Characterization of the on and Off Pathways in Human Vision

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Loyola University Chicago

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LOYOLA UNIVERSITY CHICAGO

CHARACTERIZATION OF THE ON AND OFF PATHWAYS
IN HUMAN VISION

A THESIS SUBMITTED TO
THE FACULTY OF THE GRADUATE SCHOOL
IN CANDIDACY FOR THE DEGREE OF
MASTER OF SCIENCE
NEUROSCIENCE PROGRAM

BY
LUISA ROVERI

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MAY 1995
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## PART ONE
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INTRODUCTION

The visual system processes information in several parallel pathways that code luminance, color, spatial and temporal properties of the visual stimulus. Recent anatomical and neurophysiological data support the existence of separate neural pathways for the processing of achromatic and chromatic stimulus components (Hubel & Livingstone, 1987). Although the integration of this information occurs in the visual cortex, it has been shown that these parallel pathways originate in the retina (Enroth-Cugell & Robson, 1966). This study focuses on two additional parallel pathways for which electrophysiological, anatomical and behavioral evidence has been found in animal studies: the ON- and OFF-channels. The functional role of the ON and OFF channels is still a matter of debate. Jung (1973) was among the first to suggest that the perception of brightness and darkness may depend on two different populations of cells with opposing responses: the ON and OFF cells. Brightness and darkness are terms that describe the sensation associated with light increments or light decrements. The perception of brightness and darkness depends on the spatial and temporal distribution of light in the visual field: an object appears brighter or darker according to whether spatially adjacent regions are respectively darker or brighter than the object itself. For example, the same gray ring looks much lighter against a black background than against a white one (Hering, 1920). The dependence of the brightness of one region on the relative brightness of adjacent regions is called simultaneous contrast, where contrast expresses the relative variation in luminance (Hering, 1920).

Helmholtz (in Fiorentini, Baumgartner, Magnussen, Schiller, & Thomas, 1990) observed different sensory effects of electrical polarization of the human eye. He produced light sensation by placing one electrode on the eye and another on the neck.
and applying a galvanic current. Changing the direction of current flow produced opposite effects: a brightness enhancement when the polarization was cathodic and a darkness enhancement when the polarization was anodic. Gernandt and Granit (1947) reported that cat ganglion cells which respond to light increments with an increase in their rate of discharge (ON-cells) were also activated by cathodic galvanic polarization. Cells which respond to light decrements (OFF-cells) with an increase in spike discharge were activated by anodic galvanic polarization. Comparing these results with the subjective effects of galvanic current supports the proposition that the ON and OFF systems subserve brightness and darkness sensations.

**Visual Physiology**

**Anatomy and Signal Transduction**

The retina is a multilayered nervous structure that covers the posterior internal surface of the eye and contains the neurons of origin of the visual system. The vertebrate retina contains five major classes of cells organized into three layers: the outer nuclear layer, containing photoreceptors, the inner nuclear layer containing bipolar horizontal and amacrine cells, and the ganglion cell layer. The processes of these cells are grouped in two plexiform layers where synaptic contacts occur. The outer plexiform layer contains the processes of receptors, bipolar cells and horizontal cells, while the inner plexiform layer contains the processes of bipolar, amacrine, and ganglion cells. Therefore, light has to go through the whole retina in order to reach the photoreceptors.

The first step of the visual process is the absorption of light by the photoreceptors. There are two types of photoreceptor, rods and cones, so named because of the characteristic shape of the outer segment. Rods and cones have different anatomical structure, topographical distribution and physiological properties. The
human retina contains many more rods than cones. Rods and cones are also distributed differently across the retina (Hood & Finkelstein 1986a). The cone population is at its highest density within the fovea, the region where images fall when an observer fixates. The fovea extends about 1.5-2 degrees visual angle in diameter. Cone density falls outside the fovea, reaching a minimum at around 10 degrees eccentricity, and beyond this point the retina contains a uniform distribution of cones. Rods density increases with eccentricity up to about 18 degrees and then gradually declines. However, in the far periphery the density of rods remains much greater than cones. At 15 degrees along the nasal retina there is a photoreceptor free region of the retina, called the blind spot, where the optic nerve leaves the eye.

The function of photoreceptors is mediated by the photosensitive pigments that transduce the electromagnetic energy of light into electrochemical signals for the nervous system. Unlike most other neurons, photoreceptors are depolarized in the resting state during darkness. When depolarized, photoreceptors continuously release their neurotransmitter. The depolarized state is maintained by cGMP-gated Na+ channels (Fesenko, Kolesnikov, & Lyubarsky, 1985). These channels are opened when cGMP binds to them. In response to light, the photoreceptors activate a cascade of biochemical modifications that leads to a change in the membrane potential which in turn regulates synaptic transmission to the bipolar cells. Absorption of light lowers the cytoplasmic concentration of cGMP, through the enzyme cGMP-phosphodiesterase (PDE), causing the closure of cGMP-gated channels and thus causing the photoreceptors to hyperpolarize and reduce the release of neurotransmitter. Photoreceptors respond to light in a graded fashion, as do bipolar cells and horizontal cells (Kaneko, 1970; Werblin & Dowling, 1969). Action potentials are first seen in the ganglion cells (Wunk & Werblin, 1979).

The functional differences between rods and cones has been determined by measuring their sensitivity to light across the visible spectrum. Sensitivity refers to
the ability to detect a change in visual stimulation. The minimum intensity of light
needed to detect a given stimulus is defined as the threshold intensity. Theoretical
curves have been obtained based on psychophysical data from a number of laboratories
with a variety of procedures. These measurements across wavelength, form luminous
efficiency functions which specify the relative spectral sensitivity of the visual system
under different light levels: scotopic and photopic (Hood & Finkelstein, 1986b). Vision
is said to be photopic at high levels of ambient illumination, as in daylight, and scotopic
at low level of illumination. The photopic function is assumed to represent the cone
system and the scotopic function the rod system. The cone system provides high acuity
and is characterized by three different photopigments which are responsible for color
vision. The rod system is more sensitive at low light levels, is poor at discriminating
spatial details, and does not provide color discrimination.

Separate pathways for rods and cones in the mammalian retina were
described first by Cajal (1893). Bipolar cells connect exclusively to either cones or
rods, and carry their signals in parallel to the ganglion cell level where signals
converge. Bipolar cells establish synaptic contact, directly or through the amacrine
cells, with the ganglion cells which are the output neurons of the retina. Ganglion cell
axons form the optic nerve which projects to the lateral geniculate nucleus (LGN) of the
thalamus. In turn, neurons from this structure project to the visual cortex. The LGN
receives input from both eyes due to the bifurcation of the optic nerves at the optic
chiasm: fibers from the nasal retina project in the contralateral optic tract to the
contralateral LGN and fibers from the temporal hemiretina of each eye project in the
ipsilateral optic tract to the ipsilateral LGN. The LGN of primates contains six layers of
cell bodies separated by layers of axons and dendrites. The two ventral layers of the
nucleus are known as the magnocellular (MC) layers since they contain relatively large
cells. The four dorsal layers are known as parvocellular (PC) layers because they
contain smaller cells. The LGN receives its main retinal input from different classes of
ganglion cells. Primate α cells (Pα or MC) ganglion cells project to MC layers of LGN forming the magnocellular M-pathway, and primate β cells (Pβ or PC) ganglion cells project to PC layers of LGN forming the parvocellular P-pathway. MC cells have large cell bodies, large dendritic arborizations, large receptive fields, and show relative transient responses to sustained illumination. They appear to be concerned with the analysis of gross features of a stimulus and its movement. PC cells have small receptive fields and are involved with color vision. The P- and M-pathways maintain their sharp anatomical segregation through the termination of the LGN projection in the visual cortex.

