Title
Studies of primary visual cortex and its development in cat and ferret

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Publication Date
1991

Peer reviewed|Thesis/dissertation

STUDIES OF PRIMARY VISUAL CORTEX AND ITS DEVELOPMENT IN CAT AND FERRET

by

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DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

NEUROSCIENCES

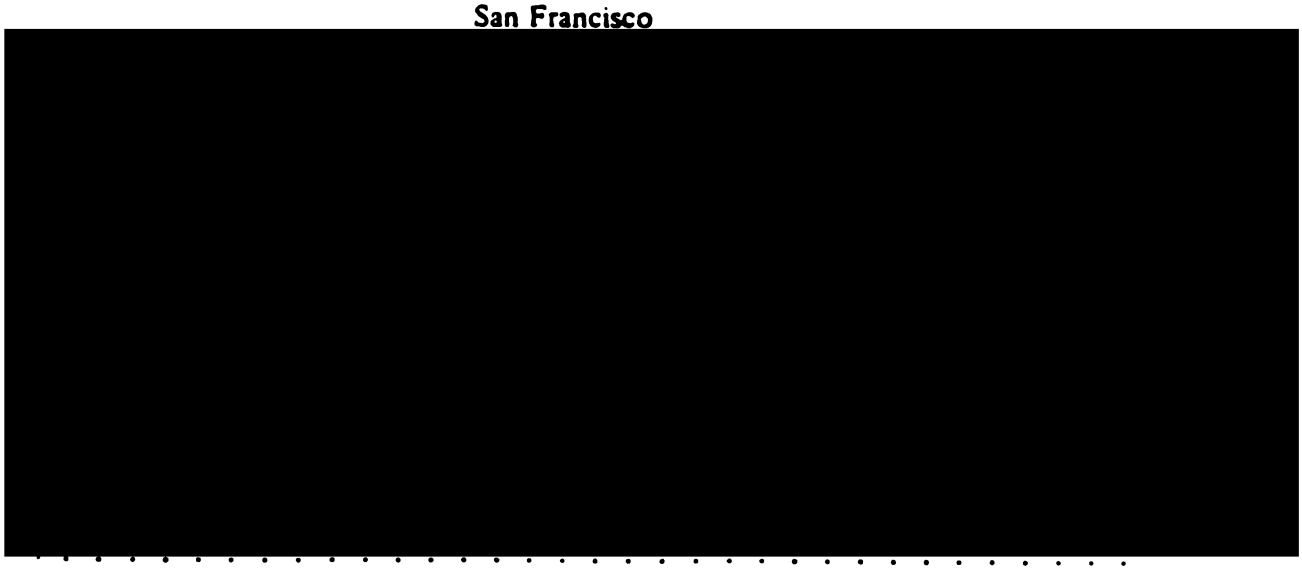
in the

GRADUATE DIVISION

of the

UNIVERSITY OF CALIFORNIA

San Francisco



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Degree Conferred: 12/31/91

This dissertation is dedicated to my mother

Anne Chapman

Acknowledgements

Michael Stryker has been all that anyone could ask for in a thesis advisor. I have greatly appreciated his dedication to teaching both in the laboratory and in the classroom, as well as his rigorous and meticulous approach to science. I am also grateful for Michael's kindness and humor which make him a pleasure to work with.

Kathy Zahs will always remain my role model as a scientist. Collaborating with Kathy was a great pleasure, and I value our friendship highly.

I thank my other collaborators, Holger Reiter, Michael Jacobson, and Ken Miller. Their and Kathy's contributions to this thesis are described in detail on the next page. I am also grateful to Ken for his patience and assistance with my computer programming.

Sheri Strickland has been an invaluable help in the lab and has turned an eternally sympathetic ear to my complaints about life. In addition I'll always fondly remember her for boating and 'shrooming outings, and for inventing the Chez Panisse Fund!

I thank my thesis committee, Steve Lisberger, Rick Van Sluyters, Allan Basbaum and Michael Merzenich for making the thesis process as pleasant as possible.

Previous publication and contributions of other authors

Michael Stryker directed and supervised the research reported in this dissertation.

Chapter 1 has been previously published (Chapman, B., M.D. Jacobson, H.O. Reiter and Michael P. Stryker (1986) *Nature* **324**:154-156). Michael Jocobson and Holger Reiter collaborated with me on the electrophysiological recordings reported in this study. I wrote the first draft of the manuscript.

Chapter 2 has been previously published (Miller, K.D., B. Chapman and M.P. Stryker (1989) *PNAS* **86**: 5183-5187). Kenneth Miller and I worked together on the surgeries and electrophysiological recordings for these experiments. Ken did most of the data analysis and wrote the first draft of the manuscript.

Chapter 3 has been previously published (Chapman, B., K.R. Zahs and M.P. Stryker (1991) *J. Neurosci.* **11**: 1347-1358). Kathleen Zahs was an equal partner in the electrophysiology and histology done in this study. I did the data analysis and wrote the first draft of the manuscript.

I certify that this is an accurate representation of the contributions of coauthors to the work presented in this dissertation.

Michael P. Stryker

Research Advisor

Abstract

Studies of Primary Visual Cortex
and its Development in Cat and Ferret
Barbara Chapman

Ocular dominance plasticity had been suggested to occur only when an animal receives behaviorally relevant visual information through the non-deprived eye. We have shown that ocular dominance plasticity can occur in the absence of vision if a large difference in the activities of the two retinas of kittens during the critical period is produced by preventing visually driven activity in one eye by monocular lid-suture and preventing all activity in the other eye by intravitreal injections of tetrodotoxin.

The cellular mechanism by which ocular dominance activity occurs is not know, but it has been suggested that the NMDA-type glutamate receptor could be involved. We have studied the role of the NMDA receptor in normal transmission of visual information in adult cat cortex. We found that selectively and effectively blocking the NMDA receptor by infusing APV into area 17 profoundly reduces visually driven activity in cortical cells. This result makes it difficult to determine whether the NMDA receptor might play a special role in plasticity, since plasticity is known to be critically dependent on neuronal activity.

There are two prevalent models of how visual cortical cells construct their orientation-selective responses from non-oriented lateral geniculate nucleus (LGN) inputs. The first model suggests that cortical cells receive excitatory synapses from a group of LGN afferents with

receptive fields aligned in visual space, while the second model states that cortical cell orientation specificity is established solely by intracortical circuitry. We have provided evidence in support of the first model by showing that the receptive fields of afferents arborizing within a single orientation column tend to be aligned in space, and that there is good correspondence between the axis of this alignment and the orientation preference of cortical cells within that column.

We have studied the time course of normal development of orientation selectivity in ferret visual cortex. We have shown that this development of orientation selectivity is occurring through an activity-dependent mechanism. If tetrodotoxin is infused directly into ferret primary visual cortex during development, the distribution of orientation selectivities remains frozen in an immature state.

Stephen G. Lisberger

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INTRODUCTION

The mammalian visual cortex is a highly organized structure where precise neuronal connections specify the response characteristics of individual cells. In the primary visual cortex of primates and carnivores, cells are arranged in columns with neurons within each column responding to similar parameters of visual stimulation. Perhaps the most basic feature of a stimulus is its position in the visual field. Primary visual cortex contains a topographic map of the visual field with neighboring columns of cells responding to stimuli at neighboring points in visual space (Talbot and Marshall, 1941; Hubel and Wiesel, 1974). This spatial map is a direct reflection of the orderly afferent input to the cortex from the lateral geniculate nucleus (LGN), which in turn receives its map of the visual world from an orderly retinal projection. Superimposed on the topographic map in the cortex are orderly representations of other features of visual stimuli. Cells are segregated into areas responding primarily to vision through one or the other eye, called ocular dominance columns, as well as areas responding to specific angles of lines or edges, called orientation columns (Hubel and Wiesel, 1962). In addition to the columnar organization of cortical cells, the visual cortex is also divided into layers which differ in their inputs and outputs. The main visual input to cortex from the LGN is received by cells in layer IV (and to a lesser extent layer VI). An idealized view of the columnar and laminar organization of striate cortex is shown in figure 1.

The experiments described in this dissertation represent our attempts at determining mechanisms involved in specifying two cortical response properties, ocular dominance and orientation selectivity, both in adult animals and during development.

Ocular Dominance

The organization of ocular dominance columns in the visual cortex, like the organization of the topographic map, reflects an ordered arrangement of afferent input from the LGN. The anatomical substrate of ocular dominance can be visualized directly by labeling the geniculocortical afferents serving one eye using degeneration techniques or any of a number of transneuronally transported marker substances injected into the vitreous humor of the eye. Such studies have shown that in adult animals the geniculocortical projections serving the two eyes form a series of alternating stripes or patches across the cortex (Hubel and Wiesel, 1972; Wiesel et al., 1974; Shatz et al., 1977). The existence of an anatomical as well as a physiological correlate of ocular dominance has greatly facilitated developmental studies. In young animals, the segregated arrangement of afferent input from the two eyes is not seen. Instead, afferents serving the two eyes form a completely overlapping pattern in cortex (Rakic, 1977; Hubel et al., 1977; Stryker and Shatz, 1978; LeVay et al., 1980). Ocular dominance columns develop gradually over time as the afferents serving the two eyes become progressively more segregated (LeVay et al., 1980).

The actual cellular mechanisms underlying the development of ocular dominance columns are not known, but several characteristics of the process have been determined. Neuronal activity is clearly involved in the segregation of afferents into eye-specific patches. When retinal activity is blocked during development by intravitreal injections of the sodium channel blocker tetrodotoxin (TTX), ocular dominance columns fail to form and afferents serving the two eyes remain overlapping in

cortex (Stryker and Harris, 1986). Neuronal activity is an active participant in the developmental process, rather than merely a permissive condition that allows afferent segregation to occur. If both optic nerves are stimulated with simultaneous activity during the critical period, ocular dominance columns fail to develop; if the nerves are stimulated alternately, normal development occurs (Stryker, 1986).

The mechanism of ocular dominance development appears to involve competition between afferents from the two eyes. If one eye is deprived of vision during a sensitive period early in life, most cortical cells no longer respond to stimulation through that eye (Hubel and Wiesel, 1963c) and the geniculocortical axons serving the deprived eye are limited to only very small patches amongst the large area of afferents serving the open eye (LeVay et al., 1980). In contrast, binocular visual deprivation has no effect on ocular dominance development, showing that the plasticity seen in response to monocular deprivation is a result of competition rather than disuse (Hubel and Wiesel, 1965).

Like ocular dominance development, ocular dominance plasticity is an activity-dependent process. Cortical plasticity is prevented when neuronal activity is blocked by infusing TTX directly into visual cortex (Reiter et al., 1986). Ocular dominance plasticity seems to depend on synchronous activation of pre- and postsynaptic cells. Postsynaptic cortical activity can be blocked without affecting the activity of presynaptic geniculocortical by infusing muscimol, a GABA-A receptor agonist, into visual cortex. After monocular visual deprivation in the presence of muscimol, an ocular dominance shift is seen in favor of the closed eye (Reiter and Stryker, 1988). When cortical cells are innactive, it is in fact the less active, closed eye whose activity is better correlated

with the postsynaptic cells, and it may be this correlation in activity which gives afferents serving that eye a competitive advantage in the cortex.

It is not certain, however, that ocular dominance plasticity is a perfect model for normal ocular dominance development. Ocular dominance development, although activity-dependent, clearly does not require visually driven activity, since ocular dominance columns form *in utero* in the monkey (Rakic, 1977; LeVay et al., 1978) and develop in cats deprived of vision by binocular lid-suture (Hubel and Wiesel, 1965). Ocular dominance plasticity, on the other hand, has been thought to require behaviorally significant visual input (Singer, 1979), suggesting that a higher order cortical process not needed for ocular dominance development might be necessary for ocular dominance plasticity to occur. The experiments described in Chapter 1 were designed to test whether ocular dominance plasticity could be produced in the absence vision by creating unequal levels of spontaneous activity in the two retinas of kittens during the critical period. We found that if all activity was silenced in one retina by intravitreal TTX injections, and visually driven activity was abolished in the other eye by lid-suture or dark rearing, cortical ocular dominance plasticity did occur.

All of the studies described above are consistent with a correlation-based, activity-dependent mechanism of ocular dominance development and plasticity (Stent, 1973; Changuex and Danchin, 1976). Such a mechanism could follow a rule (first proposed by Hebb, 1949) which specifies that synapses are strengthened when pre- and postsynaptic activities are correlated, and weakened when they are not. The most likely molecular candidate to be responsible for carrying out the Hebbian

rule is the N-methyl-D-aspartate (NMDA) receptor. The NMDA receptor only allows current flow into the postsynaptic neuron if both the pre- and postsynaptic cells are activated. The presynaptic cell must be active to release glutamate, the agonist for the NMDA receptor, and the postsynaptic cell must be depolarized to alleviate a voltage-dependent Mg^{++} block of the NMDA receptor-activated ion channels (Mayer et al., 1984; Nowak et al., 1984). The opened NMDA mediated ion channels allow Ca^{++} to enter the postsynaptic cell (Mayer et al., 1977) where it can act as a second messenger, leading to events which strengthen the synapse (for review see Nicoll et al., 1988).

The NMDA receptor is known to mediate one form of plasticity of neural connections, the long term potentiation (LTP) seen in response to repetitive stimulation in the hippocampus. NMDA receptors are found throughout cortex (Mayer and Westbrook, 1987) and are thus a candidate for the mediator of ocular dominance plasticity. The experiments described in Chapter 2 represent our investigations of the role of the NMDA receptor in the normal transmission of visual information in cat cortex. We found that infusing APV, an NMDA receptor antagonist, into adult cat visual cortex did selectively and effectively block NMDA receptors, but it profoundly reduced visually driven activation of cortical cells. This result makes it difficult to determine whether NMDA receptors might play a special role in plasticity, since interfering with the NMDA receptor interferes with cortical activity, and plasticity is known to be activity-dependent.

Orientation Selectivity

The organization of orientation selectivity in visual cortex cannot

directly be explained by the LGN afferent input which specifies topography and ocular dominance. Unlike cortical cells, which show strong selectivity for stimulus orientation, cells in the lateral geniculate nucleus have circular receptive fields with strong center-surround antagonism, but little or no selectivity for stimulus orientation (Hubel, 1960). Hubel and Wiesel originally proposed a model of how visual cortex could construct orientation selectivity from nonselective afferent input. They suggested that cells in cortical layers IV and VI, where geniculate axons terminate, might receive synapses from several geniculocortical afferents with receptive fields that are aligned in visual space (Hubel and Wiesel, 1962). Such a cortical cell would respond best to a stimulus oriented in space so as to excite all of its input afferents at the same time. Neurons in other cortical layers were hypothesized to receive convergent input from several orientation-selective cells in the input laminae of the same cortical column, thus preserving orientation preference within the column. An alternative model suggests that cortical orientation selectivity does depend on afferent input, but rather on intracortical inhibitory circuitry. This cross-orientation inhibition model suggests that cortical orientation selectivity might be established or at least refined by cortical cells receiving inhibitory input from other cortical cells with orthagonal orientation preferences (Creutzfeldt et al., 1974; Sillito, 1975). The experiments described in Chapter 3 show our attempts to provide direct evidence for or against the Hubel and Wiesel model by recording directly from the geniculocortical afferents that terminate within a single orientation column. We have found that the afferents recorded in a single penetration through cortical layer IV in ferret visual cortex have receptive fields covering an elongated region of

space, and that the axis of this elongation corresponds to the orientation preference of cortical cells in that column.

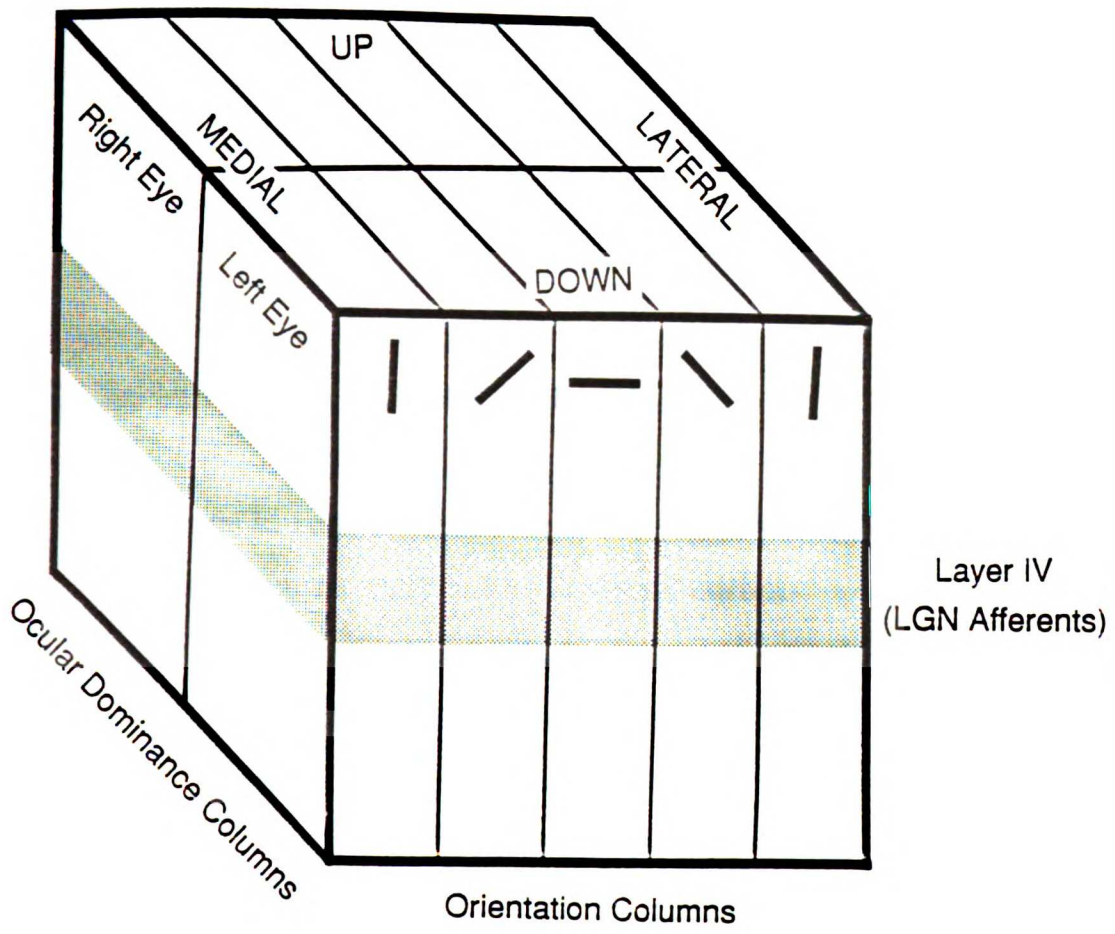
The lack of an anatomical marker for orientation selectivity has made the development of orientation selectivity difficult to study. Orientation specific responses in visual cortex are already present very early in an animals life. Orientation selectivity has been recorded at birth in the monkey (Wiesel and Hubel, 1974), and by the end of the first postnatal week in the cat (Hubel and Wiesel, 1963b), at a time when physiological studies are problematic due to the fragility of the preparation. Many attempts have been made to demonstrate a plasticity phenomenon for orientation selectivity analogous to the ocular dominance plasticity produced by monocular occlusion. If such plasticity could be produced, manipulations of the system could be used to provide insight into the normal development of orientation selectivity in the same way that ocular dominance plasticity has elucidated ocular dominance development. When a kitten is raised with visual experience limited to a pattern of parallel stripes of a single orientation, orientation-selective cells in its visual cortex were initially reported to respond only to the orientation which was seen during development (Blakemore and Cooper, 1970; but see Stryker and Sherk, 1975). If a kitten is raised with the two eyes presented with orthagonal orientations, cortical cells responsive to each eye respond only to the orientation viewed by that eye during development (Hirsch and Spinelli, 1970). This phenomenon does not, however, appear to be actual plasticity of the orientation preference of visual cortical cells. Careful analysis of the visual cortices of kittens raised in striped environments has suggested that cortical cells originally destined to respond to the orientation to which the kittens were exposed

remain responsive and orientation-selective, while cells in other orientation columns lose their responsiveness and orientation selectivity, rather than changing their orientation preference to match the visual stimulation (Stryker et al., 1978). Other studies where kittens viewed slightly different orientations of stripes in their two eyes during development (Shinkman and Bruce, 1977) also suggest that orientation selectivity shows only very limited plasticity if any. These findings have made it infeasible to attempt to use plasticity to reveal mechanisms of development for the orientation-selectivity system.

In experiments described in Chapter 4, we study the development of orientation selectivity in the ferret, an animal born at a very early developmental stage. The ferret is born approximately three weeks earlier in development than the cat (Linden et al., 1981), which made it possibly for us to make electrophysiological recordings in ferret visual cortex at a development stage approximately one week younger than the earliest recordings in cat visual cortex. We found that at the earliest age at which we could record visual activity in the cortex (postnatal day 23), very few cells showed orientation selective responses, and that orientation selectivity matured fairly abruptly during postnatal week 6. We could prevent this maturation of selectivity by infusing tetrodotoxin into the visual cortex, indicating that the development of orientation selectivity, like that of ocular dominance, is an activity dependent process.

Figure 1

Model of the organization of stimulus specificity in striate cortex. The cortical surface is represented at the top, with directions in the topographic map of visual space indicated. Ocular dominance and orientation columns extend through the depth of the cortex from the pial surface down to white matter. Cortical layer IV, which receives the main cortical input from the lateral geniculate nucleus is shown.


UP
MEDIAL
LATERAL
DOWN
Right Eye
Left Eye
Layer IV
(LGN Afferents)
Ocular Dominance Columns
Orientation Columns

CHAPTER 1

Imbalance in retinal electrical activity causes ocular dominance shift in kitten visual cortex

Introduction

Monocular lid suture during the sensitive period early in the life of a kitten disrupts normal development of inputs fron the two eyes to the visual cortex, causing a decrease in the fraction of cortical cells responding to the deprived eye (Wiesel and Hubel, 1963). Such an ocular dominance shift has been assumed to depend on patterned visual experience, because no change in cortical physiology is produced by inequalities between the two eyes in retinal illumination (Blakemore, 1976) or temporally modulated diffuse light stimulation (Singer et al., 1977; Wilson et al, 1977). A higher level process, involving gating signals from areas outside striate cortex, has been proposed to ensure that sustained changes in synaptic efficacy occur only in response to behaviourally significant visual inputs (Singer, 1979). To test whether such a process is necessary for ocular dominance plasticity, we treated four-week-old kittens with visual deprivation and monocular tetrodotoxin (TTX) injections to create an imbalance in the electrical activities of the two retinas in the absence of patterned vision. After one week of treatment, we determined the ocular dominance distribution of single units in primary visual cortex. In all kittens studied, a significant ocular dominance shift was found . In addition to this physiological change, there was an anatomical change in the lateral geniculate nucleus, where cells were larger in laminae receiving input from the more active eye. Our results indicate that patterned vision is not necessary for visual cortical plasticity, and that an imbalance in spontaneous retinal activity alone can produce a significant ocular dominance shift.

Materials and Methods

For this study eleven kittens, 28-34 days old, received intravitreal injections of 5 mM TTX in one eye to block ganglion cell activity in that eye (Stryker and Harris, 1986). Patterned visual input to the other eye was eliminated by suturing the eyelid. Six of the kittens (TTX/lid-suture group) were kept in a normal light cycle, and thus experienced no activity in one eye, and diffuse, temporally modulated light in the other. The remaining five kittens (TTX/dark group) were kept in the dark, experiencing no activity in one eye and only spontaneous activity in the other eye. The retinal action potential blockade was maintained for one week by repeating the TTX injections. The lid of the injected eye was then sutured, and the animals remained or were placed in the dark to prevent vision during recovery of retinal activity. 24-48 hours after the retinal blockade began to wear off, the kittens were prepared for electrophysiological recording as described elsewhere (Schopmann and Stryker, 1981).

To demonstrate that normal activity had returned to the previously blocked eye, 5-13 single units with central receptive fields (within 15 degrees of area centralis) were recorded in the lateral geniculate nucleus (LGN) contralateral to the injected eye. In addition, multi-unit responses were assesed every 100 microns through laminae A and A1. No difference in responsiveness was detected between cells driven by the two eyes (see figures 1 & 2), nor between multi-unit recordings in laminae responsive to the two eyes. Briskly responsive ON- and OFF-center, and sustained and transient units were recorded in both laminae.

To determine whether an ocular dominance shift had occured, we studied cortical area 17 contralateral to the injected eye. This hemisphere was chosen because normal kittens show an ocular dominance bias toward the contralateral eye (Stryker and Harris, 1986); thus if recording had been done in the hemisphere ipsilateral to the TTX-treated eye, an apparent shift in ocular dominance toward the more active eye could be caused, at least in part, by this bias. In the hemisphere contralateral to the injected eye, however, an ocular dominance distribution favoring the more active (ipsilateral) eye must be caused by the effects of deprivation. Horizontal electrode penetrations were used in order to sample cells across, rather than down, ocular dominance columns; each penetration was long enough to pass through several columns. The intended sample size was 30 cells per animal. Receptive fields of all units studied were within 15 degrees of area centralis. Ocular dominance was determined for each visually responsive unit using the standard seven point scale (Hubel and Wiesel, 1962).

Results

For both conditions, TTX/lid suture and TTX/dark, the ocular dominance histograms obtained were significantly shifted toward the more active, ipsilateral eye (figure 3 B & C). These data contrast with histograms from normal kittens of the same age, which show a slight bias in favor of the contralateral eye (figure 3 A), as well as with the more strongly shifted histogram from conventional monocularly-lid-sutured kittens (figure 3 D). The degree of the shift in each kitten, as estimated by a scalar index, indicates that our two groups are significantly different from both normal and conventionally monocularly deprived animals (Mann-Whitney U test, p's~0.01 [N1,N2=numbers of animals]), but that the results in the TTX-vs-lid-suture and TTX-vs-dark groups are not significantly different (Mann-Whitney U test, p>O.05) (figure 4A)

In seven of the original eleven kittens, cross sectional areas of cells in the LGN were measured in Nissl stained sections. LGN cell sizes are known to be smaller in the laminae responding to the deprived eye in kittens with monocular lid suture (Wiesel and Hubel, 1963; Movshon and Dursteler, 1977), or monocular TTX blockade (Kuppermann and Kasamatsu, 1983). As expected, cell sizes were smaller in laminae responding to the TTX-treated eye in both TTX/lid-suture and TTX/dark animals. These morphological results, like the physiological ones, were intermediate between those from normal and monocularly deprived animals (figure 4 B).

Although unlikely, the changes in cortical physiology observed could have been caused by damage to the TTX-treated eye that was not

detected in our geniculate recordings, rather than by the activity blockade. If such toxicity were responsible for the ocular dominance shifts, the effects should be permanent. If, however, differential activity were responsible, then the shifts should be reversible, as are those produced by conventional monocular deprivation in kittens of the same age (Movshon, 1976). To test the reversibility of our treatment, two additional kitttens were studied. These kittens experienced one week of the TTX/lid-suture treatment starting at 28 or 32 days old. After the TTX blockade wore off, the kittens remained in a normal light cycle for 9 or 11 days, with the sutured eyelid remaining closed. Thus these kittens experienced a week of no activity in the TTX-treated eye combined with diffuse light vision through a closed lid in the other eye, followed by a period of conventional monocular deprivation with patterned vision in the previously TTX-treated eye. The first week of treatment presumably produced an ocular dominance shift toward the lid-sutured eye, as occured in TTX/lid-suture animals. If TTX injections did not cause permanent damage, this shift should have been reversed during the period of monocular deprivation, resulting in a cortical bias in favor of the eye that had been blocked during the first part of the treatment and had experienced patterned vision during the second part. Single unit recordings from area 17 of the two kittens showed that such a reversal had occured. Ocular dominance histograms from both kittens were strongly shifted toward the eye that first was blocked and then had patterned vision. The degree of shift in these kitttens (contralateral bias indices 86 and 99; see figure 4 legend for index definition) was comparable to that seen in conventional reverse lid-sutured kittens of the same age (Movshon, 1976). Thus TTX injections do not cause lasting

damage to the ability of the treated eye to compete for cortical dominance.

The similarity of three of the present findings to those from conventional monocular deprivation experiments indicates that the ocular dominance shifts seen in TTX/lid-suture and TTX/dark animals were caused by the differences between activities of the two eyes, rather than by TTX-induced damage to the retinal ganglion cells of the injected eye. First, LGN responses to stimulation of the two eyes were equally good even in the most shifted animals where few or no cortical cells responded to the injected eye. Second, LGN cell sizes were smaller in laminae responding to the injected eye. Third, the ocular dominance shifts produced were readily reversible.

Discussion

We draw three conclusions from the results of these experiments. First, the significant changes in ocular dominance which occured in both TTX/lid-suture and TTX/dark animals show that patterned vision is not necessary to produce changes in cortical physiology. Secondly, the results from the TTX/dark group indicate that an imbalance in spontaneous activity alone is enough to cause an ocular dominance shift. Finally, the similar results produced by TTX/dark and TTX/lid-suture treatments suggest that the vision that occurs through a closed eyelid has little additional effect on ocular dominance.

It is generally believed that cortical plasticity is dependent on cortical cells being driven by their geniculate inputs. Silencing all cortical activity through intra-cortical TTX injections has been shown to prevent ocular dominance shifts in monocularly deprived animals (Reiter et al., 1985). Because geniculate (Eysel and Wolfherd, 1983) and cortical (Harris and Stryker, 1977) spontaneous activities are dependent on levels of spontaneous activity in the retina, spontaneous activity must drive cortical cells, however weakly. Thus the present results are consistent with a model of plasticity requiring that cortical cells be driven.

