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Inhibitory mechanisms in the LGN: A possible substrate for amblyopia?

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Abstract

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INHIBITORY MECHANISMS IN THE LGN:
A POSSIBLE SUBSTRATE FOR AMBLYOPIA?

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I. INTRODUCTION

Amblyopia comes from the Greek words amblys meaning
blunt or dull and ops meaning eye. Amplyopia is a term
applied to an eye with a visual acuity that cannot be
corrected with conventional lens to better than 20/40.
It is particularly disturbing due to the fact that there
seems to be no detectable eye disease associated with the
reduced visual acuity. It is usually monocular but can
affect both eyes. Also, an individual with an amblyopic
eye will usually have difficulty in pointing and moving
that eye. Detecting amblyopia is not always easy since
the patient can frequently call out some of the smallest
letters. They tend to skip letters in the middle of rows
but correctly call out those on the ends.

There are many etiologically classified amblyopias
but this paper will deal only with functional amblyopia.
Amblyopia ex anopsia (amblyopia attributable to nonuse
or prolonged suppression). It is usually associated with
strabismus or anisometropia and is sometimes identified as
disuse amblyopia, obligatory amblyopia or suppression
amblyopia.

Amblyopia is treatable in most cases even though the exact mechanisms of its etiology are unknown.

The first question to consider is whether the prevalence of amblyopia warrants the expenditure of valuable manpower and research funds in an effort to determine its mechanism. Flom and Neumaier[1] have reported from numerous studies that the prevalence of amplyopia (20/40 or worse) is approximately 1.8% of the population. This would mean that in the United States alone that more than 4,000,000 people have some form of amblyopia. This is certainly a significant number and, I feel, worthy of the scientific community's efforts to find a solution.

It was not until the middle and late 1950's that experimental neurophysiology had advanced to a stage where individual visual cortical cell activity could be monitored while presenting various visual stimuli.[2-6]

Hubel and Wiesel in 1959 reported the following findings from single neuron recordings in the cat striate cortex:

"1. Recordings were made from single cells in the striate cortex of lightly anaesthetized cats. The retinas were stimulated separately or simultaneously with light spots of various sizes and shapes.

2. In the light-adapted state cortical cells were active in the absence of additional light stimulation. Increasing the depth of anaesthesia tended to suppress this maintained activity.

3. Restricted retinal areas which on illumination influenced the firing of single cortical units were called receptive fields. These fields were usually subdivided into mutually antagonistic excitatory and inhibitory regions.

4. A light stimulus (approximately one second duration) covering the whole receptive field, or diffuse illumination of the whole retina, was relatively ineffective in driving most units, owning to mutual antagonism between excitatory and inhibitory regions.

5. Excitatory and inhibitory regions, as mapped by stationary stimuli, were arranged within a receptive field in a side-by-side fashion with a central area of one type flanked by antagonistic areas. The centers of receptive fields could be either excitatory or inhibitory. The flanks were often asymmetrical, in that a given stationary stimulus gave unequal responses in corresponding portions of the flanking areas. In a few fields only two regions could be demonstrated, located side-by-side. Receptive fields could be oriented in a vertical, horizontal or oblique manner.

6. Effective driving of a unit required a stimulus
    specific in form, size, position and orientation,
    based on the arrangement of excitatory and inhibi-
    tory regions within receptive fields.

7. A spot of light gave greater responses for some
    directions of movement than for others. Responses
    were often stronger for one direction of movement
    than for the opposite; in some units these asym-
    metries could be interpreted in terms of receptive
    field arrangements.

8. Of the forty-five units studied, thirty-six were
    driven from only one eye, fifteen from the ipsi-
    lateral eye and twenty-one from the contralateral;
    the remaining nine could be driven from the two
    eyes independently. In some binocular units the
    two eyes were equally effective, in others various
    degrees of dominance of one eye over the other were
    seen.

9. Binocularly activated units were driven from
    roughly homologous regions in the two retinas.
    For each unit the fields mapped for the two eyes
    were similar in size, form and orientation, and
    when stimulated with moving spots, showed similar
    directional preferences.

10. In a binocular unit excitatory and inhibitory
     regions of the two receptive fields interacted,
     and summation and mutual antagonism could be
     shown just as within a single receptive field."[3]

This study provided information about how the normal
visual cortex was organized which provided a sensitive
means of detecting and studying any modification of the
visual input to the visual cortex. Further studies into
lateral geniculate and nonstriate visual areas (18/19)
of the cat[4-6] also provided a means of studying the
changes that might take place in these structures and the
onset of such changes relative to each other.

It has been well established by Hubel and Wiesel
in their studies with cats that there is a critical
period of visual development during which amblyopia
may be induced if an eye is sufficiently deprived of
visual input.[7-12]

The initial experiments leading to this discovery
were first directed at establishing what cortical cell
organization existed in kittens and how early in life it
was operational. A Hubel and Wiesel study in 1963 reported
the following: "Responses of single cells to visual stimuli
were studied in the striate cortex of very young kittens.
Two animals, aged 8 to 16 days, had had no previous exposure
to patterned stimuli. Responses of cortical cells in these
animals were strikingly similar to those of adult cats.

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Fields were simple or complex, with a clear receptive-field orientation. Cells with similar orientations appeared to be grouped in columnar regions. The majority of cells were driven by the two eyes, with patterns of binocular interaction that were similar to those in the adult. Compared with cells in the mature cat, those in young kittens responded somewhat more sluggishly to visual stimuli, and receptive-field orientations tended to be not quite so well defined.

In two other kittens, one monocularly deprived by translucent occluder from birth for 19 days, the other a normal 20-day old, responses to patterned stimulation of either eye were entirely normal by adult standards.

It is concluded that many of the connections responsible for the highly organized behavior of cells in the striate cortex must be present at birth or within a few days of it. The development of these connections occurs even in the absence of patterned visual experience."[7]

A similar study by Wiesel reported the following about the lateral geniculate body:

"1.   Kittens were subjected to deprivation of form and light in one eye, at various ages and for various periods. Deprivation was accomplished either by suturing the lids together or by placing a translucent contact occluder over the cornea.

2. In kittens with the lids of one eye sutured from birth for three months, most geniculate cells with input from the deprived eye had normal receptive fields, with an on-center and an off-periphery, or the reverse. The normal process by which the peripheral suppression demonstrable in retinal ganglion cells is increased at the geniculate level was observed. The over-all activity of cells in layers fed by the deprived eye was, however, diminished, and a few cells had sluggish responses and receptive fields with abnormally large centers.