The human visual cortex consist of six layers of cells. Layer 4, the principal layer of inputs from LGN, is further subdivided into four sublamina: 4A, 4Cβ (projections from P pathway) and 4B, 4Cα (projections from M pathway) (Fitzpatrick, Lund, & Blasdel, 1985). The complex network of connections in primate extrastriate cortex has also been described as two basic pathways. One pathway includes areas in parietal cortex and is thought to be important for assessing spatial relationship and object motion. The other pathway includes visual area in temporal cortex and is thought to be involved in identification of colors, patterns and objects (Ungerleider & Mishkin, 1982). Differences between the parietal and temporal pathway can be seen in lesion-induced deficits, in neuronal response properties, and in anatomical connections (Desimone & Ungerleider, 1989; DeYoe, Hockfield, Garren, & Van Essen, 1990; Mishkin, Ungerleider, & Macko, 1983; Ungerleider & Mishkin, 1982; Van Essen & Maunsell, 1983). Cumulative anatomical, physiological and behavioral evidence has suggested that the contribution of the M- and P-pathways remains segregated in primary visual cortex. Each pathway connects to one of two major cortical processing streams: the M-pathway and the parietal pathway forming a subsystem and the P-pathway and the temporal pathway forming the other (Livingstone & Hubel, 1987; Maunsell, 1987). However, many lines of evidence suggest that the temporal and the
parietal cortical pathways may each receive inputs from the P- and the M-pathway (Van Essen, Anderson, & Felleman, 1992).

**Electrophysiological Response: Receptive Fields**

Due to their electrical properties and size, the ganglion cells were the first cells in the retina to be studied in terms of response to different patterns of light. Hartline (Hartline, 1938) recorded the pattern of action potentials of single ganglion cell axons in response to spots of light in the retina of the frog. He discovered three classes of fibres: ON fibres that discharge when the light is turned on, OFF fibres that discharge when the light is turned off, and ON/OFF fibres that respond to both the onset and termination of light. Each ganglion cell responds to light directed to a specific area of the retina which defines the receptive field of the cell. Later on, the development of techniques for recording from various cell types of the retina revealed a similar organization of the receptive fields at the bipolar cell level. Intracellular recordings from bipolar cells revealed the existence of two major classes of bipolar cells: ON-center bipolar cells that depolarize in response to light and OFF-center bipolar cells that hyperpolarize in response to light (Bortoff, 1964; Tomita, 1964; Werblin & Dowling, 1969). Receptive fields are circular and vary in size across the retina (Barlow, 1953; Kuffler, 1953). In the foveal region of the retina, where cone concentration and visual acuity is greatest, the receptive fields are small and have an antagonistic center-surround organization in response to light increments or light decrements (Barlow, 1953; Kuffler, 1953): center-on/surround off or center-off/surround-on. The antagonistic surround mechanism for each cell type is due to the lateral connections of horizontal and amacrine cells. In many vertebrates horizontal cells hyperpolarize to light, and it is widely believed that horizontal cells are responsible for the surround response seen in bipolar cell recording (Kaneko, 1970). The only documented site of horizontal cell interaction has been through a negative
feedback influence onto cone terminals (Lasansky, 1981). As was first shown by Baylor and his colleagues (1971), horizontal cells depolarize cones in the center of the receptive field, thereby causing an antagonistic response in the center. The center-surround structure of ganglion cell receptive fields appears to enhance the ability to detect objects that contrast only weakly with their background. Hence, the judgement of brightness relies principally on information about contrast rather than the absolute amount of light. Consequently, the appearance of an object is influenced by the contrast between the object and its surround, as reflected by the phenomenon of simultaneous contrast.

While the receptive field characteristics of neurons in the LGN are similar to those of the retinal ganglion cells (Dreher, Fukada, & Rodieck, 1976; Lee, Martin, & Valberg, 1989; Schiller & Malpeli, 1978; Wiesel & Hubel, 1966), cortical receptive fields are more complicated. Cortical cells do not have circular receptive field properties and respond to stimuli such as linear bars. Based on their responses, cortical cells were categorized into two major groups: simple and complex (Hubel & Wiesel, 1959). Simple cells receptive fields are rectangular with a specific axis of orientation and are built up from many circular fields by appropriate connections with cells in the primary visual cortex. A cell may have a rectangular ON (excitatory) zone flanked on each side by a rectangular OFF (inhibitory) zones. The effective stimulus for the ON zone of such a field must excite the specific segment of the retina and have a specific orientation. Every axis of rotation is represented for every retinal position: cells in the cortex receiving impulses from the same point of the retina have similar receptive field shapes but different axes of orientation. In comparison, complex cells do not have clearly defined ON and OFF zones, but their receptive fields also have a critical axis of orientation. Movement across the receptive field is a particularly effective stimulus for certain complex cells. Hubel and Wiesel (1978) proposed that a significant input to complex cells comes from a family of simple cortical cells that have
the same axis of orientation but slightly offset receptive field position. It seems that these cells are important for analysing the form of the visual image. Overall the visual cortex is organized in columns of cells with different axes of orientation. Detailed mapping of adjacent columns using tangential penetration with microelectrodes revealed a precise organization with an orderly shift in axis of orientation from one column to the next (10 degree every 30-100 µm). Therefore, the majority of cells in the primary visual cortex show selectivity for the orientation of edges, and many cells respond selectively to the direction of motion of these edges.

The ON and the OFF Channels

Anatomy and Physiology

The physiological basis of ON and OFF channel separation was first proposed when intracellular electrophysiological techniques identified two types of bipolar cells by the change of their membrane potential evoked by a focal light stimulus: ON bipolar cells depolarize in response to light and OFF bipolar cells hyperpolarize in response to light (Kaneko, 1970; Werblin & Dowling, 1969). Hence, the origin of these channels appeared to occur in the retina at the first level of synaptic interaction between photoreceptors and bipolar cells. Combined electrophysiological and intracellular staining techniques demonstrated that the two types of bipolar cells make synaptic contacts on the respective ganglion cells in anatomically distinct sublamina of the inner plexiform layer of the retina. The ON-center bipolar cells make contacts with ON-center ganglion cells in the inner portion of the inner plexiform layer (sublamina b), and the OFF-center bipolar cells make contacts with OFF-center ganglion cells in the outer portion of the inner plexiform layer (sublamina a), (Famiglietti, Kaneko, & Tachibana, 1977; Nelson, Famiglietti, & Kolb, 1978) (Fig. 1). This suggested that increments and decrements of light could be processed separately and independently starting in the retina. Depolarizing bipolar cells underlie the ON pathway, whereas
hyperpolarizing bipolar cells underlie the OFF channel (Miller & Dacheux, 1976; Naka, 1976). In agreement, the two classes of bipolar cells establish morphologically distinct synapses on photoreceptors (Kolb & Famiglietti, 1976). ON cone bipolar cells form invaginating synapses with photoreceptor synaptic ribbons and OFF cone bipolar cells form flat synapses with the photoreceptors. The rod pathways are organized differently than the cone pathways. In the rod system only one morphological type of bipolar cell has been found which forms invaginating connections with rods (Boycott & Kolb, 1973; Kolb, 1979; Kolb & Nelson, 1983). Rod bipolar cells do not synapse directly on ganglion cells but instead amacrine cells are interposed (Famiglietti & Kolb, 1976; Kolb & Famiglietti, 1974). The amacrine cell in turn connects to the ON pathway through gap-junctions with ON-center bipolar cells in sublamina b and to the OFF pathway through conventional synapses with OFF-center ganglion cells in sublamina a.