Our conclusion that ocular dominance shifts can be produced in the absence of patterned vision does not necessarily refute the central gating hypothesis (Singer, 1979). Although shifts in our animals did occur without behaviourally significant visual input to either eye, these shifts were not as large as those seen in conventionally monocularly deprived animals (Movshon and Dursteler. 1977; Hubel and Wiesel,

1970; Olson and Freeman, 1975), even though the differences in retinal activities were extreme. Perhaps the central gate is always slightly open, allowing some degree of shift to occur. Behaviourally significant information could further open the gate, causing the larger ocular dominance shifts seen in conventionally monocularly deprived animals. We do not know why ocular dominance shifts were produced by the present conditions but not by other rearing conditions with different inputs to the two eyes in the absence of patterned vision (Blakemore, 1976; Singer et al., 1977; Wilson et al., 1977). The actual retinal activities produced by any of these rearing conditions are, however, unknown (except that TTX does block all activity (Stryker and Harris, 1986)). Therefore either a large quantitative difference, or some temporally modulated qualitative difference between the activities of the two retinas may be necessary to produce ocular dominance shifts.

The present finding that imbalances in spontaneous activity alone can cause changes in connections to cortical cells, together with earlier work on the effects of spontaneous activity blockade on the development of ocular dominance columns (Stryker and Harris, 1986; Stryker, 1981), is consistent with the hypothesis that spontaneous electrical activity plays a role in the normal fetal development of the visual system (Stryker, 1989). Activity-dependent rearrangements of ocular dominance columns, which occur postnatally in the cat (LeVay and Stryker, 1979), begin 3-6 weeks prenatally in the monkey (LeVay et al., 1980; Rakic, 1976). If the development of ocular dominance columns follows similar principles in the two species (DesRosier et al., 1978), then it is not surprising that spontaneous activity can play this part in the cat because in the monkey in utero, vision is occluded, and the only possible

electrical activity is spontaneous. Observations that retinogeniculate axons make functionally effective binocular connections prenatally (Shatz and Kirkwood, 1984), some of which are lost before birth (Shatz and Kirkwood, 1984; Dubin et al., 1986), suggest that spontaneous activity is also involved in the refinement of these connections (Sur et al., 1985; Dubin et al., 1986)

Figure 1.

Coronal Nissl-stained section through an LGN contralateral to the TTX injected eye. The section shown is 2.88 mm caudal to the rostral pole of the LGN. An electrode track is shown with electrolytic marking lesions indicated by curved arrows. Filled arrows indicate locations of single units responding to the untreated ipsilateral eye; open arrows indicate single units responding to the previously blocked contralateral eye. Receptive fields of the units indicated by the top two arrows were at elevation 30 degrees, azimuth 9 degrees; those of the units indicated by the lower five arrows ranged between elevation 6, azimuth 4 and elevation 4, azimuth 2. Scale bar = 0.5 mm.

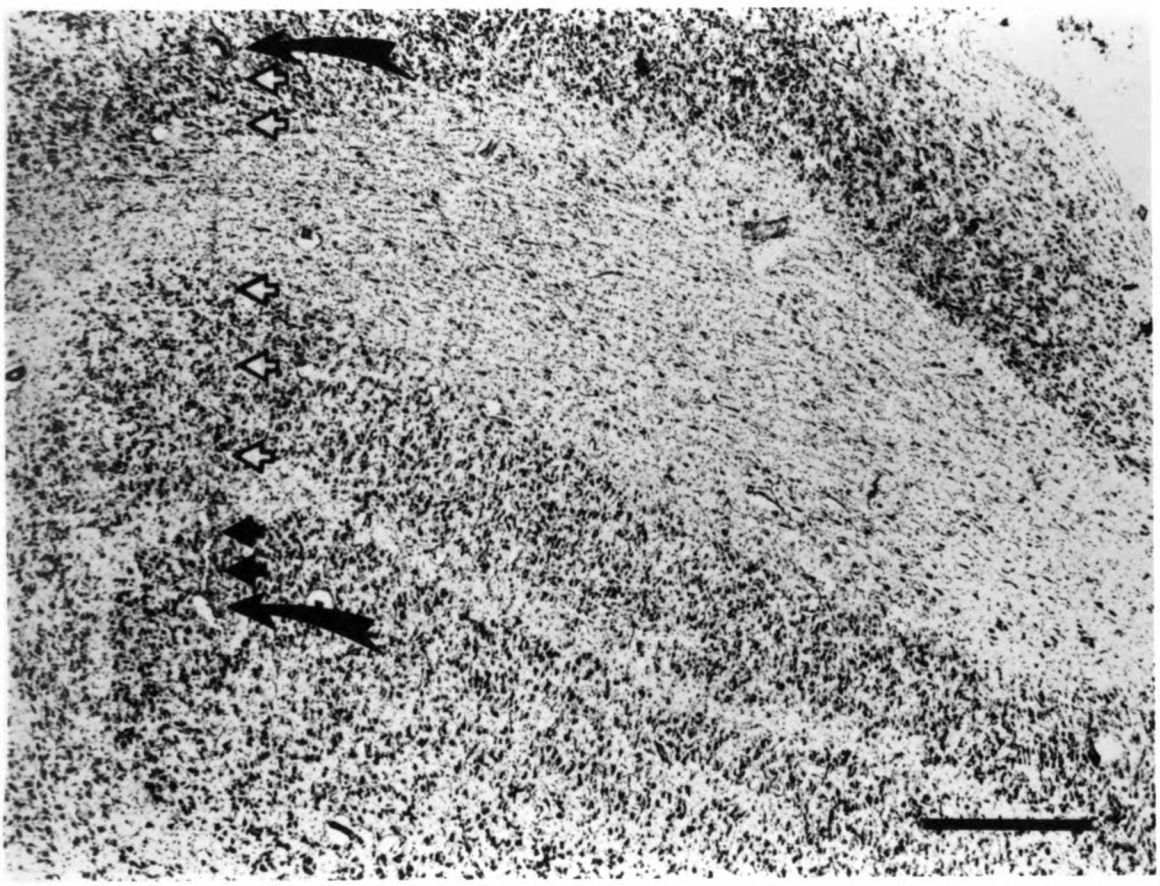


Figure 2.

Post-stimulus histograms showing examples of the flash responses of single OFF center units driven by the untreated ipsilateral eye (A) and by the previously blocked contralateral eye (B) in the same LGN. Stimulus intensity was one log unit above threshold. Y-axis = spikes/sec, X-axis = seconds. Asterisk indicates level of spontaneous activity. Stimulus time course is represented by the line above the X-axis (ON = up, OFF = down).

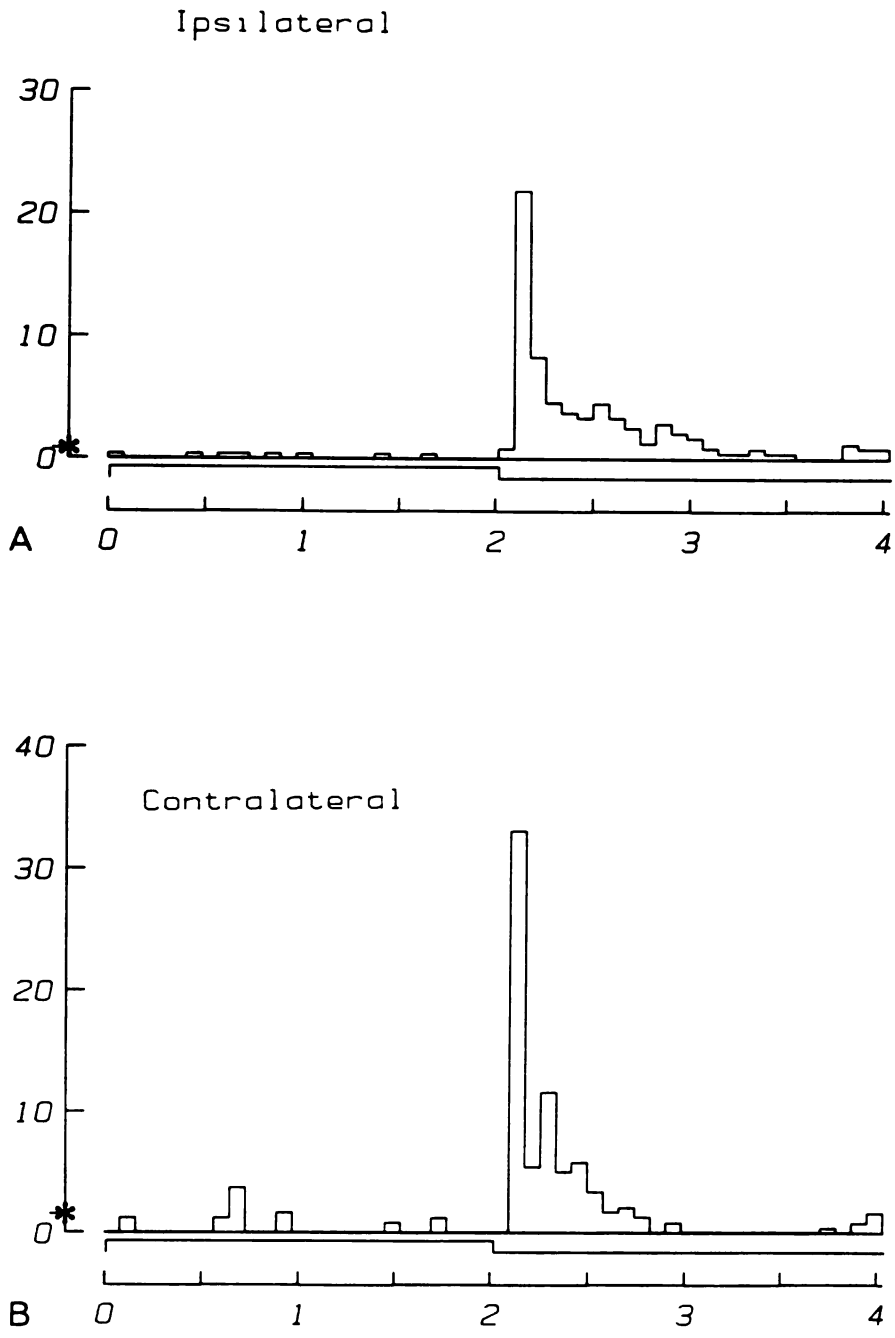

Ipsilateral
30
20
10
0
A
0
1
2
3
4
40
Contralateral
30
20
10
0
B
0
1
2
3
4

Figure 3.

Ocular dominance histograms (Hubel and Wiesel, 1962) compiled from single unit responses in area 17. An ocular dominance of 1 indicates a cell driven only by the contralateral eye, 7 indicates a cell driven only by the ipsilateral eye, and 4 indicates a cell driven equally by the two eyes. In each deprivation condition, the contralateral eye is less active. A) 372 visually responsive units from 6 normal kittens, 36-51 days old. 26 visually unresponsive units were recorded (Stryker and harris, 1986). B) 173 visually responsive units from 5 TTX/dark kittens, 37-44 days old. 12 visually unresponsive units were recorded. C) 183 visually responsive units from 6 TTX/lid-suture kittens, 36-40 days old. 19 visually unresponsive units were recorded. D) 151 units from 4 kittens monocularly deprived by lid suture for 3.5-6 days, followed by 0-2 days recovery in darkness; 29-34 days old (Movshon and Dursteler, 1987; Hubel and Wiesel, 1970; Olson and Freeman, 1975).

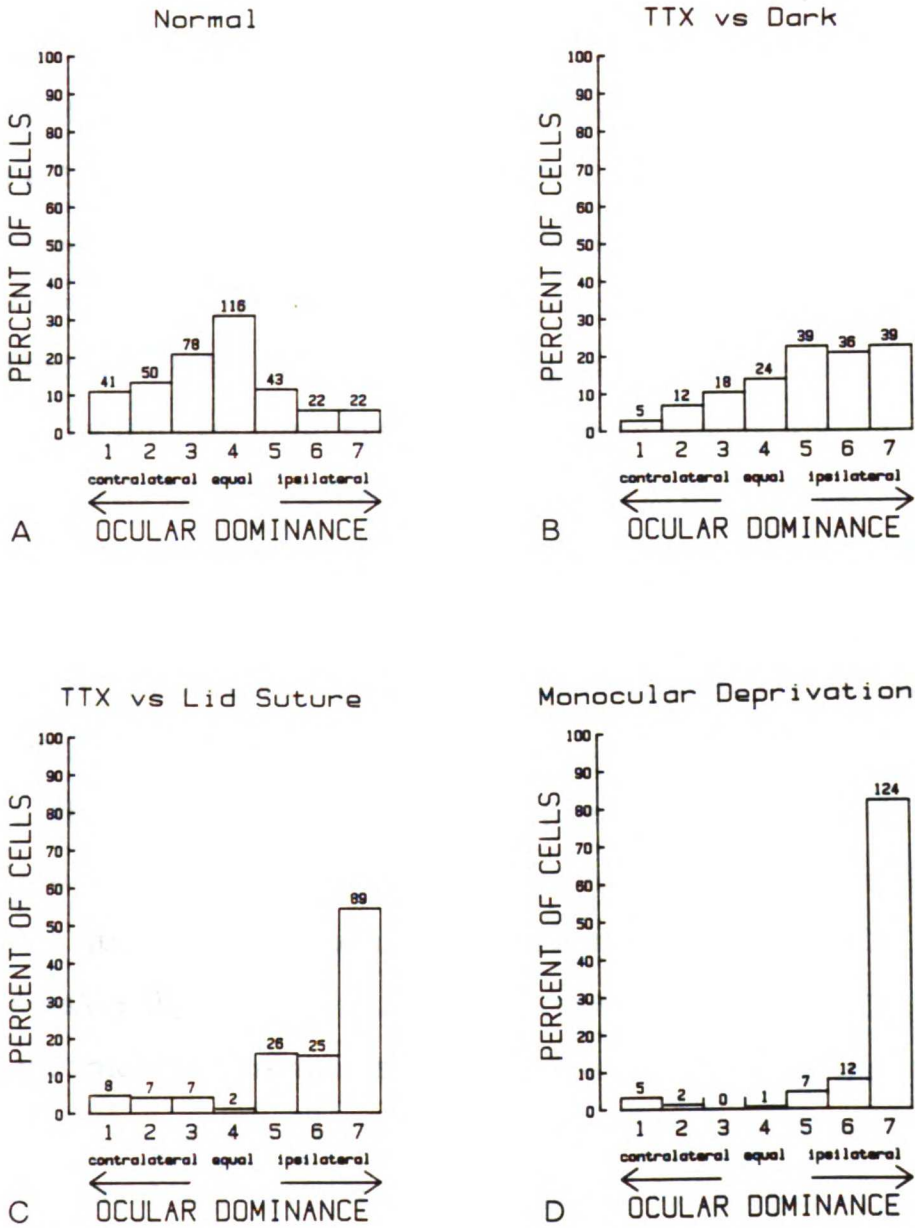

Normal
PERCENT OF CELLS
100
90
80
70
60
50
40
30
20
10
0
41
50
78
116
43
22
22
1 2 3 4 5 6 7
contralateral
equal
ipsilateral
A
OCULAR DOMINANCE
TTX vs Dark
PERCENT OF CELLS
5
12
18
24
39
36
39
1 2 3 4 5 6 7
contralateral
equal
ipsilateral
B
OCULAR DOMINANCE
TTX vs Lid Suture
PERCENT OF CELLS
8
7
7
2
26
25
89
1 2 3 4 5 6 7
contralateral
equal
ipsilateral
C
OCULAR DOMINANCE
Monocular Deprivation
PERCENT OF CELLS
5
2
0
1
7
12
124
1 2 3 4 5 6 7
contralateral
equal
ipsilateral
D
OCULAR DOMINANCE

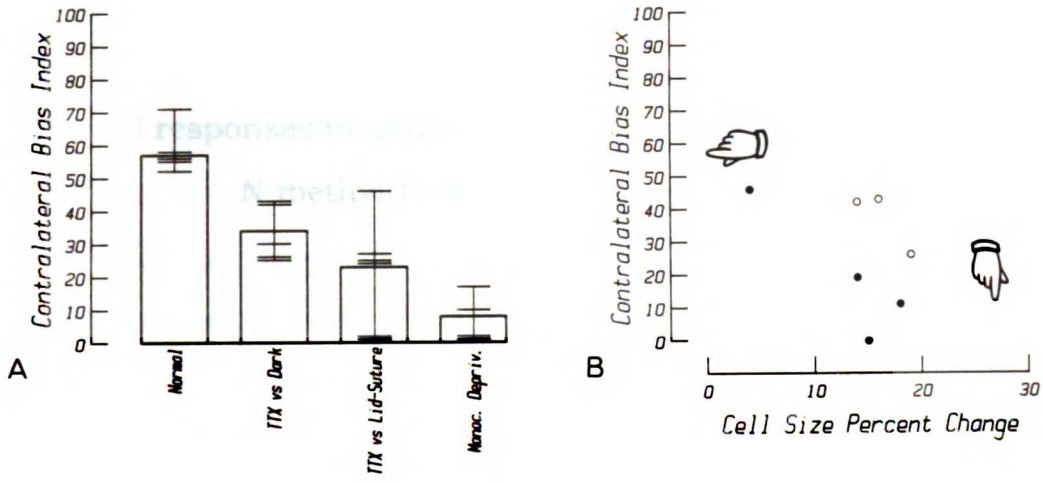
Figure 4.

A) Contralateral bias indices showing the range of findings among individual animals from figure 3. B) A scatter plot of percent change in geniculate cell size versus contralateral bias index. The index provides a single number reflecting the degree of ocular dominance shift for each animal. A) The index is calculated according to the following formula:

$$100[(\mathit{1}\text{-}\mathit{7}) + 2/3(\mathit{2}\text{-}\mathit{6}) + 1/3(\mathit{3}\text{-}\mathit{5}) + N] / 2N$$

Italicized numbers, number of units in each ocular dominance group; N, total number of visually responsive units. This index equals 100 if all cells are driven exclusively by the contralateral eye and 0 if all cells are driven exclusively by the ipsilateral eye. The greater variability among TTX/lid-suture animals is unexplained; it may be due to differences among the animals of this group in the amount of diffuse-light stimulation of the retina of their sutured eyes. B) Cels (75 per lamina) were measured in both LGNs of each animal. Measurements were made in the latero-medial middle third of coronal sections taken aproximately 2.5mm caudal to the rostral pole of the nucleus (the area coresponding to the central 5-10 degrees of the visual field)(Sanderson, 1971). All cell bodies with visible nucleoli were traced in the camera lucida under 63X or 100X oil immersion objectives at final magnifications of 1160-1840X. The areas of these tracings were then measured using a Summagraphics Bitpad Planimeter. The standard errors ranged from 4% to 6% of the mean for individual laminae. Percent change was calculated as one minus the sum of the means of the cell sizes from laminae responding to the injected eye divided by the sum of the means of the cell sizes from laminae responding to the more active eye. Open circles represent data from TTX/lid-suture animals; filled circles represent data from TTX/dark

animals. The upper finger points to the region of data from normal animals (Stryker and Harris, 1986); the lower finger points to the region of data from monocularly deprived animals (Movshon and Dursteler, 1977). The spread of these control data along the ordinate is evident from figure 4 A; the spread along the abscissa is aproximately 4% for normal animals and 7% for monocularly deprived animals (Movshon and Dursteler, 1977). Although there is no strong correlation between the change in cell size and the degree of ocular dominance shift in our animals, the extents of both morphological and physiological changes are intermediate between data from normal and conventional monocularly deprived animals. The morphological results for TTX/dark animals (average percent change = 16) are very similar to those previously reported for older TTX/dark kittens (average percent change = 17)(Kuppermann and Kasamatsu, 1983).


A
Contralateral Bias Index
100
90
80
70
60
50
40
30
20
10
0
Normal
TTX vs Dark
TTX vs Lid-Suture
Monoc. Depriv.
B
Contralateral Bias Index
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
Cell Size Percent Change

CHAPTER 2

Visual responses in adult cat visual cortex depend on N-methyl-D-aspartate receptors

Abstract

We have investigated the role of the N-methyl-D-aspartate (NMDA) receptor, a subtype of glutamate receptor, in the responses of cells in adult cat visual cortex. After intracortical infusion of the NMDA receptor antagonist D,L-2-amino-5-phosphono-valeric acid (D,L-APV) for one day, iontophoretic responses to NMDA, to kainic acid (KA) and to quisqualic acid (QUIS) revealed a receptor blockade specific to NMDA receptors and extending several millimeters from the cannula. In this region, neuronal responses to visual stimulation were profoundly suppressed, in a manner strongly correlated with the degree of NMDA receptor blockade. Neither NMDA receptor blockade nor activity suppression were caused by the inactive stereoisomer L-APV. Hence, we conclude that NMDA receptors make a major contribution to normal excitatory transmission in adult cat visual cortex.

Introduction

Excitatory synaptic transmission in visual cortex depends on glutamate receptors. Kynurenate, a general blocker of these receptors, abolishes excitatory postsynaptic potentials (EPSPs) and responses to glutamate agonists in cultured cells (Huettner and Baughman, 1988) and slices (Langdon et al., 1988) of visual cortex. An important issue for the understanding of cortical function is the contribution of each subtype of glutamate receptor to excitatory transmission.

Glutamate receptors are of two general types (Mayer and Westbrook, 1987). One type, which appears to have multiple subtypes, is the kainate/quisqualate receptor. Activation of this type of receptor leads to a conventional fast EPSP. The other type of glutamate receptor is the N-Methyl-D-Aspartate (NMDA) receptor. When it is activated, it causes a slow, long-lasting EPSP (Collinridge et al., 1988b; Poolos and Kocsis, 1987; Forsythe and Westbrook, 1988; Dale and Roberts, 1985; Wigstrom et al., 1985; Herron et al., 1986; Aram et al., 1987; Jones and Baughman, 1988).

We have studied the role of the NMDA receptor in excitatory synaptic transmission in visual cortex. Previous reports (Tsumoto et al., 1987; Hagihara et al., 1988) have concluded that NMDA receptors contribute to excitatory transmission in the visual cortex of young kittens during the critical period for synaptic plasticity, but do not do so appreciably in adult cats. To study the role of NMDA receptors in cortical synaptic transmission, several conditions must be achieved. First, one needs to produce a blockade of NMDA receptors which is

uniform at least over the full extent of an individual cell's synaptic input. Second, one must be able to assess the degree and the specificity of NMDA blockade at each cortical site studied. Then one may assess the effect of that blockade on spontaneous and visually driven activity.

To achieve a locally uniform blockade, we have chronically infused the cortex with the NMDA-receptor blocker 2-amino-5-phosphonovaleric acid (APV). We found that APV infusion created a specific blockade of NMDA receptors over several millimeters of cortex. In this region, neuronal responses to visual stimulation were profoundly suppressed, in a manner strongly correlated with the degree of NMDA blockade. We interpret these results to suggest that visual responses, in at least a critical subset of visual cortical cells, depend on a slow EPSP mediated by activation of NMDA receptors in response to high-frequency sensory inputs. An abstract of this work has appeared (Miller et al., 1987).

Materials and Methods

In six adult cats a 33-gauge cannula (200 micron outer diameter) was implanted under aseptic conditions two millimeters below the cortical surface into the representation of the central visual fields of one hemisphere of primary visual cortex (Horsley-Clarke coordinates AP -1-0, LM 2). The cannula was attached to an osmotic minipump (ALZA 2001) delivering 1 ul/hr of 50mM solution (pH 7.2-7.6) of D,L-APV (four animals) or of the active sterioisomer D-APV (two animals). Opposite hemispheres were either left untreated (three animals), or implanted with a cannula delivering saline (one animal) or the inactive stereoisomer L-APV (two animals). APV infusions continued throughout the recording sessions. Five of these animals were used to study visually-evoked and spontaneous cortical activity and responses to NMDA and KA. The sixth animal was used to study responses to NMDA and QUIS. In a seventh animal, one hemisphere was treated with L-APV and the opposite hemisphere untreated. The control data include results only from untreated and saline-treated hemispheres.

One day after implantation, animals were prepared for physiological recording. Methods are as described previously (Reiter et al., 1986), except that gallamine triethiodide rather than pancuronium bromide was used for neuromuscular blockade. Ketamine and similar anaesthetics known to affect NMDA receptors were never used.

Physiological measurements were made using tungsten recording electrodes (Hubel, 1957) glued to three-barreled glass electrodes. The glass electrodes had one barrel filled with KA or QUIS (20 mM, pH 7.2-

7.6), one with NMDA (50 mM, pH 7.2-7.6), and one with saline.

Vertical microelectrode penetrations were made into primary visual cortex at various distances anterior to the APV cannula. Neural activity was studied at regular intervals of 200 or 300 microns. At each recording site, we assessed visually evoked and spontaneous neural discharge, the threshold to activation by iontophoretic application of NMDA, and the threshold to activation by iontophoretic application of KA or QUIS. The threshold measurements delineated the region of cortex in which NMDA receptors were specifically blocked.

We studied multiunit activity at regular intervals, rather than single isolated cells, to obtain a representative sample in a situation in which many sites had little or no spontaneous or visually driven activity. We summarized the multiunit activity at each site on a six point scale: 0: Neither spontaneous nor visually driven activity of cortical neurons. 1: Spontaneous cortical activity only: no visually driven activity. 2: Unreliably driven visual responses. 3: Weakly but reliably driven, sluggish visual responses. 4: Reliable, vigorous visual responses by at least one cell. 5: Strong multiunit visual responses. Visual responses were always assessed before drug thresholds were tested.

Drug thresholds were assessed as follows and as shown in figure 1. A spike discriminator was set to detect all spikes with amplitude above the noise level. For each drug, we collected three four-second samples of spontaneous activity, or zero drug current (shown at the left of each panel in figure 1 a-d). Drug current was then increased by regular steps every four seconds, until a peak response was passed or until the limits of our equipment -- generally 1000 nanoamps -- were reached (some sites showed no response to NMDA). Step sizes ranged

from 5 to 50 nA. Threshold was assessed as that current which first gave a response equal to twice the average spontaneous level of activity and at least two spikes per second. When no response was obtained, the threshold was assigned the largest current we could test. We allowed at least three minutes between assessment of KA or QUIS threshold and assessment of NMDA threshold, and we alternated, with each site, whether KA/QUIS or NMDA was the first tested. Equal holding currents of 5-10 nA were generally used on drug-containing barrels, balanced by opposite current on the saline barrel. In each animal, drug thresholds for each electrode were assessed first in control cortex. Periodic penetrations into control cortex were made throughout each experiment to ensure that electrode characteristics were unchanged.

Results

After one day of APV infusion, a specific blockade of NMDA receptors was achieved. Typical responses to drug applications, and the method of assessment of drug thresholds, are illustrated in figure 1. In control cortex, multiunit responses to iontophoretic application of KA (fig. 1a) and NMDA (fig 1b) were similar. In D- or D,L-APV-treated cortex, responses to NMDA (fig. 1d, h-j) were profoundly blocked except with very high iontophoretic currents, while responses to KA (fig 1c) remained vigorous and normal. Responses to QUIS (fig. 1e-g) were entirely like those to KA.

Specificity of blockade is indicated by the lack of elevation of thresholds to activation by KA or QUIS even at the closest distances to the cannula. Figure 2 illustrates results from all animals as a function of distance from the D- or D,L-APV cannula. In the five animals in which responses to KA and NMDA were studied, blockade of NMDA receptors (fig. 2a) was seen within 3-4 mm from the cannula, while responses to KA were unaffected even very close to the cannula (fig. 2b). In the animal in which responses to QUIS and NMDA were studied, a blockade of NMDA receptors (fig. 2d) again was seen close to the cannula, while responses to QUIS (fig. 3e) were unaffected.

Specificity of the blockade is also demonstrated by examining the dependence of QUIS or KA thresholds on NMDA thresholds at each site (fig. 3). There is no tendency for elevation of NMDA thresholds to be accompanied by an elevation of thresholds to QUIS (fig. 3a) or KA (fig. 3b).

Vigorous neuronal activity was lacking where NMDA thresholds were elevated. Activity was assessed systematically in the five animals in which drug responses to KA and NMDA were assessed. Blockade of activity closely paralleled blockade of NMDA receptors as a function of distance from the cannula (fig. 2c). Activity was profoundly suppressed within 3-4 mm from the cannula, where NMDA thresholds were strongly elevated. Mild elevations of threshold to NMDA activation, and correspondingly mild depressions of visual responses, were seen from 4 to 7 mm from the cannula. Neural activity appeared equally suppressed in the animal in which QUIS was studied.

Activity suppression was strongly correlated with NMDA receptor blockade when data are analyzed site by site (fig. 4). Sites that lacked vigorously driven visual responses (activity ratings 0-3) had strongly elevated NMDA thresholds (fig. 4a) but normal KA thresholds (fig. 4b). This correlation did not result from mechanical damage near the cannula tip, since it was present even when, as in figure 4, sites within 1 mm of the cannula were excluded from analysis. Sites with vigorous visual responses (activity ratings 4-5) had normal NMDA and KA thresholds. Conversely, at sites with sufficient elevation of the NMDA threshold to ensure a blockade of NMDA receptors, vigorous activity was nearly absent (fig. 4c). Cells with vigorously driven activity were found in the APV-treated hemisphere at only 3 of 38 sites where thresholds were elevated by more than two standard deviations above the control side mean, and at only 1 of 30 sites where thresholds were elevated by more than 3.2 standard deviations. In contrast, at 29 of 38 control sites, and at 20 of 28 sites in APV-treated cortex with thresholds less than one standard deviation above the control side mean, cells responded

vigorously to visual stimulation. We conclude that a sufficiently complete NMDA blockade virtually abolishes normal, vigorously driven neuronal activity.