3. Marked histological changes were present in layers fed by the deprived eye. Mean cell areas were decreased by about 40% for the dorsal and middle layers and 25% for the ventral layer, and nuclei and nucleoli were also shrunken. No obvious histological changes were found in the retinas, optic nerves, superior colliculi, or straite cortex.

4. Lid closure for comparable periods in two month old, visually experienced kittens produced similar but less severe histological changes in the lateral geniculate bodies. No changes were seen in adult cats visually deprived by lid suture of one eye for three months.

5. A translucent contact occluder placed over one eye from birth for two to two and one half months produced similar histological changes, but again these were less marked, with 10-15% reduction in mean cell area, in the appropriate dorsal and middle layers. In one kitten a translucent occuluder was placed over one eye at five weeks for a three month period; there was no atrophy of geniculate cells.

6. Geniculate cells measured in a newborn kitten are smaller than those in the adult, and are even smaller than cells in the atrophic layers of kittens deprived from birth by lid suture for three months, indicating that some growth of cells occurs subsequent to birth, in spite of visual deprivation."[8]

The next logical step was to conduct an experiment to see if monocular deprivation would produce alterations in cortical cell activity and/or their receptive fields. From their previous work Hubel and Wiesel knew this about striate cortex cells:

"1. Four-fifths are binocularly influenced.

2. For any given cell the receptive fields mapped in the two eyes are similar in arrangement and occupy corresponding retinal positions, but with some cells responding preferentially to the contralateral eye while others preferred the ipsilateral eye.

3. That ocular-dominance was predominately contralateral, see ocular dominane histogram below.

Ocular Dominance Histogram

Contralateral Equal Ipsilateral

Ocular Dominance
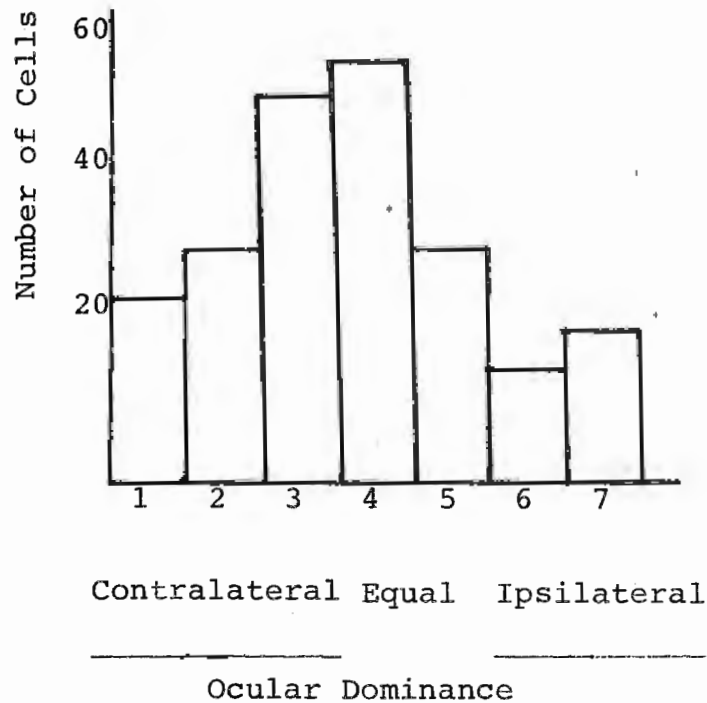
4. And that very young kittens resembled adults in all of these respects."[4]

With this information in hand they conducted their
next experiment in the sequence and reported the following:

"1. Single-unit recordings were made from striate
    cortex of kittens in which one eye had been deprived
    of vision either from birth or subsequently, and
    for various periods of time.

2. Kittens deprived from birth for two to three months
    showed profoundly defective vision in the deprived
    eye. Visual placing and following reactions were
    absent, and there was no hint of any ability to
    perceive form. Pupillary light reflexes were never-
    theless normal.

3. In kittens deprived from birth, either by suturing
    the lid of one eye or by covering the cornea with
    a translucent contact occluder, the great majority
    of cortical cells were actively driven from the
    normal eye, with normal receptive fields. On the
    other hand, only one cell out of 84 was at all
    influenced by the deprived eye, and in that cell the
    receptive fields in the two eyes were abnormal. A
    few cells could not be driven from either eye.

4. In one two month old kitten monocularly deprived with
    a translucent contact occluder, the corneal electro-
    retinograms were normal in the two eyes. On flashing
    a light in the previously occluded eye the slow-wave

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potentials evoked in the visual cortex of the two hemispheres were highly abnormal, compared with responses from the normal eye.

5. One to two months of normal visual experience prior to monocular deprivation by lid suture or with a translucent occluder reduced the severity of the physiological defect. Even though the ability of the deprived eye to influence cortical cells was still well below normal. On the other hand, three months of deprivation by lid closure in an adult cat produced no detectable physiological abnormality.

6. We conclude that monocular deprivation in kittens can lead to unresponsiveness of cortical cells to stimulation of the deprived eye, and that the effect is most severe in animals deprived from birth. The relative normality of responses in newborn kittens suggests that the physiological defect in the deprived kittens represents a disruption of connections that were present at birth."[9]

We now have the first hint of a possible neurophysiological cause for amblyopia in kittens. At this point it appeared that early visual deprivation some how disrupted innately determined connections within or between the LGN and the striate cortex. In an effort to close the loop, that is, if depriving one eye reduced its cortical responsiveness, then depriving both eyes should produce an almost

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total unresponsiveness of cortical cells to stimulation of either eye. Strangely enough this did not occur. "Responsive cells were found throughout the greater part of all penetrations, and over half of these cells seemed perfectly normal. The cortex was nevertheless not normal in that many cells responded abnormally, and many were completely unresponsive. In the fifth kitten an eye was opened and vision tested. The pupillary response was normal but the animal from its behavior appeared to be blind.

Histologically the lateral geniculate body showed changes similar to those found after monocular deprivation, but they occurred throughout all layers bilaterally: the Nissl-stained cells appeared pale, cross-sectional areas of cell bodies were reduced by about 40%, and the pale substance between cell nests was greatly reduced in volume. There were no obvious changes in retinas or cortex.