Beyond the retina, the population of ON-center and OFF-center cells is recognized in LGN in both the PC-pathway (Shapley & Reid, 1992) and MC pathway (DeValois, 1965; Gouras, 1968; Wiesel & Hubel, 1966). In several species, including tree shrew (Conway & Schiller, 1983), macaque monkey (Schiller & Malpeli, 1978), mink (LeVay & Mcconnel, 1982) and ferret (Stryker & Zahs, 1983), ON-center and OFF-center neurons have been found to be segregated into different lamina or sublamina of the LGN. De Bruyn and Casagrande (1983) have examined ganglion cells filled retrogradely by injections of horseradish peroxidase in the LGN of the tree shrew. They found that the ganglion cells projecting to the LGN layers containing ON-center cells have dendrites which ramify close to the cell body in sublamina b of the IPL. Ganglion cells which project to the LGN layers containing OFF-center cells have dendrites which extend into sublamina a of the IPL. In addition, separate ON and OFF regions are present in the tree shrew striate cortex suggesting that spatially separate parallel ON and OFF afferent channels extend in this species at least through the first synapse in the striate cortex (Norton, Rager, & Kretz, 1985).
Figure 1. Proposed ON and OFF pathways in the mammalian retina. Depolarizing ON cone bipolar cells make invaginating connections with cones and connect directly with ON ganglion cells in the inner portion of the inner plexiform layer, sublamina b (IPL (b)). Hyperpolarizing OFF cone bipolar cells make flat connection with cones and connect directly with OFF ganglion cells in the outer portion of the inner plexiform layer, sublamina a (IPL (a)). Rods bipolar, which make invaginating connections with rods, do not connect directly with ganglion cells and instead connect with AII amacrine cells. AII amacrines form electrical gap-junctions with the ON cone bipolars and synapses with the OFF cone bipolars and ganglion cells.
Cone Photoreceptors

Outer Plexiform Layer

Bipolar Cells

Inner Plexiform Layer

Sublamina a

Sublamina b

Ganglion Cells

Amacrine cell

ON

OFF
Pharmacology

The first suggestion that an excitatory amino acid might be involved in retinal neurotransmission was provided by Furukawa and Hanawa (1955). Recording from ON-center and OFF-center bipolar cells of the carp, Murakami (1975) demonstrated that exogenously applied aspartate and glutamate had opposite polarity responses: ON-center bipolar cells were hyperpolarized while OFF-center bipolar cells were depolarized. This study also indicated that bipolar cell responses are probably the result of specialization within the synaptic receptors themselves rather than through the use of multiple photoreceptors transmitters. It has been shown that glutamate produces different responses in the two classes of bipolar cells by gating different ion channels. Glutamate depolarizes OFF-center bipolar cells by opening cation channels that carry an inward depolarizing Na+ current into the cell (Saito, Kondo, & Toyoda, 1979; Toyoda, 1973). The mechanism by which glutamate hyperpolarizes ON-center bipolar cells may involve the opening of K+ selective channels or the closure of Na+ channels (Shiells, Falk, & Naghshineh, 1981; Slaughter & Miller, 1981). In a recent study, Nawy and Jahr (1990b) found that glutamate suppresses the current flow through cGMP-gated channels in ON-center bipolar cells. In the absence of the neurotransmitter, the channels are kept open by high concentration of cGMP. Glutamate appears to cause the closure of these channels in the same way as light causes the closure of cGMP-gated channels in photoreceptors namely, by activating a second messenger cascade that result in lowering of the cytoplasmic concentration of cGMP. By analogy with photoreceptors it is thought that the ON bipolar cells have a specific glutamate receptor that, like rhodopsin, activates a G-protein which in turn activates cGMP phosphodiesterase (PDE). Therefore, the ON and OFF systems subserved by different bipolar cells appear to be segregated according to the specialized synaptic receptors they contain.
During the past few years the increased availability of effective antagonists combined with an improved knowledge of the selectivity of several agonists has enhanced the understanding of the types of synaptic receptors that mediate neurotransmission in the outer and in the inner retina. Studies of the sensitivity of the photoreceptor-bipolar synapse to different glutamate analogs have yielded evidence for different types of receptors. Slaughter and Miller obtained intracellular recordings from all types of retinal neurons in the perfused retina eye-cup of the mudpuppy (Slaughter & Miller, 1981). The ON-bipolar synapses were blocked by 2-amino-4-phosphonobutyrate (APB), an analogue of the neurotransmitter glutamate that produces a prolonged hyperpolarization without significant interaction on the OFF-center bipolar cells. All OFF-center cells showed only disfacilitatory hyperpolarization following the administration of APB. Whereas the OFF-bipolar synapse is not much affected by APB, it can be blocked by either PDA (2,3-piperidine dicarboxylic acid) (Slaughter & Miller, 1983a) or KYN (kynurenic acid) (Slaughter & Miller, 1983b), two other glutamate analogues that bind to a different receptor.

The nature of the rod bipolar cell response has been studied in several species with different results. Recording from rod bipolar cells in the rabbit retina, Dacheux and Raviola (1986) found that all rod bipolar cells depolarize in response to light. Similarly, the majority of investigators believe that rod bipolar cells depolarize upon being stimulated (Wässle & Boycott, 1991). Wässle and his colleagues have used APB to show that all signals in the rod pathway in the cat pass through the APB receptor and therefore demonstrated that there is only one type of rod bipolar cell. Since APB blocked responses in both ON and OFF ganglion cells in the dark-adapted state, and since APB generally acts on the synapse between photoreceptors and depolarizing bipolar cells, the conclusion was that rod bipolar cells depolarize in response to light. The existence of only depolarizing bipolar cells has also been supported by the observation that under scotopic conditions all light-mediated responses in the monkey are eliminated following
APB injection (Dolan & Schiller, 1989).

Application of APB, in addition to evaluating retina physiology of the ON and OFF pathways, has been useful in a number of studies that explored the separation of the ON and OFF channels at different levels. Schiller (1984) examined the effect of retinal APB on the receptive field organization of different classes of cells in the LGN of the monkey. APB effectively blocked ON-center cells response whereas OFF-center cells were not significantly affected. He also observed that PC cells were blocked at lower concentration and for longer time than MC cells. Furthermore he found that the surround response of OFF-center cells is not affected by APB, suggesting that the center-surround organization of the receptive field of ganglion cells and LGN cells is not a result of interaction between the ON and OFF systems, thereby strongly supporting the idea of the existence of two separate and independent channels. Similar data were found in records from LGN of cats following vitreal APB infusion (Horton & Sherk, 1984) suggesting that the ON and OFF channels remain separate in the thalamus. At the level of striate cortex in monkey (Schiller, 1982) and in the cat (Sherk & Horton, 1984) directionality and orientation selectivity of the cortical cells are unaltered by APB infusion, suggesting that these attributes are not produced by interaction between the ON and the OFF channels. Nevertheless, inhibitory interactions between these channels were evident with light-edge response carried by ON-center cells and dark-edge response produced by OFF-center cells (Schiller, 1982). In fact, APB infusion eliminated the light edge response of cortical cells revealing a dark edge response in some cells. These studies indicate that the ON and OFF systems remain largely segregated in the primate geniculostriate system until they reach the striate cortex where inhibitory interactions occur between the ON and OFF channels.
Behavioral Studies

Animal

To elucidate the functional role of the ON- and OFF-channels in vision, behavioral studies have been performed in monkeys before, during and after the ON-channel was blocked with retinal APB (Schiller, Sandell, & Maunsell, 1986). To accomplish this, monkeys were trained to make saccades to a variety of detection and discrimination tasks. Under light-adapted conditions, monkeys treated with APB have difficulty in detecting light increments but not light decrements. Reaction time for saccades to increments were doubled after APB injection, and the proportion of correct responses dropped from 100% to chance level. To date, this has been one of the strongest studies to show that the primary function of the ON and OFF pathways is to facilitate detection of increments and decrements of light, independently.

The role that the ON and OFF pathways play in dark-adapted vision was examined in a study by Dolan and Schiller (1989). They investigated the effect of APB on visual behavior in the dark-adapted monkey. The monkeys were trained to make saccades to either an incremental or decremental stimulus after a period of dark adaptation. Performance was assessed by determining the percentage of correct trials and the reaction time latencies for the correct trials. Under scotopic conditions all monkeys showed an impaired ability to detect both incremental and decremental stimuli following APB administration. Based upon these results, they inferred that there is only one class of rod bipolar cells in the monkey. Furthermore, the APB suppression of scotopic vision is consistent with the physiology that has been reported for the mammalian rod pathway, where rods connect only to ON-center bipolar cells (Müller, Wässle, & Voight, 1988).

These animal studies are consistent with previous human psychophysical evidence that supports the notion of separate channels for discriminating bright and dark targets (Antis, 1967; Cavanagh & Antis, 1986; Hanly & MacKay, 1979; Krauskopf,
1980). However, the perception of brightness is affected by both the absolute luminance of the stimulus and the relative luminance of the background, a phenomenon known as simultaneous contrast. According to Jung's hypothesis (Jung, 1973), the phenomenon of simultaneous contrast is the result of lateral inhibition between the brightness and darkness channels. Recently, Dolan and Schiller (1994) examined the perceptual consequences of ON channel blockade in the monkey on processing of luminance stimuli relative to the contrast of the background. Following ON channel blockade with APB, Dolan and Schiller found that the brightness of a stimulus is still determined by the contrast with its background. This suggests that lateral inhibitory mechanisms that underlie simultaneous contrast are still intact, disproving the Jung hypothesis. In another experiment they examined the role of the ON channel in detection of stimuli that result from luminance modulation of either the background or the foreground. They found that the ON channel selectively conveys incremental stimuli regardless of whether it appears as incremental or decremental temporal event as long as the spatial features define it as incremental. This result also reinforces previous evidence from monkey that the center-surround organization of the receptive field is not created by interaction between the ON and OFF channels (Schiller, 1984).

