The Representation of Brightness in Primary Visual Cortex

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Although neurons in primary visual cortex are sensitive to the spatial distribution and intensity of light, their responses have not been thought to correlate with the perception of brightness. Indeed, primary visual cortex is often described as an initial processing stage that sends information to higher cortical areas where perception of brightness, color, and form occurs. However, a significant percentage of neurons in primary visual cortex were shown to respond in a manner correlated with perceived brightness, rather than responding strictly to the light level in the receptive fields of the cells. This finding suggests that even at the first stage of visual cortical processing, spatial integration of information yields perceptual qualities that are only indirectly related to the pattern of illumination of the retina.

Although perception generally provides an accurate reflection of the physical world, it is far from a simple reconstruction. This fact is clearly evident in the mammalian visual system in which numerous distinct cortical areas alter and repackage visual information in different ways. A major goal of visual neuroscience is to understand where in these cortical areas, and by what processes, neural responses to light energy are transformed into responses associated with perception. Two key aspects of visual perception are contour (form) perception and surface perception (the brightness and color of areas between contours). Primary visual cortex is often described as a preprocessor that projects to "higher order" visual areas where computations are performed culminating in the perception of form, brightness, and color. However, we now show that many neurons in primary visual cortex respond in a manner correlated with surface brightness, suggesting that perceptual responses may occur at the first stage of cortical processing.

Surface brightness is an advantageous probe for investigating perceptual mechanisms because it can be easily and dramatically manipulated in a manner that dissociates it from the actual light level (luminance). For instance, in brightness induction (*1*), a gray piece of paper can be made to look light gray if it is placed on a black background and a much darker gray if it is on a light background (Fig. 1A). Brightness induction is a simple example of the extensive perceptual interactions that occur between areas throughout the visual field. These interactions are thought to underlie brightness and color constancy (*2*), in which our judgment of these attributes is largely immune from overall changes in illumination (such as the difference between the light outside on a sunny day and the light inside an artificially lit room). The normalization processes underlying these perceptual constancies are of crucial behavioral importance in order for brightness and color to reliably signal information necessary for object recognition.

To study the responses of cortical neurons we used a dynamic version of brightness induction (Fig. 1B). By modulating the luminance of areas flanking a gray patch on a computer monitor, we created the perception that the brightness of the gray patch oscillated between light and dark (*3*). Several features of this dynamic perceptual induction are relevant to our study. First, large variations in brightness are produced by induction only if the luminance of the static gray patch is intermediate between the minimum and maximum luminances of the modulated flanks. Second, the brightness of the induced patch varies roughly in antiphase to the modulation of the flanks. Third, as the modulation rate of the surround is increased, the dynamic brightness induction stops at temporal frequencies higher than a few hertz (*4*, *5*), roughly 10 times lower than the highest rate at which brightness variations produced directly by luminance changes are perceived (*6*).

We made extracellular recordings from individual neurons in primary visual cortex of anesthetized cats (*7*) and used elongated slits of light to determine the borders of the receptive field and standard receptive field properties such as orientation tuning and end stopping. Subsequently, the computer monitor used for visual stimulation was positioned such that a uniform gray patch of light encompassed the cell's receptive field. This central patch usually extended 3° or more to each side of the receptive field borders (including any end-stopping regions). Flanking stimulus patches were placed either to the sides of the central patch or above and below it. A computer presented a set of stimuli, some involving brightness induction and others serving as controls (*8*).

A set of peristimulus-time histograms for a representative neuron with a receptive field 6° wide is shown in Fig. 1C. The induction stimulus was configured so that the central gray region was 14° across, extending 4° to each side of the receptive field. The top histogram shows that in the induction condition the response was mod-