It thus appears that at the cortical level the results of closing one eye depend upon whether the other eye is also closed. The damage produced by monocular closure may therefore not be caused simply by disuse, but may instead depend to a large extent on interaction of the two pathways."[11]

We now have a second variable - (interaction between the two eyes) and the first one, that of early visual deprivation, as possible causes of amblyopia.

In an effort to get closer to producing an amblyopia

in cats that more closely paralled the human situation,

an artificial strabismus was produced in kittens by cutting

an extraocular muscle. This procedure would also provide

for an intact visual system from the retina to the cortex

which was not possible with suturing or occlusion. This

study reported the following: "In four kittens the right

medial rectus was severed at about the time of normal eye

opening, producing an obvious divergent squint. The animals

were raised under normal conditions for periods of three

months to one year. When the two eyes were then tested

separately, no behavioral visual defects were seen. Record-

ings from the striate cortex were normal, except for a marked

decrease in the proportion of binocularly driven cells:

instead of about 80%, only 20% could be influenced from the

two eyes. The cortex appeared normal microscopically. In

a given penetration there was a marked tendency for cells

driven from a particular eye to occur in long uninterrupted

sequences. These results suggest that the strabismus caused

cells to shift in their ocular dominance, a given cell coming

to favor more and more the eye that dominated it at birth,

ultimately losing all connections with the nondominant eye.

We conclude that a lack of synergy in the input from the two

eyes is sufficient to cause a profound disruption in the

connections that subserve binocular interaction. In two

kittens an opaque contact occluder was placed over one

eye one day and the other eye the next, alternating eyes

each day from shortly after birth to an age of ten weeks.

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This kept the eyes from working together without introducing the possibility of antagonistic interaction between them. Vision in either eye seemed normal. Penetrations in the striate cortex gave results similar to those obtained in squint anaimals; if anything, the shift in ocular dominance was more extreme, 91% of cells being driven by only one eye. Again cells were spatially aggregated according to ocular dominance.

Recordings from normal adult cats indicate that besides being grouped according to receptive-field orientation, cells in the striate cortex are grouped by ocular dominance into regions of ipsilateral, contralateral, and mixed dominance. The exaggeration of eye dominance of individual cells, in animals raised with squint or alternating monocular occlusion, produces an accentuation of these cortical subdivisions."[10]

They had now produced an amblyopic model that more closely paralled what is found in the human amblyope aside from the fact that there is still a huge step to take if one is to draw a conclusion concerning the human amblyope.

Their next study was to determine if deprivation by occlusion was transient or permanent. This study was summarized as follows: "In kittens, monocular or binocular deprivation by lid suture for the first three months of life leads to virtual blindness, marked morphological

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changes in the lateral geniculate body, and a severe

deterioration of innate cortical connections. In seven

kittens whose eyes had been sutured at birth for three

months, six unilaterally and one bilaterally, an attempt

was made to assess the extent of recovery by reopening

an eye and allowing the animals to live for another three

to fifteen months. In two of the monocular closures the

deprived eye was opened and the normal eye closed.

In all kittens there was some slight behavioral

recovery during the first three months, but the animals

remained severely handicapped and never learned to move

freely using visual cues. There was no morphological

improvement in the lateral geniculate body. Our previous

impression that atrophy can develop with deprivation

beginning at three months was confirmed. In monocularly

deprived animals a few cells in the striate cortex may

have recovered responses to stimulation to the originally

deprived eye, but in many of these cells the responses

were abnormal. In the binocularly deprived kitten there

was a marked increase in the proportion of cells responding

abnormally to the eye that was reopened, without any

obvious increase in the total number of cells responding

to that eye. We conclude that the animal's capacity to

recover from the effects of early monocular and binocular

visual deprivation, whether measured behaviorally, morpholo-

gically, or in terms of single-cell cortical physiology,

is severely limited, even for recovery periods of over a

-15-

year."[12] The last paragraph of this report, unfortunately, was adopted by many in the ophthalmologic, optometric and textbook writing fields. It has been shown[13] that deprivation amblyopia, is treatable and it is not age dependent.

It was thought at this time that the visual system of the cat was not similar enough to the human visual system to make any statements about the mechanism of deprivation amblyopia in humans from the foregoing data. So the next logical step was to produce deprivation amblyopia in a sub-human primate whose visual system more closely matched that of man's. "The rhesus monkey was chosen since its visual system is virtually identical to that of man." 6-7" [14]

Von Noorden[14] conducted essentially the same experiment as Wiesel and Hubel[12] except that he used rhesus monkeys and from this study he concluded the following: "As to the site of the lesion in unilateral deprivation amblyopia, it becomes necessary to refer to data collected since the beginning of this century in lower animals, since no such information is available in primates.[12-13] With the exception of one group of investigators who found a decrease of acetylcholinesterase in the retina of rats with pattern vision deprivation,[14] all authors agree on normalcy of the retina and optic nerve in such studies. The most profound morphologic changes have been described in the lateral geniculate body associated with the deprived eye and consist of a marked decrease of cells on that side.[2,15-17]

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At this time we can only conclude from our study that the defect is not in the distal part of the retina since the ERG's of the normal and the deprived eye were indistinguishable. Normalcy of the ERG was also reported by Wiesel and Hubel[3] in kittens after pattern vision deprivation. However, Ganz, et al.[16] found a 40% reduction of the b wave after pattern vision deprivation in kittens. Ophthalmoscopic anomalies, such as optic atrophy, described by Chow and his coworkers[18] in chimpanzees reared in complete darkness, were not found in our amblyopic monkeys."[14]

Hubel and Wiesel[10] were unable to produce amblyopia in artificially induced exotropia in cats. Von Noorden, however, was able to produce amblyopia in artificially induced esotropia in rhesus monkeys, but again not in exotropic monkeys.[15]

At this point in his investigations Von Noorden was very interested in establishing what the critical time period was during development when deprivation or strabismic amblyopia could be induced and what duration of occlusion or strabismus during this critical period was necessary to produce the amblyopia. He was anxious to establish this because he felt that the rhesus monkey was a very good model from which he could extrapolate to the human system with a high degree of confidence. He suspected that some of the amblyopia he observed in his human clinical population may have been due to inadvertent occlusion during the human critical period.