Identical treatment in three animals with the inactive stereoisomer L-APV did not suppress activity (except in regions within 750 um of the cannula, where mechanical damage was possible), indicating a stereo-specific effect. L-APV treatment did not elevate NMDA or KA thresholds at any distance studied.

Discussion

The results of this study demonstrate that APV infusion at a concentration sufficient specifically to block the NMDA receptors of all cells at a site leads to profound suppression of cortical activity. The measure of NMDA threshold used here is the current at which at least one cell in the local population begins to respond to NMDA application. Similarly, the measure of activity deems sites to have vigorous responses if at least one cell in the local population responds vigorously to visual stimulation. Thus at sites where at least one cell responded normally to NMDA, at least one cell generally responded vigorously to visual stimuli; while conversely, at sites where no cell responded normally to NMDA, in general no cell responded vigorously to visual stimuli.

Several observations indicate that the suppression of cortical activity produced by APV infusion was due to a specific blockade of NMDA receptors. The fact that no such suppression was seen with infusion of L-APV demonstrates that the effect is stereo-specific. A stereo-specific action of D-APV at non-NMDA glutamate receptors does not appear to play a role for two reasons. First, no elevation of either QUIS or KA thresholds were seen even at the closest distances to the cannula or at the highest NMDA thresholds. Concentration decreased many times, as judged by decrease of NMDA thresholds, within the regions in which suppression of activity was seen. Therefore even if slight, undetected effects on QUIS or KA receptors occurred at the closest distances, there should have been no such effects at further distances where activity suppression persisted. Second, if the activity blockade

were due to a nonspecific effect seen only at high APV concentrations, we would expect to have found a region with markedly elevated NMDA thresholds but normal activity. Instead, activity was very poor wherever NMDA thresholds were elevated (fig. 4c).

An additional argument reinforces these conclusions. L-APV, which is a weak blocker of NMDA receptors, did not elevate NMDA thresholds even 0.5 mm from the cannula. Based on pharmacology of spinal cord cells, this indicates that the highest concentrations of APV achieved with our protocol, at the closest distances to the cannula studied, were at most 1/8 (with D-APV) or 1/20 (with D,L-APV) of that required to block either QUIS or KA receptors (Jones et al., 1984; Evans et al., 1982).

We have found a critical role of the NMDA receptor in excitatory transmission in the adult: at sufficiently elevated NMDA thresholds, normal visual responses are nearly abolished. In contrast, Tsumoto and colleagues (Tsumoto et al., 1987; Hagihara et al., 1988) found that, while local iontophoretic application of APV significantly suppressed visual responses in 71% of cells studied in kitten visual cortex, only 34% of cells in adult cat visual cortex were significantly suppressed. Most or all of these cells did not have visual responses completely suppressed. More recent results suggest that iontophoresis of APV in adult animals suppresses visual responses of many cortical cells in superficial layers but of few cells in granular and deep layers (Fox et al., 1989), although the previous work found the strongest suppression in layer 5 (Hagihara et al., 1988).

We see two probable explanations for the discrepancy between these results and ours. First, our method suppresses both cells that are

directly dependent on NMDA receptors for their activation and cells that are indirectly dependent via corticocortical connections, whereas iontophoresis affects only a very local region of cortex. The suppression of deep layer cells we have found cannot be explained simply by the suppression of superficial layer cells (Fox et al., 1989), since silencing of superficial layers by cortical cooling, or destruction of them by cryogenic lesions, leaves the responses of deep layer cells intact (Schwark et al., 1986). Methods of widespread drug application (Kleinshcmidt et al, 1987; Cline et al., 1987) such as the one we have used are necessary for long-term studies of the role of NMDA receptors in development, plasticity, and overall cortical function.

Second, individual cells may see lower and less uniform concentrations of APV with iontophoretic application. Iontophoresis produces an extreme spatial gradient of concentration, varying strongly over 100 microns (Fox et al., 1989; Herz et al., 1969). In contrast, cortical dendrites may extend over many hundreds of microns from the cell body (Lund et al., 1979; Katz, 1987). Thus iontophoresis of APV may fail to block NMDA receptors completely over the entire dendritic tree of the cell being studied. Incomplete blockade may also be produced by iontophoretic application of APV if synaptic concentrations of NMDA are much larger than the concentrations achieved by NMDA iontophoresis, since blockade of the response to NMDA iontophoresis is used to calibrate the iontophoretic currents of APV. In some systems, it has been shown that substantially greater APV currents may be needed to block the synaptic responses mediated by NMDA receptors than to block responses to NMDA iontophoresis (Davies and Watkins, 1982; Davies et al., 1986). Incomplete blockade may be indicated by Tsumoto and

colleagues' report (Tsumoto et al., 1987) that, in the same adult cats in which the effects of APV iontophoresis were studied, visual responses were suppressed in only 71% of cells by iontophoretic application of kynurenate (which abolishes synaptic EPSPs and responses to all glutamate agonists in cultured cells (Huettner and Baughman, 1988) and slices (Langdon et al., 1988) of visual cortex).

We have studied cells after exposure to APV for one day, whereas iontophoretic methods study the effects of acute application. Hence, receptor regulation in response to blockade may play a role in the greater degree of activity suppression that we see.

Our results indicate that NMDA receptors participate in, and are required for, the normal responses of many mature visual cortical cells. The NMDA receptor mediates a slow, long-lasting EPSP that rises to peak in 10-75 msec (Forsythe and Westbrook, 1988; Dale and Roberts, 1985; Wigstrom et al., 1985; Herron et al., 1986; Aram et al., 1987; Jones and Baughman, 1988). This slow EPSP is seen under physiological conditions particularly in response to high-frequency stimulation (Herron et al., 1986; Collinridge et al., 1988b; Poolos and Kocsis, 1987). Visual cortical cells receive appropriately high-frequency and maintained inputs (Creutzfeldt and Ito, 1968) to evoke such a slow EPSP, and they integrate these inputs over long and varied time periods: latencies between responses in lateral geniculate nucleus and visual cortex are greater than 20 msec (Raushecker et al., 1986), compared to conduction times of 2-3 msec. Thus, visual cortical responses may depend upon the buildup of a slow baseline depolarization mediated by NMDA receptors. Such a slow EPSP could provide a base upon which continuing subthreshold input, mediated by non-NMDA receptors, would become suprathreshold.

Intracellular recordings of responses of visual cortical cells to stimulation by light show a similar time course: a slow (15 to 60 msec) baseline depolarization must build before individual fast EPSPs become supratheshold, and this depolarization persists throughout the post-synaptic spike response (Creutzfeldt and Ito, 1968). This depolarization was attributed to summation of fast EPSPs, but results in other systems (Dale and Roberts, 1985; Herron et al., 1986) demonstrate that such apparent summation can represent slow, NMDA-activated EPSPs, abolished by APV.

There is clear precedent for the notion that NMDA receptors are involved in normal, mature sensory activation. In ventrobasal (somatosensory) thalamus, APV antagonizes responses to maintained sensory stimulation, but not to transient sensory stimulation (Salt, 1986; Salt, 1987). More recently a preliminary report that sensory activation of visual thalamic cells depends upon NMDA receptors has appeared (Moody and Sillito, 1988). In hippocampus, it is known that NMDA receptors can carry a significant fraction of the synaptic current (Forsythe and Westbrook, 1988), and are involved in responses to sustained (Herron et al., 1986; Collinridge et al, 1988b), but not transient (Collinridge et al., 1983; Collinridge et al., 1988a), electrical stimulation. Little is known of the role of NMDA receptors in hippocampal responses to natural events. In general many factors, including local excitatory and inhibitory circuitry and the strength and temporal pattern of afferent activation, may determine whether excitatory transmission depends upon NMDA receptors in a particular location and situation. In this regard, it may be of interest for cortical transmission that there appears to be a thalamic system capable of modulating NMDA

receptor activation in deep layers of cortex in a widespread, diffuse manner (Fox and Armstrong-James, 1986).

Chronic application of APV has been shown to prevent or reverse activity-dependent developmental plasticity in the visual system (Kleinschmidt et al., 1987; Cline et al., 1987). It is, however, well known that disruption of the pattern of activity can itself profoundly alter plasticity (discussed in (Stryker and Harris, 1986)). Visual responses in cortex are more strongly suppressed by APV iontophoresis in kittens than in adult cats (Tsumoto et al., 1987; Fox et al., 1989). Thus chronic APV application in kittens is likely to suppress activity at least to the degree that we have found in adults, wherever concentrations become sufficient to block NMDA receptors. The same may be true in other developmental systems. Hence, the developmental results may reflect the effects of suppressing postsynaptic activity, rather than of specifically blocking a mechanism of plasticity.

In visual and perhaps other regions of cortex, integration over times comparable to that of NMDA-mediated slow EPSPs appears to be the normal mode of response to natural stimuli. Patterns of activation among inputs which are high-frequency and maintained over many tens or hundreds of milliseconds are also the norm. NMDA receptors may thus be used, at various locations and times, for either or both of two distinct tasks: to regulate synaptic plasticity; and to regulate the temporal patterns of input that can activate post-synaptic cells.

Figure 1

(a-d): Multi-unit responses to drug applications. Bins with asterisks indicate threshold. Drug current was steadily increased every four seconds after three four-second bins of zero current. Each bin represents response in one such four-second interval. (a and b) Drug responses at a typical unblocked site, 2.4 mm anterior to a cannula pumping the inactive stereoisomer L-APV. KA was stepped by 10 nA, NMDA by 20 nA. Threshold was 20 nA for KA, 60 nA for NMDA. Activity had been rated 5 at this site. (c and d) Typical drug responses where NMDA receptors were blocked, 1.4 mm anterior to the D-APV cannula in the same animal as in a and b. KA response, assessed in 10 nA steps, was normal and vigorous; the KA threshold was 10 nA. NMDA threshold was assessed in 50 nA steps, because threshold had been found to be highly elevated. NMDA threshold response was achieved at 550 nA. Activity had been rated 1 at this site. (e-j) Raw multiunit records where NMDA receptors were blocked, 1.2 mm anterior to DL-APV cannula in another animal. To left are responses to QUIS iontophoresis at 0 nA (e), 25 nA (f, threshold) and 65 nA (g, peak response. To right are responses to NMDA iontophoresis at 450 nA (h), 500 nA (i, threshold) and 1000 nA (j, peak response). At unblocked sites, NMDA records were indistinguishable from KA or Quis records. Calibration bars correspond to 20 uV and 20 msec. The threshold and peak response currents were consistent and reproducible. After being excited by drugs, some cells in the population desensitize quickly; others require longer times or higher currents before desensitization. Hence, the shapes of the post-peak part of the histogram are extremely variable and without significance. In the blocked regions, the entire population may not be excited near the

threshold of first response. In these regions, responses can be spread over a much broader range of currents, as shown.

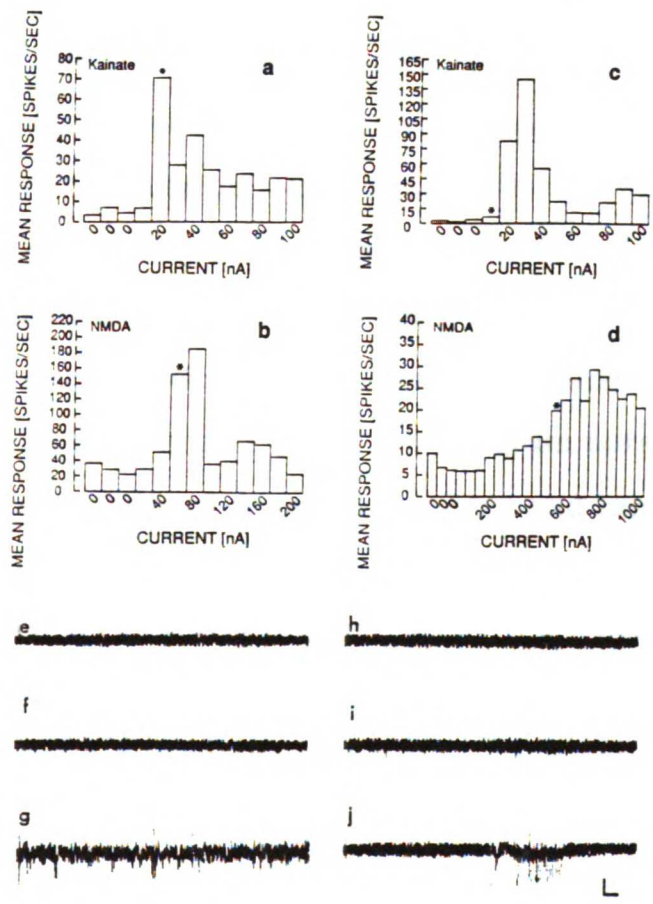

MEAN RESPONSE [SPIKES/SEC]
Kainate
a
CURRENT [nA]
MEAN RESPONSE [SPIKES/SEC]
Kainate
c
CURRENT [nA]
MEAN RESPONSE [SPIKES/SEC]
NMDA
b
CURRENT [nA]
MEAN RESPONSE [SPIKES/SEC]
NMDA
d
CURRENT [nA]
e
f
g
h
i
j

Figure 2

Blockade of NMDA, compared to responses to KA (a and b, five animals) or QUIS (d and e, one animal), and to visual response (c, same five animals as a and b) (ordinates). All are shown as a function of distance anterior to the cannula delivering APV (abscissae). c includes 79 sites (69 in APV hemispheres, 10 in control hemispheres) at which only activity was studied, as well as 123 sites (95 in APV hemispheres, 28 in control hemispheres) at which both activity and drug thresholds were studied. In QUIS animal, penetrations 8.3 mm anterior to cannula and control hemisphere were both controls for normalization of thresholds and statistical tests. Conventions for figures 2 and 4: NMDA and KA or QUIS currents for each animal and each electrode have been normalized to the mean threshold current seen in the control hemisphere for that drug, electrode, and animal. For a, b, d and e, histogram bars show mean, error bars indicate standard error of the mean, and numbers above the error bars indicate the number of sites. For c, bars represent the percent of sites in each bin with the indicated activity: hatched bars show activity ratings 0-2 (lacking reliable visually driven activity), solid bars show activity ratings 4-5 (vigorous visual responses). Difference from 100% in each bin is made up by sites with activity rating 3. Numbers above bars indicate total number (100%) of sites. Statistical significance of elevation of thresholds (a,b) or depression of activity (c), in comparison to pooled control data, was assessed by Kolmogorov-Smirnov two-sample test or Mann-Whitney U test or, for figures (c) only, by chisquared test. ***: $P < 0.001$; **: $P < 0.01$; *:$P < 0.05$.

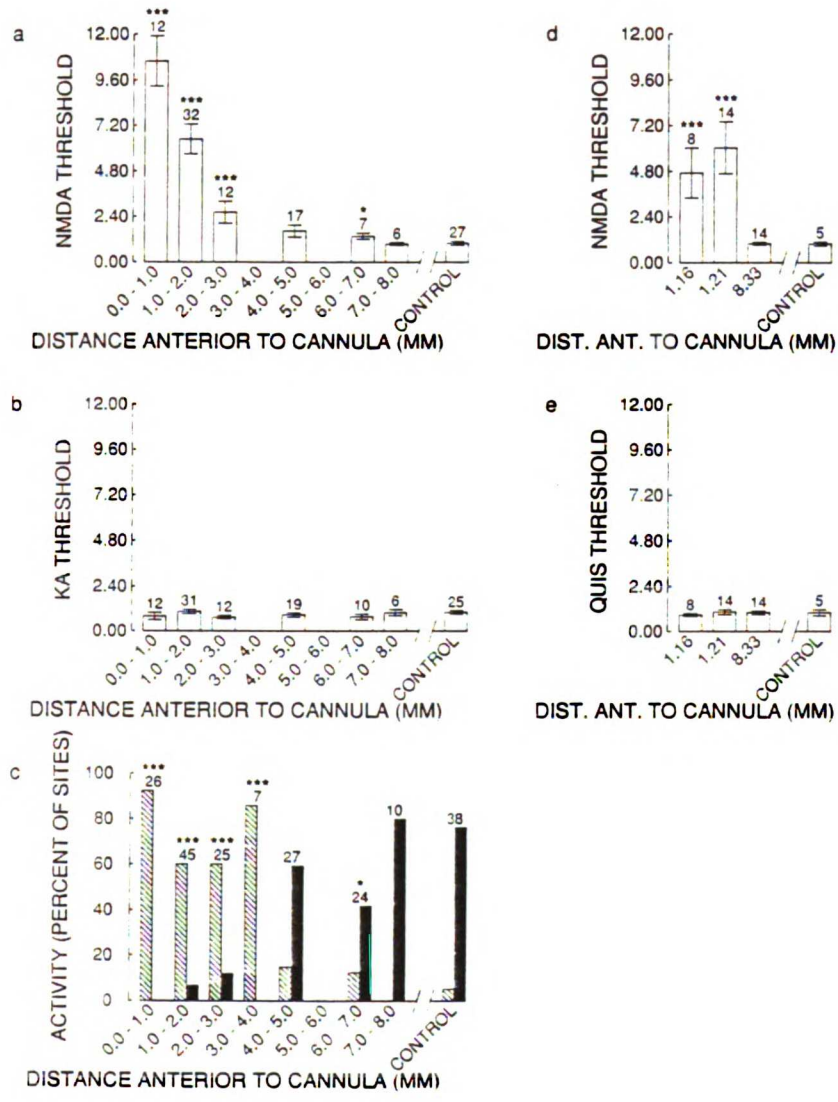
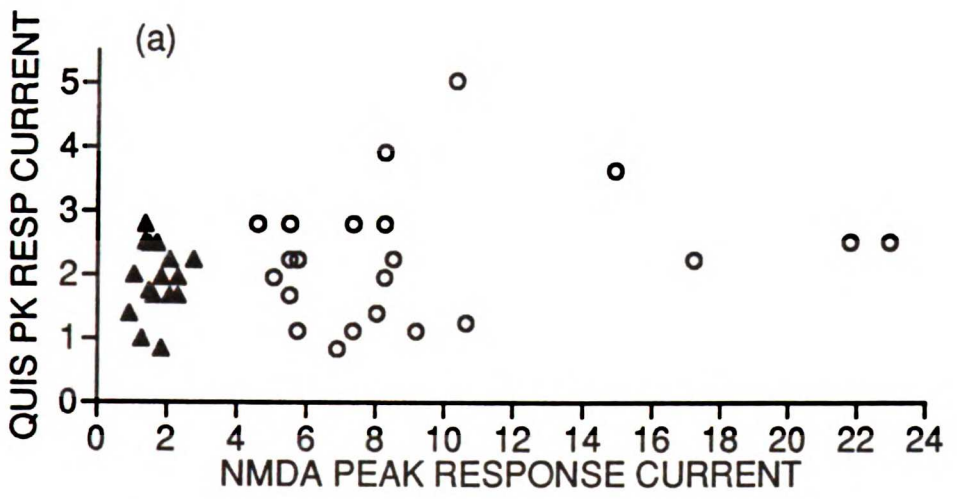

a
NMDA THRESHOLD
12.00
9.60
7.20
4.80
2.40
0.00
12
32
12
17
7
6
27
0.0 - 1.0
1.0 - 2.0
2.0 - 3.0
3.0 - 4.0
4.0 - 5.0
5.0 - 6.0
6.0 - 7.0
7.0 - 8.0
CONTROL
DISTANCE ANTERIOR TO CANNULA (MM)
d
NMDA THRESHOLD
8
14
14
5
1.16
1.21
8.33
CONTROL
DIST. ANT. TO CANNULA (MM)
b
KA THRESHOLD
12
31
12
19
10
6
25
DISTANCE ANTERIOR TO CANNULA (MM)
e
QUIS THRESHOLD
8
14
14
5
DIST. ANT. TO CANNULA (MM)
c
ACTIVITY (PERCENT OF SITES)
100
80
60
40
20
0
26
45
25
7
27
24
10
38
DISTANCE ANTERIOR TO CANNULA (MM)

Figure 3

Comparison of QUIS (a) or KA (b) thresholds to NMDA thresholds on a site-by-site basis, for the data shown in figures 2 and 4. Symbols code distance of a site from the cannula: filled triangles represent sites in the control hemisphere or more than 7 mm anterior to the cannula; open triangles represent sites 4-7 mm anterior to the cannula; open circles, 1-4 mm anterior; open squares, 0-1 mm anterior to the cannula. Thresholds normalized as in figure 2.


(a)
QUIS PK RESP CURRENT
0
1
2
3
4
5
0 2 4 6 8 10 12 14 16 18 20 22 24
NMDA PEAK RESPONSE CURRENT

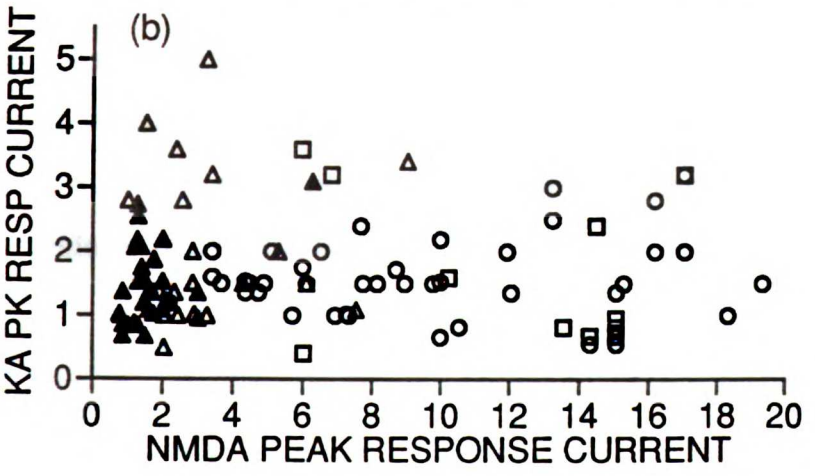
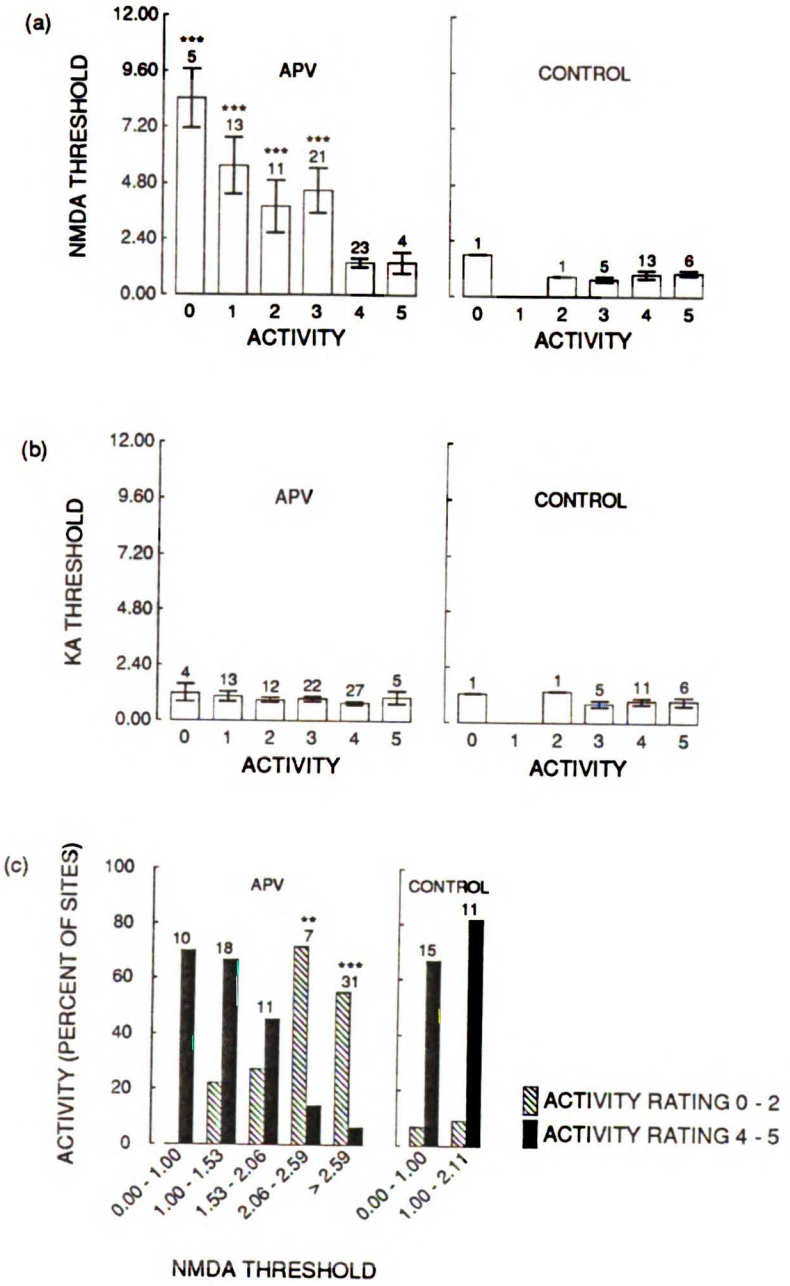

(b)
KA PK RESP CURRENT
0
1
2
3
4
5
0 2 4 6 8 10 12 14 16 18 20
NMDA PEAK RESPONSE CURRENT

Figure 4

Site-by-site comparisons of thresholds with activity. Conventions as in figure 2. (a) NMDA threshold as a function of multiunit activity rating. (b) KA threshold as a function of multiunit activity rating. c. Multiunit activity rating as a function of NMDA threshold. Bins on the APV side represent standard deviations (0.53) above the control side mean threshold of 1.0. Points above 1.0 on the control side have been collapsed into a single bin, as only three points had threshold elevated by more than one standard deviation, and as no variation in activity with threshold was apparent. Because of the possibility of nonspecific damage very close to the cannula, this figure includes only data from sites > 1 mm anterior to the cannula. The 12 excluded sites at distances < 1 mm all had activity ratings < 3 and NMDA thresholds > 4.26; their inclusion would only have made the results appear to support our conclusions more strongly.


(a)
NMDA THRESHOLD
12.00
9.60
7.20
4.80
2.40
0.00
APV
CONTROL
***
5
***
13
***
11
***
21
23
4
1
1
5
13
6
0 1 2 3 4 5
ACTIVITY
(b)
KA THRESHOLD
4
13
12
22
27
5
1
1
5
11
6
(c)
ACTIVITY (PERCENT OF SITES)
100
80
60
40
20
0
10
18
11
**
7
***
31
15
11
0.00 - 1.00
1.00 - 1.53
1.53 - 2.06
2.06 - 2.59
> 2.59
0.00 - 1.00
1.00 - 2.11
NMDA THRESHOLD
ACTIVITY RATING 0 - 2
ACTIVITY RATING 4 - 5

CHAPTER 3

Relation of cortical cell orientation selectivity to alignment of receptive fields of the geniculocortical afferents that arborize within a single orientation column in ferret visual cortex

Abstract

Neurons in the primary visual cortex of higher mammals are arranged in columns and the neurons in each column respond best to light-dark borders of particular orientations. The basis of cortical cell orientation selectivity is not known. One possible mechanism would be for cortical cells to receive input from several lateral geniculate nucleus neurons with receptive fields that are aligned in the visual field (Hubel and Wiesel, 1962). We have investigated the relationship between the arrangement of the receptive fields of geniculocortical afferents and the orientation preferences of cortical cells in the orientation columns to which the afferents provide visual input.

Radial microelectrode penetrations were made into primary visual cortex of anaesthetized adult sable ferrets. Cortical cells were recorded throughout the depth of the cortex and their orientation preferences were determined. Cortical cell responses were then eliminated by superfusion of the cortex with either kainic acid (Zahs and Stryker, 1988) or muscimol. After the drug treatment, responses from many single units with distinct receptive fields were recorded. These responses were presumed to be those of geniculocortical afferents, because they had the response properties characteristic of lateral geniculate nucleus neurons, and because they could be recorded only in cortical layers which receive geniculate input. In 16 of 18 cases the afferent receptive fields recorded in a single penetration covered an elongated region of visual space. In these penetrations the best-fit line through the centers of the afferent receptive fields generally paralleled the preferred orientation of cortical

cells recorded at the same site in cortex.

These results are consistent with the Hubel and Wiesel (1962) model for the construction of oriented visual cortical receptive fields from geniculate inputs with aligned receptive fields.

Introduction

Each cell in the primary visual cortex of higher mammals responds best to light-dark borders at a particular orientation. This orientation selectivity must be produced at the level of the cortex since the principal visual input to these cortical cells is from the lateral geniculate nucleus (LGN), where neurons have round receptive fields and do not exhibit marked orientation preferences. When they first described cortical orientation specificity Hubel and Wiesel (1962) proposed a model to explain how cortical cells with simple-type receptive fields, which predominate in the layers of carnivore cortex that receive direct geniculate input, could acquire orientation selectivity from their non-orientation selective inputs. According to this model, a given cell in the visual cortex would receive excitatory inputs from geniculate cells the receptive fields of which are aligned in the visual field. A stimulus falling along a line of the correct orientation to excite all of these geniculate cells simultaneously would produce the best response in the cortical neuron. Stimuli at other orientations would excite only a few of the aligned geniculate cells at a time, and would produce little or no response in the cortical neuron. Over the past 28 years it has proved difficult to provide strong evidence for or against this model.