The results of these behavioral studies suggest a possible role for the ON and OFF channels in vision. The performance of the visual system is enhanced when information to the central nervous system is transmitted with an excitatory process such as an increase in rate of discharge. The properties of each type of channel are best suited to signaling either a light increment or a light decrement as a modulation of the spontaneous activity of the ganglion cells. Since ganglion cells are already firing spontaneously at a low rate, the same cell could not signal with the same efficiency two opposite changes such as a light increment and decrement. In fact, the dynamic range carried by decreases in firing rate would be lower than that carried by increases in
firing rate. Therefore, two independent channels would provide an efficient transmission of both light increments and decrements.

**Human**

A number of psychophysical experiments in humans reported asymmetries in detection threshold between light increments and light decrements. Short (1966) and Patel (1968) found that threshold for a light decrement was consistently lower than threshold for a light increment. Similar results were reported in more recent studies (Bowen, Pokorny, & Smith, 1989; Bowen, Pokorny, Smith, & Fowler, 1992; DeMarco, Smith, & Pokorny, 1994). Comparing the sensitivity to light increments and decrements at different luminance levels, Bowen (1992) found a progressively greater sensitivity for decremental stimuli. This result suggested that light adaptation progressively increases the sensitivity difference between the ON- and OFF-pathways.

Psychophysical studies have also investigated the temporal properties of the visual system in response to increments and decrements of light. It has been shown that the subjective brightness of the light phases and the subjective darkness of the dark phases of a flickering light stimulus depend upon flicker frequency and that in the lower frequency range brightness enhancement and darkness enhancement may be observed. Magnussen and Glad (1975), reported an asymmetry in brightness and darkness judgments made by human observers to suprathreshold flicker. Darkness enhancement was found to be greater than brightness enhancement. This result appeared to be in agreement with a number of former studies (Cleland, Levick, & Sanderson, 1973; Enroth-Cugell & Robson, 1966; Ikeda & Wright, 1972) reporting, in the cat, a higher proportion of transient to sustained off-center neurons than for on-center neurons, indicating a more transient character of the system involved with darkness judgment. Asymmetry in the temporal response of the ON and OFF systems to different waveforms of luminance modulation has also been reported. Results from a psychophysical study of
temporal sensitivity indicated that temporal edges are crucial for activating the ON and OFF pathways (Bowen, et al., 1992).

**Purpose of the Study**

Taken together, these studies suggest that brightness and darkness perceptions are processed by two different pathways. Whereas studies have addressed the functional properties of the ON and OFF channels in the primates, relatively few studies have investigated the characteristics of these two channels at the visual cortex level human subjects. There is much interest today in describing the function of the various parallel pathways found in human, both for basic science and clinical knowledge. The studies to be presented examined different aspects to provide additional information on the ON and OFF pathways in human subjects.

One direct way to test hypotheses about the function of subclasses of neurons is to inactivate selected classes of neurons and observe the effects on visual function. Blocking the activity of ON center neurons by APB is an example of a method that can be applied in experiments with animals. In humans, the technique that comes closest to neural blocking is selective adaptation, which has proven to be useful in mapping stimulus selective mechanisms in the spatial and temporal domains (Sekuler and Blake 1985). The logic of selective adaptation is to temporarily reduce the sensitivity of the neuronal mechanisms mediating the detection of a stimulus. The degree with which the test stimulus is affected by adaptation is an indication of the stimulus specificity for the detecting mechanism. When more than one pathway is present, selectively desensitizing one pathway may reveal activity from the other(s). The first part of the present study examined the effect of adaptation to light increments and decrements. This part included two experiments. The first experiment examined the psychophysical effect of adaptation over a range of luminance levels. In the second experiment, the electrophysiological response of the ON and OFF systems to adaptation was studied.
The purpose of the second part of the study was to characterize the electrophysiological properties of the ON and OFF pathways in humans. The VEP has been used in previous studies as a measure of the contrast sensitivity function (CSF) (Campbell & Maffei, 1970). Campbell and Robson (1968) have proposed that the visual system contains a number of elements each selectively sensitive to a limited range of spatial frequency. Subsequently, Blakemore and Campbell (1969), in a psychophysical study, reported that the human visual system may possess channels selectively sensitive to spatial frequencies with narrow bandwidth, analogous to the electrophysiological observations in animal studies (Campbell, Cooper, & Enroth-Cugell, 1969). This original suggestion was confirmed also electrophysiologically. Campbell and Maffei (1970) have reported human electrophysiological evidence that there exist channels selectively sensitive to spatial frequency. The knowledge of differences in the spatial tuning of the visual system suggested that this aspect could have helped to characterize possible differences between the ON- and the OFF-pathways.
PART ONE

ADAPTATION TO LIGHT INCREMENTS AND DECREMENTS
CHAPTER I
RATIONALE FOR THE RESEARCH METHOD

Psychophysical studies measuring sensitivity change following adaptation support the idea that increments and decrements of light are processed by independent visual pathways (Hanly & MacKay, 1979; Krauskopf, 1980). Before studying the properties of the ON and OFF pathways in human vision it is necessary to generate appropriate stimuli which will be processed independently by each pathway. The sawtooth waveform, which is characterized by a rapid transient followed by a ramp component, might represent such a stimulus (Fig. 2). Using temporal sawtooth modulation of luminance, Hanly and MacKay (1979) produced selective adaptation. They found greater elevation of threshold when the test stimulus and the adaptation stimulus had waveforms of the same polarity. They also found threshold elevation for the test stimulus of opposite polarity, although only half as much as that for the stimulus of the same polarity. Based upon previous evidence that most retinal ganglion cells are sensitive to rates of change of luminance (Hughes & Maffei, 1966) this effect would have been expected since the sawtooth waveform combines a slow change with a rapid one of the opposite polarity. Cavanagh and Anstis (1986) proposed the existence of separate channels to process the ramp-phase and the step-phase of the sawtooth. The perception of brightness shift seen when a ramp grating is in motion was explained as the sum of a sustained and a transient response. The ramp and the step phase of the sawtooth waveform combine opposite polarity. In the hypothesis that the temporal response to the step-phase is not linear and therefore reaches a saturation level, they suggested that the on- and off-response to the two phases of the sawtooth no longer balance and favor the
The results of a recent electrophysiological study strongly suggest that the response to rapid-on and rapid-off luminance sawtooth stimuli will be mediated by ON and OFF pathways, respectively. Kremers (1993) measured the responses of macaque retinal ganglion cells to sinusoidal, square, rapid-on and rapid-off sawtooth waveforms. Both ON-center and OFF-center ganglion cells displayed responses to sinusoidal stimulation, but with a phase shift of 180 degrees. ON-center and OFF-center cells also responded to the square waveform, but with opposite sign responses to the rapid transitions in the wave. However, ON-center cells responded to the rapid increment portion of the rapid-on sawtooth waveform, with little response (slight inhibition) to the rapid-off waveform. Likewise, OFF-center cells responded best to the rapid-off sawtooth, and poorly to the rapid-on sawtooth. The characteristic and unique responses of these cells to the sawtooth waveforms support the idea that sawtooth stimuli can be used to preferentially excite cells belonging to the ON and OFF pathways.
Figure 2. Example of the sawtooth waveform.
CHAPTER II

EXPERIMENT 1: PERCEPTUAL ADAPTATION TO LIGHT INCREMENTS AND DECREMENTS

Subjects
One subject participated in the experiment. Corrected acuity was at least 20/20.