Von Noorden reported "The finding that only brief
periods of unilateral occlusion during visual immaturity
may cause severe, irreversible behavioral defects in
the adult monkey is of special interest. Alerted from
our monkey experiments, we have recently observed three
patients in whom a similar mechanism may have been a factor
in causing the amplyopia."[16] Upon histological studies
of these monkeys he reported "Since only diffuse light
can enter the eye after lid closure, the only common
factor shared by deprivation and strabismus appears to
be the dissimilarity of input received by the two eyes.
In the case of visual deprivation this may lead to competi-
tion, and perhaps interaction on a geniculate or cortical
level, between the input received by the occluded eye and
the non-occluded eye. With strabismus, the visual message
received by the deviated eye perhaps competes in a similar
manner with that of the fixing eye."[17]

Van Noorden, now, has arrived at the same conclusion
is his experiments with rhesus monkeys as Hubel and Wiesel
had in their experiments with cats, that is, that there
appears to be an inhibitory effect initiated by the seeing
eye on the amblyopic eye. For the next five years several
investigators[18-20] corroborated the data previously collec-
ted by Hubel-Wiesel and Von Noorden. The next logical
step then would be to design an experiment that might
inhibit the inhibitory action of the seeing eye on the
amblyopic eye. Duffy attempted just such an experiment

and reported his findings: "Several lines of evidence suggest that inhibition in the cat visual system may be mediated by γ-aminobutyric acid (GABA). Of particular importance are the reports that bicuculline, a GABA-receptor blocker,[4] is able to alter receptive field (RF) properties of normal visual neurones.[5-8] We, therefore, attempted to restore binocularity in monocularly deprived cats by intravenous administration of bicuculline. This drug was able to restore binocular input to more than half the cortical neurones tested.

We postulate that the restoration of binocular input by bicuculline occurs by reduction of inhibitory mechanisms by way of GABA-receptor blockade. This mechanism is compatible with available information about the cat visual system. First, inhibitory input has been proposed as an important determinant of the normal response characteristics of visual neurones[14-15]. Second, GABA has been implicated as a visual inhibitory transmitter[16,17]. Finally, bicuculline alters the receptive fields of normal visual neurons in a manner consistent with reduction of inhibition[5-8]. If our interpretation is correct, then the total dominance by the normal eye results from active inhibition of the relatively intact input from the amblyopic eye and suggests that feline amblyopia may not involve a permanent, irreversible loss of visual function."[21] Duffy admitted that his experiment was not definitive enough to indicate where bicuculline was acting.

Kratz and Spear[22,23] attacked the binocular inhibition question in a different way. They reared monocularly deprived kittens and then after the critical period, enucleated the seeing eye. Kratz reported the following: "In this case, the release (due to enucleation) must occur transynaptically through the DLG since the locus of inhibition would be principally in the striate cortex itself. It is possible that some inhibition could occur in the DLG, contributing to the marked reduction in cells in the deprived laminae which have Y-type responsive properties.[49] However, the major component of the inhibition would have to occur in striate cortex since many DLG cells receiving inputs from the deprived eye continue to respond normally[18,19,49,50,56]".[22]

Spear reports, "The results suggest that the reduced cell-size may be secondary to the changes in striate cortex which I have described. However, the precise relationship between the striate cortex and the lateral geniculate cell-size changes still are not fully understood. For example, we do not know the relationship between which cells change in size and which cells maintain functional, but suppressed, inputs to striate cortex. We also do not yet know the relationships between the suppressed inputs to striate cortex and the X- and Y-cell functional classes in the dorsal lateral geniculate."[22]

It appears that this is not the end of the saga because Maffei and Fiorentini reported, "We conclude that visual deprivation of one eye during the early period of life in cats impairs, on the average, the spatial resolution of the neurones in the LGN on to which the deprived eye projects."[24]

Upon recording from the optic tract of the same animal they found: "The data for penetration 8 in Table 1 were obtained by recording from optic tract fibers projecting to a restricted area of the visual field. They look very much like the statistics evaluated for similar samples of neurons of the LGN, suggesting that the retinal neurones of the deprived eye might be involved in the loss of visual acuity observed at the LGN level. A similar hypothesis was proposed previously.[3] Further recordings from retinal fibers would be required to assess this point."[24]

At the present time we know that there are abnormal receptive fields and action potentials all the way from the optic tract to the striate cortex, that there is a binocular inhibitory effort by the seeing eye on the deprived eye, and that the LGN shows a reduction in cell size in the layers associated with the deprived eye.

It would seem to me that the next step would be to determine where in the visual system these abnormal fields and action potentials initiate and what role the LGN plays in the inhibition exhibited by the seeing eye.

'

II. <u>METHODS</u>

A. Facility Preparations

In order to investigate the possible neural mechanisms associated with amblyopia as discussed in the previous section, it is necessary to begin with an animal model. As the previous studies showed, the cat, is a good animal to use. Their visual system is quite similar to the human visual system, they are relatively inexpensive, easy to handle, propogate sufficiently and are a relatively easy animal to maintain. Equipment and instrumentation to completely immobilize the animal and provide for most of its vital functions is required. These include maintaining its core body temperature, respiration, monitoring its heart rate and function, providing for focusing and alignment of its eyes during the recording portion of the experiment. A craniotomy must be performed to expose the proper portion of the brain to be recorded from, a tracheotomy must be performed to provide for artificial respiration. A screen of some type is needed to project a visual stimulus and also to map out receptive fields. But even before any of the above can be actually put into practice, an approved animal housing facility must be available.

1. Animal Care Facility

What was referred to as the animal quarters when I
first started working with Doctor Cool was nothing
more than four filthy rooms above the third floor
on the northeast wing of Jefferson Hall. They were
used mostly for storage of apparatus or equipment
from previous experiments. One room was used to
house a colony of RCS rats. In one room we put in
a false wall to seal off openings to an exercise
area that had been used in the past. We removed all
the stored equipment, replaced a sink, fixed the
plumbing, dismantled old and dirty cages, fixed locks
on doors, shaved off the bottoms of doors to equalize
pressure to the different rooms. We also plastered
and painted the floors and walls in all the rooms
including the ceiling in the main room. We cleaned
out previously clogged floor drains, removed, rewired
and added lighting fixtures, repaired the timing
mechanisms on the room lighting switches. We sealed
off windows to provide for a darkroom in which to
dark rear cats. An intercom system was installed
to the main lab. Last but not least, we had installed
a heating and air conditioning unit with medical filters
to control the environment in the animal quarters. At
the same time that these renovations were being accom-
plished, Doctor Cool designed a cat breeding cage,

12' x 8' x 4' to be built out of 500 pounds of 1/4"
angle iron and fencing wire. All the angle iron
was ordered in pre-determined lengths from solid
stock. Every piece had to be hand cleaned, drilled,
deburred and painted before it could be assembled.
All of this seems very straight forward, but at every
turn there were unique problems presented that required
a great deal of patience and sometimes an ingenious
solution. Only after all this was accomplished, could
a federal inspector be called in to inspect and approve
the facility. A licensed Veterinarian's services are
also required by law to ensure that any sick or diseased
animals are properly cared for. All of this is necessary
because according to federal law, any animal that is to
participate in an experiment, must be held for twenty-
four hours in an approved facility before it can be used.