The orientation selective cells in primary visual cortex are arranged in columns. Within one column all cells have the same orientation; cells in neighboring columns have similar preferred orientations such that the orientation changes across the surface of the cortex are gradual and progressive (Hubel and Wiesel, 1962). This orderly cortical

representation of orientation allows an alternative model for cortical cell orientation preferences. Orientation might not depend on the arrangement of excitatory inputs to cortical cells from LGN afferents. Instead, the cross-orientation inhibition model relies on intra-cortical inhibitory connections between neurons with orthogonal orientation preference to account for cortical cell orientation specificity (Blakemore and Tobin, 1972; Creutzfeldt et al., 1974). Experimental tests of the cross-orientation inhibition model have provided mixed results. In support of the model, treatment of visual cortex with pharmacological agents designed to remove intracortical inhibition tends to decrease orientation selectivity in the majority of cells (Tsumoto et al., 1979; Sillito et al., 1980). However, the cross-orientation inhibition model suggests that intracellular recordings from oriented cortical cells would reveal that inhibitory post-synaptic potentials were preferentially oriented orthogonal to the preferred orientation of the cell. Such cross-oriented IPSPs were not found (Ferster, 1986).

In order to study neuronal connections underlying orientation selectivity in visual cortex, we have attempted to look at the geniculocortical input to single orientation columns in ferret primary visual cortex. The arrangement of cortical orientation columns in the ferret (studied physiologically by Waitzmann and Stryker, unpublished, and with deoxyglucose by Redies et al., 1990) is similar to that seen in the more familiar carnivore, the cat (studied physiologically by Hubel and Wiesel, 1963a, and with deoxyglucose by Singer, 1981, and Schoppmann and Stryker, 1981). We have recorded from cortical cells and from a collection of geniculocortical afferents that terminate at the same location in cortex. In earlier experiments (Zahs and Stryker, 1988)

geniculocortical afferents recorded in radial electrode penetrations through ferret primary visual cortex were commonly found to be dispersed over elongated regions of the visual field. We now relate the axis of this elongation to the orientation preference of cortical cells recorded along the same electrode penetration.

Methods

Animals

Fourteen adult sable ferrets were used in these studies. Ferrets were obtained from Marshall Farms (New Rose, NY) and were housed in the University of California, San Francisco animal care facility under a light cycle of 14-17 hours of light, 7-10 hours of dark.

Surgery

Ferrets were initially anesthetized with an intramuscular injection of a mixture of acepromazine (0.04 mg/kg) and ketamine (40 mg/kg), and were given atropine sulfate (0.8 mg) every 12 hours to prevent mucus accumulation in the trachea. Following femoral vein and tracheal cannulation, anesthesia was maintained at surgical levels with intravenous thiopental sodium (35mg/kg). Lidocaine was applied to all wound margins. Each ferret was then positioned in a modified cat stereotactic apparatus. A midline incision was made through the scalp, and the muscles overlying the skull were retracted. In most animals 0.2 - 0.5 ml cerebro-spinal fluid was withdrawn through cisternal puncture using a 22 ga. needle. A craniotomy was performed extending from approximately Horsely-Clarke anterior-posterior -3.0 to the caudal pole of the cortex, and from approximately Horsely-Clarke lateral-medial 1.5 to 8.5. The exposed dura was kept moist with gauze soaked in 0.9% saline. Neuromuscular blockade was produced using gallamine triethiodide (1 ml/kg-hr, 10 mg/ml in 5% dextrose ringers). Thereafter, the animal was artificially ventilated with a 3:1 mixture of nitrous oxide

to oxygen. For the first 30 minutes, the animal was hyperventilated to prevent cortical edema, following which the dura was retracted to expose cortical area 17.

The respiratory rate and volume were set to maintain peak expired carbon dioxide between 3.8% and 4.2%. Peak inspiratory pressure was always kept below 1.5 kPa. A skull screw was placed over the hemisphere contralateral to the craniotomy, and the electroencephalogram (EEG) was monitored throughout the experiment. Intravenous thiopental sodium (approximately 2 mg/kg-hr) was administered whenever there was any sign of EEG desynchronization. The animal's heart rate was also monitored throughout the experiment, and its temperature was maintained at 37.5°C using a feedback system. Prior to fitting the animal's eyes with contact lenses of appropriate refractive power, pupils were dilated with 2% atropine sulfate and nictitating membranes were retracted with 10% phenylephrine hydrochloride.

Kainate treatment

In 2 ferrets, the cortical surface was photographed using a Nikon 35 mm camera mounted on a Zeiss operating microscope. Photos were printed at a final magnification of approximately 28X, allowing for accurate notation of the positions of microelectrode penetrations with respect to cortical vasculature. Cortical cell responses in several radial penetrations were then recorded (see below). Following recording of cortical units, a piece of filter paper (Whatman #4, approximately 2mm X 7mm) was soaked in kainic acid (Sigma k0205, 10mM in 0.9% saline) and placed over the caudal portion of the exposed cortex. Kainic acid

was superfused onto this filter paper at a rate of 60ml/hr for 12-14 hours through silastic tubing (Dow Corning) attached to a syringe held in an infusion pump. The anterior portion of exposed cortex was covered with a separate piece of filter paper soaked in 0.9% saline. Saline-moistened cotton was placed around the exposure and covered with saran wrap (Dow Chemical) to create a moist chamber over the exposed cortex.

Muscimol Treatment

In eleven ferrets, a piece of gel foam (approximately 0.2 mm thick, 2mm X 7mm) was placed over the caudal portion of the exposed cortex. A recording electrode (see below) was then positioned perpendicular to the brain surface with its tip just touching the cortical surface through a hole in the gel foam. A piece of silastic tubing containing muscimol (Sigma M1523, 50mM in 0.9% sterile saline) attached to a syringe held in an infusion pump had its terminal 2cm filled with 0.9% saline and its end placed on the gel foam less than 1mm away from the recording electrode. Following the recording of cortical units in one radial penetration (see below), the microelectrode was withdrawn to approximately 200 mm above the presumed location of layer IV. Muscimol was then infused through the silastic tubing at a rate of 74 ml/hr for approximately 2 hours.

Electrophysiological Recording

Lacquer-coated tungsten microelectrodes (Hubel, 1957) were used to record extracellularly. Impedances ranged between 1.0 and 2.0 MW, and electrode tips tapered from a diameter of 10-15 mm to a very sharp

point over approximately 30 mm. Electrodes were connected to a pre-amplifier (Grass P15). The signal was capacity-compensated and band-pass filtered 100Hz - 10kHz, then displayed on an oscilloscope (Tektronix 5115) and played on an audio monitor (Grass AM8). Radial electrode penetrations were made into area 17. Cortical cell receptive fields were studied at 200 mm intervals. Receptive fields were first hand-plotted using moving bars of light produced by a Zeiss hand-lamp to determine location, ocular dominance and preferred orientation. Then an orientation tuning histogram was compiled, using a computer to drive an optical display that swept light bars across the receptive fields at 36 different orientations, randomly interleaved, and to record unit responses to each stimulus presentation.

In kainate-treated ferrets, several radial electrode penetrations were performed and their locations marked on the photograph of the brain surface. Cortical units were recorded and their receptive fields plotted throughout the depth of the cortex. The microelectrode was then withdrawn from the brain. After kainate infusion, microelectrode penetrations were made as close as possible to the penetrations where cortical units had been recorded.

In order to determine whether it was possible to return to the same orientation column following the 12 hour time lapse necessary for the kainate infusion, 2 microelectrode penetrations were made into the non-kainate treated hemisphere of one animal, and receptive field locations and orientation preferences were recorded. Twelve hours later penetrations were made at these same locations, and cortical cell receptive field properties were again assessed. Receptive field centers in the later penetrations were all within 3° of those recorded earlier in the

same penetration, and preferred orientations never varied by more than 10° from those recorded earlier.

In muscimol-treated ferrets, data relating afferent receptive fields to cortical cell orientation resulted from only one penetration per animal. After recording from cortical cells throughout the depth of the cortex, the microelectrode was withdrawn to 200mm above the presumed location of the top of layer IV, based on the locations at which afferent-like "swish" could be recorded. After muscimol infusion the microelectrode remained in the same penetration in which cortical responses had been recorded.

Data were discarded from any penetration in which the preferred orientation of cortical units at the top of layer 2 differed by more than 10° from the preferred orientation of units in layer IV.

Kainate or muscimol infusion was stopped when little or no activity could be recorded in supra-granular layers. The microelectrode was advanced in 40 mm steps until vigorous visually-driven activity from presumed geniculocortical afferents was encountered. The electrode was then advanced in 20 mm steps and the receptive fields of units at each site were studied using flashing or rapidly moving spots of light produced by a Zeiss hand lamp, or in 1 animal by the computer-driven optic bench. For each afferent receptive field, location, size, center-type, and the eye through which a response could be elicited were noted. Units were assessed for orientation preference using moving bars of light. The recordings were terminated after the electrode had been advanced 200 mm below the last recorded afferent. In 10 cases the electrode was then slowly withdrawn up through layer IV and the afferent receptive fields were plotted again. Electrolytic marking lesions were made in each penetration, usually at both ends of the region within which presumed

afferent units had been recorded.

In 11 animals receptive fields were plotted using a 'blind' procedure: that is, the person plotting the afferent receptive fields did not know the location or orientation preference of the cortical cell receptive fields previously recorded at the same site.

Lateral Geniculate Nucleus Stimulation

In one ferret, two concentric bipolar electrodes (Rhodes SNE100) were placed in the LGN. The electrodes were aligned rostro-caudally, 2mm apart with the caudal electrode in a region of LGN where recorded units had centrally located receptive fields. A tungsten recording electrode (see above) was placed in area 17. Cortical unit responses to light and to LGN stimulation (50 mA - 1.5 mA, 100 - 200 msec duration, 100 - 500 Hz) were recorded. Muscimol was then infused onto the cortex as described above. After cortical units were silenced, the responses of presumed geniculocortical afferents to light and to LGN stimulation were recorded.

Histology

The brains of 7 animals (2 kainate-treated, 5 muscimol-treated) were processed for histological reconstruction of electrode penetrations. After the recording session, the animals were very deeply anesthetized with intravenous pentobarbital (50 - 100 mg/kg) and perfused intracardially with 0.9% saline followed by 10% formol saline. After post-fixing in 10% formol saline the brain was blocked and the occipital cortex was sunk in 30% sucrose formalin and embedded in albumin-gelatin. Coronal sections were cut at 40 mm on a freezing cryostat, mounted on

slides, and stained with cresylecht violet to locate electrode tracks and marking lesions.

Quantitative Analysis

Experiments where few afferents were recorded were not suitable for analysis since the aim of the study was to relate the arrangement of a group of afferent receptive fields to the cortical cell orientations. Data were therefore analyzed only from the 17 penetrations where 8 or more afferent units were recorded (data from one penetration with 6 afferent units were discarded; 3 or fewer afferents were recorded in all other penetrations that were not analyzed). Afferent receptive fields of different center-type from the same penetration were analyzed separately. A total of 30 afferent receptive field arrays were analyzed: 18 from this study and an additional 12 from previous experiments (Zahs and Stryker, 1988) where afferent receptive fields were mapped but cortical cell orientation was not determined.

A quantitative analysis was performed to determine how well geniculocortical afferent receptive fields from a single electrode penetration were aligned with one another in visual space. For each array of afferent receptive fields, the principal axis of elongation of the afferent centers was calculated, along with its correlation coefficient (r^2). The principal axis of elongation is not simply the regression line of the receptive field elevations on their azimuths, because neither coordinate is a dependent variable. Furthermore, if a standard regression line were calculated, correlation coefficients would be zero for afferent receptive fields aligned vertically or horizontally. The principal axis of afferent receptive field arrays was therefore calculated as the axis which, when

rotated to 45°, produces the maximum correlation coefficient. This maximum correlation coefficient was the r^2 used in the analysis.

To calculate the extent to which this measured alignment was significantly different from chance, a Monte Carlo analysis was performed as follows. For each array of afferent receptive fields, 10000 simulated arrays of receptive fields were generated on a DECstation 3100 computer. Each simulated array contained the same number of afferent receptive fields as were encountered in the electrode penetration, but each receptive field was assigned a random location while maintaining its distance from the center of the array. r^2 was calculated as above for each of the 10000 simulated arrays. The percentile occupied by the experimental r^2 in the distribution of randomized r^2s was calculated and called the "alignment significance".

Results

In all penetrations analyzed in this study, visual cortical cells were orientation selective, and the preferred orientations of the cortical cells did not change significantly within the penetration which extended from the surface of the cortex down to white matter. An example of orientation tuning histograms for single cortical units recorded in different layers of cortex within one penetration is shown in figure 1.

After superfusion of kainate for 12-14 hours (as shown previously, Zahs and Stryker, 1988) or muscimol for 2-3 hours, neuronal activity in supragranular layers of area 17 was almost entirely eliminated. Occasionally small amplitude units could be recorded which responded to rapidly moving stimuli but not to light flashes, and which lacked orientation preference to slowly moving light bars but did not exhibit center-suround receptive field organization. In general, however, neither spontaneous nor visually driven activity could be recorded in the upper layers. Within layer IV spontaneous activity was high and visual responses were easily elicited. Several single units could be isolated at most recording sites based on their non-overlapping receptive fields and on differences in their action-potential wave forms. These units were presumed to be the terminals of geniculocortical afferents because they had the response properties identical to those of neurons recorded in the LGN and were recorded only after the electrode had reached cortical layer IV, and not above. The presumed afferents were monocularly driven; they responded reliably to repeated light flashes or rapidly moving stimuli within receptive fields of appropriate size; they showed no

preference for stimulus orientation (figure 2A) in contrast to the tightly orientation-tuned responses seen in cortical cells (figure 2B); they exhibited center-suround antagonism (fig. 2C), they had very fast (< 200ms) small (20-100mV, usually < 50 mV) action potentials (fig. 2D), they consistently followed high-frequency electrical stimulation of the LGN (fig. 2D), and they were located in layer IV in all 7 penetrations reconstructed histologically (fig. 2E). Afferent unit recordings stopped abruptly at the bottom of layer IV and electrical signals were almost completely absent in layer V. When the electrode entered layer VI geniculocortical afferent units were again encountered; the receptive fields of these layer VI afferents were not quantitatively studied.

Figure 4 shows the receptive field of a cortical cell recorded at the top of layer IV, the preferred orientation of that cell determined from its orientation tuning histogram, and the afferent receptive fields recorded in each microelectrode penetration. Because the ferret has segregated patches of on- and off-center geniculate afferents in visual cortex (Zahs and Stryker, 1988), most electrode penetrations contained afferents of only one center type. However, in one penetration more than 8 afferents of each center type were recorded. This allowed 18 afferent receptive field arrays to be analyzed from the 17 penetrations which were performed. Figure 4 illustrates the ranges observed in the degree of elongation of afferent receptive field arrays, and in the fit between the preferred orientations of the cortical cells in layer IV and the principal axes of elongation of the afferent arrays. These plots, which constitute the primary data of this experiment, are further analyzed below.

Cortical cell receptive fields and afferent receptive field arrays showed varying degrees of overlap. In some penetrations (figure 4 F,G

and M) there appeared to be no overlap between the receptive fields of the cortical cell and those of the afferents. Possible explanations for this variable degree of overlap are discussed below.

Results of the Monte Carlo analysis that tests the significance of afferent receptive field alignment are shown in figures 5 and 6. The histograms in figure 5 show the distributions of r^2s calculated for the 10000 simulated afferent arrays derived from each of 3 electrode penetrations. The arrows point to the r^2s obtained from the experimental data and the insets show the experimentally obtained afferent receptive fields for each case. The Monte Carlo measure of the significance of receptive field alignment is the percentile occupied by the experimental r^2 in the distribution of randomized simulation r^2s. For arrays of receptive fields that appear by eye clearly to be elongated (fig. 5A) r^2 for the experimental data falls well outside the distribution of values of r^2 for the random simulated arrays, making the degree of alignment high. For clustered arrays of afferents (figure 5C) the experimental r^2 falls well within the distribution, showing that the degree of alignment is not significant. An intermediate case is shown in figure 5B. A histogram showing the alignment significance for all of the experimental data is shown in figure 6. Sixteen of the 18 afferent receptive field arrays are significantly aligned with > 90% confidence. This distribution of alignment significances is similar to the distribution calculated from afferent receptive field arrays obtained in a previous study (Zahs and Stryker, 1988) where cortical cell orientation was not determined; 27 out of 30 penetrations in the total data set have alignment significance greater than 90%.

Two possible artifacts might have caused afferent receptive field

arrays recorded in this study to appear to be aligned, but both of these artifacts can be discounted. First, observed alignment could be a reflection of the retinotopic map on the cortex. An electrode penetration which failed to travel radially through the cortex along a line of projection could yield an elongated array of afferent receptive fields as the electrode moved across the retinotopic map. In that case, however, the receptive fields of successively encountered afferents would be expected to progress in an orderly fashion across the visual field. No such progression was present in any electrode penetration in these experiments. Instead, receptive fields of successively encountered afferents zigzagged back and forth through visual space as the electrode advanced. Figure 3 shows a typical example of this, with the receptive fields numbered in the order in which they were encountered, and with arrows connecting successively encountered receptive fields. The second artifact which could account for observed alignment of afferent receptive fields would be eye movements occurring during the recordings of the afferents. However, eye movements are not consistent with the data since, afferent fields at both ends of an array were often recorded simultaneously at one depth in cortex. Also, in those penetrations where receptive fields were recorded both as the electrode advanced down through layer IV and as the electrode was withdrawn, many of the same afferent receptive fields were plotted at the same depth on the way down and on the way up.

No relationship is seen between the alignment significance of afferent receptive field arrays and the degree of orientation tuning seen in the cortical cells recorded at the same site. Figure 7 shows the tuning of cortical cells, measured as the average half-width at half-height of the

tuning histograms of all the supragranular cells recorded in each penetration, plotted against the alignment significances of the afferent receptive field arrays. The 2 sites where afferent receptive fields were clustered (figure 4 P,Q), producing low alignment significances, have cortical cell orientation tunings slightly better than the mean of tuning at all the cortical sites.

The 2 clustered afferent receptive field arrays could represent cortical locations where the afferents are arranged differently than at the other locations studied, or they could merely result from randomly sampling a small number of afferents from a larger population that is actually elongated. In order to determine how likely it is that such clustered arrays would be recorded from a large population of afferents forming an elongated array, the following analysis was performed. A universe was constructed consisting of all of the experimentally recorded afferent arrays placed in register such that their principal axes and their geometric centers lined up. Differences in the distances between receptive fields due to differences in cortical magnification factor between penetrations could affect the outcome of the simulation. Therefore the analysis was performed using random samples drawn from a universe in which the experimental receptive field arrays were not only in register, but also normalized for magnification factor. Random samples of receptive fields were drawn from this universe shown in figure 8A. The sizes of the random samples were matched to the numbers of afferents recorded in the experimental electrode penetrations; each real penetration was represented by 10 samples of the same size in the simulation. The alignment significance of each random sample was calculated, and the distribution of alignment significances was compared

to that of the experimental data. The results of this analysis are shown in figure 9 which plots the cumulative percentage of real and simulated arrays occurring at each alignment significance. Although a few arrays with low alignment significance are seen in the simulated data, these represent a much smaller percentage of the total simulated data than the percentage represented by the 2 clustered arrays in the experimental data. In addition, the two experimentally observed arrays with the lowest alignment significance are more closely clustered than any of the simulated arrays, having a smaller mean distance of receptive field centers from the geometric center of the array than do any of the simulated arrays. The same result was seen to a lesser degree when neither afferent receptive field arrays nor simulated data were normalized for magnification factor (data not shown). This analysis suggests that the 2 afferent receptive field arrays with very low elongation significance are unlikely to have been evident merely because of the small sample size. Rather, they appear to represent sites in visual cortex at which geniculocortical afferent organization differs from that found in most locations.

Figure 8B shows a universe of possible afferent receptive field positions constructed as in figure 8A, excepting that only the 18 afferent arrays recorded in this study were used, and the data from each penetration have been rotated to align the cortical cell preferred orientations rather than the afferent receptive field array principal axes. This universe is similar to that shown in figure 8A.

The universes shown in figures 8A and 8B represent our best estimates of the aggregate receptive field shape of the collection of geniculocortical inputs to a single orientation column.

The correspondence between cortical cell orientation and the spatial arrangement of geniculocortical afferent receptive fields at the same cortical site is illustrated in figure 10. The principal axis of elongation of the afferent receptive field centers is plotted versus the preferred orientation of layer IV cortical cells as measured from orientation tuning histograms. Data from all 16 penetrations in which the alignment significance is greater than 90% are included. The diagonal line (x=y) indicates the predicted result if cortical cell and afferent orientations were to match perfectly. In the majority of experiments the afferent receptive fields were plotted with no knowledge of the cortical cell orientation previously recorded in the penetration. This 'blind' procedure resulted in an equally good match between cortical cell and afferent orientations as was seen in the rest of the experiments. Although there are two sites where the mismatch between cortical cell orientation and afferent receptive field array axis is nearly 30 degrees, in 12 of 16 cases the match is closer than 10 degrees. Such a match is essentially perfect, since cortical cell orientation was measured in 10 degree steps.

Discussion

Receptive fields of individual geniculocortical afferents can be recorded in ferret area 17 following pharmacological suppression of cortical cell activity. Treatment of the cortex with either the glutamate agonist kainate which acts as an excitotoxin, or with the GABA agonist muscimol which mimics intracortical inhibition silences the action-potential firing of visual cortical neurons. Units recorded after such suppression of cortical activity are clearly geniculocortical afferents: They have the same receptive field properties as LGN neurons, they are recorded only in the geniculocortical input layers of cortex, and they respond quickly, without failures, and with consistent response latency to electrical stimulation of the LGN. That the two functionally distinct methods of silencing cortex result in identical recordings of LGN-like units provides further evidence that these units are in fact afferents rather than degraded responses of cortical cells. The recorded afferents appear likely to terminate in, and provide visual input to, the orientation column within which they were encountered. Although afferent fibers are known to pass through layer V, no afferent responses could be recorded within that layer in these experiments. This suggests that geniculocortical afferent action potentials are only large enough to record at their axon terminal arbors using the electrodes employed in this study.

Geniculocortical afferent receptive fields recorded in single radial penetrations through layer IV of ferret area 17 form arrays which often appear to be elongated in visual space. A Monte Carlo analysis confirms

that in 16 of 18 cases studied the afferent arrays from single penetrations were significantly aligned. The axis of this observed alignment closely paralleled the preferred orientation of cortical cells recorded in the same electrode penetration.

A surprising feature of several penetrations was the apparent lack of overlap between cortical cell receptive fields and afferent arrays. There are a number of possible explanations for this observed lack of overlap. First, it is possible that the animal's eyes may have moved between the time of recording the cortical cell receptive fields and the time that pharmacological silencing of cortical activity had progressed sufficiently to record afferent receptive fields. Note that this period of drug infusion was often many hours long, much longer than the period of actual recording of afferent receptive fields (during which time the animals eyes could not have moved, since the same receptive fields were recorded at the same depth in layer IV at the beginning of the recordings as the electrode was advanced and then again at the end of the recordings as the electrode was retracted). Eye position during the experiments was monitored by plotting optic disk location, and no eye movements were observed. The possibility of eye movements could not completely be ruled out because of the considerable uncertainty in plotting the location of the optic disk in the small eye of the ferret. Secondly, the lack of overlap could be due to the normal scatter of receptive fields of cortical cells recorded in a single penetration through layer IV. It is possible that the plotted cortical cell receptive field might be at one edge of this scatter while the afferents plotted might provide the input to a cell at the other edge. The cortical cells plotted, those at the top of layer IV, have receptive fields that are among the smallest found in area 17, and thus

tend to have the largest scatter between receptive fields (Hubel and Wiesel, 1974). In the cat, cells in layers V and VI have receptive fields 2-10 times the size of cells in the upper layers (Gilbert, 1977). Therefore afferents with receptive fields that do not overlap the receptive fields of the layer IV cell plotted may well provide inputs to neighboring cells in the same column. Finally, the non-overlapping cortical and afferent receptive fields might result from the necessary compression of the whole eye map onto the segregated ON- and OFF-center afferent patches seen in ferret cortex (Zahs and Stryker, 1988).

As discussed above, the methods used in this study cannot exclude the possibility that eye movements may have occurred between the times of cortical and afferent unit recordings. It is therefore possible that some of the mismatches seen in figure 10 between the cortical cell preferred orientation and the angle of the principal axis of the afferent receptive field arrays could be due to uncontrolled torsional eye movements.

The fraction of the geniculocortical afferent inputs to an orientation column that was recorded in these experiments is not known. In the present study we recorded between 8 and 30 afferent units per radial penetration through cortical layer IV. It is clear that not all the afferents present were studied, since additional afferents with electrical signals too small to be isolated were present at many recording sites (note the 20mV spikes in figure 2D). Comparisons between the numbers of geniculate cell receptive fields covering a region of visual space, the sizes of geniculocortical afferent arbors and the magnifications of geniculate and cortical maps of the visual field have suggested that in the cat, hundreds of geniculocortical afferent terminal arbors overlap a single vertical

electrode penetration (Martin, 1988; Humphrey et al., 1985). Although these calculations have not been done for ferret area 17, the afferents we recorded presumably represent only a sample of those present. Nevertheless there is no reason to assume that the sample encountered is not random with respect to receptive field position.

The observed match between the axis of elongation of the afferent receptive field array and the cortical cell orientation preference is consistent with Hubel and Wiesel's (1962) model of cortical simple cell orientation derived from geniculocortical input from LGN cells with receptive fields aligned in visual space. The present results cannot directly confirm this model, because they do not provide evidence about the geniculocortical afferents that converge onto a single cell. The afferent receptive field arrays recorded in this study indicate only that in most cases the aggregate geniculate input to a single orientation column is itself oriented, and that the orientation of geniculate input matches the orientation of cortical cells in the column.

A quantitative implementation of Hubel and Wiesel's (1962) model has shown that the orientation tuning seen in cortical cells could in principle be accounted for by geniculate inputs with aligned receptive fields (Ferster, 1987). An alternative model has shown that cortical cell orientation specificity could be produced by simple cells' receiving input from two LGN cells of different center type with slightly offset receptive fields (Heggelund, 1986). This model would not predict any alignment of geniculate inputs to a cortical orientation column.

The arrangement of geniculocortical afferent inputs to single cortical cells has been studied by several techniques. Intracellular recordings from simple cells revealed that both excitatory and inhibitory

post-synaptic potentials were greatest for elongated stimuli at the cells preferred orientation (Ferster, 1986), but these recordings did not reveal which of the excitatory potentials were of geniculate origin.

Cross-correlation analysis of LGN and cortical neuron responses has revealed little evidence for elongated collections of geniculocortical inputs to single simple cells, but only a small number of inputs to a given cell could be found, and the most powerful of these inputs accounted for only an average of 11% of the cortical cell's discharge, suggesting that each cortical cell receives at least 9 afferent inputs, and likely many more (Tanaka, 1983). Only if a comparable number of inputs had been found in the cross-correlation studies would it have been possible to determine whether the collection of inputs was significantly aligned.

The alignment of geniculocortical inputs may not be the sole basis of simple cell orientation. Other factors, including cross-orientation inhibition may also influence orientation selectivity. In two electrode penetrations in this study, cortical cell receptive fields were well oriented, but the afferent receptive field arrays were not elongated. Statistical analysis suggests that the lack of afferent receptive field alignment seen at these sites is real, not merely a result of sampling bias. It is possible that at such sites in cortex (figure 4 P and Q) orientation selectivity is primarily due to inhibitory inputs from cortical neurons at orthogonal orientations. At other sites where afferents are well aligned (e.g. figure 4A) cortical cell orientation may result primarily from excitatory geniculocortical inputs aligned in visual space. Such a range of influence of excitatory and inhibitory factors on orientation selectivity has been seen previously in studies of cross-orientation inhibition

(Pettigrew and Daniels, 1973; Sillito, 1975; Tsumoto et al., 1979; Sillito et al. 1980; Albus and Baumfalk, 1989; Eysel et al. 1989). When inhibitory inputs to oriented cells in cat visual cortex are abolished through application of GABA antagonists, results range from orientation selectivity somewhat reduced in 60% of simple cells (Albus and Baumfalk, 1989), to orientation selectivity abolished in 100% of simple cells (Sillito et al. 1980). Although some of the between- and even within-study variability in those results may be due to differences in the degree of GABA antagonism produced, it may also reflect a real difference in the degree of remaining orientation selectivity produced by different degrees of alignment of geniculate inputs at different sites in cortex.

Cross-correlation analysis of corticocortical connections has not revealed selective inhibition from orthogonal orientations (Toyama et al., 1981; Hata et al., 1988), but has shown surprisingly strong common inputs to orientation columns selective for the same orientation and separated by as much as almost 3mm (T'so et al., 1986). It is not clear whether cross-correlation techniques as applied have the sensitivity to detect relatively weak or diffuse inhibitory inputs (Moore et al., 1970), which may be powerful only in the aggregate (Hess et al., 1975).

