice below (dotted line) and the point of entry of the electrode (asterisk)(Figure 10A).

To identify the visual regions in which individual Hubel-Wiesel cells lay, these reconstructions were overlaid on anatomically-corresponding slices from our functional MR atlas. These overlays allowed us to identify the visual regions recorded from by an electrode as it advanced through the cortex over the course of an experiment. Figure 10B illustrates the MR atlas overlay for the penetration illustrated in 8A. This overlay shows that the electrode penetration likely entered in V2 (blue) and progressed into the buried annectant gyrus, where it reached V3 (green).
Within this penetration, individual cells could be reliably identified using records of their depth within the penetration and by the lesions visible in the histology. The locations of the three lesions made within this penetration are marked with arrows (Figure 10C). Two of them are clearly visible in this slide. Figure 10D illustrates this electrode penetration with single-cell resolution: individual cells are marked with notches along the electrode path and numbered by their sequence in the electrode penetration. This particular penetration recorded from numerous stereo cells, which are indicated in red.

We generated alignments between the Hubel-Wiesel electrode reconstructions and the functional MR atlas for 28 penetrations in extrastriate cortex of 18 animals, in which Hubel and Wiesel recorded from a total of 584 cells. (We were unable to generate alignments for three experiments as the histology and electrode path reconstructions were unavailable). Six representative registrations are illustrated in Figure 11, with areas in which Hubel and Wiesel identified disparity-tuned cells labeled in red. These alignments indicate that Hubel and Wiesel’s binocular depth cells lay not only in V2, but also in areas V3 and V3A.
Figure 9. Retinotopic mapping of visual areas in the alert macaque. (A) Response to flickering checkerboards along vertical v. horizontal meridians in alert macaque (left) and in central 3° v. peripheral visual field (right). (B) Retinotopically-defined areas labeled on anatomical MR. Sagittal (left) and horizontal (right) sections are shown. Black lines mark plane of section. Scale bars represent 1 cm.
Figure 10. Localization of Hubel-Wiesel cells using electrode path reconstructions. Histological slides and line drawings from Hubel-Wiesel stereo experiments were aligned to a retinotopically-defined MRI atlas. (A) Hubel-Wiesel histological slide and line drawing detailing electrode path and recording sites. Asterisk marks site of electrode penetration; black triangle indicates V1-V2 border. (B) Hubel-Wiesel line drawing registered to corresponding sagittal slice from MRI atlas. (C) Lesions (indicated with arrows) and electrode track (indicated with triangle) provide a guide for electrode path reconstruction. (D) Electrode path reconstruction enables location of individual stereo cells. Notches on electrode path indicate individual cells; disparity-tuned cells are labeled in red. Numbers indicate sequence of cells in electrode penetration.
Figure 11. Six registrations of Hubel-Wiesel electrode path reconstructions to MRI atlas. Left: Hubel-Wiesel line drawings representing experiments in six animals. Red lines indicate sections of electrode penetration in which Hubel and Wiesel identified disparity-tuned cells. Center: corresponding sagittal slices from retinotopically-defined MRI atlas. Right: registration of Hubel-Wiesel line drawings to atlas slices.
Disparity tuning by visual area

Using these localizations, we were able to describe the properties of Hubel and Wiesel’s disparity-tuned cells by visual area. Figure 12A shows a bar plot of the proportion of cells tuned to binocular disparity within areas V2, V3, and V3A. The error bars on this plot represent high and low estimates for percent of cells disparity tuned by area, which were generated by using conservative and liberal designations of area boundaries for each visual area.

In V2, the total number of stereo-tuned cells was 55 of 245 (22%). With a liberal definition of cells residing in V2 – one that included all cells that might conceivably be assigned to the area – the number of stereo-tuned cells in V2 would be 66 out of 266 (25%). With a strictly conservative definition of cells residing in V2 - excluding all cells that might have been assigned to V3 - the total number of stereo-tuned cells would be 54 out of 244 (22%). Due to minimal overlap between our labels for V2 and V3, assignments of cells to V2 were made with little ambiguity.