Methods
The stimuli were generated by a custom built optical system already present in the lab. A sawtooth waveform was used to generate repetitive light increments and decrements about the mean luminance. Light modulation of the stimulus was achieved via a high-speed galvanometer. The mirror galvanometer was driven by waveforms generated by D/A converters installed in a Macintosh Quadra 800 computer. The stimulus field consisted of a 2 deg spot. The subject viewed the stimulus field through a 3 mm artificial pupil. Data were collected for a range of retinal illuminance from 250 to 4500 Trolands (Td). The experiment included pre- and post-adaptation thresholds measurements for sawtooth modulation of both polarity. Thresholds were obtained using a double-random staircase technique. One staircase presented a rapid-on sawtooth and the other staircase presented the rapid-off sawtooth. The subject controlled the beginning of the trial by pressing a button. To determine pre-adaptation thresholds for each luminance, the observer was required to respond yes or no depending on whether he saw the stimulus. The procedure was repeated until there were six reversals for each staircase and the average of ten settings for each luminance level was taken as the
threshold. Post-adaptation thresholds were obtained after an adaptation period designed to preferentially desensitize either the ON- or OFF-pathway by adapting to either a rapid-on or rapid-off sawtooth, respectively. The sensitivity of a particular pathway was then verified during a test period where the same or opposite sign sawtooth was presented. There were four experimental conditions defined by the factorial combination of the two types of adaptation with the two types of test as follow: adapt to rapid-on sawtooth/test with rapid-on sawtooth, adapt to rapid-on/test with rapid-off, adapt to rapid-off/test with rapid-off, adapt to rapid-off/test with rapid-on (Fig. 3). The subject controlled the beginning of the trial by pressing a button and at the end of an adaptation period of 10 seconds to either the rapid-on or rapid-off sawtooth at a temporal frequency of 2 Hz, a beep signaled the beginning of the test stimulus represented by 1 cycle of either sawtooth. The observer was required to respond yes or no depending on whether he saw the test. The procedure was repeated until there were six reversals for each staircase. Because the effect of adaptation was found to decay rapidly (Hanly & MacKay, 1979), re-adaptation periods were interposed between test stimuli. The average of 10 sessions for each luminance level was taken as the threshold. The relative threshold elevation, which is a measure of the effect of adaptation, was obtained comparing pre- and post-adaptation thresholds.

A t-test was performed to evaluate difference in threshold of each test after adaptation of the same and of the opposite polarity.
Figure 3. Paradigm of the adapting and testing conditions of experiment 1 and 2.
Results

Figure 4a shows pre-adaptation thresholds for the on- and off-sawtooth, at each luminance level. In the absence of adaptation, baseline thresholds were comparable for the on- and off-test below 900 Td. Above this luminance level, the subject showed greater sensitivity for the off-test.

The adaptation effect produced on the on- and off-sawtooth tests is represented in figure 4b and 4c respectively. The on-sawtooth adaptation produced a greater increase in threshold for the on-sawtooth test at all luminance levels (Fig. 4b). The adaptation to the off-sawtooth appeared to be selective for the off-sawtooth test only at luminance levels below 900 Td (Fig. 4c). The significance of the adaptation effect was evaluated comparing the threshold of each test after adaptation of the same and of the opposite polarity. The star symbols in figure 4 indicate that below 900 Td the selectivity of the adaptation effect was statistically significant (p<.001).
Figure 4. (a) sensitivity (± 1 S.E.M.) of the on- and off-response before adaptation, at different luminance levels. (b-c). Adaptation effect (± 1 S.E.M.) on the on- and off-test, respectively. Results are described in the text.
CHAPTER III

Experiment-2: ELECTROPHYSIOLOGICAL ADAPTATION

Subjects

Three subjects, aged 27-36, participated in the experiment. All had corrected acuity of at least 20/20.

Methods

The electrophysiological response has been investigated by measuring the visual evoked potential (VEP) to light increments and light decrements after adaptation. The VEP is the expression of the electrical activity of the occipital cortex and provides an objective measure of visually elicited cortical activity.

Stimulus generation and data collection was performed with a Neuroscientific VENUS model 1020, coupled to a Data Translation DT2821 data acquisition board. Stimuli were presented on a calibrated Mitsubishi Diamond Scan 16 monitor. The VEP was obtained from electrodes placed on each subject at Oz, Cz and Fz according to the 10-20 system (Jasper, 1958). The electrode contact resistance was maintained below 5kΩ. Signals were amplified by a Grass P-5 pre-amplifier with a gain of 50,000 and a bandpass of 1-100 Hz. Stimuli were viewed binocularly with natural pupils.

The same adaptation/test protocol as described in the behavioral procedure was used. However, since the adaptation effect was expected to be relatively small, in order to increase the amplitude of the VEP response, a pattern stimulus was used. The stimulus consisted of square checks of 1.5 cycles/degree presented on a gray background of 16x17 degrees at a luminance of 25 cd/m² (Fig. 5). The luminance of the checks was
temporally modulated as a rapid-on and rapid-off sawtooth waveform at 2 Hz about the mean luminance. The modulation contrast of the checks was varied by varying the amplitude of the sawtooth waveform. Contrast was calculated as \( \frac{L_{\text{max}} - L_{\text{min}}}{L_{\text{max}} + L_{\text{min}}} \), where \( L_{\text{max}} \) and \( L_{\text{min}} \) equaled the maximum and minimum luminance, respectively, for a given modulation amplitude.

Each trial of the experiment was divided into an adaptation period and a test period during which the VEP response was collected. The adaptation period consisted of 8 cycles at 20% contrast of rapid-on or rapid-off sawtooth modulated checks. Following this period, the VEP response to 4 cycles of the test stimulus at 10% contrast was recorded. The test period was set at 4 cycles to make sure that the duration of the test period was not long enough so that the response could have not exclusively reflect the adaptation effect which is known to decay. During the acquisition there was no time lag between adaptation period and test period.

Each experimental session was composed of 40 trials. One of the two adaptation paradigms combined with one of the two test stimuli designated a trial, yielding a total of 40 trials for each session. Data for at least two sessions were collected for each subject. For a particular trial, the VEP was averaged over 20 stimulus presentations, and a recording epoch of 400 msec after the onset of each stimulus presentation was stored for subsequent off-line analysis.

Data analysis was performed on each trial. The amplitude of the first component, measured from the baseline, was taken as a measure of the VEP response. The effect of adaptation was estimated comparing the amplitude of the P1 component of each of the two tests after adaptation of each polarity.
Figure 5. Example of the stimulus pattern used in experiments 2 and 3. Bright checks appear at the peak of the rapid-on transient. Dark checks appear at the trough of the rapid-off transient.
paradigms: in this period as well which is characteristic of modulation about the background. Therefore a second adaptation period the on- and off-set of the luminance of the background was applied. The observation was a clear change of VEP morphology as seen in figure 5. Namely, when the test stimuli were presented above the apparent inverted, in this condition a contrast effect although quite small and not as a third condition. Due to the contrast stimuli were used. During the checks was made following a rapid-on sawtooth presentation of the positive and negative contrast stimuli. Figure 5 is an example of the negative component was of the same polarity indicating that the adaptation effect was selective.
**Results**

Pilot data revealed that the VEP response was affected by different adaptation paradigms. In this study, three conditions have been tested. In the first condition the checks were modulated about the mean luminance of the background during the adaptation period as well as during the test period. Figure 6 is an example of the VEP response which is characterized by a positive component (P1) around 100 msec. The data did not show either a consistent or a specific adaptation effect. One possible explanation for this observation was that the stimulus contained both increments and decrements, because of modulation about the background, and was unable to produce selective adaptation. Therefore a second adaptation paradigm was used. Here, during the adaptation period the on- and off-sawtooth stimuli were modulated about the mean luminance of the background while during the test period the stimuli were modulated above or below the background; other parameters remained unchanged. The first observation was a clear change of VEP morphology as seen in figure 6. Namely, when the test stimuli were presented above or below the background, the classic P1 component appeared inverted. In this condition, the VEP response began to show some adaptation effect although quite small and not selective (Fig. 7). Based upon these pilot data it seemed appropriate to try a third condition. To improve the procedure, only positive and negative contrast stimuli were used. During both the adaptation period and the test period the luminance of the checks was modulated either above the luminance of the background (positive contrast: rapid-on sawtooth) or below it (negative contrast: rapid-off sawtooth). Although this presentation of the stimuli contained different luminance levels for the positive and negative contrast, it produced an adaptation effect of some specificity. Figure 8 is an example of the VEP response to this paradigms. In this condition, the amplitude of the negative component decreased more when the adaptation and the test sawtooth were of the same polarity. The t-test showed that the decrease in amplitude of the negative component was significantly greater (p< .001) when the test followed adaptation of the same polarity indicating that the adaptation effect was selective.
Figure 6. VEP response of one representative subject. Adaptation and test stimuli were modulated about the mean luminance of the background. Results are described in the text.
Adaptation and Test about the background