Recent reports in the literature demonstrate that the use of particular kinds of visual stimuli can make LGN cells appear to be orientation selective (Vidyasagar and Urbas, 1982; Shou and Leventhal, 1989). This apparent geniculate orientation selectivity is unlikely to contribute to the orientation tuning of cortical neurons for two reasons. First, geniculate afferents show no orientation preference for stimuli such as the light bars used in this study. Figure 2A is representative of the orientation tuning histograms of the geniculocortical afferents we

recorded, and all recorded afferents responded to all orientations of light bars when tested by hand. Secondly, the apparent orientation selectivity of most LGN cells appears to merely reflect the fact that geniculate receptive fields are elliptical rather than perfectly circular. Thus high spatial frequency stimuli beyond the resolving power of the longer axis of the receptive field are resolved when the stimulus is rotated to be perpendicular to the shorter axis (Shou and Leventhal, 1989).

The existence of oriented collections of geniculate inputs to orientation columns in the adult cortex does not reveal the mechanism by which orientation selectivity is established during development. Although it is possible that the oriented arrays of afferents are present very early in development and drive the formation of cortical orientation columns by providing the cortical cells with oriented input, it is also possible that geniculate input is not oriented early on, and rather that intra-cortical circuitry is responsible for first establishing orientation columns. Anatomical tracing techniques show that long intracortical connections, similar to those that connect columns of same orientation preference in adults (Gilbert and Wiesel, 1989), are present during the time that orientation columns are developing (Luhmann et al., 1986; Calloway and Katz, 1990). If such specific intracortical circuitry causes the formation of orientation columns, activity-dependent models such as those that have been used successfully to simulate the development of ocular dominance columns (e.g. Miller et al., 1989) would suggest that the geniculate inputs would then rearrange by a Hebbian mechanism (Hebb, 1949) into the aligned arrangement found in the adult (Miller, 1990). Orientation selective cortical cells are present at birth in the monkey (Hubel, Wiesel and LeVay, 1977), develop without visual

experience in the cat (Hubel and Wiesel, 1963b; Sherk and Stryker, 1976), and appear from our preliminary studies to be present before the time of natural eye-opening in the ferret. Thus if the early development of cortical orientation selectivity is activity-dependent, it is likely to depend on spontaneous neuronal discharges rather than on visual stimulation.

Figure 1

Experimental set-up and cortical cell orientation tuning histograms. A radial microelectrode penetration through the depth of cortical area 17 is illustrated. Orientation tuning histograms for 4 single-unit recordings from cells located at the arrow tails are shown on the right. Each histogram shows the mean response in spikes per second to 3 presentations of 36 randomly interleaved orientations of moving light bars swept across the cell's receptive field. Orientation conventions are as described in figure 2. Following these recordings, the electrode was withdrawn to approximately the depth shown, just above layer IV. 50 mM muscimol was then superfused onto gelfoam surrounding the electrode to silence cortical cell activity prior to recording the responses of geniculocortical afferents.

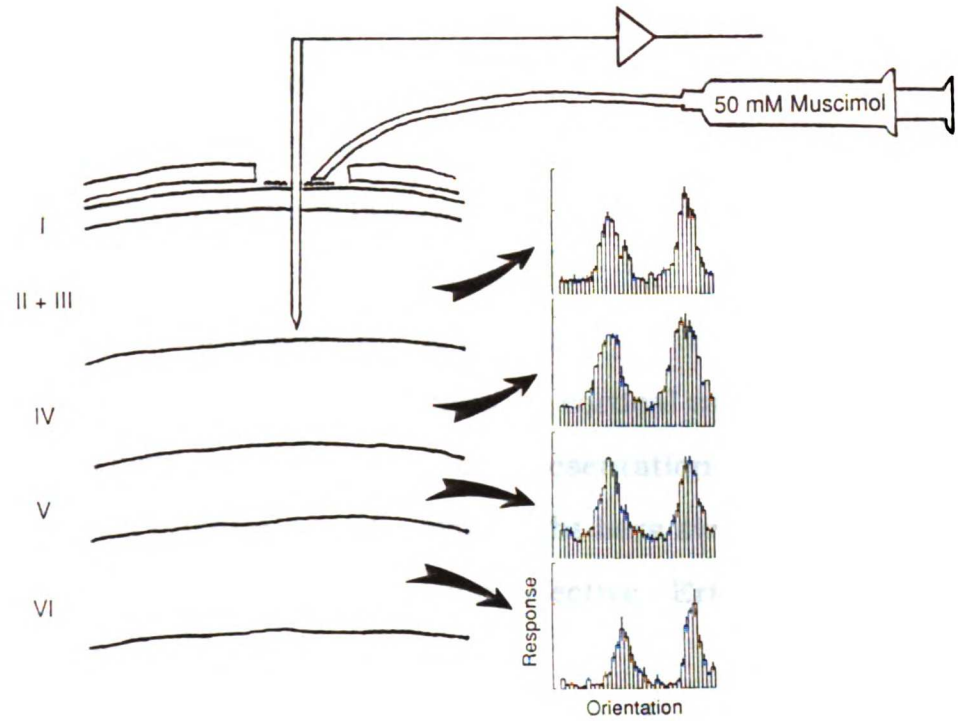

50 mM Muscimol
I
II + III
IV
V
VI
Response
Orientation

Figure 2

Geniculocortical afferent responses and location of afferent recordings. For orientation tuning histograms 0 represents a vertical bar moving leftward, 90 represents a horizontal bar moving downward.

A. Orientation tuning histogram for geniculocortical afferent. Mean response in spikes per second is shown to 3 presentations of 12 randomly interleaved orientations of moving light bars swept across the afferent's receptive field. The unit shows no orientation preference. Error bars indicate standard error of the mean.

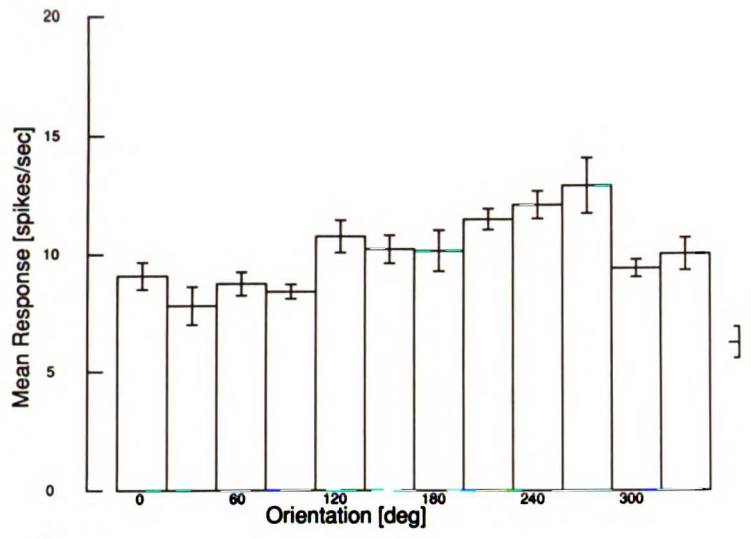
B. Orientation tuning histogram for an area 17 cortical unit. Mean response in spikes per second is shown to 3 presentations of 36 randomly interleaved orientations of moving light bars swept across the cell's receptive field. The unit is orientation selective. Error bars indicate standard error of the mean.

C. Stimulus size series for geniculocortical afferent. Mean response in spikes per second to 3 presentations of 10 randomly interleaved sizes of light spot flashed onto the afferent's receptive field. The afferent shows center-surround receptive field organization, responding best to a stimulus large enough to fill the center of the receptive field, and poorly to larger stimuli which impinge on the inhibitory surround of the field. Error bars indicate standard error of the mean.

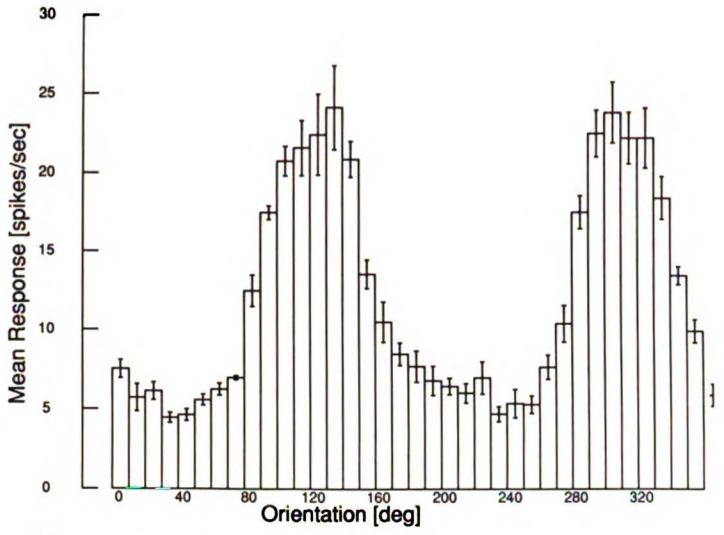
D. Visually and electrically driven action potential of a geniculocortical afferent. The top trace shows the response of an afferent recorded in cortical layer IV to the 450 Hz electrical stimulation of the LGN shown just below. The lower trace, also recorded in cortical layer IV, shows the response of an afferent to visual stimulation with a flashing spot of light. The visual stimulus was on continuously during the interval shown. The

unit studied here produced a particularly large electrical signal. Note that smaller spikes from other afferents are also evident in the visual response.

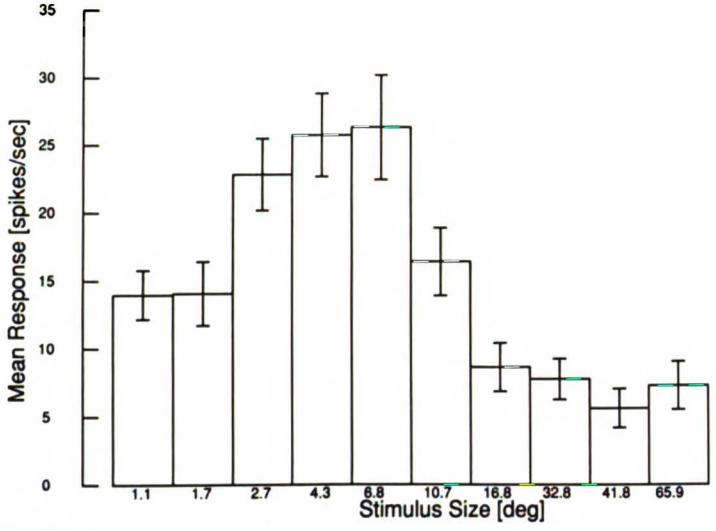
E. Histological reconstruction of electrode penetration. A photomicrograph of a nissl-stained section through area 17 is shown. The open arrows mark the upper and lower boundaries of the region from which afferent responses were recorded following muscimol superfusion. The filled arrow points to an electrolytic marking legion. Note that all afferent recordings were within layer IV. Scale bar is 200m.


Mean Response [spikes/sec]
0
5
10
15
20
0
60
120
180
240
300
Orientation [deg]

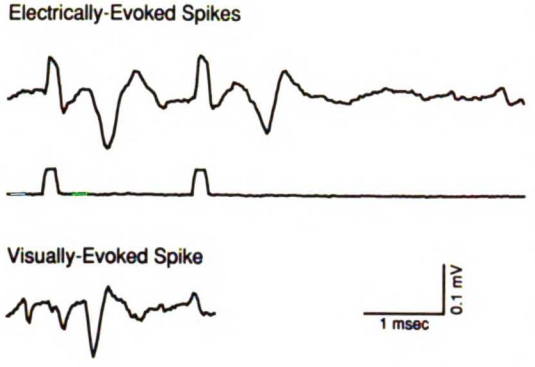
A


Mean Response [spikes/sec]
0
5
10
15
20
25
30
0
40
80
120
160
200
240
280
320
Orientation [deg]

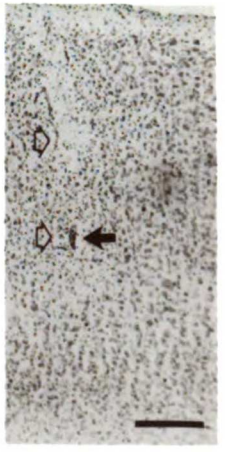
B


Mean Response [spikes/sec]
0
5
10
15
20
25
30
35
1.1
1.7
2.7
4.3
6.8
10.7
16.8
32.8
41.8
65.9
Stimulus Size [deg]

C


Electrically-Evoked Spikes
Visually-Evoked Spike
0.1 mV
1 msec

D



E

Figure 3

Receptive field positions of successively encountered afferents recorded in one microelectrode penetration. Numbers indicate the order in which the receptive fields were encountered as the electrode advanced down through layer IV; arrows connect successively encountered receptive fields. No orderly progression of receptive fields across visual space is seen, indicating that the elongated arrays of afferents found do not merely reflect microtopography of the retinotopic map on the cortex. Scale bar = 2 degrees.

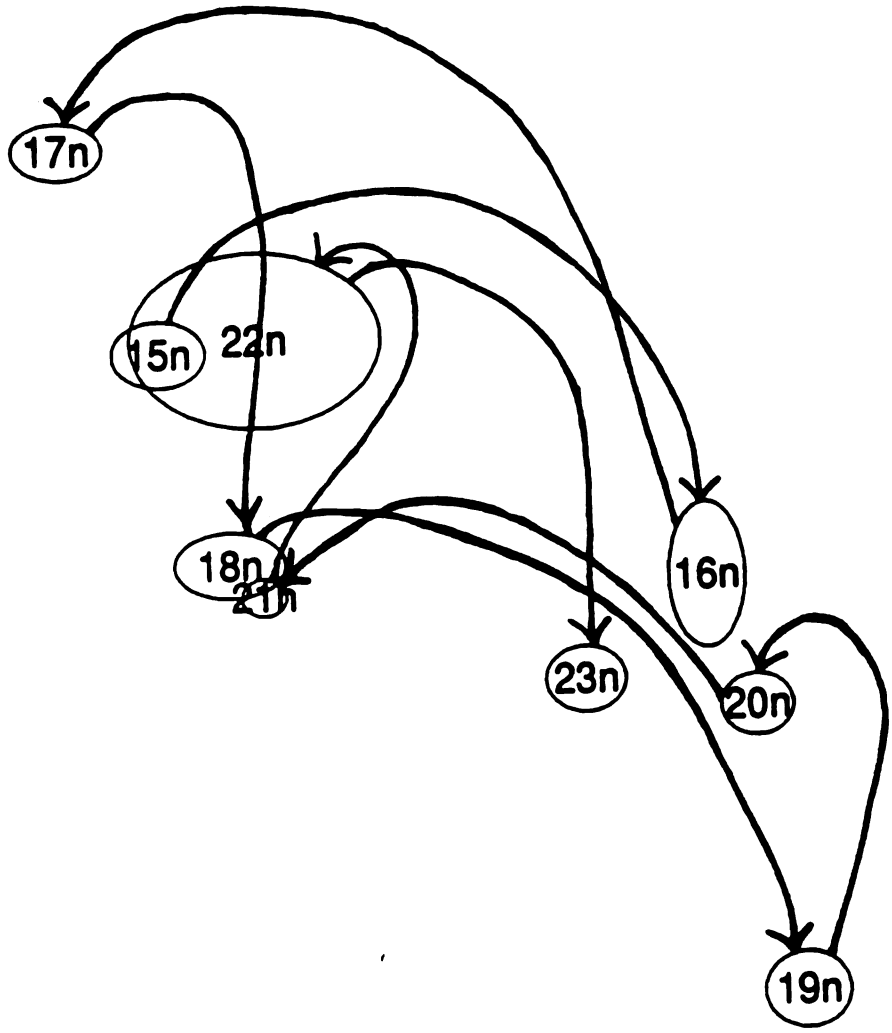
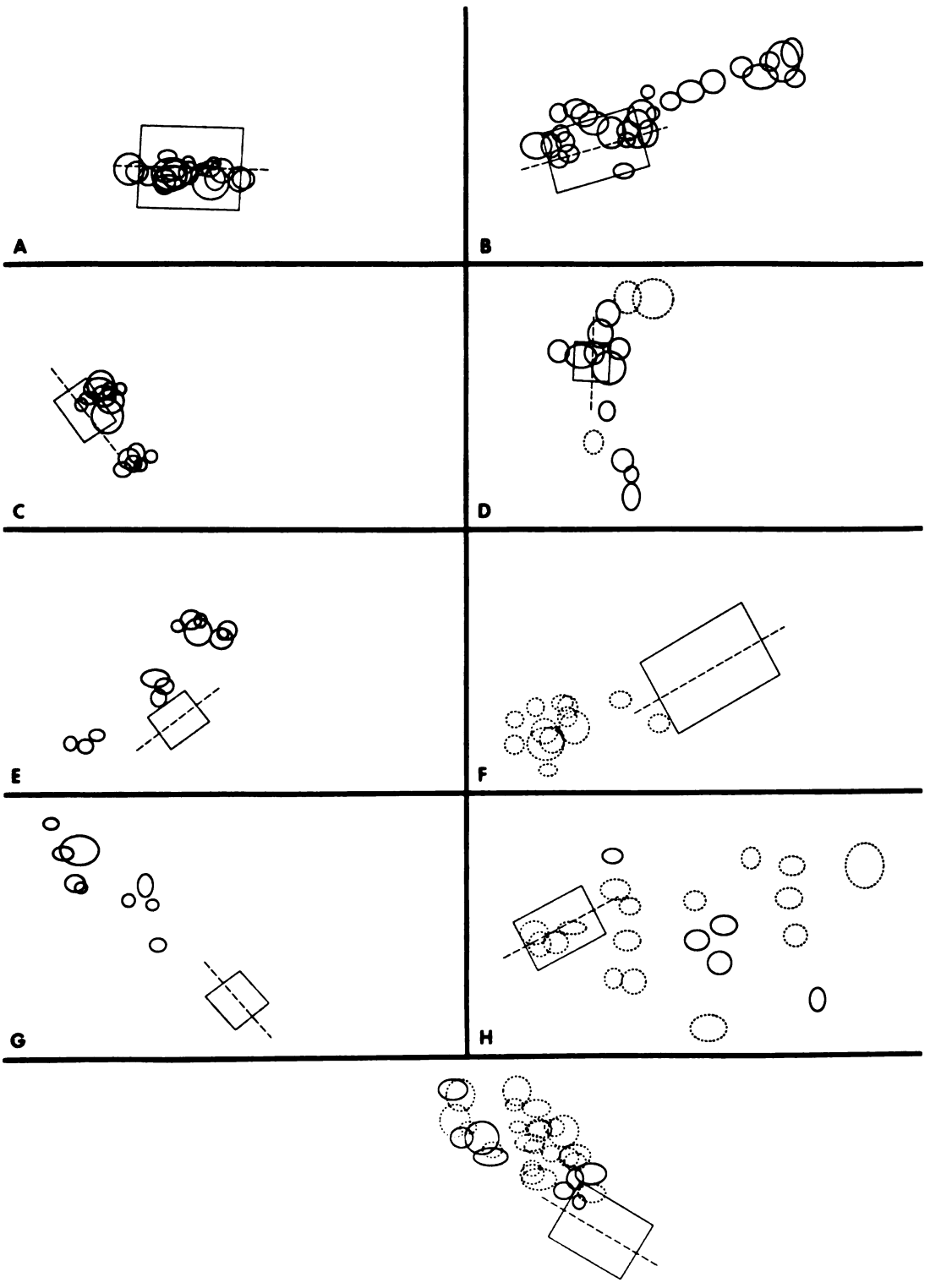

17n
15n
22n
18n
21n
16n
23n
20n
19n

Figure 4

Receptive fields of cortical cells and afferents recorded in all 17 microelectrode penetrations. On-center afferents are shown as solid ovals, off-center afferents as dashed ovals. For each penetration the receptive field of a cortical cell recorded at the top of layer IV is shown as a rectangle, with its preferred orientation, determined from its orientation tuning histogram, shown as a dashed line. Scale bar = 2 degrees.


A
B
C
D
E
F
G
H
I

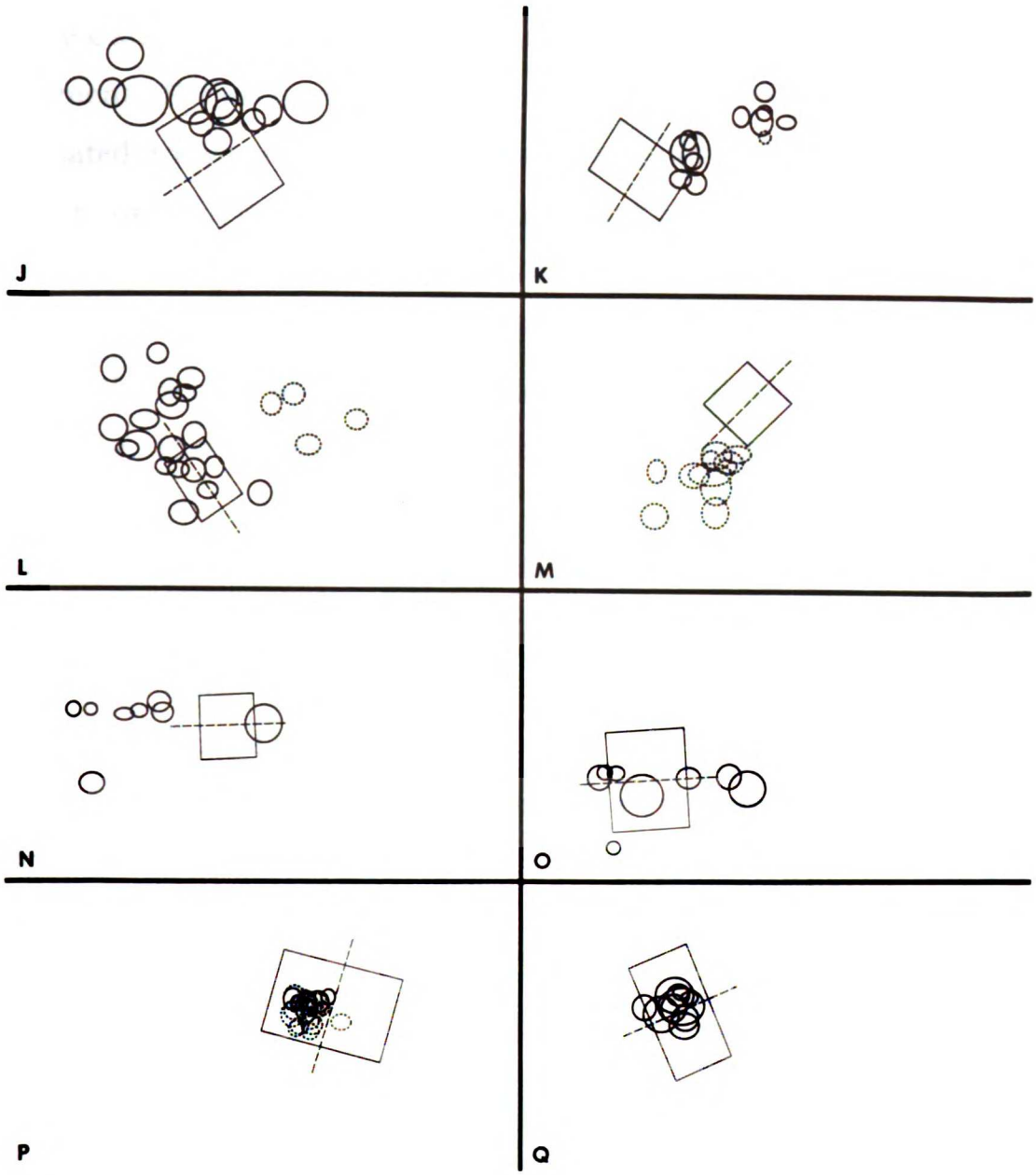

J
K
L
M
N
O
P
Q

Figure 5

Monte Carlo analysis of the significance of afferent receptive field alignment. For each set of recorded afferent receptive fields, 10000 simulated arrays of receptive fields were generated by assigning receptive fields to random locations while maintaining each field's distance from the center of the array. r^2 was then calculated for each of the simulated arrays. The histograms show the distributions for r^2 obtained in 3 cases. The arrows point to the r^2 obtained from the experimental data in each case. Insets show the experimentally obtained afferent receptive field locations.

A. For arrays which appear clearly elongated, r^2 for the experimental data falls well outside of the distribution of values of r^2 for the simulated random arrays.

B. An intermediate case.

C. For a clustered array, the experimental r^2 falls well within the distribution.

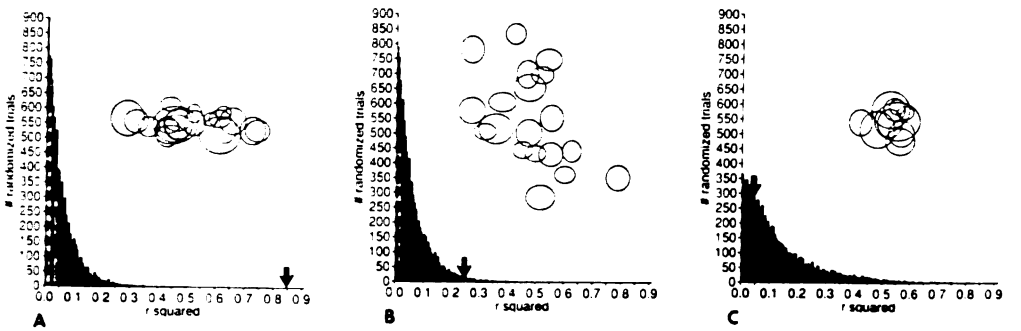

randomized trials
r squared
A
B
C

Figure 6

Alignment significance for all 18 afferent receptive field arrays.

The Monte Carlo measure of the significance of receptive field alignment is the percentile occupied by the experimental r^2 in the distribution of r^2s obtained in the simulation illustrated in figure 5. This alignment significance is shown for the 18 afferent receptive field arrays analyzed in this study. 16 of 18 arrays are significantly aligned with greater than 90% confidence.

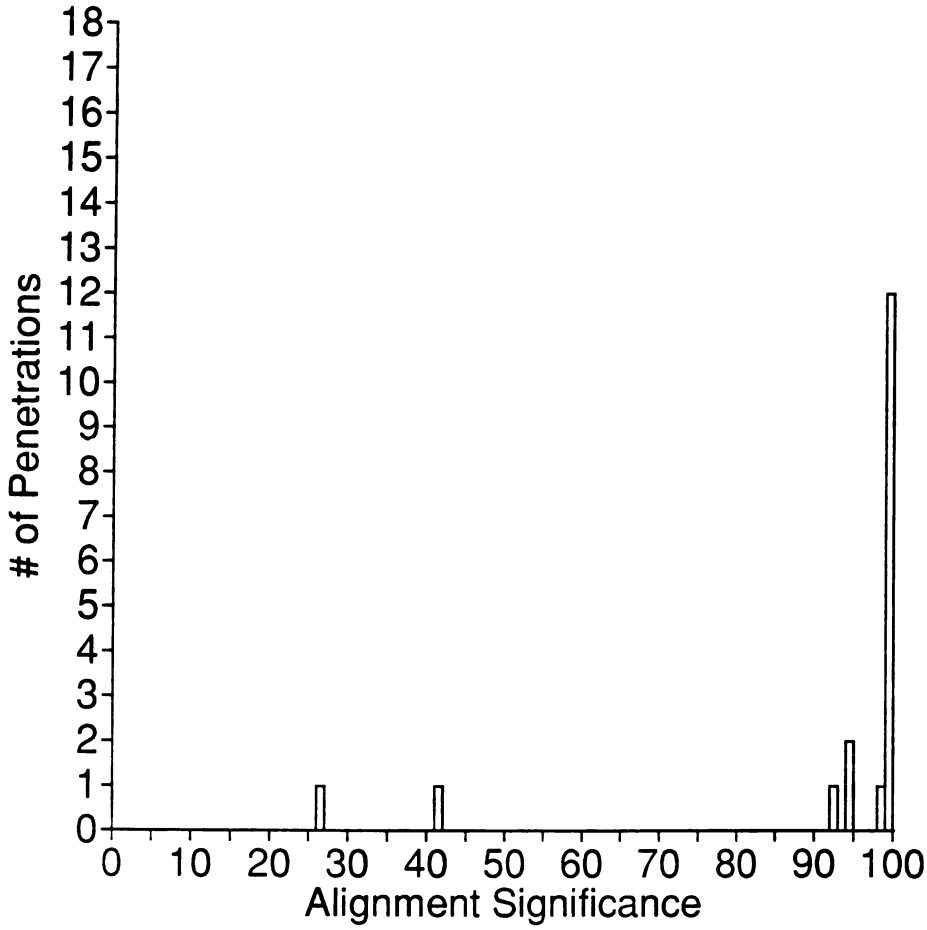

# of Penetrations
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
0 10 20 30 40 50 60 70 80 90 100
Alignment Significance

Figure 7

Relationship between alignment significance of afferent receptive fields and orientation tuning of cortical cells recorded at the same site. Alignment significance is plotted against mean half-width at half-height for supragranular cortical cells recorded in each penetration. There appears to be no relationship between the degree of alignment of afferents and the strength of cortical orientation tuning. Note that the cortical cells are nicely tuned at the 2 locations where afferent receptive field arrays are not elongated.