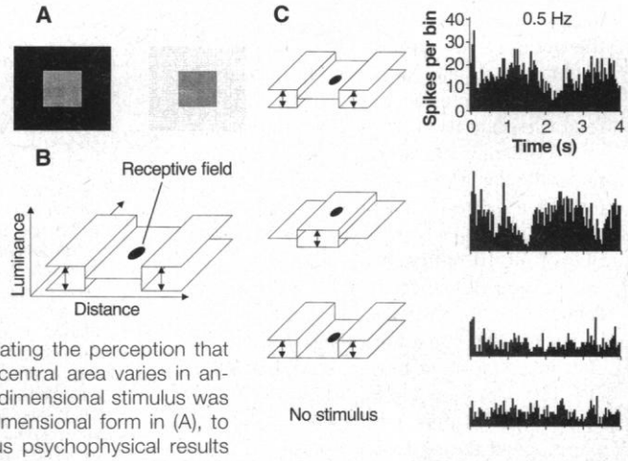

Fig. 1. (**A**) In brightness induction, also called simultaneous contrast, a gray square looks lighter on a black background (left) than on a light gray background (right). (**B**) Luminance profile of the stimulus used in the present study. In this dynamic version of brightness induction, the luminance of areas flanking a central gray area is modulated (arrows in figure), creating the perception that the brightness of the static central area varies in antiphase to the flanks. A one-dimensional stimulus was used, rather than the two-dimensional form in (A), to allow comparison to previous psychophysical results (*5*). Neural responses possibly associated with brightness can be examined by configuring the stimulus so that a cell's receptive field is in the large static gray region. (**C**) A neuron in striate cortex studied with luminance-modulated stimuli. The receptive field was 6° wide by 2.5° high and was centered on a uniform gray region 14° by 14°, with flanks of the same size to the sides. When the luminance of the flanks was modulated at 0.5 Hz and the central area was gray, the neural response was modulated at 0.5 Hz (top histogram). Luminance modulation of the central area also produced a modulated response, but the temporal phase of the response was shifted by approximately 180° (second histogram). When the flanks were modulated and the central area was black, the response of the neuron (third histogram) was similar to the spontaneous firing rate (bottom histogram).

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ulated by luminance variations in the surrounding flanks even though these were well outside the receptive field. The firing of the neuron was phase-locked to the flank modulation at 0.5 Hz, being maximal when the surround luminance was lowest. The neuron was also driven by modulating the luminance of the central area containing the receptive field (second histogram). However, in this center modulation condition, the phase of the response was shifted relative to the induction condition: the response was greatest when the modulated area was brightest rather than darkest. Importantly, response modulation was not detected when the uniform area containing the receptive field was black rather than gray—activity was similar to the spontaneous firing of the cell (third and fourth histograms). A comparison of the responses in the different conditions suggests that the firing of the neuron was correlated more with the brightness in the area covering the receptive field than with the luminance of any particular portion of the stimulus. This correlation was seen in the strong response modulation when the stimulus center was gray (a condition that yields perceptual induction) compared to the weak response when the center was black (a condition that does not cause induction). A correlation with brightness is also seen by comparing the responses to luminance modulation within the receptive field and luminance modulation in the flanks. When light covering the receptive field was modulated, the response was maximal when the central area was brightest (and the luminance of the modulated light was highest). When the flanks were modulated, the response was again greatest when the central area was brightest, but in this instance the luminance of the modulated flanks was lowest. The response was clearly not determined by the overall amount of light present in the stimulus.

In our sample of 160 neurons, 120 (75%) had responses that were modulated and phase locked to the luminance changes outside the receptive field (according to the criterion defined in Fig. 2). There are several ways in which modulation of luminance outside the receptive field might have produced a neural response, and it is critical to distinguish between these. The simplest possibility—that we underestimated the boundaries of the receptive field, and the luminance-modulated flanks were actually within the receptive field—is unlikely, as we were quite conservative in our estimate of receptive field size and placed the flanks at least several degrees away from the receptive field boundaries. Another possibility is that the modulated areas were outside the receptive field, but scatter of light within the eye produced stimulation of the receptive field. This, too, is unlikely for two reasons. First, the addition of small artificial pupils in front of the eyes often did not diminish the responses even though it should have minimized the likelihood of scattering. Second, if scattering were the cause of the response modulation, it should have been most apparent when the center of the stimulus was black rather than gray, because the lower light level on the retina would have increased the sensitivity of the retinal receptors.