2. Laboratory Facility

The laboratory where the actual recordings were to
take place required a considerable amount of modifi-
cation to accommodate such an experiment. The
instrumentation to be used to monitor, record and
power the experiment had to be housed in a concise,
organized and convenient manner. It had to be con-
venient in that the electrical connections would not
be remote and that all monitors and control switches
were easy to access. (See Figures 1 and 2.)

Figure 1

Rack and Equipment Used for Biomedical Monitoring

Figure 2

Microcomputer and Audio Equipment

During a recording session the electrical activity
of the brain can be obscured by other electrical
activity in the room or building so it is necessary
to have a separate area in the room electrically
shielded from the building and the rest of the room.
(See Figure 3.)

Figure 3

Electrically Shielded Room

It is also necessary to isolate the animal during a
recording session from any external building or room
vibrations. Therefore, the animal is affixed to a
stereotaxic apparatus which is secured to a 2 x 3 foot
square piece of 1/2" boiler plate which rests on
multiple layers of foam rubber which itself is on top
of a 700 pound marble table resting on a 2 x 4 foot

piece of 1/2" plywood on a 1/2" thick rubber floor covering.   (See Figure 4.)

Figure 4

Vibration Dampening Table

Numerous cabinets were ordered, assembled and mounted to accommodate the many vials, containers, towels, chemicals and miscellaneous parts and equipment that any well and self-sufficient lab must have. Additional workbenches were ordered, assembled and wired with electrical outlets. (See Figures 5 and 6.)

Figure 5

Workbenches and Racks for Miscellaneous Parts

Figure 6

Storage Cabinets

Since professionally manufactured recording electrodes
are expensive and sometimes unreliable, it was decided
that we would make our own tungsten electrodes. This
required a fume hood and a piece of equipment designed
and built by Doctor Cool to properly etch and coat
the electrodes. We used the procedures to etch and
coat the electrodes as outlined by Cool and Crawford.[25]
(See Figure 7.)

Figure 7

Fume Hood and Electrode Etching Equipment

There were, of course, other almost innumerable small
projects and activities that supported these major
laboratory modifications.

B. Experimental Procedures

   1. Surgical Preparations

      a. General

The procedures and equipment used in the actual data
gathering process are the same as those outlined by
Crawford and Cool[26]. When an experiment is to be run,
the animal is deprived of food and water approximately
twelve hours before the experiment is to begin.
The experiment is begun by retrieving the animal from
the animal facility, weighing it and anesthetizing
with an intraperitoneal injection of 40 mg. of Nembutal
per kiolgram of body weight. When the animal is sedated
the top of its head, its throat, two spots on its chest
just lateral to the anterior nipples, and the inside of
both thighs are shaved. (See Figures 8 and 9.)

Figure 8

Top of Head Shaved

Figure 9

Ventral Shaved Areas

This is to accommodate respectively the craniotomy, tracheotomy, cardiac monitoring and access to the femoral vein for an intravenous anesthesia to be administered for the duration of the experiment.

b. Craniotomy

Following this, an incision is made down the midline of the skull and the tissues separated sufficiently to allow for an opening through the skull of approximately 10 mm in diameter. This opening is made over the posterior lateral gyrus which reveals the posterior termination of the sulcus lateralis laterally and the central sinus medially. This allows access to the

visual cortex and lateral geniculate nucleus. This
opening is gently filled in with surgical bone wax
to maintain the integrity of the brain in an intact
skull. (See Figures 10, 11 and 12.)

Figure 10

Mid Line Incision
With Skull Exposed

Figure 11

10 mm Trephined Craniotomy

Figure 12

Craniotomy After Being Fi led W tn Surgica Bone Wax

Two holes are then drilled in o he frontal sinuses,

one in each sinus Tnese openings allow two screws

to secure a bracket tc tne sku This bracket

provides for an attachmen to the stereotaxic apparatus

which is the sole suppo t and alignment mechanism

for the head and eyes. (See Figure 13.)

Figure 13

Support and Alignment Bracket in Place

c. Tracheotomy

Next an incision is made down the midline of the
animal's throat and the tissues separated to expose
the trachea. An incision is then made down the
midline of the trachea to accommodate the insertion
of a tube which will allow for artifical respiration.
(See Figure 14.)

Figure 14

Intubated Trachea

2. Immobilization - Vital Signs

    The animal is then secured and aligned in the stereo-
    taxic apparatus which is inside the electrically
    shielded room. The tubes from the artificial
    respirator are secured to the tracheal insertion.

    (See Figure 15.)

Figure 15

Immobilized Animal Secured to Stereotaxic Apparatus

The EKG electrodes are inserted under the skin
on the chest and taped to hold them in place.
An anal thermometer is inserted to record core
body temperature. The thermometer is connected to
a recording device which automatically turns a
heating pad on or off as the animal's core tempera-
ture deviates from normal core temperature. An
IV of Flaxedil in lactated ringers (5 mg/cc) is
inserted in the femoral vein; this is a synthetic
curare and completely immobilizes the animal. When
normal respiration stops, the artifical respirator

is turned on. Vagus innervation to the heart is
slowly replaced by the heart's own pacemaker. Under
optimum conditions, the animal can be maintained in
this condition for forty-eight hours.