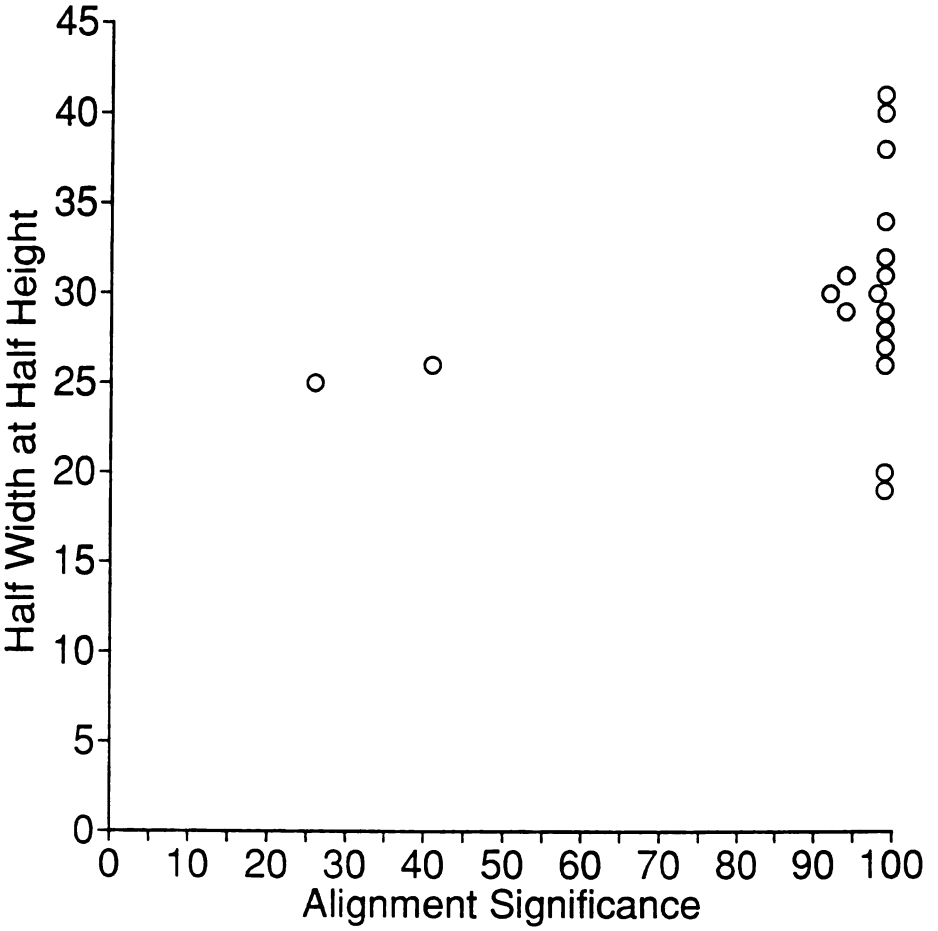

Half Width at Half Height
45
40
35
30
25
20
15
10
5
0
0 10 20 30 40 50 60 70 80 90 100
Alignment Significance

Figure 8

A. A universe of possible afferent receptive field locations was constructed from the 18 afferent receptive field arrays obtained in this study, plus 12 afferent receptive field arrays from a previous study (Zahs and Stryker, 1988). Afferent receptive field locations were normalized for magnification factor by calculating the mean radius of the receptive fields encountered in each penetration, and then multiplying the distance of each receptive field center from the center of the array by the average mean radius for all penetrations divided by the mean radius for that particular penetration. All the arrays were placed in register such that their principle axes of elongation were line up horizontally and their geometric centers were superimposed. Afferent receptive field centers (not the whole fields) are shown. Receptive field centers from penetrations where afferents were significantly aligned are shown as open circles, unaligned arrays are shown as filled circles.

B. Universe of possible afferent receptive fields constructed as in figure 8A, excepting that only the 18 afferent arrays from this study were used, and each array is rotated to align the preferred orientations of cortical cells rather than the principal axes of the afferent arrays.
Tic-marks represent 1 degree of visual angle.

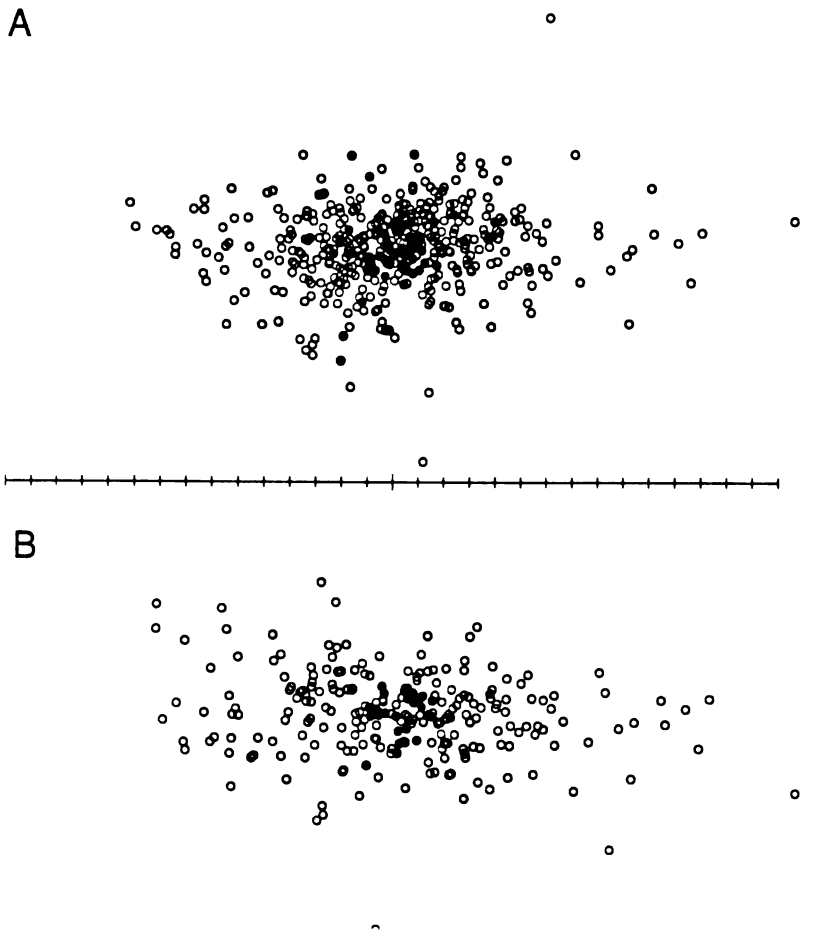

A
B

Figure 9

Alignment significance for experimental afferent arrays, and for simulated arrays of afferents randomly chosen from an aggregate afferent array universe. Random samples of afferents were drawn from the universe shown in figure 8A, with the sizes of samples matched to the numbers of afferents recorded in the experimental arrays. Each experimental array was represented by 10 size-matched samples in the simulation. Alignment significance was calculated for each real array (normalized for cortical magnification as described in figure 8 legend) and each simulated array. Cumulative percentages of penetrations are plotted at each alignment significance for the real data (closed symbols) and the simulated data (open symbols). The percentage rises faster for the real data, showing an over-representation of sites with low alignment significance in the real data as compared to the simulation. This suggests that 2 recorded sites where afferents were clustered are not due to sampling bias.

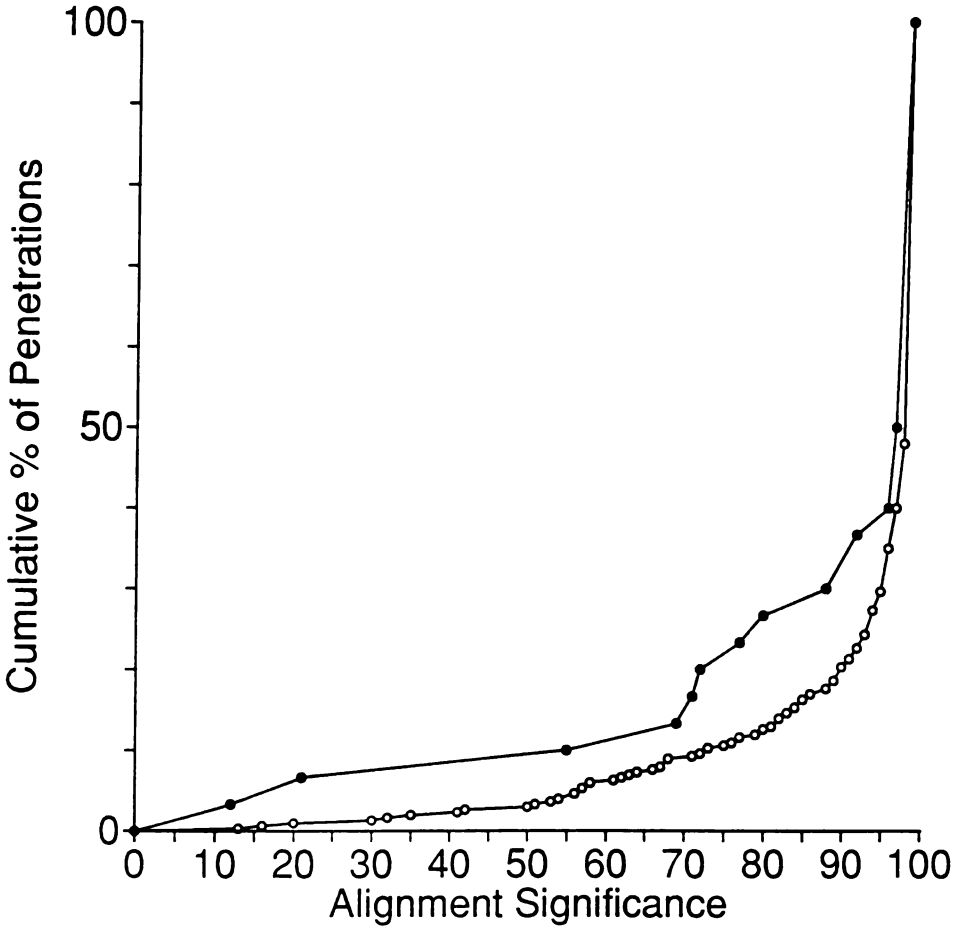
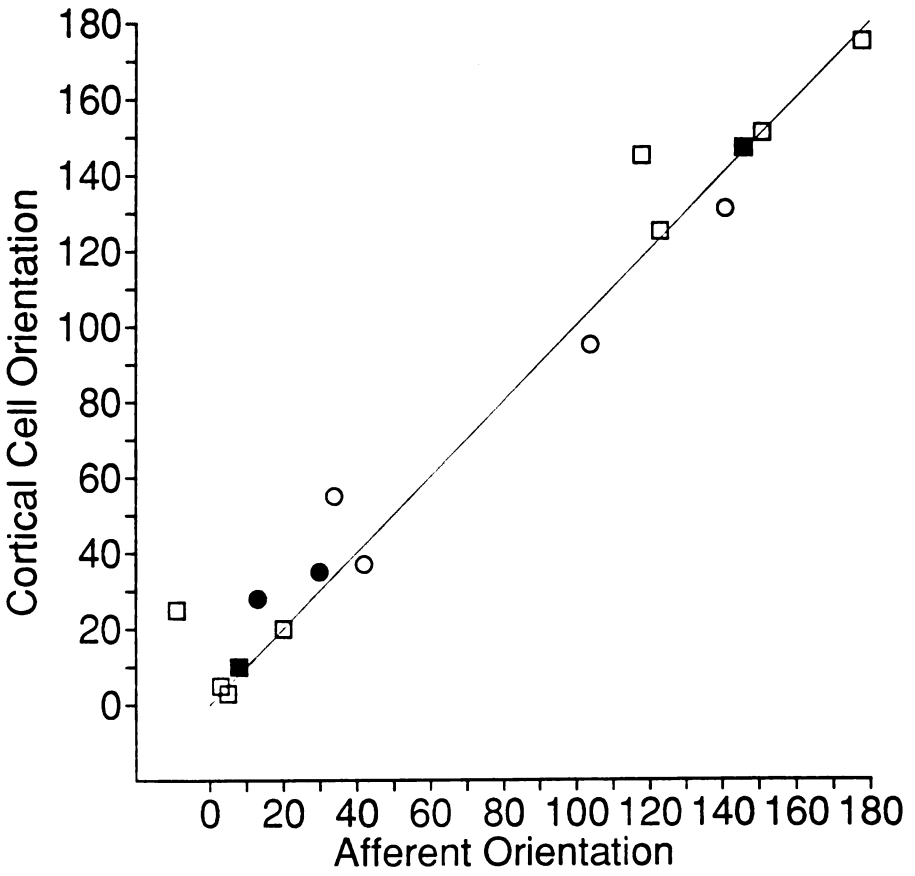

100
50
0
Cumulative % of Penetrations
0 10 20 30 40 50 60 70 80 90 100
Alignment Significance

Figure 10

Correspondence between the cortical cell orientation preference and the angle of the principle axis of afferent receptive field arrays. Orientations are plotted following the normal mathematical conventions for angles: zero degrees represents horizontal, with other orientations measured counterclockwise from horizontal. Open symbols represent on-center afferents; filled symbols represent off-center afferents. Squares represent data collected under the blind protocol, circles represent data collected when the experimenter plotting afferent receptive fields knew the orientation preference of the cortical cells at that site. The diagonal line (x=y) indicates the predicted result if cortical and afferent best orientations matched perfectly.


Cortical Cell Orientation
180
160
140
120
100
80
60
40
20
0
0 20 40 60 80 100 120 140 160 180
Afferent Orientation

CHAPTER 4

Development of orientation-specific neuronal responses in ferret primary visual cortex

Abstract

The orientation selectivity of cells in ferret primary visual cortex during normal development and in animals deprived of vision or of visual cortical activity was studied. In normal animals from the age when visual responses were first recorded (postnatal day 23) through postnatal week 5, only about 25% of cells showed orientation-selective responses. By week 7, cortical responses had matured to an adult-like state with approximately 75% of cells clearly selective for orientation.

Silencing visual cortical cell action potentials early in development by infusing tetrodotoxin intracortically blocked the maturation of orientation selectivity. Visual deprivation by bilateral lid suture also impaired maturation of orientation selectivity, but only in cortical layer VI: binocularly deprived animals showed normal development of orientation specificity in supragranular layers.

We conclude that the maturation of orientation selectivity in ferret visual cortex requires cortical neuronal activity, and that normal development requires visually driven activity.

Introduction

Neurons in the primary visual cortex of higher mammals respond best to stimuli consisting of light-dark borders presented at particular angles in the visual field. The development of this cortical orientation specificity has been widely studied, with somewhat conflicting results reported in the literature. Orientation-specific responses appear to be present at birth in the macaque monkey (based on a small number of cells in one animal; Wiesel and Hubel, 1974). In the cat, there is some disagreement as to the degree of orientation specificity found in cells in striate cortex of very young individuals. In kittens studied near the end of the first postnatal week, before the time of natural eye-opening, the reported percentage of orientation-selective cortical cells ranges from zero (Barlow and Pettigrew, 1971; Pettigrew, 1974) through 25-30% (Blakemore and Van Sluyters, 1975; Buisseret and Imbert, 1976; Fregnac and Imbert, 1978; Albus and Wolf, 1984), to 100% (Hubel and Wiesel, 1963b). These discrepancies may be due to the difficulties involved in electrophysiological recording in such young animals. The cells in area 17 of one week old kittens have low spontaneous activity, respond sluggishly to stimulation, and habituate rapidly (Hubel and Wiesel, 1963b). These response characteristics make it difficult to determine a cell's orientation specificity. An additional problem in recording from young animals is the fragility of the preparation. The specificity of responses of kitten visual cortical neurons is dependent on the physiological condition of the animal: small changes in blood pressure or expired carbon dioxide levels can make a previously

orientation-selective cell become non-specific or unresponsive (Blakemore and Van Sluyters, 1975). These difficulties in recording would tend to decrease the reported percentage of orientation-selective cells, suggesting that studies showing no orientation specificity in very young kittens may be invalid (Stryker, 1977).

The cat therefore has two major disadvantages as a model system for studying the development of cortical cell orientation selectivity. First, it is difficult to maintain young kitten visual cortex in an adequately responsive state to judge the degree of selectivity of cortical neuron responses. Secondly, orientation-specific responses may be already present at birth, making it difficult to perform experimental manipulations during the time that orientation specificity is developing. These disadvantages could potentially be avoided by studying development of orientation selectivity in the visual cortex of the pigmented ferret. The ferret visual system is quite similar to that of the cat (Law et al., 1988), but the ferret is born approximately three weeks earlier in development (Linden et al., 1981). The ferret might thus provide a system in which the development of cortical orientation selectivity occurs after birth, and individuals are relatively hardy when orientation-selective cortical responses first develop.

We have studied the normal development of orientation selectivity in the ferret by recording the responses of primary visual cortex neurons to moving oriented bars of light in animals at different ages. In order to determine whether the development of orientation-selective responses was dependent on the presence of neuronal activity, as is the development of normal ocular dominance in visual cortex (Stryker and Harris, 1986), we studied ferrets deprived of cortical activity by the

infusion of the sodium-channel blocker tetrodotoxin (TTX) directly into area 17. To study the role of visual experience in development of orientation selectivity, we examined animals deprived of vision by binocular lid suture from before the time of natural eye opening.

Methods

Animals

Pigmented ferret adults and timed-pregnant females were obtained from Marshall Farms (New Rose, NY). For the studies of normal development, a total of 19 animals ranging in age from postnatal day 23 through adult were studied. In addition, data are presented from 8 adult ferrets used in a previous study (Waitzman and Stryker, unpublished). For the study of effects of activity blockade on orientation development 7 animals were used. The visual deprivation study involved 2 animals. Animals were housed in the University of California at San Francisco animal care facility under a light-dark cycle of 18 hours light, 6 hours dark.

Surgery

The techniques of surgery, anaesthesia and monitoring used on animals 6 weeks old through adult were described previously (Chapman et al., 1991). Certain modifications in these procedures were necessary in animals under 6 weeks old. The young animals were initially anaesthetized with an intramuscular injection of 0.04 mg/kg acepromazine and 40 mg/kg ketamine, and received 0.4 mg atropine sulfate to prevent breathing difficulties from mucous accumulation. Due to the small size of the femoral vein in these young animals, a jugular vein cannulation was performed to allow for maintenance of anaesthesia with intravenous thiopental sodium. Tracheal cannulation, scalp incision, cisternal puncture and craniotomy were done as in the older

animals. Since the animals' skulls were not yet fully calcified, they could not be held using ear bars in a stereotactic frame, but rather were held by a metal rod attached to the skull rostral to the craniotomy using self-tapping stainless steel screws (Small Parts, Inc.) and dental cement (Fastcure, Kerr Laboratory Products). Due to the young animals small lung volume, peak expired carbon dioxide could not be monitored. Therefore, these animals were allowed to breath freely rather than being paralyzed and respirated. This allowed for direct monitoring of anaesthetic state by testing for withdrawal reflex to a hard pinch of the toes. Heart rate was monitored throughout the experiments, and temperature was maintained at 37.5 degrees using a feedback system. In most cases, eyes were immobilized using eye rings attached to the conjunctiva near the scleral margin with 8-0 ophthalmic silk (Ethicon). In other animals, eye movements could be monitored during the recording session by watching the computer-generated raster of times of unit firing; if eye movements occur, the position of the receptive field outlined in the raster moves. Data were discarded from any stimulus run during which the eyes moved. Atropine sulfate (2%) drops were administered to dilate the pupils, and the eyes were covered with 30K silicone oil to protect the cornea.

Tetrodotoxin Treatment

7 ferrets, aged 21-23 days, were anesthetized with Halothane (1-2%) in a mixture of 2:1 nitrous oxide: oxygen. Under aseptic conditions, the scalp was retracted, and a 30 gauge stainless steel cannula was implanted through the skull into visual cortex in one hemisphere. The cannula was located approximately 8 mm rostral to the caudal pole and

3 mm lateral to the midline, and angled approximately 30 degrees caudally in the parasaggital plane. The cannula was immobilized and attached to the skull using dental cement (Fastcure, Kerr Laboratory Products). Attached to the cannula was an osmotic minipump (Alzet 2002, Alza) which was implanted under the skin between the animals shoulder blades. The minipump delivered 0.5 ul/hr 5 uM TTX (6 animals) or 0.9% NaCl (1 animal) through the cannula into visual cortex. Following surgery, animals were returned to their mothers. The osmotic minipumps have a pumping duration of 14 days; after 12-14 days, pumps were replaced under aseptic conditions, and the infusion was continued to produce a total of 23-26 days of TTX treatment. In 2 TTX-treated animals, electrophysiological recordings were then performed immediately in order to determine the area of activity blockade in the cortex produced by the TTX infusion. Osmotic minipumps were removed from the other animals, and the animals survived an additional 3-4 days in order to allow the TTX to wear off and neuronal activity to recover before electrophysiological recordings were performed.

Binocular Deprivation

Two 30 day old ferrets, whose eyelids had not yet opened, were anaesthetized with Halothane (1-2%) in a mixture of 2:1 nitrous oxide: oxygen. Both eyelids were gently pulled apart, the lid-margins were surgically removed, and the eyelids were sutured shut. The animals were returned to their mother and allowed to mature under a normal light cycle to an age of 88 or 90 days, at which time electrophysiological recordings were performed

Electrophysiological Recording

Extracellular recordings were made in ferret primary visual cortex using laquer-coated tungsten electrodes (Hubel, 1957). Microelectrode penetrations were radial or angled in the coronal plane. Cortical cell receptive fields were studied at approximately 100 um intervals. Receptive fields were first plotted by hand using a Zeiss lamp. All of the units recorded in these studies had centrally located receptive fields, within 15 degrees of area centralis. An orientation tuning histogram was compiled for each cell using a computer driven display of moving light bars swept across the receptive field. The light bars were presented at either 12 (for 4 young animals from this study and the 8 adult animals from a previous study) or 36 (for all other animals) different orientations. There were 3 repetitions of each stimulus, and presentations were randomly interleaved. For each stimulus condition the average firing rate of the cell was determined across the portion of the sweep where firing rate was elevated. The spontaneous activity of each cell in the absence of visual stimulation was also determined.

Data Analysis

Prior to analysis of orientation tuning, the spontaneous firing rate for each cell was subtracted from the firing rates in response to stimulation. Since orientation tuning is a circular function with a period of 180 degrees, the orientation selectivity of a neuron can be determined by taking a Fourier transform of the data from the orientation tuning histograms and examining the amplitude of the second harmonic component (Worgotter and Eysel, 1987). The amplitude at the second harmonic was normalized by dividing by the sum of the DC level (average

elevation in firing rate of the cell produced by the stimuli) and the amplitude of the second harmonic component. This number was then multiplied by 100 to produce the orientation selectivity index (OSI) which varies from 0 (no orientation specificity) to 100 (very tightly tuned for orientation). Examples of orientation tuning histograms labeled with their calculated OSIs are shown in figure 1. Tuning curves with OSIs of less than 25 show little if any evidence of orientation specificity.

To determine whether the two different stimulus presentation paradigms used in this study had an effect on the OSI which would be calculated for a given cell, orientation tuning histograms from a set of 50 neurons spanning the whole range of orientation selectivities were examined. The neurons, which had originally been stimulated with 36 different orientations of moving light bars at 10 degree intervals were re-analyzed looking only at the responses to 12 orientations at 30 degree intervals. The orientation selectivity indices for each cell determined with these two analyses were then compared. In no cell was the difference in the calculated index for the two conditions more than 10, and in all but 4 cells the difference was less than 5. There was no bias toward higher numbers in either analysis. Additional evidence for the lack of effect of the stimulus presentation paradigm on the calculated degree of orientation specificity was provided by a comparison of the distribution of OSIs calculated for 80 neurons where stimuli were presented at 10 degree intervals in the two adult ferrets used in this study with the distribution calculated for the 74 neurons where stimuli were presented at 30 degree intervals in 8 adult ferrets from a previous study (Waitzman and Stryker, unpublished). These two distributions were statistically indistinguishable from each other (Mann-Whitney U

test, $p = 0.26$).

Histology

After the electrophysiological recording sessions, animals were given an over-dose of pentobarbitol (50-100 mg/kg) administered intravenously, and were perfused intracardialy with 0.9% saline followed by 10% formol saline. The brain was blocked and the occipital cortex was post-fixed in 10% formol saline. 50 um coronal sections were cut on a vibratome, mounted on slides and stained with cresyl violet in order to visualize electrode tracks and electrolytic marking lesions.

Results

Normal Development of Orientation Selectivity

The earliest age at which visually evoked neuronal responses could be recorded in ferret primary visual cortex was postnatal day 23. In an additional 4 animals, aged between postnatal day 20 and postnatal day 22, spontaneously active cells could be recorded, but did not respond to visual stimulation. At postnatal day 23, and continuing through postnatal week 5, the majority of cells in area 17 were not tuned for orientation, with about 75% of cells having orientation selectivity indices of less than 25. Even at the age when cortex was first responsive, however, some cells showed quite strong orientation tuning (the maximum OSI at postnatal day 23 was 47). The distribution of orientation selectivities of cortical cells began to change during postnatal week 6. At this time, only about 35% of cells remained untuned for orientation (OSI less than 25), and some cells had very tight orientation tuning (the maximum OSI was 63). By postnatal week 7 the distribution of orientation tuning was statistically indistinguishable from that seen in adults (Mann-Whitney U test, $p = 0.28$). In adult ferret primary visual cortex, about 25% of cells are unselective for stimulus orientation. The distributions of the orientation selectivity index for neurons in area 17 of ferrets of different ages are shown in the histograms in figure 2. These distributions are replotted as cumulative percentages of cells at each OSI in figure 3 in order to facilitate comparisons between the different distributions.

Effects of Activity Blockade and Visual Deprivation

In preliminary experiments in two animals, intracortical infusion of tetrodotoxin (5 uM, 1 ul/hr) starting during postnatal week 4 and continuing through postnatal week 7 was found to silence neuronal action potentials in an area extending approximately 10 mm caudally from the cannula tip, with a lateral-medial extent of approximately 5 mm (data not shown). The cortical surface within the silenced area was slightly lighter in color than the surrounding cortex, with good correspondence between the edges of the blanched region and the edges of the electrophysiologically assessed activity blockade. In later experiments where the TTX was allowed to wear off for several days before recording, the blanched area of cortex was still seen. In order to ensure that the cells recorded in these experiments had been previously silenced by the TTX treatment, all penetrations were made well within the blanched area.

TTX treatment begun during postnatal week 4 (when cortical cells are poorly selective for orientation) and continued through postnatal week 7 (when cortical cells would normally have an adult-like distribution of orientation tuning) appeared to completely prevent the development of orientation selectivity. The distribution of orientation selectivity seen in TTX-treated cortex was very similar to the distribution seen in normal 4 week old animals (figure 4A and B; Mann-Whitney U test $p = 0.46$). In contrast, orientation selectivity had developed normally in these animals' opposite (untreated) cortical hemispheres (figure 4C), and in cortex treated with 0.9% saline (figure 4D).

Visual deprivation by binocular lid suture begun before the time of natural eye opening and continued through postnatal week 13 had less

severe effects on development of orientation selectivity than did actvity blockade. Approximately 50% of cells in binocularly deprived animals were unselective for orientation (orientation selectivity index less than 25) compared to 75% untuned cells in 4 week old normal or in TTX-treated animals and 25% in normal adults. A few of the best tuned cells in binocularly deprived animals were as well orientation-tuned as those in normal adults. Histograms showing the distributions of orientation selectivity found in TTX-treated, binocularly deprived and normal animals are shown in figure 5. For ease of comparison of the distributions, these data are replotted as cumulative percentages of cells versus orientation selectivity index in figure 6.

The results described above are not dependent on the particular analysis of orientation tuning that we have used. The differences in orientation specificity between age and treatment groups are evident from the orientation tuning histograms themselves. This is illustrated in figure 7, which presents two orientation tuning histograms from each group. One histogram represents the average selectivity (median OSI), and one represents high selectivity (90th percentile OSI) for that group of animals. Both of the tuning histograms from the adult and from postnatal week 7-8 animals show more orientation selectivity than the corresponding histograms from postnatal week 6 animals, which in turn are more tightly tuned than the histograms from postnatal week 4-5. Histograms from TTX-treated ferrets are similar to those from week 4-5 animals, and histograms from binocularly deprived animals are similar to those from postnatal week 6.

Since visual cortical cells are known to become poorly responsive and to lose their stimulus specificity when the animal's health is

compromised or when physiological recording conditions are suboptimal (Blakemore and Van Sluyters, 1975), it was important to examine the responsiveness of cells in TTX-treated and in binocularly deprived animals. If the lessened orientation selectivity of cells in these animals were due to lessened responsiveness (which could be caused by possible non-specific damage to cortical cells or their connections by the experimental treatments), then the least responsive neurons would be expected to be the least well orientation-tuned. This relationship was not found. Figure 8 shows each neuron's maximum response to visual stimulation plotted against its orientation selectivity index for several age groups of normal ferrets and for the TTX-treated and binocularly deprived animals. In no group is there any significant correlation between orientation selectivity and responsiveness (regression analysis, $p > 0.25$ in all cases). In addition, the distribution of firing rates for TTX-treated cortex is similar to that seen in normal animals at the age at which the treatment started, and the distribution for binocularly deprived animals is similar to that seen in age-matched controls. Therefore activity blockade and visual deprivation do not noticeably impair the responsiveness of visual cortex, but instead appear specifically to interfere with the development of orientation selectivity.

Laminar Analysis of Orientation Selectivity

Electrode penetrations were reconstructed from the histological sections of brains from different age groups and from TTX-treated and binocularly deprived ferrets in order to determine in which cortical layer each recorded cell was located. The percentages of cells recorded in each lamina were fairly constant across animal groups, with the most cells

recorded in layers II/III and layer VI, somewhat fewer cells recorded in layer IV and many fewer recorded in layer 5 (see table 1). This pattern is probably a reflection of the relative thickness of the cortical laminae, since neurons were recorded at approximately 100 um intervals in all electrode penetrations. The distribution of orientation selectivity found in each cortical layer within each group was determined. These distributions for layers II/III, IV, and VI are shown in figure 9. Data are not shown for layer V due to the small numbers of cells recorded there. In postnatal week 4-5 ferrets and in TTX-treated ferrets, where the majority of cells were unselective for orientation, and no very tightly-tuned responses were recorded, there were no significant differences seen among the distributions of orientation selectivity in the different laminae (figure 9A and E). During postnatal week 6, cells in the supragranular layers have become significantly more orientation selective than cells in layer IV or layer VI (figure 9B). This same pattern is seen in the binocularly deprived animals (figure 9F). By postnatal weeks 7-8 (figure 9C), and through adulthood (figure 9D), the distribution of orientation selectivity found in layer VI matches that of the supragranular layers, while cells in layer IV remain relatively poorly tuned for orientation, with a distribution only slightly more orientation-selective than that seen in postnatal week 4-5 animals.