In V3, the total number of stereo-tuned cells was 33 of 96 (34%). With a liberal definition of the area, this number was 93 of 205 (45%); with a conservative definition, it was 5 of 23 (22%) - due to the high proportion of penetrations that passed along the V3/V3A border, very few penetrations passed within unambiguous V3 as defined by our functional atlas.

The total number of stereo-tuned cells in V3A was 98 of 217 (45%). With a liberal definition of cells residing in V3A, the number of stereo-tuned cells was 115 of 269 (43%). This inclusion of more cells in the liberal definition of V3A decreases the prevalence of stereo-tuning, as the added cells – though potentially V3A – likely belonged to V3, which
generally exhibited a lower prevalence of stereo-tuning than V3A in the Hubel-Wiesel data. With a strictly conservative definition of cells residing in V3A - excluding all cells that might have been assigned to V3 - the total number of stereo-tuned cells was 39 of 109 (36%). The relatively small number of cells included in the conservative definition of V3A reflects the large number of penetrations along the V3/V3A border.

Because of the high number of cells recorded from along the V3/V3A border, we combined the two regions for comparison with V2. The total number of cells that were stereo-tuned in V3 or V3A was 134 of 313 (42%), which was significantly greater than the proportion of stereo-tuned cells in V2 (Chi-squared value 12.879, p=0.00033229, df =1), strongly suggesting a functional specialization for stereopsis in V3/V3A.

To assess disparity sensitivity among early visual areas, we used fMRI to compare responses to zero-disparity vs. disparity-rich stimuli. Two alert monkeys viewed zero- and non-zero disparity drifting random-dot checkerboards with disparities of .22° near to .22° far. Stimulus blocks included conditions with zero disparity, combined near and far disparities, and near and far disparities only. Our stimuli were generated to replicate those used by Tsao et al. (2003). Our results, quantified using a disparity selectivity index, confirm prior description of a significant bias for non-zero disparity in V3A (Tsao 2003), and show a non-significant bias for disparity in V3 (Figure 12B). We also show a bias for non-zero disparity in the caudal intraparietal sulcus (CIPS), confirming the previous finding of Tsao et al. (2003).

Figure 12C shows a population histogram of preferred disparities of stereo-tuned cells. Disparity-tuned cells in V2, V3, and V3A demonstrate a strong bias among disparity-tuned cells for disparities near zero (which can be accounted for as a foveal bias). More
subtly, they also demonstrate a bias for near over far disparities (Chi-squared value 11.701, 1 degree of freedom, p = 0.0006). For statistical analysis, near and far disparities were defined as disparities greater than +/- 0.2° (5 mm).

This bias for near disparities is evident not only in single cells, but in fMRI data as well. Figure 12D illustrates the near bias in V1 and extrastriate regions through the near disparity sensitivity index for each area, which quantifies preference for near over far disparities. Near disparity sensitivity indices are greater than far in all visual areas assessed, indicating a global bias for near disparity in early visual areas. However, this preference for near over far is greatest for areas V2, V3, and V4, and less pronounced in V1 and dorsal areas V3A, MT, and CIPS (Figure 12D).
Figure 12. Disparity tuning by visual area: Hubel-Wiesel electrophysiology and fMRI data. (A and B) fMRI and Hubel-Wiesel single-unit data reveal bias for non-zero disparity in V3 and V3A. (A) The proportion of disparity-tuned cells is greater in V3 and V3A than in V2. (B) fMRI disparity sensitivity index reveals bias for non-zero disparity in V3A and CIPS. Disparity sensitivity index was calculated as [(response to drifting random-dot checkerboard stereograms - response to zero-disparity checkerboards)/ (response to drifting random-dot checkerboard stereograms + response to zero-disparity checkerboards)]. Error bars represent standard error. Asterisks indicate p<0.05 (two-tailed t-test). Inset shows disparity index on inflated cortex (LH = left hemisphere, RH = right hemisphere). (C and D) Hubel-Wiesel single-unit data illustrates bias for near over far disparity in V2, V3, and V3A; fMRI confirms near bias in V1 and extrastriate regions. (C) Histogram of preferred disparities of disparity-tuned cells (Hubel-Wiesel data) demonstrates bias for disparities near zero, and near over far disparity, in V2, V3, and V3A. (D) fMRI near disparity bias index reveals a bias for near over far disparity in V1 and extrastriate regions. Near disparity bias index was calculated as [(response to near disparity stereograms - response to far disparity stereograms)/(response to near disparity stereograms + response to far disparity stereograms)]. Error bars represent standard error. Asterisks indicate p<0.05 (two-tailed t-test).
**Receptive field size and eccentricity in V2, V3, and V3A**