3. Refraction

Plano contact lenses are placed on the animal's
corneas after it has been dilated with atropisol (1%),
and efricel (10%). Retinoscopy is performed to
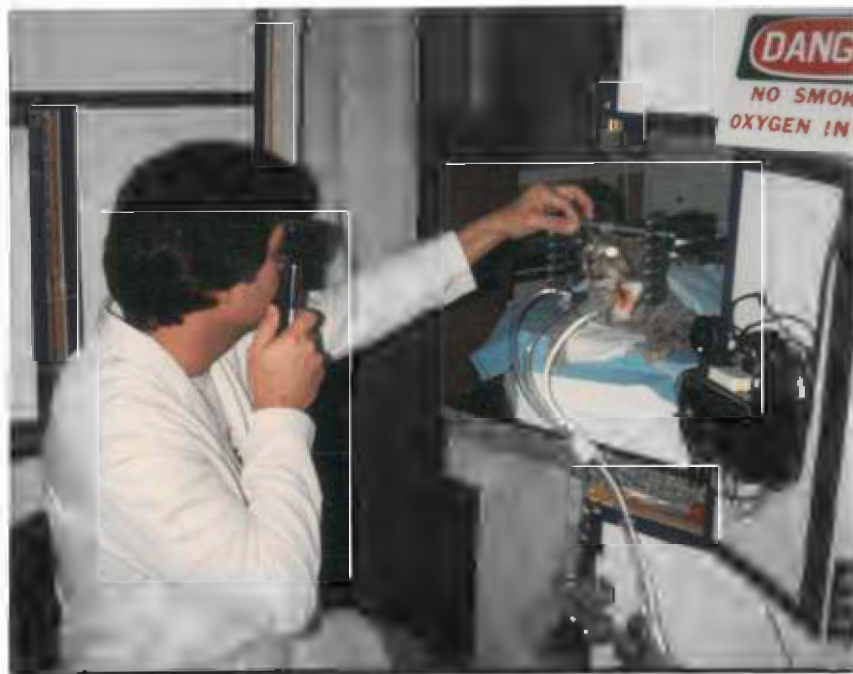establish the animal's refractive status. (See Figure 16.)

Figure 16

Retinoscopy

Once this is determined, the appropriate power contact lens is placed on each cornea to fix the animal's far point at one meter. An opthalmoscope with a plano lens in place is secured to a holder and its light is directed at the optic disc of each eye which is then projected onto a screen one meter away. The details of this procedure are outlined by Fernald, R., and Chase, R.[27] and Pettigrew, J.D., Cooper, M.L., Blasdel, G.G.[28]. If the animal's far point is truly one meter, the disc will be in sharp focus on the screen. Its borders and accompanying vessels are traced out on the back side of the screen. From here area centralis of each eye can be mapped out on the screen. (See Figure 17.)
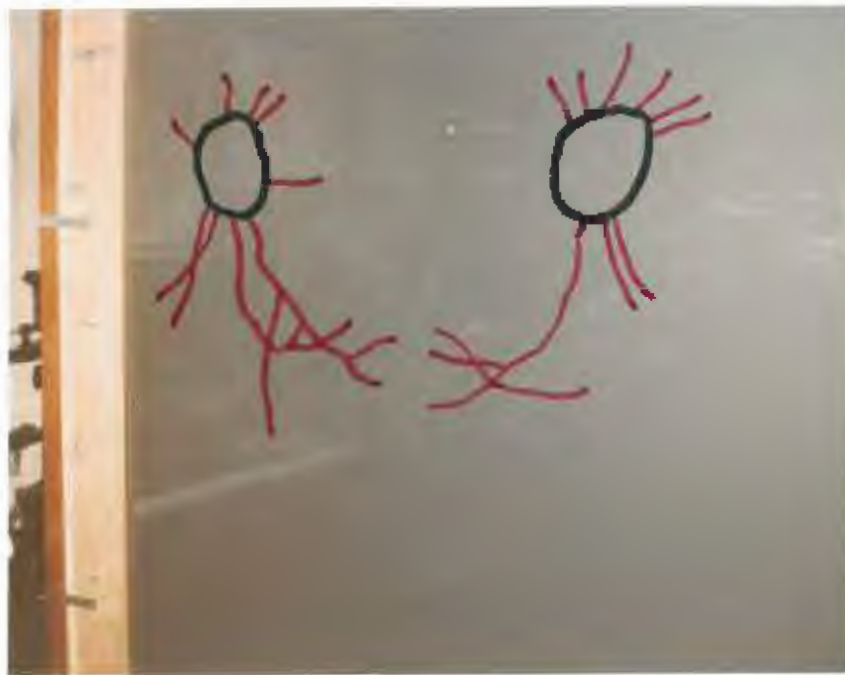

Figure 17

Optic Discs, Retinal Vessels and Areas Centralis
Mapped out on Tangent Screen

4. Single Cell Recording and Receptive Field Mapping

A tungsten electrode is then secured to a DKI 607w
hydraulic microdrive. The electrode is positioned
above the bone wax which is covering the opening
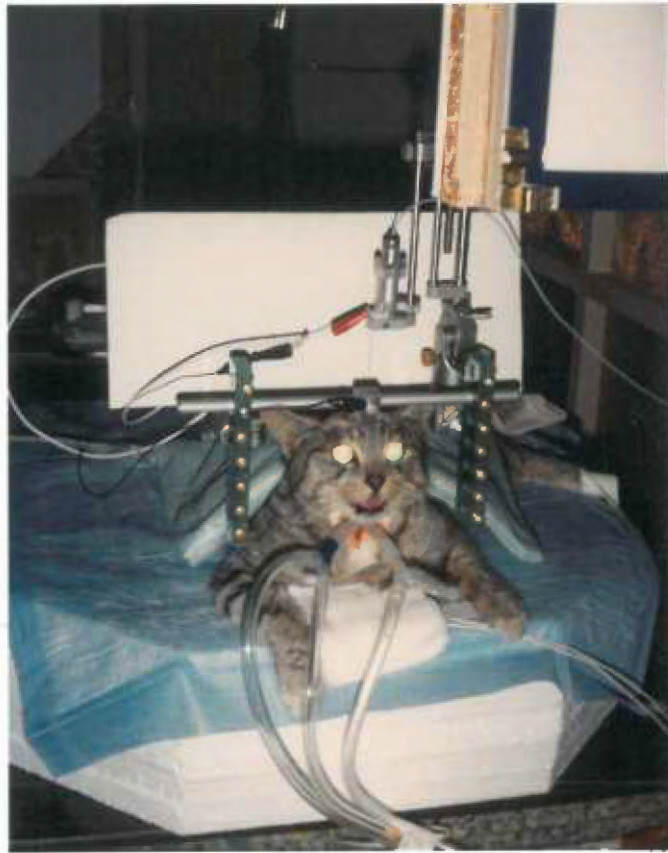of the skull. (See Figure 18.)