- On-Test
- AdOn-TestOn
- AdOff-TestOn

Adaptation and Test about the background

- Off-Test
- AdOff-TestOff
- AdOn-TestOff

Amplitude (µV)

Time (sec)
Figure 7. VEP response of the same subject of figure 6. Luminance modulation: about the background during the adaptation period; above or below the background during the on- and off-sawtooth test respectively. Results are described in the text.
Adaptation about/Test above the background

Adaptation about/Test below the background
Figure 8. VEP response in the third condition of adaptation when luminance was modulated above or below the background during both the adaptation and test periods. Results are described in the text.
Psychophysical experiments measure the selectivity of the adaptation effect as change in perception threshold. Experiment 1 of this study added further evidence on the existence of two separate pathways involved with brightness and darkness perception. In a recent psychophysical study, Bowen (1992) measured the effect of mean luminance and temporal frequency on the contrast sensitivity function for sawtooth and sine waveform. It was found that at higher luminance levels and low temporal frequencies sawtooth sensitivity increasingly exceeds sine sensitivity. Moreover, rapid-off (decremental stimuli) sawtooth showed progressively greater sensitivity than rapid-on (incremental stimuli) sawtooth. Higher luminance levels progressively increased the sensitivity difference between the ON and OFF pathways as reported in the present study. The gradual increase in detection threshold (or decrease in sensitivity) with increasing luminance level might reflect the effect of increasing the noise of the cell response and or reducing the gain of the cell response. The observation that, at higher luminance levels, on- and off-adaptation produce an equal effect on the off test, in spite of a greater sensitivity of the off-response, indicates the convergence of signals from the ON- and OFF-channels. It might also suggest that the reciprocal interaction between the two channels is not exclusively independent.

The issue of the site of visual adaptation has also been addressed with the psychophysical experiment. The electrophysiological approach used in this study was used to provide additional information on the characteristics of the adaptation mechanism. The psychophysical and electrophysiological approaches to visual adaptation
are expected to generate mutually consistent accounts of the characteristics of the ON- and OFF-pathways at higher levels in the visual system. Assuming the existence of two independent pathways, the logical framework of adaptation is that an adapting stimulus that excites one pathway would not modify sensitivity for a test stimulus that excites another pathway. If it can, then the adaptation process must occur at a site in the visual system where signal from both pathways converge. If it cannot, then any modification of sensitivity has been introduced at a stage of processing where the signals from the different pathways remain separate. However, both the psychophysical and electrophysiological results from the present study suggest that a third alternative might be possible. On one hand, the interaction between adaptation and test of opposite sign indicate some convergence of the two pathways at the cortical level. Nevertheless, the selectivity of adaptation suggests that the ON- and OFF-mechanisms can still operate on cortical cells with some degree of independence. Possibly, the different responses to the various adaptation paradigms could be an expression of the degree of interaction between the ON- and OFF-channels. According to anatomical, physiological and behavioral studies, there is evidence of a clear separation between the ON and OFF pathways up to the level of the LGN. In the striate cortex the two pathways seem to converge. Schiller (1986) examined the effect of APB infusion on the light- and dark-edge response of cortical cells in the monkey. Complex cells respond to both the leading light-edge and the trailing dark-edge of a moving light bar, whereas simple cells respond only to the light-edge. In complex cells, APB eliminated only the light-edge response, suggesting that it is produced by the input from the ON-channel. In simple cells infusion of APB eliminated light-edge response and uncovered a dark-edge response. These observations suggested that in addition to convergent excitation, significant inhibitory interactions can also occur between the ON- and OFF-channels in the striate cortex. The interpretation of psychophysical results or of cortical responses might not be directly correlated to models derived from single cell electrophysiology.
Instead they represent complex responses to which various subpopulations of neurons probably contribute in different degrees and which involve processing at several levels of the visual system. For instance, one issue is to consider the relative contribution to brightness perception of neural mechanisms with transient or sustained responses (Cavanagh & Antis, 1986). A change in threshold is not the only consequence of adaptation. Anstis (1967) described a dynamic brightness aftereffect following a stimulus modulated with a sawtooth temporal profile. The aftereffect appeared as a gradual change in brightness in the direction opposite to that of the ramp phase of the adapting sawtooth. Thus, when the adapting stimulus was a slowly increasing luminance ramp, the observed aftereffect was a slowing decreasing luminance ramp. This aftereffect was interpreted as retinal rather than central because no interocular transfer was observed. Psychophysical evidence for contrast polarity selective adaptation at the level of the visual cortex came from experiments on spatial aftereffects. DeValois (1977) studied the size adaptation with single black and white bars slowly drifting back and forth across the visual field. Differences in width between pairs of adapting and test bars were observed only when the contrast of the adapting and test bar had the same sign. Visual aftereffects have been explained proposing that the resulting perception becomes biased toward the percept carried by the less adapted channel. Previous psychophysical studies, where adaptation to the fast and slow phases of the sawtooth has been adopted, reported controversial results (Krauskopf, 1980; Krauskopf & Zaidi, 1986). Dolan and Schiller (1994) proposed that the sawtooth would differentially adapt the ON and OFF channels so to create a non-linearity in the response. They tested the hypothesis that ramp changes could be selectively processed by a single channel to a greater extent than the step changes. Studying the effect of APB on detection of incremental ramps versus steps in the monkey, they found that ON channel blockade with APB does not affect the detection of ramps more than the detection of steps. These data are in agreement with previous evidence that sawtooth modulation does not simply
adapt one channel (Hanly & MacKay, 1979). They also support the existence of a
temporal channel for processing the step phase of the sawtooth and a sustained channel
for processing the ramp phase with different saturation in response to adaptation, as
proposed by Cavanagh & Anstis (1986). It is likely thus, that the modulation of
different temporal channels contributes to the adaptation effect. Bowen (1992), in a
recent psychophysical study, provided further evidence of this concept. In one
experiment of this study contrast sensitivity for sawtooth and sine waveforms was
measured over a range of temporal frequencies. At low temporal frequencies, rapid-on
and rapid-off sensitivities decreased only slightly in contrast to sine sensitivity. In
light of these results, they proposed that the visual response reflects the temporal
characteristics of the sawtooth waveform, in particular the presence of step changes in
luminance.

Overall, these findings seem to support the existence in the human visual
system of some parallel processing of brightness and darkness in the visual cortex.
Whether or not the ON- and OFF-pathway in humans are kept separate through all stages
of processing is still not clear.
PART TWO

SPATIAL TUNING OF THE ON AND OFF SYSTEMS
The possibility that differences exist in the spatial tuning of the ON and OFF channels was first suggested by the data of Schiller (1986) who isolated the responses of the OFF pathway by injecting APB into the vitreal chamber of the eyes of monkeys. Relative to the untreated eyes, detection responses for the ON pathway were significantly reduced and latencies were slowed for intermediate spatial frequencies (between 0.75 and 2.5 c/d) but showed no change at higher spatial frequencies (above 4 c/d). These data suggest that there may be differences in the spatial tuning of the ON and OFF channels, with the ON channel showing a narrower bandwidth than the OFF pathway. A similar result was obtained by Zemon (1988) by means of visual evoked potential (VEP) recording in humans to patterns of bright and dark checks sinusoidally modulated relative to a gray background. The tuning of the responses as a function of check size (spatial frequency) was substantially narrower for the bright checks than for the dark checks. Both spatial tuning curves had a bandpass characteristic but they were different in shape. The response to dark checks was found to peak at a higher spatial frequency and to be greater in amplitude than the response to bright checks. On the basis of this observations they inferred that the OFF system has receptive fields of smaller diameter than the ON system, and that the OFF pathway has greater contrast sensitivity than the ON pathway.