In addition to analyzing the disparity tuning of the Hubel-Wiesel cells by visual area, we also compared the relationship between receptive field size and eccentricity between V2, V3, and V3A. While the size of receptive fields in V2 increased very little with distance from the fovea, V3 and V3A receptive field size increased with eccentricity at a much greater rate, so that at a particular eccentricity receptive field area tended to be greatest in V3A, less in V3, and least in V2 (Figure 13).

![Figure 13. Receptive field size v. eccentricity in V2, V3, and V3A.](image)

V2, V3, and V3A can be distinguished on the basis of their receptive field size as eccentricity increases. Blue circles indicate V2 cells; green triangles, V3 cells; yellow diamonds, V3A cells. Values for single cells in each visual area were fit with second-order polynomials (lines).
Characteristics of stereo cells: orientation, spatial organization, binocularity

In their original report of these data, Hubel and Wiesel noted that disparity-tuned cells with orientations within 15° of the horizontal are less common than a random distribution of orientations preferences would predict (1970). Figure 14 presents the data to support this conclusion: histograms of preferred orientations of cells tuned to binocular disparity (A) and not tuned to binocular disparity (B). For each group, preferred orientations of individual cells are marked as dots within the arc at top; the center shows a smoothed histogram of the same data (bin size = 5°). The preferred orientations of cells in V2, V3, and V3A tuned to binocular disparity are not evenly distributed: they underrepresent orientations near the horizontal meridian (Chi-squared value 54, 17 degrees of freedom, p= 0.00001). By contrast, the preferred orientations of cells in the same regions not tuned to binocular disparity are evenly distributed (Chi-squared value 6.8, 17 degrees of freedom, p=0.986).

Another property of disparity-tuned cells Hubel and Wiesel documented – but did not publish – was spatial clustering among cells by disparity preference. Figure 15A shows this clustering in a line drawing of four electrode penetrations within one experiment, in which disparity-tuned cells are labeled by preference for near (N), zero (0), and distant (D) disparities. Figure 15B illustrates a schematic of receptive fields for disparity-tuned cells in the penetration marked with an arrowhead in 15A. For cells preferring distant disparity, the cell peaked with the left eye’s receptive field (solid line) deviated to the right of the right eye’s (dashed line); for cells preferring near disparity, the optimal stimulus disparity was the left eye’s receptive field displaced to the left of the right eye’s. We quantified the clustered organization of disparity-tuned cells by plotting each disparity cell’s preferred
disparity – categorized as near, zero, or distant – against the disparity of the stereo-tuned cell next following it within a penetration. The majority of stereo-tuned cells match their neighbors in disparity tuning type, a result indicated by darker gray (higher cell count) along the x=y diagonal.

Hubel and Wiesel also documented a then-novel characteristic of disparity-tuned cells in extrastriate cortex: the prevalence of cells that demonstrated minimal response to monocular stimulation. All of these “obligate binocular” cells were tuned to binocular disparity. Figure 16 shows a population histogram of ocular dominance for cells tuned to binocular disparity. Ocular dominance was quantified on a scale from 1 to 7, where 1 indicates exclusive response to the contralateral eye, 7 indicates exclusive response to the ipsilateral eye, and 4 indicates equal response from both eyes. Obligate binocular cells were assigned to the “X” category. Within the Hubel-Wiesel data, many stereo-tuned cells displayed characteristics of obligate binocularity (Figure 16B), while none of the non-stereo cells were classed as obligate binocular (Figure 16A).