In many neurons, scattering could easily be ruled out because the response was greatly reduced in the center black condition compared to the induction condition. However, in some neurons significant response modulation occurred when the stimulus center was black or gray. The response of these neurons might be partly associated with brightness, but this could not be proven in the presence of what appeared to be scattering. We chose the conservative criterion that neurons were considered for further analysis only if the modulation amplitude in the induction condition was more than twice that in the center black condition. This left 49 (31% of the total of 160) neurons that had responses not easily accounted for by scattering, suggesting that the responses to modulation of the stimulus flanks involved neural interactions from beyond the classical receptive field (9). These neurons were located in all layers 2 to 6 and were of both simple and complex types. For these 49 neurons, the phase of the temporal response and the response across temporal frequencies were analyzed for possible correlations with brightness perception.

One of the hallmarks of the dynamic brightness induction effect is that brightness changes are perceived only at quite low modulation rates in comparison to the rates of direct luminance modulation that elicit brightness variations (*4*, *5*). Physiologically, we also detected differences in the amplitudes of response modulation in the induction and center modulation conditions. In the induction condition, the response of the neuron was largest at low temporal frequencies and decreased as the rate of flank modulation was increased above 1.0 Hz (top row of histograms in Fig. 3A). However, when the luminance of the central area was modulated, the response amplitude progressively increased with increasing temporal frequency (second row of histograms). A significant difference in the induction and center modulation conditions is evident in the averaged data (Fig. 3B).

Another characteristic of dynamic induction is that the perceived temporal phase of the brightness variations is in approximate antiphase to the luminance variations in the flanks. Of course, if the luminance of the area covering the receptive field is modulated, brightness and luminance changes covary (that is, they are in phase). Therefore, perceptually, brightness changes are 180° out of phase between the induction condition and the center modulation condition. For the neurons with responses not attributable to light scattering, the response phase differences between the induction and luminance modulation conditions were diverse, but the great majority were near either 0° or 180° (Fig. 4).

Our physiological data suggest that a significant fraction of neurons in striate

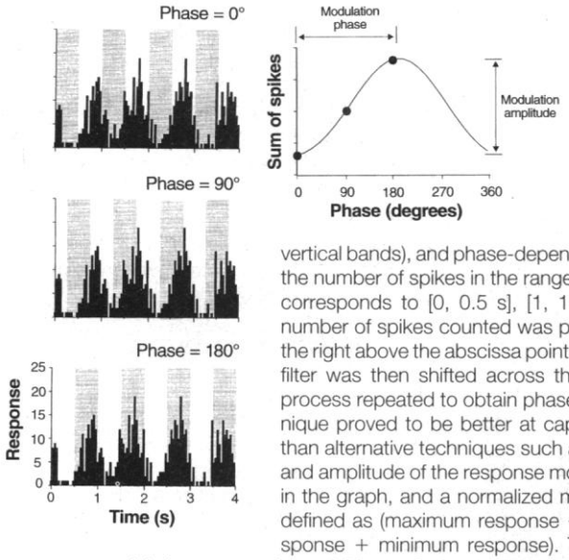


Fig. 2. Procedure used to quantify the extent of modulation in peristimulus time histograms. In the example shown, the stimulus flanks were modulated at 1 Hz, and the histogram suggests modulation at this temporal frequency. A sliding filter with the temporal period of the flank luminance modulation was placed over the histogram (shaded vertical bands), and phase-dependent activity was measured by adding the number of spikes in the ranges [0, π], [2π, 3π], [4π, 5π], . . . which corresponds to [0, 0.5 s], [1, 1.5 s], . . . (top histogram). The total number of spikes counted was plotted on the ordinate in the graph to the right above the abscissa point indicating the starting phase of 0. The filter was then shifted across the histogram in 4-ms steps and the process repeated to obtain phase-dependent spike counts. This technique proved to be better at capturing modulation in the histograms than alternative techniques such as fast Fourier transforms. The phase and amplitude of the response modulation were quantified as indicated in the graph, and a normalized measure of response modulation was defined as (maximum response − minimum response)/(maximum response + minimum response). To determine the significance of the response modulation, we performed Monte Carlo simulations to estimate the probability that the modulation occurred by chance. Neurons were considered to have significant modulation if the normalized measure of response modulation satisfied the $P < 0.05$ level of significance.