In recent morphological studies Dacey (1993; 1992) used intracellular staining on human retina to characterize the relationship of dendritic field size of midget and parasol ganglion cells to retinal eccentricity. Midget and parasol cells project,
respectively, to the PC and MC layers of the LGN. The midget and parasol ganglion cells of the human retina have been distinguished at all retinal eccentricities by their dendritic morphology. Midget cells have small dendritic fields and high density in the central retina. Parasol cells have much larger dendritic fields and a lower spatial density. Distinct functional properties also characterize the two pathways. The midget-parvocellular pathway is responsible for color vision and the perception of fine details (Schiller, Logothetis, & Charles, 1990), as found in lesions of the parvocellular pathway (Merigan, Katz, & Maunsell, 1991). Neurons of the magnocellular pathway have, instead, higher sensitivity to low levels of contrast (Merigan & Maunsell, 1990). The midget and parasol cells are further divided into ON-center and OFF-center types. The receptive field of human ON-center parasol (magnocellular projections) and midget (parvocellular projections) cells was reported to be 30 to 50% larger in diameter than the OFF-center cells suggesting a distinct asymmetry in the human ON-OFF visual pathways. A similar difference has been reported for the parasol cell equivalent in the rat retina (Peichl, 1989) but in other mammalian retinas, including the macaque, dendritic fields size differences between ON- and OFF-center ganglion cells have not been observed (Peichl, Ott, & Boycott, 1987). It has been suggested that the increased sensitivity of the magnocellular pathway to low contrast is a result of their larger receptive fields. As well, the higher resolution of the parvocellular pathway might reflect the small dendritic field of midget cells.

The purpose of this study was to characterize the electrophysiological properties of the ON and OFF pathways in humans to spatial and temporal stimuli over a range of contrast levels. Based upon previous studies, the two pathways are supposed to show some asymmetry.
CHAPTER VI

EXPERIMENT-3: VEP SPATIAL-CONTRAST SENSITIVITY FUNCTION OF THE ON AND OFF PATHWAYS

Subjects

Three subjects, aged 27-36, participated in the study. All had corrected acuity of at least 20/20.

Methods

Stimulus generation and data collection was performed with the same system described in experiment 2.

The stimulus consisted of square checks of different spatial frequencies in the range of 0.2-3.0 cycles/degree presented on a gray background of 16x17 degrees at a luminance of 25 cd/m². The luminance of the checks was temporally modulated as a sawtooth waveform at 2 and 6 Hz about the mean luminance. Both rapid-on and rapid-off sawtooth modulation was used. The modulation contrast of the checks was varied between 5 and 30% by varying the amplitude of the sawtooth waveform. Contrast was calculated as \( \frac{L_{\text{max}} - L_{\text{min}}}{L_{\text{max}} + L_{\text{min}}} \), where \( L_{\text{max}} \) and \( L_{\text{min}} \) equaled the maximum and minimum luminance, respectively, for a given modulation amplitude.

Each experimental session was composed of a complete set of contrasts, spatial frequency and temporal frequency for the rapid-on and rapid-off stimuli. One of the six spatial frequencies combined with one of the five contrast levels and one of the two temporal frequencies designated a trial, yielding a total of 30 trials for each session. Data for at least 6 sessions were collected for each subject. Stimulus contrast was
varied by an ascending method of limits. Presentation of rapid-on and rapid-off stimuli was alternated for each contrast level and spatial frequency at 2 and 6 Hz.

For a particular trial, the VEP was averaged over 240 stimulus presentations, and a recording epoch of 400 msec after the onset of each stimulus presentation was stored for subsequent off-line analysis. Under these conditions, the VEP response was characterized by an initial negativity (N1) around 70 msec followed by a positivity (P1) around 100 msec. However, the P1 amplitude appeared to be the most consistent parameter upon which to base the analysis across the different conditions.

The analysis of the data was performed as follows. For each trial, the amplitude of the P1 component was measured from the baseline and plotted as a function of contrast at each spatial frequency for both rapid-on and rapid-off stimuli. The variation of sensitivity over the range of spatial frequencies is described by the spatial-contrast sensitivity transfer function. The contrast sensitivity as a function of spatial frequency was obtained by plotting the contrast at which the VEP response to each spatial frequency reached 50% amplitude, for both the on- and off-response at 2 and 6 Hz (an example of the method is represented in figure 10). The 50% amplitude criteria used in the present study was based upon the observation of the VEP contrast-amplitude function. The VEP response revealed a very low signal to noise ratio at low contrast (below 5%) particularly at 2 Hz. Campbell and Maffei (1970) measured the VEP amplitude over a range of contrasts and found that the VEP amplitude at low contrast was an approximately linear function of log contrast. Extrapolation of this function to zero contrast provided an electrophysiologically determined threshold for cortical activity that corresponded well with the psychophysical threshold. It is often assumed that the electrical activity of the brain recorded from the scalp should reflect the properties of the visual system as inferred from psychophysics. Nevertheless, Cannon (1983) also found that the VEP-derived threshold systematically underestimated psychophysical
threshold. Furthermore, the VEP response reflects the activity of visual system mechanisms that operate over a range of suprathreshold contrasts, whereas threshold measurements are characteristic of psychophysical studies. Consequently it seemed that extrapolation of the VEP response to zero amplitude as a criteria to estimate threshold would not have been appropriate given the signal to noise ratio of the VEP response.

A two-way ANOVA was performed to evaluate the statistical significance of the results.

**Results**

The analysis of the VEP waveform in response to the on- and off-sawtooth stimulus revealed different characteristics of the response relative to the contrast. Response to the rapid-on sawtooth will be referred to as an on-response, and response to the rapid-off sawtooth will be referred as an off-response. As expected, there was a progressive increase in amplitude as the positive and the negative contrast increased. As well, the waveform became morphologically better defined. However, for both the rapid-on and rapid-off sawtooth stimuli, whereas the N1 component was not reliable, the P1 component was consistently present (Figure 9).

Figures 10 through 15 show the averaged contrast-amplitude response for each subject at the temporal frequencies of 2 and 6 Hz. At both temporal frequencies the on-response to high spatial frequencies (3.0 and 1.5 c/d) saturated above 20% contrast, whereas at spatial frequencies below 1.5 c/d the response saturated between 10 and 20% contrast. The off-response appeared to have a similar trend except for the spatial frequency of 1 c/d that saturated above 20% contrast. No consistent differences in amplitude between the on- and off-responses were observed.

The contrast sensitivity as a function of spatial frequency at 2 and 6 Hz for the three subjects is represented in figures 16a through 16f. At 2 Hz the on-response showed a decrease in sensitivity at spatial frequencies above 0.75 c/d in all subjects.
The off-response presented a similar trend in two subjects. Sensitivity reached a maximum between 0.5 and 1.0 c/d for both the on- and off- response. Only one subject showed a sharp peak in sensitivity. Between 0.75 and 1.5 c/d the on-response appeared more sensitive than the off-response. However, the statistical analysis indicated that only in subject PD the on-response at the spatial frequency of 1 c/d was significantly (p<.05) more sensitive than the off-response. At the temporal frequency of 6 Hz the CSF function for all subjects was shifted toward higher contrast indicating that the VEP response was less sensitive at this temporal frequency (p<.001). Greater sensitivity was still observed between 0.5 and 1.0 c/d for both the on- and the off-response. The two subjects with the smoother curve at 2 Hz showed instead at this temporal frequency two sharp peaks in sensitivity. The third subject showed a trend basically similar at both temporal frequencies however, at the lowest spatial frequency the on-response was much more sensitive than the off-response (p<.001). Subject PD showed a greater sensitivity of the on-response at 0.5 c/d (p<.001) and of the off-response at 1 c/d (p<.001). No difference in peak sensitivity of the on- and the off-response was observed in subject LR. Although the differences described above reached statistical significance, considering the inter-subject variability, the effect of these differences is not completely clear. What seems to be consistent among subjects is a substantially similar tuning of the on- and off-response and a trend for the on-response to be slightly more sensitive than the off-response between 0.75 and 1.5 c/d at 2 Hz and between 0.2 and 0.75 c/d at 6 Hz.
Figure 9. VEP waveforms in response to rapid-on (a) and rapid-off (b) sawtooth of progressively increasing contrast for one representative subject. Temporal frequency 2 Hz; spatial frequency 1.5 c/d. For both rapid-on and rapid-off sawtooth stimuli the amplitude of the VEP response progressively increased and became morphologically better defined.
VEP: on-sawtooth 2Hz 1.5 c/d