We further examined the obligate binocularity of early visual areas by contrasting responses to binocular and monocular viewing of zero disparity and disparity-rich stimuli (thus isolating binocularity from stereo-tuning). All areas showed a greater response to binocular vs. monocular viewing, even V1 (Figure 16C, inset). However, for CIPS, response was almost entirely suppressed for monocular conditions, showing that the region is obligate binocular. V3A also showed strong suppression in the monocular viewing condition. This preference for a binocular stimulus is quantified for V1 and extrastriate areas in Figure 16C. Preference for binocular viewing was greatest in V3A and CIPS for both disparity and zero-disparity conditions.
Figure 14. Orientation tuning of disparity-tuned cells underrepresents the horizontal. (A) Histogram of preferred orientations of disparity-tuned cells. Circles indicate preferred orientations of individual cells; at center is a smoothed histogram (bin size = 5°) of the same data. (B) Histogram of preferred orientations of cells not tuned to disparity.
Figure 15. Spatial analysis of Hubel-Wiesel cells reveals clustering by preferred disparity.

(A) Line drawing of electrode penetrations show spatial grouping of stereo cells preferring near, zero, and distant (far) disparities (marked with “N”, “0”, and “D”, respectively). (B) Schematic of receptive field maps for recordings highlighted in (A). Triangles indicate penetration highlighted. Dashed lines indicate receptive field for left eye; solid lines indicate receptive field for right eye. Horizontal marks indicate the position of the right fovea relative to each receptive field, numbers indicate the sequence in which cells were recorded from. (D) Spatial analysis quantifies correspondence between disparity preference of cells in V2, V3, and V3A and their nearest recorded neighbors.
Figure 16. Obligate binocularity: single-cell and fMRI results. Ocular dominance quantified using Hubel-Wiesel scale: 1 indicates exclusive response to the contralateral eye; 7 indicates exclusive response to the ipsilateral eye; 4 indicates equal response to both eyes. X indicates obligate binocularity: no response to either eye stimulated alone. (A) Cells not tuned to disparity do not exhibit obligate binocularity. (B) Disparity-tuned cells exhibit high prevalence of obligate binocularity. (C) Binocular component of response to zero v. non-zero disparity reveals a strong preference for binocular stimuli in V3A and CIPS. Inset shows sample time courses for V1, V3A, and CIPS for monocular (bottom) and binocular (top) viewing of zero disparity (left) and non-zero disparity (right) random-dot stereograms.
Discussion

In 1970, Hubel and Wiesel published the first description of cells tuned to binocular disparity in primates (Hubel and Wiesel, 1970). As disparity is sufficient to induce perception of stereoscopic depth, these cells were understood to play a critical role in computations of stereopsis. This initial paper was brief and left many of their findings undescribed; however, Hubel and Wiesel never published a full account of their results, as they suspected their recordings from “area 18” had encompassed multiple visual areas (Hubel and Wiesel, 2004).

We identified the contemporary visual areas from which Hubel and Wiesel had most likely recorded in their early experiments on stereopsis, using alignment of Hubel and Wiesel’s original electrode penetrations and histology to slices from a functional atlas of macaque early visual areas. These localizations illustrate that Hubel and Wiesel’s data describe not only V2, as has been historically believed, but also V3 and V3A (Figure 11). Furthermore, V3 and V3A cells recorded by Hubel and Wiesel were more often disparity-tuned than those in V2 (Figure 12A). Using fMRI, we also assessed response to disparity among early visual areas, confirming prior findings of a strong bias for disparity in V3A and in the caudal intraparietal sulcus (CIPS) (Figure 12B).

In addition to this analysis of disparity tuning by area, we here present unpublished Hubel-Wiesel data documenting characteristics of disparity-tuned cells in V2, V3, and V3A. Hubel and Wiesel’s data show a bias for near over far disparities, which we confirm with fMRI (Figure 12C-D); a bias against orientations near the horizontal meridian (Figure 14); a spatial clustering by disparity tuning (Figure 15); and a prevalence of cells insensitive to monocular stimulation (Figure 16).
Specialization for stereopsis in V3/V3A

By aligning Hubel and Wiesel’s reconstructions of their electrode penetrations to a functional atlas of macaque early visual areas, we identified the probable locations of 584 of 627 cells recorded by Hubel and Wiesel from extrastriate cortex. Our localizations demonstrate that in V2, 22% of neurons in the Hubel-Wiesel data are tuned to absolute binocular disparity, while in V3/V3A, this proportion is significantly greater, at 42% (Figure 12A). (While Hubel and Wiesel’s initial paper described a prevalence of 43% of stereo cells within “area 18,” further analysis of binocular depth cells was restricted to include only those cells that showed clear excitatory peaks in response to a specific disparity.) These data are consistent with a specialization for absolute disparity in areas V3 and V3A, a conclusion that echoes those of previous electrophysiology (Poggio et al., 1988) and fMRI studies (Tsao et al., 2003).