Discussion

Visually driven responses could first be recorded in ferret primary visual cortex on postnatal day 23, when the ferret visual system is at a developmental stage equivalent to the day of birth in the cat (Linden et al., 1981). These recordings are thus almost a week earlier in development than the earliest reported visual responses in kitten cortex, which were recorded near the end of the first postnatal week (for review see Fregnac and Imbert, 1984; Movshon and Van Sluyters, 1981). Neurons in the postnatal day 23 ferret cortex were quite responsive and could routinely be recorded at 100 um intervals along an electrode penetration (table 1). This result is in sharp contrast to the reports of the recordings in 1-2 week old kitten cortex, where visual responses were difficult to elicit and habituated markedly, allowing for the recording of only a few cells in each electrode penetration from cortical surface down to white matter (Hubel and Wiesel, 1963b; Pettigrew, 1974; Blakemore and Van Sluyters, 1975; Buisseret and Imbert, 1976; Albus and Wolf, 1984; Braastad and Heggelund, 1985).

The orientation selectivity of neurons in very young ferret primary visual cortex is similar to that seen in the majority of studies of young kitten visual cortex. In the ferret during postnatal weeks 4-5, approximately 75% of cells lacked orientation specificity (figures 2 and 3), comparable to the 70-75% of cells reported to lack orientation selectivity in kitten cortex at the end of the first postnatal week (Blakemore and Van Sluyters, 1975; Buisseret and Imbert, 1976; Fregnac and Imbert, 1978; Albus and Wolf, 1984; but see also reports of

significantly higher (Hubel and Wiesel, 1963b) and lower (Barlow and Pettigrew, 1971; Pettigrew, 1974) percentages). The relatively poor orientation selectivity seen early in development in both species is unlikely to be due merely to the poor optical quality of young animals' eyes, since orientation selectivity in adults appears to be invariant when the retinal image is severely blurred (Bonds, 1979). In addition, some cells in young animals do show fairly strong orientation tuning, suggesting that the maturation of orientation tuning occurs centrally rather than merely reflecting maturation in the periphery.

The laminar distribution of responsiveness and orientation selectivity in the young ferret may be different than that seen in the cat. In postnatal week 4-5 ferret cortex, cells are responsive (table 1) and equally (poorly) orientation-selective (figure 9) in all cortical layers. Cells in young kitten cortex have been reported to be most responsive and most selective either in layer IV (Albus and Wolf, 1984) or in layer V (Tsumoto and Suda, 1982). The disagreement between these two studies, taken together with the fact that both studies show good correlation between responsiveness and selectivity, suggest that the reported laminar differences in orientation selectivity in the cat may merely reflect poor responses due to the fragility of the preparation.

The development of orientation selectivity appears to follow approximately the same time course in the ferret as it does in the cat. The distribution of cortical cell orientation specificity has reached an adult-like level by postnatal week 7 in the ferret (figures 2 and 3), and by postnatal week 4, the equivalent developmental stage, in the cat (Pettigrew, 1974; Blakemore and Van Sluyters, 1975). The adult ferret cortex, however, contains significantly more cells that are non-specific for

stimulus orientation than are found in the cat. Approximately 25% of cells in adult ferret cortex lack orientation specificity, compared to fewer than 10% in the adult cat (Bishop and Henry, 1972).

In the ferret, the maturation of orientation specificity appears to occur in two temporally separated stages, with selectivity in supragranular layers reaching adult levels during postnatal week 6, while layer VI cells remain poorly orientation-selective until postnatal week 7 (figure 9). Different results have been reported in the cat, with maturation of orientation selectivity in supragranular layers lagging behind that of layers IV and VI (Albus and Wolf, 1984), or maturation of orientation selectivity in layers II/III and layer VI occurring after maturation in layer IV (Tsumoto and Suda, 1982). These results are difficult to interpret, however, since activity in the less orientation-selective laminae was also reported to remain poor during the time that development of orientation selectivity was immature.

The maturation of orientation selectivity in ferret primary visual cortex depends on neuronal activity. Blocking activity in area 17 by infusing tetrodotoxin into the cortex appears to freeze orientation selectivity in the immature state (figure 4). It is not clear, however, whether the initial development of orientation selectivity is activity dependent. Some degree of orientation selectivity is clearly present in ferret cortex as early as we were able to record visual responses in an anaesthetized animal (figure 2 and 3), but it is possible that cortical cells could be responsive earlier in development in the awake, behaving animal. Spontaneous activity could be involved in the original establishment of orientation selectivity *in utero* in the cat and before eye-opening in the ferret. In the cat and rat patterned spontaneous activity

is present in the retina prenatally, (Maffei and Galli-Resta, 1990; Meister at al., 1991); a model has been proposed which shows that cortical orientation selectivity could develop using the information in such spontaneous retinal activity (Miller, 1990). The hypothesis that the original establishment of orientation selectivity in ferret visual cortex depends on neuronal activity could in principle be tested by infusing TTX into visual cortex in neonatal ferrets. However, there are technical difficulties involved in attaching a drug delivery system to the small, rapidly growing and completely non-calcified skull of a very young ferret, as well problems in ensuring the viability of immature animals in the presence of such an invasive procedure. The effect of cortical activity blockade on the establishment of cortical orientation specificity in the cat is not known. Experiments where kittens received binocular intravitreal TTX injections beginning at 2 weeks of age, when cat cortical cells show some orientation-tuning but are not yet as selective as those in the adult, show that retinal activity blockade does seem to impede the full maturation of orientation selectivity, but such studies do not address the issue of earlier development (Stryker and Harris, 1986).

The dependence of orientation selectivity maturation on visual experience has been widely studied in the kitten, with rather conflicting results. A few weeks of visual deprivation by binocular lid suture or by dark rearing has been reported to freeze cat cortical orientation selectivity in an immature state (Pettigrew, 1974; Bonds, 1979), but has also been observed to have no effect on the development of orientation selectivity (Sherk and Stryker, 1976; Braastad and Heggelund, 1985). Longer term deprivation appears to lead to a progressive degradation of orientation specificity in kitten visual cortex (Blakemore and Van

Sluyters, 1975; Buisseret and Imbert, 1976). Visual deprivation in the cat does cause cortical cell responsivity to remain poor, with cells as difficult to record from as they are in very young kittens (Pettigrew, 1974; Blakemore and Van Sluyters, 1975; Sherk and Stryker, 1976; Braastad and Heggelund, 1985). Thus the different results reported for the effects of visual deprivation may again be explainable by differences in the responsiveness of the cortical neurons in animals studied by different experimenters.

Visual deprivation by binocular lid suture does not appear to affect cortical cell responsiveness in the ferret (table 1). This may be because in the ferret, unlike in the cat, even very young animals have nicely responsive cortical cells. Binocular deprivation in the ferret does affect the development of orientation selectivity, but dramatically so only in cells in layer VI, where cortical cell orientation selectivity appears to be frozen in the immature state (figure 9). Cells in supragranular layers develop normal orientation selectivity.

One might speculate that the laminar specificity of the deprivation effects in the ferret is due to the different connectivities of the cortical layers. Assuming that cortical circuitry in the ferret is similar to that found in the cat (Gilbert and Wiesel, 1981), layer IV cells get their primary input directly from LGN afferents, supragranular cells receive input from layer IV, but also have extensive, orientation-specific intralaminar excitatory connections (Gilbert and Wiesel, 1989), and layer VI cells receive direct LGN input as well as input from layer V, which in turn gets its input from the supragranular layers. This pattern of cortical connectivity could account for the distributions of orientation selectivity seen in our experiments, as described below.

The relatively poorly orientation-selective cells seen in layer IV of ferrets of all ages might be due to layer IV cells acquiring their orientation selectivity only from LGN afferents whose receptive fields cover an elongated region of visual space (Hubel and Wiesel, 1962; Chapman et al., 1991), rather than from any intracortical circuitry. If this is true, the poor orientation selectivity seen in all cortical layers in normal postnatal week 4-5 ferrets might indicate that at this early age all cortical orientation preference merely reflects the arrangement of afferent input, and no orientation-specific intracortical connections are yet present; in the cat at the same developmental stage patchy cortical connections in the supragranular layers have not yet formed (Luhmann et al., 1986; Calloway and Katz, 1990).

During postnatal week 6, orientation selectivity in layers II/III of ferret visual cortex matures, which might reflect maturation of the patchy connections in the supragranular layers. When cortical activity is blocked by TTX infusion, the maturation of orientation selectivity is prevented, possibly by interfering with the maturation of these intralaminar connections. During postnatal week 7, orientation selectivity matures in ferret cortical layer VI, possibly reflecting the maturation of connections between layers II/III and layer V or between layer V and layer VI. Without visual experience, mature orientation selectivity does not develop in layer VI. This could be because under these conditions the activity of layer V cell input to layer VI might be less vigorous when compared to direct LGN input than in a normal animal. This could give a competitive advantage to the LGN afferents, and allow layer VI cell orientation selectivity in deprived animals to remain a reflection of afferent input alone.

These hypotheses suggest a number of further experiments. To test whether the orientation selectivity seen in adult layer IV could result from the afferent input alone, layer IV neuron responses to oriented stimuli could be simulated by summing the responses of their inputs, the elongated arrays ofrom the afferent input alone, layer IV neuron responses to oriented stimuli could be simulated by summing the responses of their inputs, the elongated arrays of LGN afferents recorded in a single orientation column (Chapman et al., 1991). The distribution of orientation selectivity in these simulated responses could be compared with the distribution recorded in adult ferret layer IV. To see whether orientation selectivity in young ferret visual cortex might also be merely a reflection of afferent input, the receptive fields of geniculocortical afferents in a single cortical column could be recorded in postnatal week 4-5 ferrets. These afferents would be expected to cover an elongated region of space as they do in the adult (Chapman et al., 1991).

To test whether the maturation of orientation selectivity in ferret supragranular layers might be due to the development of patchy intralaminar connections between columns of like orientation preference, the normal time-course of development of these connections could be studied using anatomical tracers. The patchy connections could also be studied in TTX-treated and binocularly deprived animals. If the hypothesis outlined above is correct, supragranular intralaminar patchy connections should not develop in animals where cortical activity is blocked by TTX infusion, but should develop normally in animals deprived of vision by binocular lid-suture. Note that in the cat, the patchy connections do develop in binocularly deprived animals (Luhmann et al., 1986; Calloway and Katz, 1991) but may not become as

refined as in normal animals (Calloway and Katz, 1991).

The hypothesized effects of visual deprivation on connections to layer VI cells would be difficult to test directly. An indirect test, would be to infuse TTX into ferret cortex during postnatal week 6, when supragranular orientation specificity is mature, but layer VI specificity is not. This would determine whether the development of layer VI orientation selectivity in an otherwise already orientation-selective cortex is activity-dependent.

Figure 1

Orientation tuning histograms for 8 neurons recorded in adult ferret primary visual cortex. Each histogram shows mean response of the cell in spikes/sec to three presentations of each of 36 randomly interleaved orientations of the moving light bar stimulus swept across the cell's receptive field. 0 represents a leftward-moving vertical bar, 90 represents a downward moving horizontal bar. Error bars are standard error of the mean. Spontaneous activity level is shown at the right of each histogram. The orientation selectivity index calculated from the data in each histogram is shown.

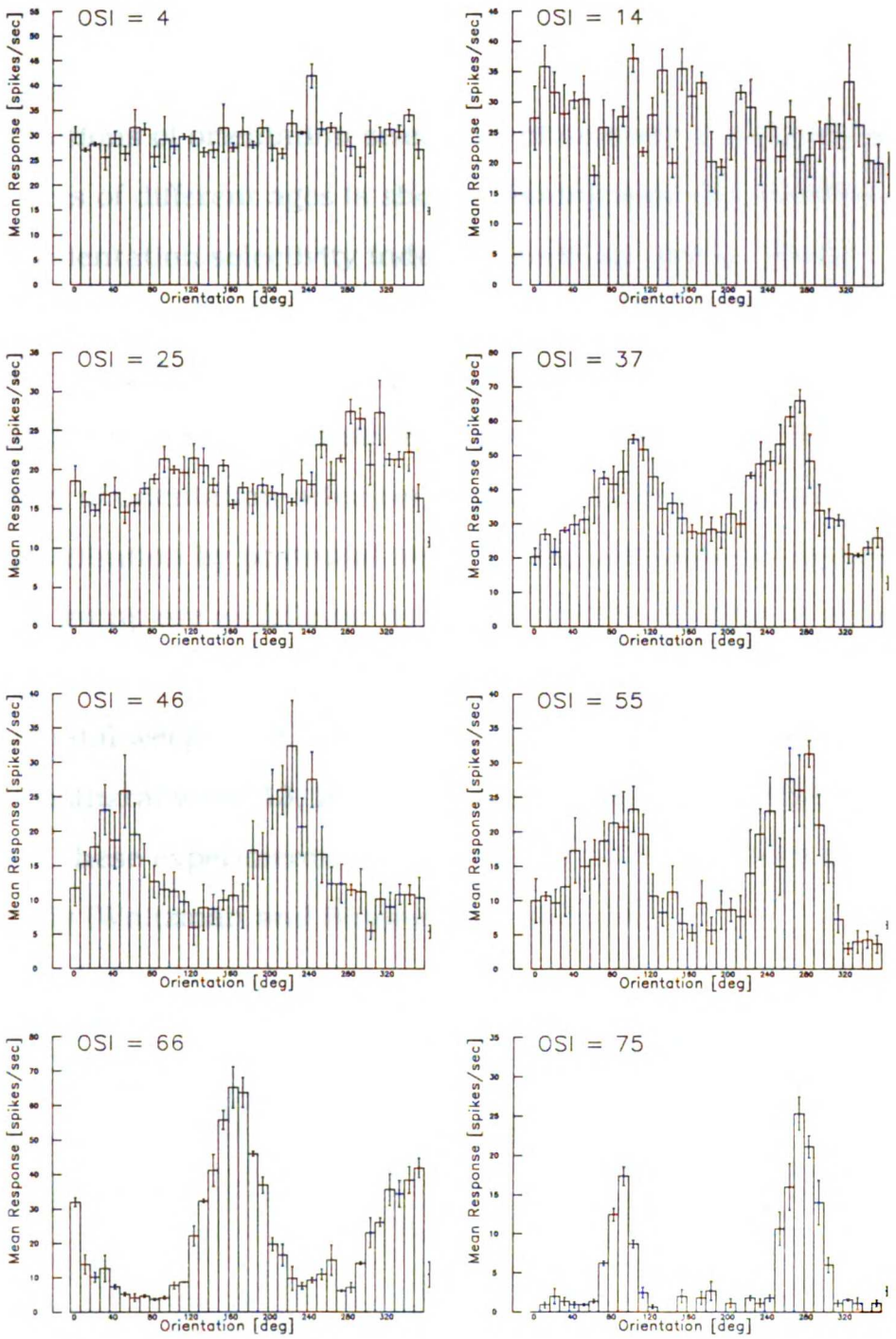

OSI = 4
OSI = 14
OSI = 25
OSI = 37
OSI = 46
OSI = 55
OSI = 66
OSI = 75
Mean Response [spikes/sec]
Orientation [deg]

Figure 2

Distributions of orientation selectivity of neurons in primary visual cortex of ferrets of different ages is shown. Histograms plot numbers of cells versus orientation selectivity index for each age group. At the earliest age at which visually driven responses could be recorded in area 17 (postnatal day 23), and through postnatal week 5, few neurons show clear orientation selectivity in their responses. During postnatal week 6, the proportion of orientation-selective responses increases, reaching an adult-like distribution by postnatal week 7. The numbers of animals and cells for each group are as follows: postnatal week 4, 38 cells, 4 animals; postnatal week 5, 55 cells, 7 animals; postnatal week 6, 36 cells, 1 animal; postnatal week 7, 18 cells, 1 animal; postnatal week 8, 61 cells, 3 animals; postnatal week 13, 24 cells, 1 animal; adult, 80 cells in 2 animals from these experiments plus 74 cells in 8 animals from a previous study (Waitzman and Stryker, unpublished).

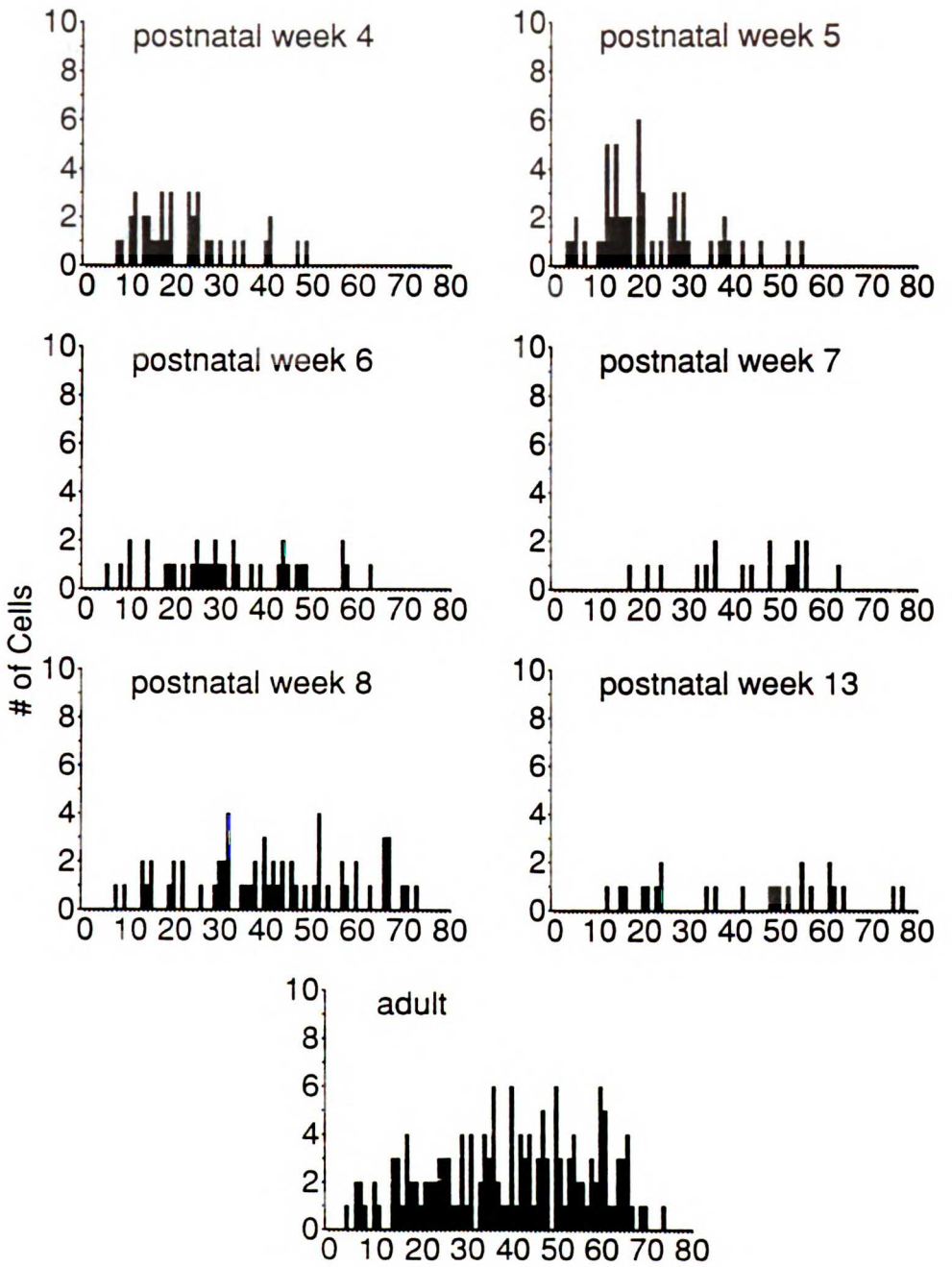

postnatal week 4
postnatal week 5
postnatal week 6
postnatal week 7
postnatal week 8
postnatal week 13
adult
# of Cells
0 10 20 30 40 50 60 70 80
0 2 4 6 8 10

Figure 3

Data from figure 2 are replotted to show the cumulative percentages of cells at each degree of orientation selectivity. Statistical analysis (Mann-Whitney U test) confirms that these distributions fall into three distinct groups. Distributions at 4 and 5 weeks are different from that seen at 6 weeks ($p < 0.001$), and the 6 week distribution is different from adult ($p < 0.005$). The 4 and 5 week old distributions are not significantly different from each other ($p = 0.38$), and the distributions for animals aged 7 weeks or older are statistically indistinguishable from the adult distribution ($p > 0.25$ in all cases).

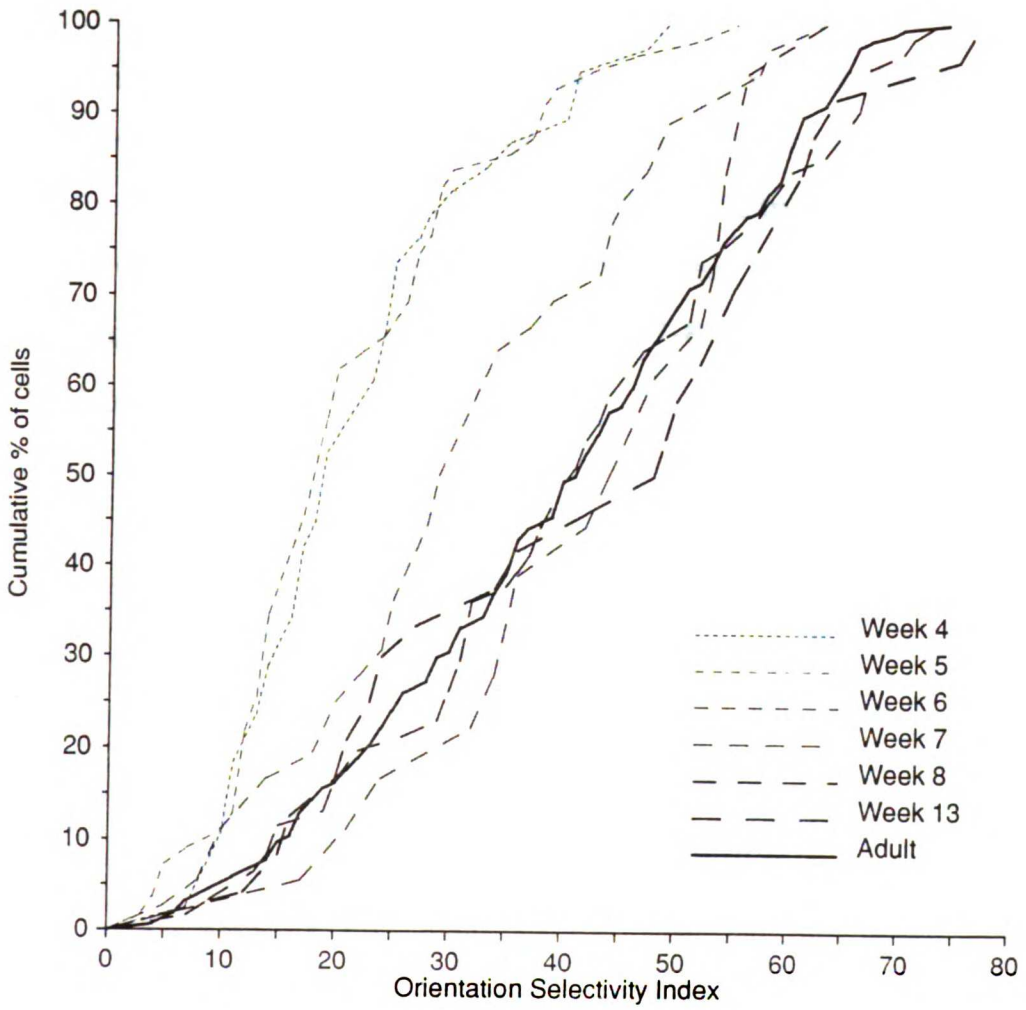

Cumulative % of cells
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
80
Orientation Selectivity Index
Week 4
Week 5
Week 6
Week 7
Week 8
Week 13
Adult

Figure 4

Orientation selectivity distributions from experiments silencing cortical activity. A. Distribution of orientation selectivity seen in cells in normal ferret area 17 during postnatal week 4 (data from figure 2). B. Distribution of orientation selectivity in animals treated with TTX during postnatal weeks 4 through 7 or 8. Data are from 56 cells in 4 animals. C. Distribution of orientation selectivity in untreated ferret visual cortex at postnatal weeks 7-8. Data are from 73 cells recorded in the opposite (untreated) cortical hemisphere of 3 of the TTX treated animals. D. Distribution of orientation selectivity in 28 cells in 1 animal treated as in B., but with 0.9% saline instead of TTX. These data are statistically indistinguishable from data from normal 7-8 week old ferrets (Mann-Whitney U : $p = 0.25$).

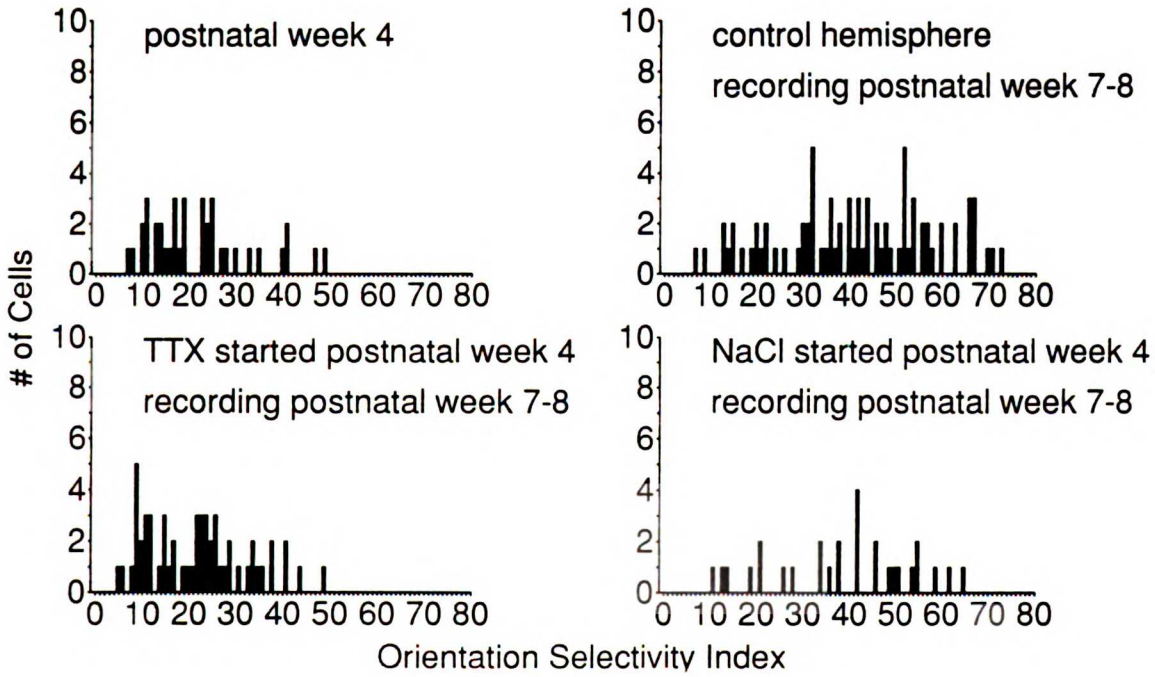

postnatal week 4
control hemisphere
recording postnatal week 7-8
TTX started postnatal week 4
recording postnatal week 7-8
NaCl started postnatal week 4
recording postnatal week 7-8
# of Cells
Orientation Selectivity Index
0 10 20 30 40 50 60 70 80
0 2 4 6 8 10

Figure 5

Orientation selectivity distributions from visual deprivation experiments. A. Distribution of orientation selectivity in animals deprived of cortical activity by TTX infusion (data from figure 4). B. Distribution of orientation selectivity in animals deprived of visual experience by binocular lid suture performed during postnatal week 4, before the time of natural eye opening. Recordings were made during postnatal week 13. Date are from 58 cells in 2 animals.
C. Distribution of orientation selectivity in normal adult ferret area 17 (data from figure 2).