Figure 18

Electrode Secured to Microdrive

The microdrive (as the name implies) can be advanced
or retracted in micrometer increments (one millionth
of a meter). The electrode is electronically connected
to an oscilliscope which has an analog display of any
electrical activity near the electrode. The electrode

is advanced into the brain while a moving light is
being displayed on the screen in front of the animal.
This is continued until a large enough signal can be
detected and its receptive area mapped out on the
screen. The signal may be of a binocular nature and
then two separate receptive fields are mapped out
representing corresponding retinal areas on the two
retinas.

III. PRELIMINARY RESULTS

At the time of this writing, we have run six separate
preparations and each animal contributed to more efficient
and better organized surgical and laboratory procedures.
They also allowed us to work out any electronic problems
associated with the equipment, shielding and the electrods.
In the first animal we were unable to elicite any electrical
activity in the brain. This was due to faulty electrodes
which were made in our laboratory.

In the second animal, three probes were made and we got
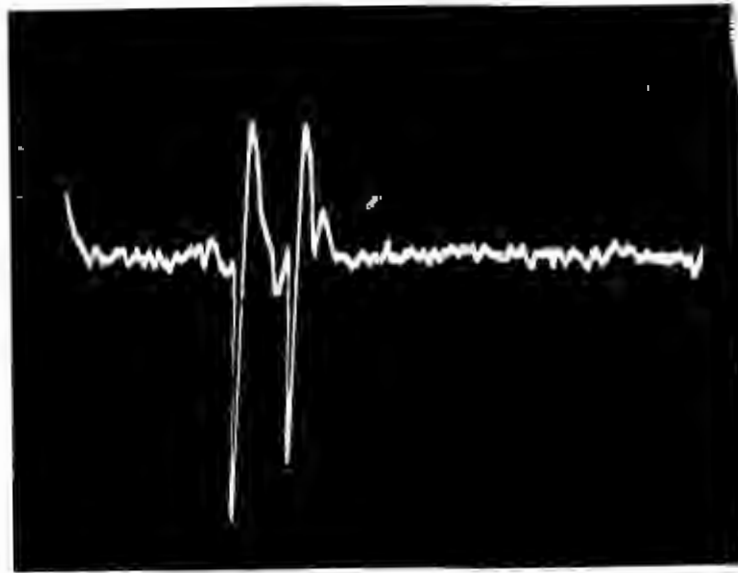a spike at a depth of 1.918 mm. (See Figure 19.)

Figure 19

Animal Number Two - Depth 1.918 mm

Two good responses were elicited from the third animal. The first response was at a depth of 1.575 mm and had a small and difficult to define receptive field. (See Figure 20.)
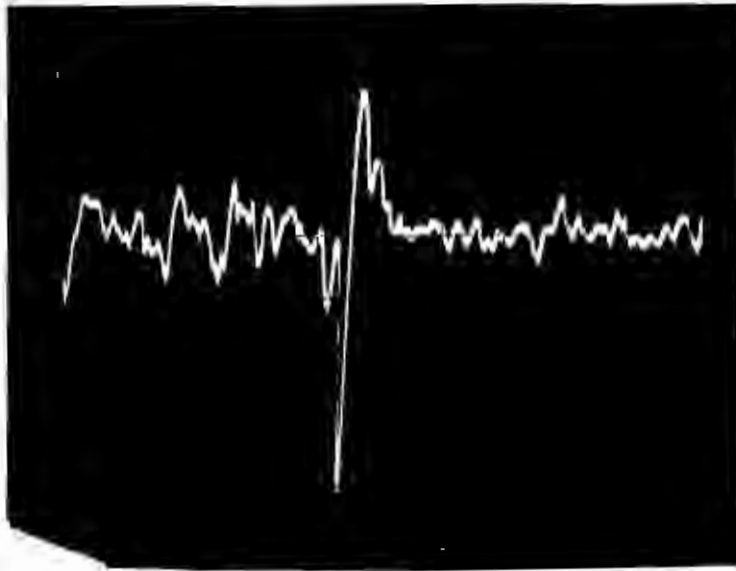
Figure 20

Animal Number Three - Depth 1.575 mm

On the same penetration, but at a depth of 1.942 mm, an on-off binocular response was elicited and mapped out. (See Figure 21.)

Figure 21

Animal Number Three - Depth 1.942 mm

The next animal experienced a cardic arrest early in
the recording session and apparently was severe enough
to depress most cortical activity.   However, we did get
good isolation of optic radiation fibers.

The fifth animal yielded two responses on a single
penetration, one at .759 mm; its receptive field was
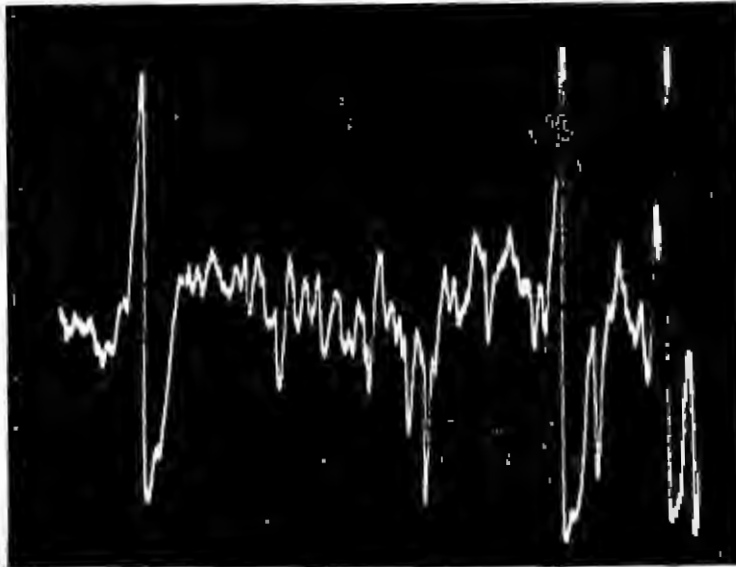superior temporal and binocular.   (See Figure 22.)