However, counter to our finding that V3 and V3A are specialized for disparity computations, a recent meta-analysis from the DeAngelis group has asserted that V3 and V3A are no more specialized for disparity than V1 or V4 (Anzai et al., 2011). The conflict of these results with those described in this paper may be reconciled in part by sample bias in studies of data collected from few penetrations or few animals. However, it seems more probable that a significant cause of the conflict between these results is the variation in the boundaries of extrastriate regions depending on whether the study in question defines visual areas using a functionally- or anatomically-defined (cytoarchitectonic) atlas of the brain. Our analysis employed a functional atlas of visual areas, while Anzai et al. used an anatomical atlas to define the boundaries of V3 and V3A. In addition, our analysis of the Hubel-Wiesel data was restricted to cells that demonstrated clear excitatory peaking in
response to a specific disparity, while the analysis of Anzai et al. included cells with a much broader range of disparity tuning preferences.

To complement our analysis of disparity tuning by area within Hubel and Wiesel’s data, we used fMRI to examine responses to disparity in V1 and extrastriate regions (Figure 12). Our results reveal a bias for non-zero disparity in V3A and CIPS, confirming the previous findings of Tsao et al. (2003). In contrast, we found no bias for non-zero disparity in V2 or V3. As these regions contain a ventral component, while V3A and CIPS do not, our data are consistent with a specialization for processing of disparity in the dorsal stream, as proposed by Hubel and Livingstone (1987b).

The absence of a significant fMRI response in V3 for the non-zero disparity condition is somewhat surprising, given that our analysis of Hubel and Wiesel’s data indicates that V3 contains a high proportion of stereo-tuned cells. However, these findings are not as contradictory as they may seem. One explanation for the difference is that our fMRI disparity sensitivity index measures response to non-zero vs. zero disparity. Using such an index, responses from populations of cells tuned to zero disparity are indistinguishable from those of populations that are not disparity-tuned. Thus, our results are compatible with the Hubel and Wiesel data if V3 contains a relatively high proportion of stereo cells tuned to zero disparity.

In addition, our experiments used different stimuli than Hubel and Wiesel’s: while Hubel and Wiesel used bar or edge stimuli, we used random-dot stereograms. Poggio et al. (1988) observed that within V3/V3A, 60% of cells exhibit disparity-tuning for only a random-dot or a solid bar stereogram, and not both. In light of this finding, it is possible that fMRI responses to stimuli more similar to Hubel and Wiesel’s would be more likely to
implicate V3 in disparity processing. Furthermore, Hubel and Wiesel evaluated only absolute disparity in their experiments, and our disparity stimuli contained both absolute and relative disparities.

Finally, V3 was not the only region in which our fMRI results seemed to conflict with established electrophysiology data on disparity tuning. Like Tsao et al. (2003) and Backus et al. (2001), we found no fMRI bias for non-zero disparity in MT, while electrophysiology studies of macaque MT indicate that most individual cells are disparity-tuned (Maunsell et al. 1983; DeAngelis and Newsome, 1999; DeAngelis and Uka, 2003). In certain recording sites, spiking activity is a poor predictor of blood-oxygen-level-dependent (BOLD) response (Heeger and Ross, 2002); it may be that V3 and MT are among these sites. Further study of the functional organization of early visual areas will be necessary to resolve these apparent conflicts between fMRI and electrophysiology results.