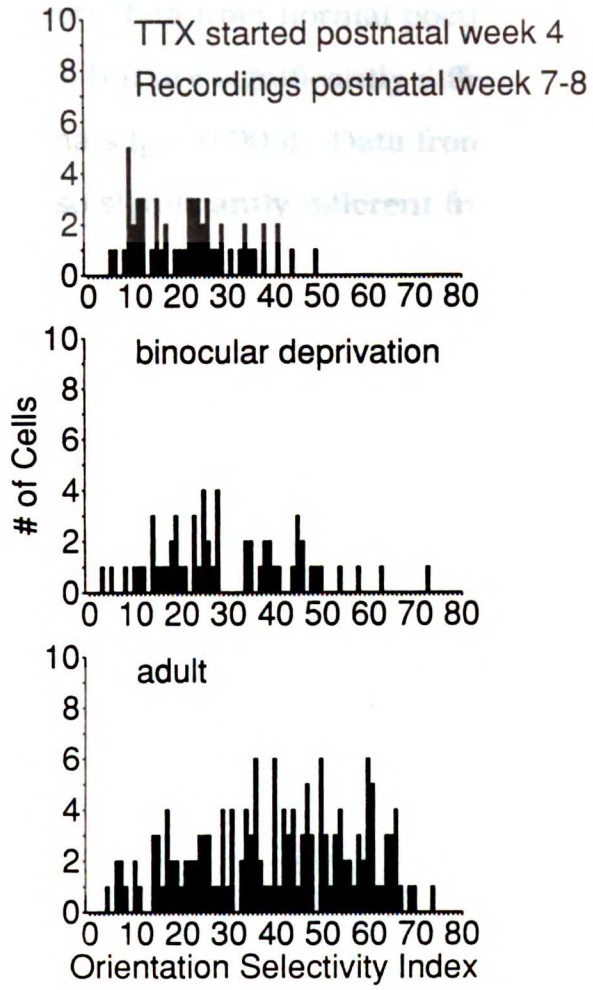

TTX started postnatal week 4
Recordings postnatal week 7-8
binocular deprivation
adult
# of Cells
0 10 20 30 40 50 60 70 80
Orientation Selectivity Index

Figure 6

Data from figure 5 are replotted showing cumulative percentages of cells at each degree of orientation selectivity from TTX treated animals, binocularly deprived animals and normal adults. Data from animals receiving intracortical TTX infusion beginning during postnatal week 4 are statistically indistinguishable from data from normal postnatal week 4 ferrets (Mann-Whitney U: $p = 0.46$) but are significantly different from data from binocularly deprived animals ($p < 0.005$). Data from binocularly deprived animals are also significantly different from data from normal adults ($p < 0.001$).

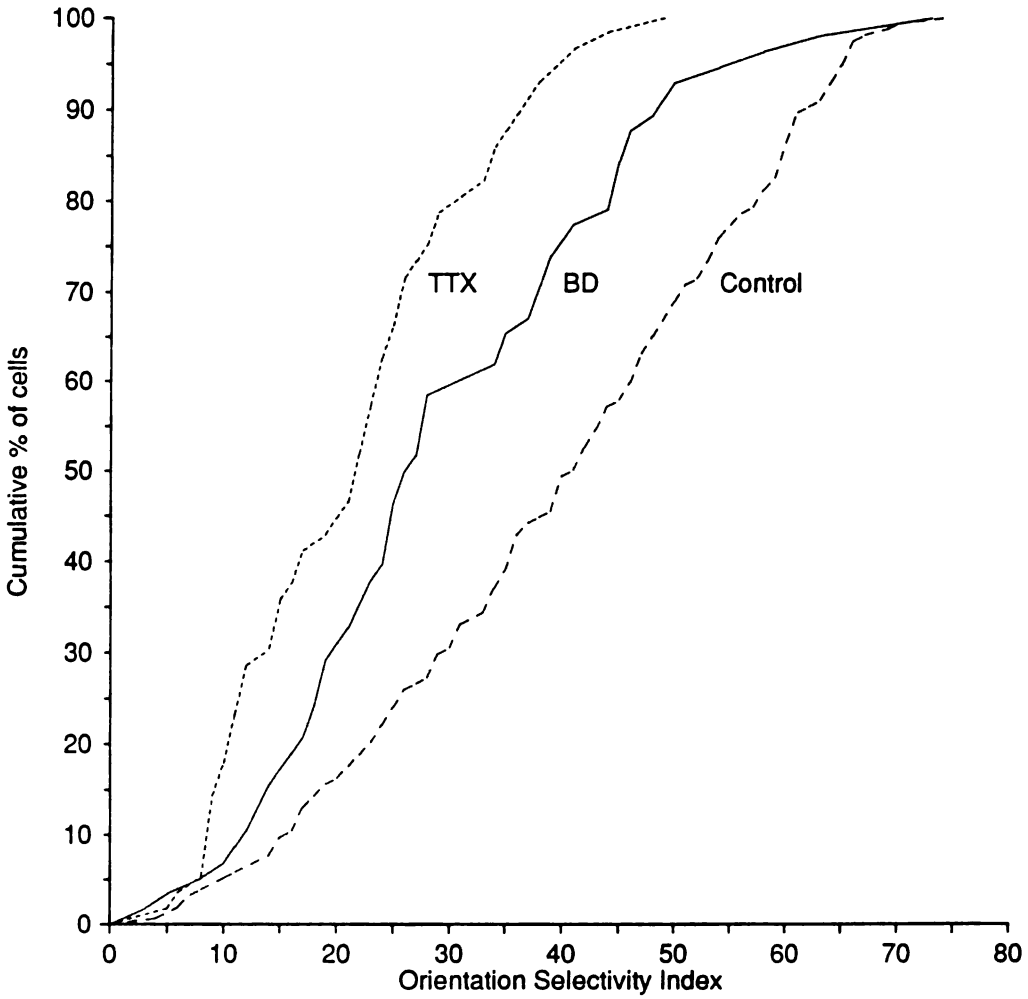
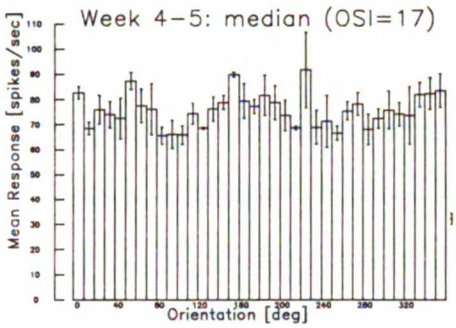
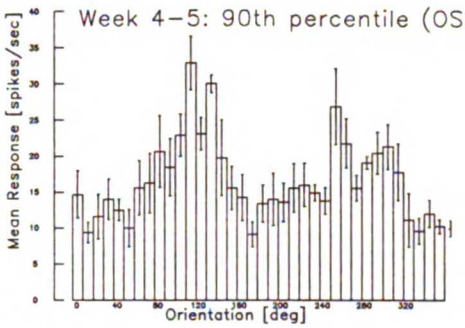
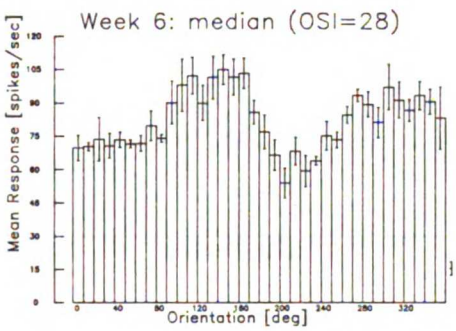

100
90
80
70
60
50
40
30
20
10
0
Cumulative % of cells
TTX
BD
Control
0
10
20
30
40
50
60
70
80
Orientation Selectivity Index

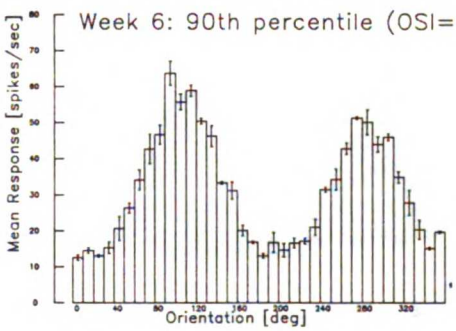
Figure 7

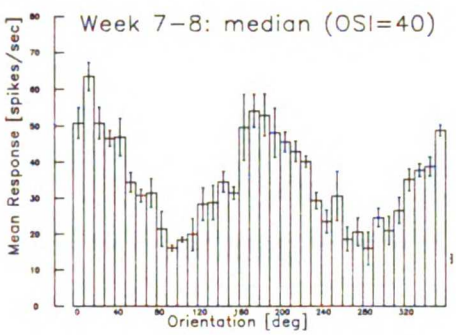
Orientation tuning histograms (see figure 1 legend for conventions) for cells of average (median) and high (90th percentile) degrees of orientation selectivity from ferrets of different age groups and from TTX-treated and binocularly deprived animals.

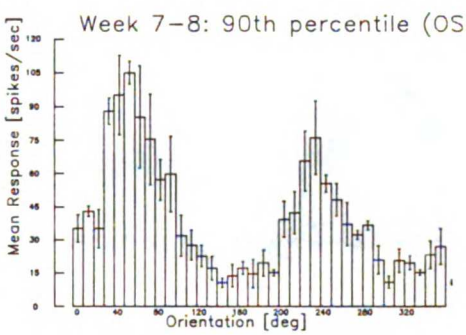

Week 4–5: median (OSI=17)
Mean Response [spikes/sec]
Orientation [deg]

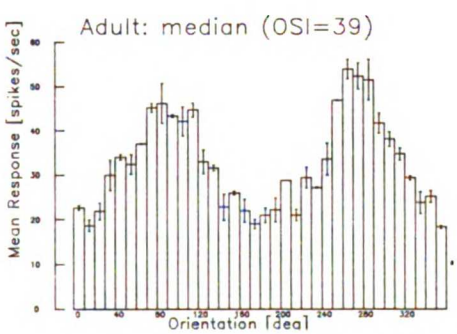

Week 4–5: 90th percentile (OSI=37)
Mean Response [spikes/sec]
Orientation [deg]

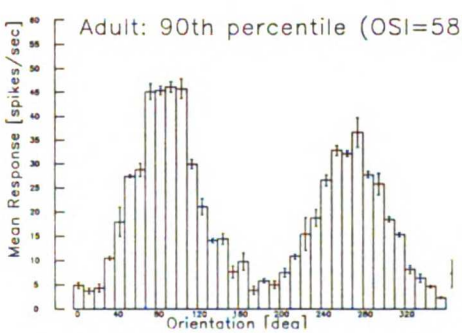

Week 6: median (OSI=28)
Mean Response [spikes/sec]
Orientation [deg]


Week 6: 90th percentile (OSI=49)
Mean Response [spikes/sec]
Orientation [deg]


Week 7–8: median (OSI=40)
Mean Response [spikes/sec]
Orientation [deg]


Week 7–8: 90th percentile (OSI=57)
Mean Response [spikes/sec]
Orientation [deg]


Adult: median (OSI=39)
Mean Response [spikes/sec]
Orientation [deg]


Adult: 90th percentile (OSI=58)
Mean Response [spikes/sec]
Orientation [deg]

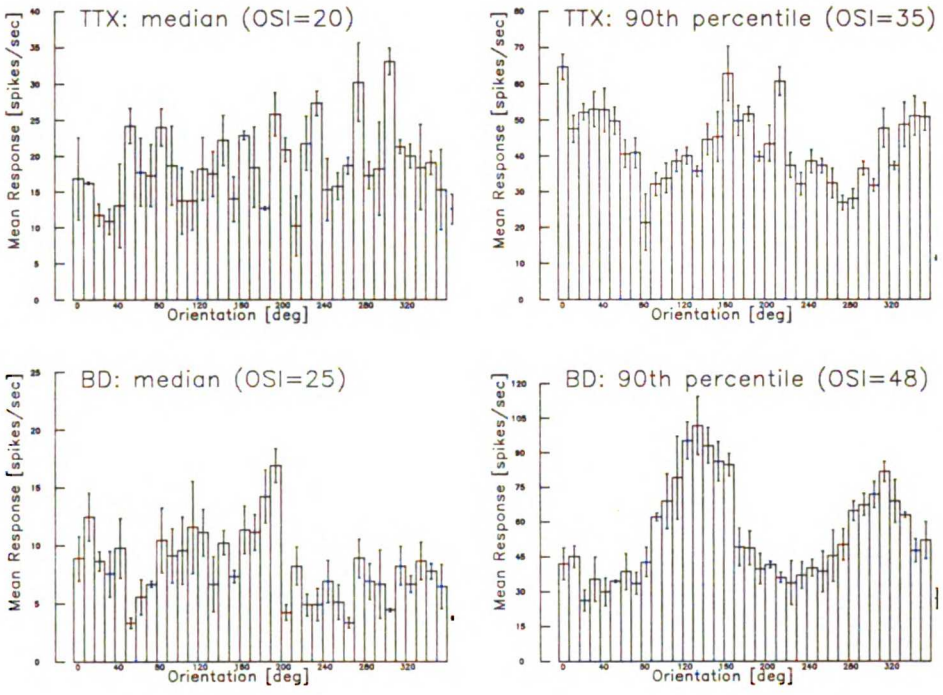

TTX: median (OSI=20)
TTX: 90th percentile (OSI=35)
BD: median (OSI=25)
BD: 90th percentile (OSI=48)
Mean Response [spikes/sec]
Orientation [deg]

Figure 8

Peak firing rate of cells plotted versus their orientation selectivity index for each group. r2s for correlation analysis of each plot are shown. In no group was a statistically significant correlation between cell responsiveness and orientation selectivity found ($p(F) > 0.25$ in all cases).

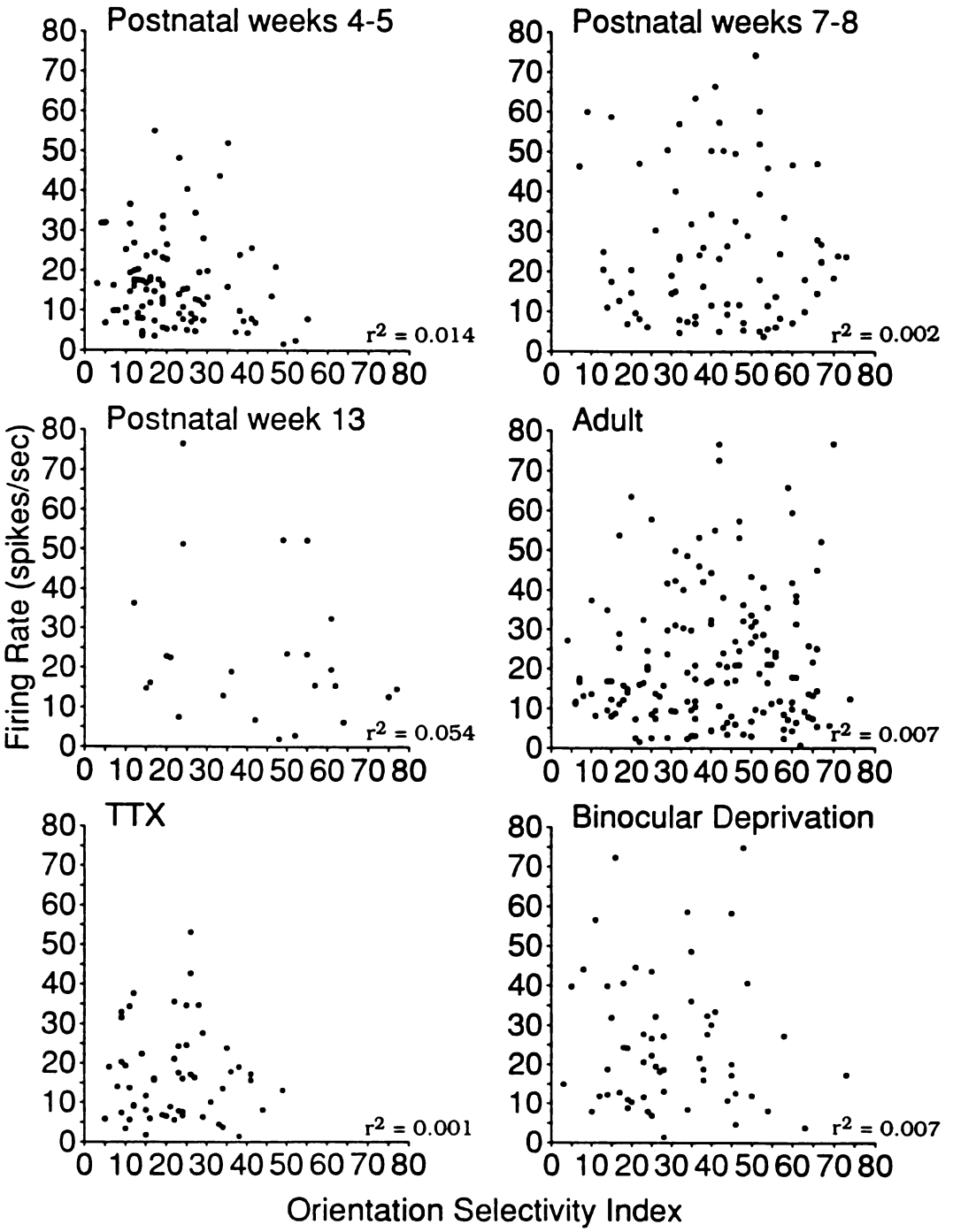

Postnatal weeks 4-5
Postnatal weeks 7-8
Postnatal week 13
Adult
TTX
Binocular Deprivation
r2 = 0.014
r2 = 0.002
r2 = 0.054
r2 = 0.007
r2 = 0.001
r2 = 0.007
Firing Rate (spikes/sec)
Orientation Selectivity Index
0 10 20 30 40 50 60 70 80

Figure 9

Laminar analysis of orientation selectivity. Cumulative percentages of cells at different orientation selectivities in the different cortical laminae are shown for animals from different age or treatment groups. The solid lines represent cells in layers II/III, dotted lines represent layer IV and dashed lines represent layer VI. Numbers of cells in each group are shown in table 1.

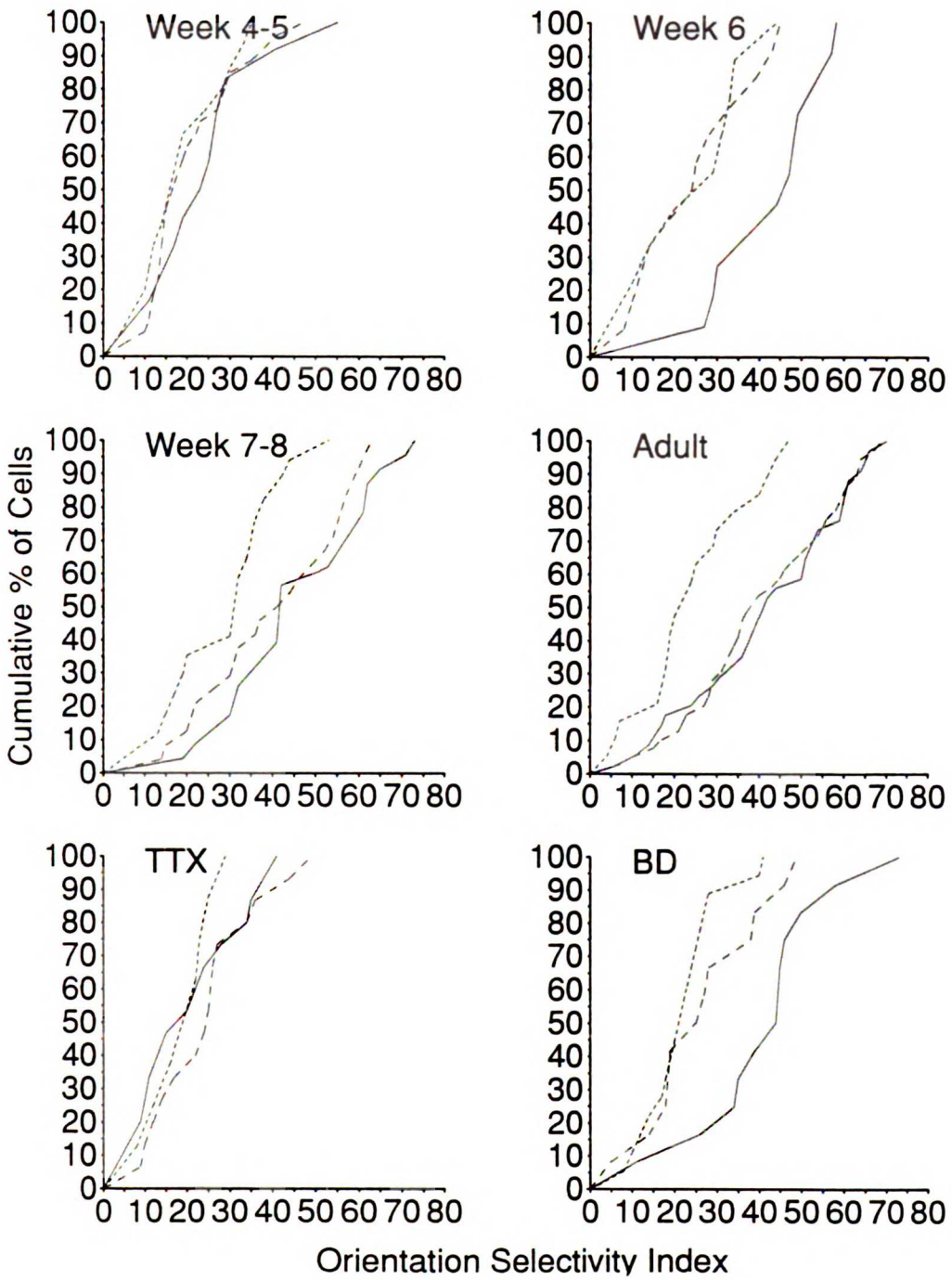

Week 4-5
Week 6
Week 7-8
Adult
TTX
BD
Cumulative % of Cells
Orientation Selectivity Index
0 10 20 30 40 50 60 70 80
0 10 20 30 40 50 60 70 80 90 100

Table 1

Laminar analysis of responsiveness. For each group of animals, total number of cells whose laminar positions were reconstructed is shown. Percentages of these totals recorded in each cortical lamina are given. Mean responsiveness is calculated as the average of the mean firing rate (in spikes/sec) above spontaneous at the preferred orientation of each cell.

Condition	Total N	Layers II/III	Layer IV	Layer V	Layer VI
Week 4-5	60				
% recorded		25%	25%	10%	40%
responsiveness		18.0	20.4	17.4	19.7
Week 6	35				
% recorded		31%	29%	11%	29%
responsiveness		32.4	28.1	31.1	27.5
Week 7-8	73				
% recorded		33%	23%	12%	32%
responsiveness		31.5	21.2	27.1	21.5
Adult	124				
% recorded		27%	25%	16%	32%
responsiveness		29.8	27.5	19.2	21.9
TTX	44				
% recorded		36%	19%	10%	36%
responsiveness		16.2	15.1	18.8	21.4
BD	50				
% recorded		24%	36%	16%	24%
responsiveness		25.0	20.1	19.4	31.2

DISCUSSION

The experiments described in this dissertation have provided several new insights into the properties of visual cortical neurons.

Ocular dominance shifts in the absence of visual experience

In Chapter 1, we show that ocular dominance plasticity can occur in the absence of visual experience in either eye. Kittens reared for one week during the critical period with no visual input to one eye and no retinal activity in the other eye showed a cortical ocular dominance shift toward the eye which experienced spontaneous retinal activity. The magnitude of this ocular dominance shift is smaller than that seen in kittens raised with monocular lid suture and normal vision in the other eye (Hubel and Wiesel, 1970; Olson and Freeman, 1975; Movshon and Dursteler, 1977).

Our result refutes a strictly interpreted central gating hypothesis (Singer, 1979) which states that behaviorally relevant visual information, acting through pontine and mesencephalic areas which mediate arousal, is necessary to allow changes in synaptic strength in visual cortex. Our results, together with the finding that in binocularly deprived kittens geniculocortical afferents from the two eyes are clearly segregated, but overlap somewhat more than in kittens raised with normal vision (Swindale, 1981; Kalil, 1982; Mower et al., 1985), are consistent with behaviorally relevant visual information playing a special role in facilitating changes synaptic strengths in visual cortex. Our results, however, are also consistent with a strictly correlation-based, activity-dependent model of development and plasticity in the visual cortex. The exact levels of activity in retina and cortex under different deprivation paradigms are not known, but certainly visually driven activity in the

retina drives cortical cells better than does spontaneous activity, and cortical cells are almost completely silent in the absence of any retinal activity (M.P. Stryker, personal communication). Thus in the case of monocular deprivation, cortical cells are very well driven through the open eye, causing correlated pre- and postsynaptic activity which would strengthen the synapses serving that eye. Cortical cells are poorly driven through the closed eye, but the afferents serving the closed eye are active, and this uncorrelated activity would weaken synapses serving the closed eye. In the case of our TTX/visually deprived animals, cortical cells are driven poorly by information from the spontaneously active retina, but are not driven at all by the TTX-treated retina, giving a competitive advantage to the spontaneously active eye at all synapses onto binocularly driven cells. Cortical cells which would normally be driven predominantly through the silenced retina, however, would remain silent. This might allow some synapses serving the silenced retina to remain strong, since there is no uncorrelated activity at these synapses. Thus the ocular dominance shift would be less complete in TTX-treated, visually deprived animals than in the case of monocular deprivation. This mechanism by which the TTX-treated eye retains some influence in the cortex may be similar to that seen in monocularly deprived animals treated with intracortical infusions of muscimol, where synapses remain strong between the silenced cortical cells and the afferents serving the less active eye (Reiter and Stryker, 1988).

Role of NMDA receptors in visual cortex

Our experiments described in Chapter 2 show that the N-methyl-D-aspartate class of glutamate receptor is involved in the normal transmission of visual information in adult cat area 17. If the antagonist

APV is infused into cortex at a concentration sufficient to fully but selectively block NMDA receptors, cortical activity in response to visual stimuli is profoundly suppressed. This result calls into question the suggestion that the NMDA receptor plays a special role in developmental plasticity of the visual cortex (Kleinschmidt et al., 1987), since it is known that cortical plasticity is dependent on cortical activity (Stryker and Harris, 1986). Our result also raises questions about the roles played by the different classes of glutamate receptors in the transmission of sensory information in the cortex. Further pharmolcological experiments are being done in the Stryker lab to examine the role of the kainate/quisqualate class of receptor. Preliminary results suggest that blocking kainate/quisqualate receptors in kitten area 17 suppresses spontaneous activity but does not greatly supress cortical responses to visual stimulation (Defrietas and Stryker, 1990).

Geniculocortical afferent input to an orientation column

Our experiments described in Chapter 3 show that geniculocortical afferents arborizing within a single orientation column in ferret area 17 usually have receptive fields which cover an elongated region of visual space, and that the axis of the elongation matches the preferred orientation of cortical cells within that column. This result is consistent with Hubel and Wiesel's model of the orientation preferences of cortical cells in layers IV and VI being determined by afferent input from LGN cells with receptive fields aligned in space (Hubel and Wiesel, 1962). It remains to be determined what are the relative contributions to cortical cell orientation specificity of this afferent input versus intracortical circuitry, including excitatory connections between like-oriented columns

(Gilbert and Wiesel, 1981; T'so et al., 1986; Calloway and Katz, 1990) and inhibitory connections whether between columns of orthogonal (Creutzfeldt et al., 1974; Sillito, 1975), similar (Hata et al., 1988) or identical (Heggelund, 1986) orientation selectivity.

Future experiments in our lab will attempt to determine whether the alignment of geniculocortical afferents pre-dates the appearance of cortical cell orientation selectivity during development.

Additional developmental studies are suggested by the second half of Hubel and Wiesel's model of cortical orientation selectivity which proposes that cortical cells in layers II/III and V have their orientation preference specified by their connections from layer IV and VI cells within the same orientation column (Hubel and Wiesel, 1962). This model does not appear to hold true in the adult cat, where silencing cortical cell responses in layer IV (by injecting cobalt chloride into the LGN A laminae) does not effect the orientation selectivity of cells in the supragranular layers (Malpeli, 1983). Malpeli's result suggests that in the adult, orientation selectivity within a single column must be established by at least two different systems: one driven by LGN A laminae projections to layer IV, and the other presumably driven by LGN C laminae projections or intracortical connections to the supragranular layers. This would necessitate a developmental mechanism to ensure that the orientation preference established in layers II/III matches the orientation preference established in layer IV of the same column. An activity-dependent, correlation-based model for such a developmental process has been proposed (Blakemore and Van Sluyters, 1975). Early in an animal's life, supragranular cells might depend on input from layer IV for their orientation selectivity, and the parallel system which specifies

supragranular orientation in the adult might not yet be strong enough to drive the cortical cells. When a visual stimulus of a particular orientation is viewed, the layer IV cells selective for that orientation would be active, and would drive the layer II/III cells. At the same time whatever inputs to layer II/III from C laminae or extrastriate cortex might be selective for the same orientation would also be active, and this correlated activity could strengthen these synapses. This model could be tested by repeating the Malpeli (1983) experiment in very young animals at the age when orientation-selective first occur. One would expect that at this age, silencing layer IV activity might abolish orientation selectivity in layers II/III.

Development of orientation selectivity

Chapter 4 describes our studies of the development of orientation-selective responses in ferret visual cortex. We have shown that at the earliest age that we could record responses in ferret visual cortex (postnatal day 23) only a very few cells had orientation-specific responses. Responses remained poorly tuned through postnatal week 5. Very well tuned units were first recorded during postnatal week 6, and the distribution of orientation selectivity reached adult-like levels by postnatal week 7. This development of orientation selectivity is activity dependent, and was completely blocked by intracortical TTX infusions. Maturation of orientation does not depend on visual experience, since some cells in binocularly deprived animals are as well tuned as any cells found in normal adult cortex. However, the development of orientation selectivity in layer VI was severely impaired by binocular lid suture, indicating that visual activity may play a role in the normal development

of orientation-selectivity in at least some cortical cells. This may be analogous to the development of ocular dominance columns, where afferents from the two eyes remain completely overlapping in the absence of retinal activity (Stryker and Harris, 1986), but clearly segregate in the absence of visual experience, although the segregation in binocularly deprived animals is not as complete as in normal animals (Swindale, 1981; Kalil, 1982; Mower et al., 1985). We have planned future experiments on the development of orientation selectivity which are discussed in Chapter 4.

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