cortex had responses correlated with brightness. (i) The neural responses were modulated at the frequency of the surrounding luminance modulation. (ii) The response modulation occurred in conditions that elicited brightness induction but not in similar conditions that did not produce induction. (iii) The temporal response mirrored brightness perception in that the amplitude of the response modulation associated with brightness induction greatly decreased as temporal frequency was raised, unlike the response modulation produced directly by luminance changes. And (iv), for many cells there was a complete phase shift between induction and center modulation conditions. However, certain qualifications to this simple correlation are necessary. For example, we have compared human psychophysics to the response properties of neurons in cat visual cortex. Although there are no behavioral data showing that cats perceive the effects we have described, there is considerable similarity in the visual systems of cats and humans up to the level of striate cortex (*10*), and cats have been shown to see other brightness effects such as illusory figures (*11*). Another concern is that not all the neurons that responded to flank modulation exhibited 180° phase shifts between induction and center modulation conditions, as one might predict from the perception of brightness in these conditions. If neurons must satisfy all three of the brightness criteria (that is, response modulation with center gray but not black, antiphase response in induction condition, and relatively rapid decline in response modulation with increasing temporal frequency) to be categorized as brightness-associated responses, then roughly 10% of the 160 neurons we examined had brightness responses. However, >25% of all neurons had responses that appeared to be correlated with brightness, except that they exhibited in-phase responses in the induction condition. Given that the link between neuronal activity and perception is unknown, these neurons may also play some role in brightness perception, particularly because the response phases are not evenly distributed across all phases. Therefore, the percentage of neurons with responses that are partially or completely correlated with brightness is in the range of 10 to 31%.

Our findings imply that a perceptual quality of surfaces—brightness—may be synthesized at the earliest stages of processing in striate cortex (*12*). The representation of surface information is surprising because, since the early work of Hubel and Wiesel (*13*), striate cortex has been studied primarily in terms of its responses to light and color edges. The representation of surfaces has remained largely a mystery. Our findings complement other results suggesting that information about perceived motion (*14–16*), illusory figures (*17–19*), and color constancy (*20, 21*) is explicit in extrastriate visual areas. For example, in macaque area V4, a large silent area beyond neurons' classical receptive field appears to be involved in color contrast and the coding of surface color (*20*). Our results suggest that an extensive silent surround may contribute to brightness contrast and a neural representation of surface brightness in striate cortex. Our findings do not mean that the neurons we have studied are brightness "detectors." These same neurons were also selective for other stimulus attributes such as orientation and spatial frequency; the cells were multiplexing information about multiple stimulus properties. However, our results imply that information about surfaces, such as their brightness, is represented in the responses of the same neurons that are sensitive to contours.

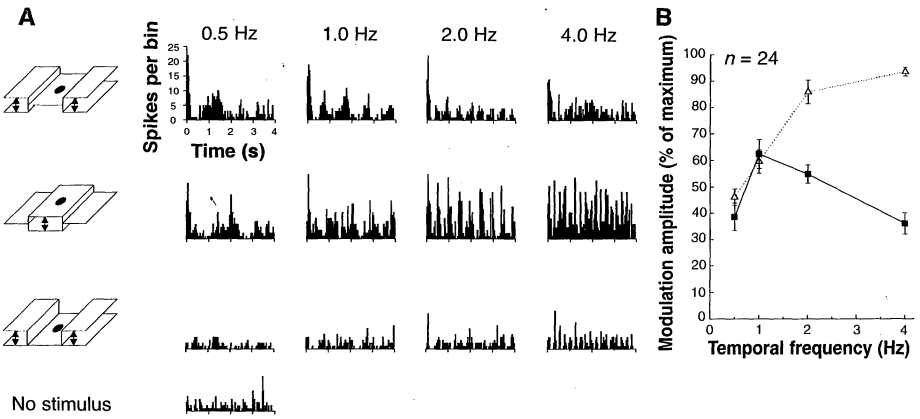
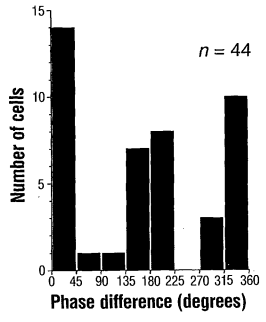