**Receptive field size and eccentricity in V2, V3, and V3A**

We also used localizations of the Hubel-Wiesel data to examine the retinotopic organization of their cells by visual area, finding that we could distinguish between cells in V2 and V3/V3A on the basis of the relationship between receptive field size and eccentricity in each area. At comparable eccentricities, receptive fields tended to be smallest in V2, larger in V3, and largest in V3A (Figure 13). This result is consistent with the traditional location of these regions in a hierarchical visual pathway, in which V2 precedes V3 and V3 precedes V3A. Our finding that receptive field size in V3 is larger than in V2 at similar eccentricities confirms that of previous work (Felleman and Van Essen, 1987); however, the distinction between receptive field sizes in V3 and V3A seems to be less
certain, since the assignment of cells between these areas was somewhat ambiguous (see Figure 12A).

**Near bias among disparity-tuned cells**

Among the stereo cells that Hubel and Wiesel recorded from V2, V3, and V3A, we found a significant bias for near over far disparities (Figure 10A). Preference for near over far disparities has been previously documented among stereo-tuned cells in V3 (Adams and Zeki, 2001), MT (DeAngelis and Uka, 2003), and IT (Tanabe et al., 2005).

We further examined prevalence of near disparity bias in V1 and extrastriate regions using fMRI. Our results indicate a bias for near over far disparities in all regions studied: V1, V2, V3, V3A, V4, MT, and CIPS (Figure 10B). However, the near bias was only significant in extrastriate areas with a ventral component: V2, V3, and V4. Biases for near in dorsal areas – MT, V3A, and CIPS – were not significant. These results indicate a bias for processing of near disparities in the ventral stream, a finding that corresponds to the conclusions of a meta-analysis by Anzai et al. (2011).

In human behavioral studies, near disparities are more salient than far (Jansen et al., 2009), indicating a functional specialization for detection of near disparities in the primate visual system. This bias for computations of near disparity may be adaptive, as objects that lie in front of the plane of fixation are more likely to be relevant to a viewer's decision-making than objects behind it.

**Orientation tuning of disparity-tuned cells**

In their initial description of these data, Hubel and Wiesel noted that the depth cells preferring orientations within 15° of the horizontal were more rare than a random distribution of orientation preferences would predict (Hubel and Wiesel, 1970). Here, we
present the data on which this observation was founded (Figure 14). The under-representation of stereo cells preferring orientations near horizontal remains a novel finding.

Given that the eyes are offset along the horizontal axis, one might predict visual cortex to specialize in detection of horizontal disparities. This horizontal displacement of the eyes generates more horizontal than vertical disparities in natural viewing (Read and Cumming, 2004). The energy model of disparity tuning predicts that disparity-tuned cells will be narrowly tuned in the direction orthogonal to their preferred orientation (Ohzawa et al., 1990). This model predicts that a population of cells specialized for horizontal disparity detection ought to overrepresent vertical, and underrepresent horizontal orientations (Read and Cumming, 2004). This prediction is a product of the assumption that disparity-tuned cells perform a linear combination of inputs from oriented receptive fields in each eye, prior to computing disparity (Read and Cumming, 2004). According to the energy model, a single cell specialized for horizontal disparity should have an orientation near vertical and exhibit narrow tuning to disparity along the horizontal axis.

Recent work has challenged the energy model’s prediction that disparity tuning is dependent on orientation: cells in primary visual cortex are broadly tuned to horizontal disparities regardless of orientation preference (Cumming, 2002). This broad tuning to horizontal disparities has been interpreted as a specialization for horizontal disparity, as it enables sensitivity to a broad range of horizontal disparities (Cumming, 2002). The energy model can be modified to explain this horizontal specialization by introducing independent calculations of orientation and disparity (Read and Cumming, 2004). Such computations could be accomplished either by cells receiving input from multiple receptive field subunits
with different position disparities, or by suppression of single-eye response in inhibitory regions of receptive fields (Read and Cumming, 2004). But each of these modified energy models predicts that disparity tuning should be unrelated to orientation tuning when cell responses are tested with stimuli that are not isotropic, like the bar stimuli used by Hubel and Wiesel (Uka and DeAngelis, 2002; Read and Cumming, 2004).