Figure 22

Animal Number Five - Depth .759 mm

The second was at a depth of 1.063 mm; its receptive
field was also superior temporal and binocular.   (See
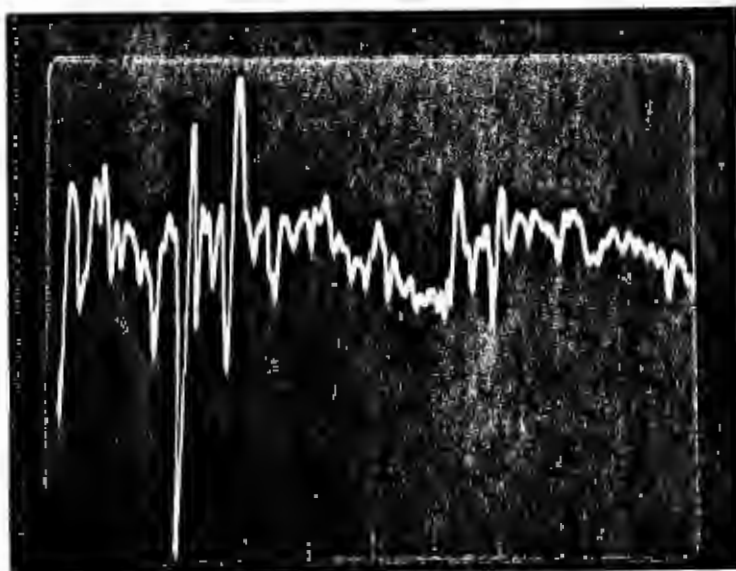Figure 23.)

-45-

Figure 23

Animal Number Five - Depth 1.063 mm

The sixth and last animal yielded some very interesting
data. On the twelfth penetration at a depth of .179 mm
in the brain, two cells were activated. Cell number one
and cell number two had amplitudes of 100 μV and 135 μV
and durations of .25 msec and .125 msec respectively.
Their receptive fields were binocular. The right eye's
receptive field was mapped out as being 4.5° temporal
from the center of the temporal disc margin and 14°
superior from the center of the temporal disc margin.
The left eye's receptive was 14.5° temporal from the
temporal disc margin and 12.5° superior from the center
of the temporal disc margin. A bar of light 2.5° long and
.25° wide was moved from lower right to upper left in
the receptive field. Both cells showed a strong right
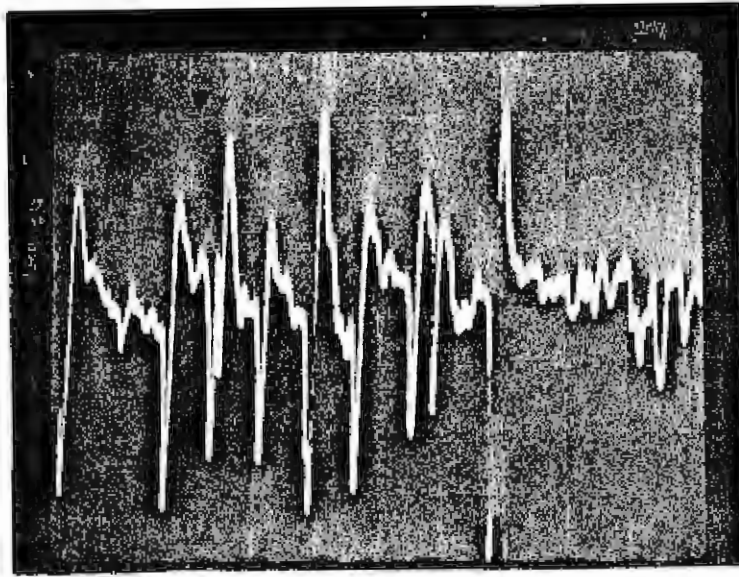eye dominance. (See Figure 24.)

Figure 24

Animal Number Six - Depth .179 mm

A response from a third cell on the same penetration at
a depth of 2.416 mm was recorded. It was a hypercomplex
cell with its receptive field in the center of area
centralis of the right eye. It was extremely sensitive
to a vertical bar approximately .5° long and very narrow.
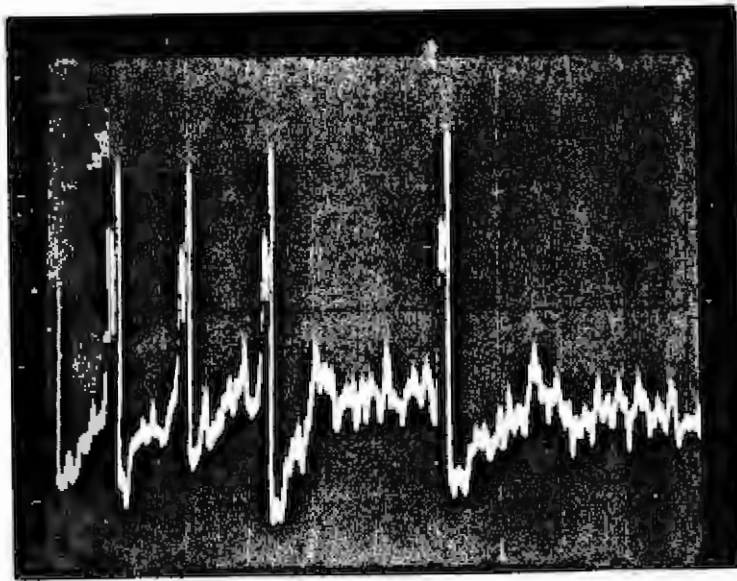(See Figure 25.)

Figure 25

Animal Number Six - Depth 2.416 mm

IV. Discussion

All of the previously recorded activity was made from the visual cortex or optic radiation fibers. Next it will have to be demonstrated that these laboratory set-ups and procedures are adequate to record from the lateral geniculate nucleus (LGN).

A breeding colony will have to be started so that kittens will be available to be reared under special conditions that will make them amblyopic. When the research reaches this stage, it will then start addressing the possibility of inhibitory mechanisms in the LGN as they relate to deprivation amblyopia.

It is very exciting to activate and map out a receptive field under these conditions. It is one thing to read about how the visual cortex is organized and how certain cells will respond to a particular shape of light, or direction and speed of movement, or a simple on-off response, but it really drives home the fact when you can actually make the cell respond by manipulating the light yourself.

This has been a very valuable experience for me to learn more about amblyopia and to be involved with the many details associated with setting up a laboratory in order to investigate a hypothesis that involves animal models. I feel that optometry should do more of this type of research. PUCO can now offer students a first-hand look at this kind of research and this exposure may draw those students who have a predilection for research into the field.

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