Fig. 3. (**A**) The influence of temporal frequency on the response to brightness induction and luminance modulation. This neuron had a receptive field of 3° by 3°. The central region and the flanks were each 14° by 14°. The response was modulated when induction was produced by oscillating the luminance of the flanks with a gray central area (top row) and when the luminance of the central area was oscillated (second row). When the area covering the receptive field was black, the response to flank modulation (third row) was similar to spontaneous activity (bottom). At 0.5 and 1.0 Hz, the temporal phase of the responses to center luminance modulation and induction were in approximate antiphase. Increasing the temporal frequency from 0.5 to 4.0 Hz affected the response differently in the center modulation and induction conditions. With modulation of the luminance covering the receptive field, there was a progressive increase in the modulation amplitude as temporal frequency was raised (second row). In the induction condition, the modulation amplitude peaked at 1.0 Hz and fell off at higher temporal frequencies (top row). There also appear to be phase changes at the higher temporal frequencies, but it is not known whether such shifts occur perceptually. (**B**) Averaged normalized modulation amplitudes for 24 neurons studied at 0.5, 1.0, 2.0, and 4.0 Hz. Modulation amplitudes for each cell are expressed as a percentage of the highest amplitude obtained in any stimulus condition for that cell. At the lower temporal frequencies, response modulation was similar in the center modulation conditions (Δ) and induction conditions (■). At the higher temporal frequencies, the modulation amplitude increased for center modulation but decreased for induction.

Fig. 4. Temporal phase of the response to luminance modulation of flanks with a central gray area, relative to the response phase to modulation of light covering the receptive field. Phase was estimated as described in Fig. 2. The response of most neurons shifted by approximately 0° or 180°. A phase difference of 180° means that if a cell responded most to luminance modulation in the receptive field when the area covering the receptive field was brightest (and the luminance was highest), then in the induction condition it also responded most when the area covering the receptive field was brightest (and the luminance in the surround was lowest). A phase difference of 0 means that in the center modulation and induction conditions the response was highest in one condition when the receptive field area was brightest and highest in the other condition when this area was darkest.



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7. Animals were anesthetized with continuous intravenous infusion of thiopentothal sodium and paralyzed with atracurium besylate. Depth of anesthesia was maintained by adjusting the rate of pentothal infusion as indicated by electroencephalography and electrocardiography, which were continuously monitored. Animals (n = 20) were artificially respirated through a tracheal cannula, and end tidal CO_2 and rectal temperature were monitored and maintained at 4% and 38°C, respectively.
8. Stimuli were presented for 4 s, and conditions involving different stimulus configurations, sizes, and temporal frequencies were randomly interleaved.
9. Fifty-two percent of the neurons had a modulation amplitude in the induction condition that was equal to or greater than the amplitude in the center black condition.
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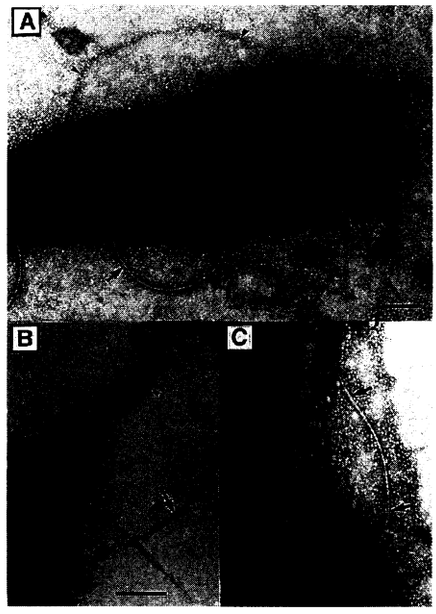

Fig. 1. *Agrobacterium tumefaciens* A348 producing pili. A348 was grown at 19°C in the presence of 200 μM acetosyringone (*10, 11*). All scale bars indicate 200 nm. Arrowheads, pili; arrows, the flagella.