Counter to these recent findings, Hubel and Wiesel's data on disparity tuning support the energy model: their binocular depth cells demonstrate narrow tuning orthogonal to their orientations (Figure 8). Furthermore, Hubel and Wiesel documented among disparity-tuned cells a strong link between orientation and disparity tuning: the population of their disparity-tuned cells underrepresents orientations near horizontal and overrepresents those near vertical. These data are in line with the classic energy model's prediction that single-cell specialization for horizontal orientation is achieved through narrow tuning to horizontal disparity.

Columnar organization of disparity-tuned cells

The Hubel-Wiesel data illustrate that disparity-tuned neurons in V2, V3, and V3A are spatially clustered by preference for near, zero, or far disparity (Figure 15). Although these data do not independently provide evidence for a columnar organization, they support previous findings of columnar organization of cells tuned to binocular disparity. Columnar organization by preferred disparity has been documented in V2 (Hubel and Livingstone, 1987a; Ts'o et al., 2001) V3/V3A (Poggio et al., 1988; Adams and Zeki, 2001; Anzai et al., 2011), V4 (Watanabe et al., 2000) and MT (DeAngelis and Newsome, 1999).

Columnar organization within the cortex provides evidence for functional specialization, as it places a constraint on the computations that can be performed within
local neural circuitry (Mountcastle, 1997). Hence, the spatial grouping of disparity-tuned cells that Hubel and Wiesel document within V2, V3, and V3A may indicate that these regions, as a group, are specialized for calculations of binocular disparity. However, comparison of columnar organization between visual areas would require further analysis of the Hubel-Wiesel data. A meta-analysis of columnar organization by disparity tuning in V3, V3A, V4, and MT found the most columnar organization in V3A and MT, less in V3, and least in V4 (Anzai et al. 2011). These data suggest that areas V3A and MT, as well as V3, are functionally specialized for disparity. A regional analysis of the Hubel-Wiesel data on spatial grouping by disparity preference may offer further insight regarding the specialization of V3 and V3A for computations of stereopsis.

**Obligate binocularity in disparity-tuned cells**

One of the most striking features of stereo-tuned cells encountered by Hubel and Wiesel in their early study of disparity tuning was the tendency of disparity-tuned cells to be insensitive to single-eye stimulation, a tuning property they termed obligate binocularity. This suppression of monocular response is consistent with a functional specialization for binocular calculations. All of the obligate binocular cells that Hubel and Wiesel encountered were also disparity-tuned (Figure 16). To identify binocularity of early visual areas, we used fMRI to evaluate responses to monocular and binocular viewing of random-dot stereograms. V3A and CIPS showed the strongest preference for binocular over monocular viewing, even in the zero disparity condition, indicating that these regions are specialized for binocular computations.

Though Hubel and Wiesel were first to document the presence of obligate binocular cells, they were later documented in areas V2, V3, V3A and V4 (Zeki, 1978). Obligate
binocular cells have since been described in visual areas beyond those examined by Hubel and Wiesel in their early study of binocular depth cells. IT, which contains disparity-tuned cells, also contains a relatively large proportion of cells insensitive to monocular stimulation (Uka et al., 2000). However, not all regions in which stereo cells have been identified contain obligate binocular cells: a majority of cells in MT are stereo-tuned, but very few are obligate binocular (Maunsell and Van Essen, 1983). While obligate binocularity indicates functional specialization for binocular calculations, it is not a requirement for disparity-tuning; indeed, most of the binocular depth cells described by Hubel and Wiesel were not obligate binocular.

**Conclusion**

Though 43 years old, the Hubel-Wiesel data provide novel insight on contemporary questions regarding the specialization of extrastriate cortical areas for disparity computations and the characteristics of disparity-tuned cells. Our localizations of their data illustrate that Hubel and Wiesel’s 1970 paper described binocular depth cells not only in V2, but also in V3 and V3A – furthermore, that cells in V3 and V3A were more often stereo-tuned than those in V2. This information enables a richer comparison of the Hubel-Wiesel data with contemporary work on calculations of stereopsis in extrastriate cortex. In addition, we show that the Hubel-Wiesel binocular depth cells demonstrated bias for near disparities, tendency to prefer vertical orientations, grouping by disparity preference, and requirement of binocular stimulation to elicit responses – properties consistent with a functional specialization for the perception of stereoscopic depth.
References