Amplitude (µV)

Time (sec)

30% contrast
20% contrast
10% contrast
5% contrast
3% contrast

10 µV

0 0.1 0.2 0.3 0.4 0.5

VEP: off-sawtooth 2Hz 1.5 c/d

Amplitude (µV)

Time (sec)

30% contrast
20% contrast
10% contrast
5% contrast
3% contrast

10 µV

0 0.1 0.2 0.3 0.4 0.5
Figures 10 to 15. For each subject, on- and off- averaged contrast-amplitude response (± 1 S.E.M.) to each spatial frequency at the temporal frequencies of 2 and 6 Hz. Results are described in the text.
Figure 10

LR 2Hz

Contrast (%)
Figure 11  LR 6Hz

Contrast (%)
Figure 12

NP 2Hz

Contrast (%)
Figure 13  NP 6Hz

Contrast (%) vs. Amplitude (µV) for different contrast levels:
- 3 c/d
- 0.75 c/d
- 1.5 c/d
- 0.5 c/d
- 1 c/d
- 0.2 c/d
Figure 14  PD 2Hz

Contrast (%)
Figure 15

PD 6Hz

Contrast (%)

Amplitude (µV)

3 c/d
0.75 c/d
1.5 c/d
0.5 c/d
1 c/d
0.2 c/d
Figure 16. (a-f) For each subject, contrast sensitivity (±1 S.E.M.) of the on- and off-response as a function of spatial frequency at 2 Hz. (d-f) For each subject, contrast sensitivity of the on- and off-response as a function of spatial frequency at 6 Hz. Results are described in the text.
Contrast Sensitivity Functions

Spatial Frequency (c/d)

(a) LR 2Hz
(b) NP 2Hz
(c) PD 2Hz
(d) LR 6Hz
(e) NP 6Hz
(f) PD 6Hz
CHAPTER VII
DISCUSSION: PART TWO

Former studies, both psychophysical (Tyler, Chan, & Liu, 1992) and electrophysiological (Zemon, et al., 1988) reported asymmetries in sensitivity and spatial tuning of the ON and OFF pathways. The results of the present study are not in agreement with the conclusions from a previous VEP study (Zemon, et al., 1988). No substantial difference was observed in the spatial tuning of the on- and the off-response. In addition, the off-response did not show greater sensitivity to higher spatial frequencies or greater contrast sensitivity than the on-response. Two considerations seem appropriate to explain the disagreement. The first point is the difference in the stimulus characteristics not only with regard to the temporal modulation (sinusoidal versus sawtooth) but also with regard to the luminance condition (modulation above or below the background versus modulation about the background). Previous electrophysiological and psychophysical investigations (Bowen, et al., 1989; Kremers, et al., 1993) support the idea that the two opposite sign sawtooth preferentially stimulate one pathway or the other. Unlike the sine waveform which is characterized by smooth temporal changes, the sawtooth waveform contains step changes in luminance. It is possible thus, that the spatial frequency sensitivity measured in the present study reflects the response of a transient channel whereas the spatial frequency sensitivity measured in Zemon (1988) might be the expression of a sustained channel. The choice to keep the mean luminance constant was made so to avoid possible influence of the difference in luminance in the response, although it is possible that a modulation above or below the background, which corresponds to a positive or to a negative contrast, represents a stronger stimulus for the respective pathway. In fact, it has been recognized that brightness and darkness are distinct sensations and recent
psychophysical studies have reported evidence for independent and differential processing of positive and negative contrast stimuli which elicit the perception of brightness and darkness (DeValois, 1977; Magnussen & Glad, 1975). The second consideration is that Zemon examined the VEP response to only 30% contrast. The observation of the contrast-response amplitude functions from the present study indicates that at 30% contrast the VEP amplitude for most spatial frequencies has reached saturation, and previous studies (Jones & Keck, 1978) have shown that the amplitude of the VEP response is a function of contrast. Therefore, there is the possibility that the pathway that has greater amplitude at saturation, in fact, it might be less sensitive if it saturates more slowly than the other pathway. However, the data from the contrast-amplitude function of this study show that this does not seem to be the case, at least with the adopted stimulus.

The results of this study share some analogy with the psychophysical data reported by Tyler (1992). The spatial tuning of sensitivity, obtained using Gabor modulation of luminance, showed a substantially greater sensitivity for the positive Gabor between 0.5 and 1.5 c/d, whereas at lower spatial frequencies the sensitivity became greater for the negative Gabor. At higher spatial frequencies the sensitivity to the positive and the negative Gabor converged to similar values.

The implication of the findings described above is that the difference observed in the spatial frequency tuning function for the two stimulus polarity might reflect asymmetry in the receptive field of the ON- and OFF-pathways. The perceptive field refers to the area of the retina that when stimulated by a visual stimulus produces a change in the behavioral response as measured in psychophysical experiments. There is a good agreement in monkey between perceptive field sizes and anatomical measurements of dendritic field size (Perry, Oehler, & Cowey, 1984). The ON-OFF dendritic field size asymmetry found in the human retina (Dacey, 1993; Dacey & Petersen, 1992) would predict that the OFF-center cells have a smaller receptive fields and thus have a
greater resolving power than the ON-center cells in agreement with the finer spatial tuning of the OFF-pathway found by Zemon (1988). In humans, it is difficult to specify the levels where the interactions described as perceptive fields occur in the visual system. Moreover, these interactions may represent the integrated activity of several partially overlapping receptive fields. On the other hand, the same ON-OFF dendritic field size asymmetry could suggest that the ON-pathway would have a greater contrast sensitivity especially at low spatial frequency as found in the present study. In a psychophysical study Bowen (1989) measured the contrast sensitivity function for sawtooth and sine waveform. It was found that at low temporal frequencies sawtooth sensitivity increasingly exceeds sine sensitivity. Moreover, rapid-off (decremental stimuli) sawtooth showed greater sensitivity than rapid-on (incremental stimuli) sawtooth. However, inter-subject variability in sensitivity and spatial tuning of the ON- and OFF-channels have been reported (Bowen, et al., 1992; Tyler, et al., 1992). Although there seems to be enough evidence for the existence of separate ON and OFF pathways, the characteristics of these two pathways at the level of visual cortex are not yet understood. Differences among the various studies and inter-subject variability suggest that more appropriate stimuli should be chosen and probably a larger number of subjects should be studied.
CHAPTER VIII

CONCLUSIONS

The data presented in this study looked at different aspects of the on- and off-response at the visual cortex level. The first part of the study showed that a selective adaptation effect can be obtained from the electrophysiological response of the visual cortex, suggesting that some degree of independence between the ON- and OFF-channel is present at this stage of the visual pathway. The second part of the study revealed a substantial equivalent spatial tuning of the on- and off-response. Although greater sensitivity occurred at different spatial frequencies for the on- and off-response, inter-subject variability make difficult the interpretation of this observation. Taken together, these results suggest that although there is not a substantial difference in the spatial-temporal characteristics of the ON- and OFF-pathways at the level of the visual cortex, the adaptation technique represent a non invasive method to study the ON and OFF pathways in human subjects.
REFERENCES


VITA

The author, Luisa Roveri, was born on August 6, 1966 in Mantova, Italy. In 1985 she entered the Medical School at University of Milan, Italy, and graduated in 1991. During her residency in Neurology, which she began in 1991 at the University of Milan, she became a research fellow in the Department of Neurology at Loyola University Chicago. During her fellowship, she entered the Neuroscience Master's program at Loyola.
The thesis submitted by Luisa Roveri has been read and approved by the following committee:

Paul J. DeMarco, Ph.D., Director
Assistant Professor, Neurology
Loyola University Chicago

Neal S. Peachey, Ph.D.
Assistant Professor Neurology
Loyola University Chicago

Edward J. Neafsey, Ph.D.
Professor, Cell Biology, Neurobiology, and Anatomy
Loyola University Chicago

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the thesis is now given final approval by the committee with reference to content and form.

The thesis is, therefore, accepted in partial fulfillment of the requirements for the degree of M.S..

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Date

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Director's Signature