Pilus Assembly by *Agrobacterium* T-DNA Transfer Genes

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Agrobacterium tumefaciens can genetically transform eukaryotic cells. In many bacteria, pili are required for interbacterial DNA transfer. The formation of pili by *Agrobacterium* required induction of tumor-inducing (Ti) plasmid–encoded virulence genes and growth at low temperature. A genetic analysis demonstrated that *virA*, *virG*, *virB1* through *virB11*, and *virD4* are the only Ti plasmid genes necessary for pilus assembly. The loss and gain of pili in various mutants correlated with the loss and gain of transferred DNA (T-DNA) transfer functions, which is consistent with the view that *Agrobacterium* pili are required for transfer of DNA to plant cells in a process similar to that of conjugation.

Agrobacterium tumefaciens is a Gram-negative bacterium with the capability of transforming eukaryotic cells. This pathogen normally infects plants, which results in the neoplastic disease crown gall, but the bacterium can also stably transform yeast cells (*1, 2*). During infection of plant cells, a segment of the *Agrobacterium* Ti plasmid, termed the T-DNA, is transferred into the plant cell nucleus, where it is integrated into the plant genome (*1*). Although this transformation system has been widely applied in studies of plant molecular biology and crop plant improvement, fundamental questions remain regarding the mechanism by which *Agrobacterium* introduces DNA into eukaryotic cells. Here we report on one crucial component of the process.

At least 10 operons, *virA* through *virJ*, on the Ti plasmid are involved in processing and transfer of T-DNA (*1*). These *virA* through *virJ* genes are regulated by the VirA-VirG two-component regulatory system and are transcribed only when the bacteria are exposed to chemical inducers found in the wound site of a plant (*3*). T-DNA is transferred to the plant cell as a single strand covalently attached to the protein VirD2 (*1*). The VirE2 protein is also transferred either separately or in association with T-DNA (*1, 4*). Transfer of T-DNA and VirE2 requires at least 12 additional proteins, VirB1 through VirB11 and VirD4 (*4, 5*), which are related to transport proteins involved in the assembly of conjugative sex pili and export of toxins by the human pathogens *Bordetella pertussis* and *Helicobacter pylori* (*6, 7*). On the basis of these homologies and subcellular fractionation studies, VirB1 through VirB11 and VirD4 are thought to assemble into a membrane-anchored transfer complex that functions similarly to bacterial conjugation (*6*). This transfer machinery performs optimally at low temperatures (*8*), as does conjugative transfer of IncH1 plasmids, which fail to form H pili when grown at the nonpermissive temperature (*9*).

To establish whether A. *tumefaciens* forms pili at low temperatures, we grew cells at 19°C on solid medium under conditions that activate *vir* gene transcription (*10*), washed the plates with 10 mM $MgCl_2$, and prepared the plates for electron microscopy (EM) (*11*). In eight separate preparations, wild-type strain A348 produced pili visible by EM (Fig. 1). The pili were 3.8 nm in diameter and were distinguishable from the flagella, which were 12 nm in diameter (Fig. 1). Pili were never seen in cells of strain A136, which lacks a Ti plasmid (Table 1), and this demonstrated that the observed structures are not cellulose fibrils, which are plant-induced structures present on A136 cells (*12*). The pili varied in length, but pili longer than the length of the bacterium were observed (Fig. 1A). In many cases, pili were present in clumps extending from a single electron-dense region on the bacterial surface (Fig. 1B). In other cases, long individual pili were observed (Fig. 1, A and C).

Pili depended on induction of *vir* genes because pili were never seen on cells grown on agar plates lacking acetosyringone (Ta-

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