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Effects of pharmacologic postsynaptic blockade on synaptic plasticity in kitten visual cortex

by

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A Thesis

Submitted in partial satisfaction of the
requirements for the M.D. with Thesis Program

of the
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I thank my research advisor, Michael Stryker, for his teaching and for his patience with me over these past four years. Perhaps he will think twice before accepting another medical student "just for the summer."

I would also like to thank Sheri Strickland, Barbara Chapman, and the other members of the Stryker lab, who were and are terrific teachers and friends.

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ABSTRACT

Synaptic plasticity, the rearrangement of connections within the nervous system, is vital to the brain's development. Models of plasticity commonly assume that the strength of a synapse is determined by the correlation between the activity of the postsynaptic cell and that of the presynaptic cell that triggered its firing. We examined the role of postsynaptic cells in synaptic plasticity by selectively inhibiting them with continuous infusion of the γ-amino-butyric acid ($GABA_A$) agonists 3-aminopropanesulfonic acid and isoguvacine. Our model system was kitten primary visual cortex, where monocular occlusion during the critical period causes a dramatic decrease in cortical responsiveness to input from the occluded eye. Single-cell recordings after discontinuation of the inhibitory infusion showed *no shift* in responsiveness to stimulation from either eye, while untreated regions of cortex showed the usual shift in favor of the more-active inputs from the open eye. This result differs from that found in earlier experiments using the $GABA_A$ agonist muscimol, which induced greater responsiveness to inputs from the less-active inputs from the closed eye. Our results support the hypothesis that the postsynaptic cell plays a vital role in ocular dominance plasticity; and suggest that the changes in the condition of the postsynaptic cell caused by the different agonists can cause different types of changes in synaptic strength.

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INTRODUCTION

Synaptic plasticity: theories and studies

Plasticity, the process of rearrangement of connections within the brain, is as vital as it is mysterious. Without the ability to change the connections laid down before birth, we could neither learn nor develop normally (1). The experiments that are the basis for this thesis investigate some of the cellular mechanisms governing plasticity during development of the visual system.

Various mechanisms governing synaptic plasticity have been suggested over the last half-century. The most influential was postulated by D.O. Hebb in 1949 in *The Organization of Behavior* (2):

> When an axon of cell A is near enough to excite a cell B and repeatedly or persisitently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased.

Thus, the strength of a synaptic connection depends on its activity. Stent, in 1973, formulated the converse of Hebb's postulate (3):

> When the presynaptic axon of cell A repeatedly and persistently fails to excite the postsynaptic cell B while cell B is firing under the influence of other presynaptic axons, metabolic changes take place in one or both cells such that A's efficiency, as one of the cells firing B, is decreased.

Later investigators (1, 4) refined Hebb's and Stent's rules to state that: (i) Synaptic contacts between sychronously active pre- and postsynaptic neurons are selectively reinforced, and (ii) Synaptic contacts between asychronously active pre- and postsynaptic neurons are selectively depressed or eliminated.

These various models of synaptic plasticity do not explain the types and locations of the changes in the synapse. That is, are the changes anatomic (e.g., axon dies off) or physiologic (e.g., change in level of neurotransmitter production); what neurotransmitter-receptor systems are involved; and what are the exact roles of the pre- and postsynaptic cells? As I will describe below, we addressed this last question in our experiments.

The N-methyl-D-aspartate (NMDA) receptor has emerged as the most likely mediator of "Hebbian," correlation-based synaptic plasticity. In visual cortex, specific blockade of the NMDA receptor alters the process of synaptic plasticity (5). On the level of the single synapse, the NMDA receptor mediates a process known as long-term potentiation (LTP), where matched pre- and postsynaptic activity strengthens the synapse between the cells (4, 6-7). The induction of the LTP, however, is a function of the *postsynaptic* ionic fluxes. Recently, the phenomenon of long-term depression (LTD), the opposite of LTP, was also found to be mediated by activation of the NMDA receptor, with the same stimulus but with smaller and probably slower changes in postsynaptic calcium concentrations than those that induce LTP (8). So, while correlation of pre- and postsynaptic activities are important for NMDA-mediated change in synaptic strength, the state of the postsynaptic cell may determine the type and duration of the change.

We investigated the role of postsynaptic neuronal activity in synaptic plasticity, using kitten primary visual cortex as our model system (fig. 1). Mature mammalian primary visual cortex is organized in **ocular dominance columns** (fig. 2), with cells in each column driven preferentially by one eye or the other (11, 12). These columns are not present at birth in non-primates, but rather develop as the young animal grows (11). While there may be some residual plasticity in the adult system, ocular dominance plasticity is most

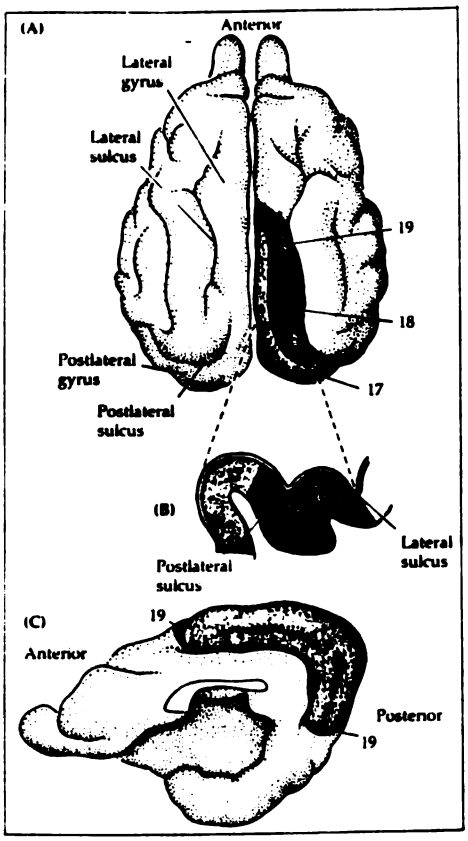

Figure 1
Cat visual cortex, viewed from above (*A*), coronally (*B*), and medially (*C*). Primary visual cortex is labelled as area 17. From Kuffler, Nicholls, and Martin, reference (9)

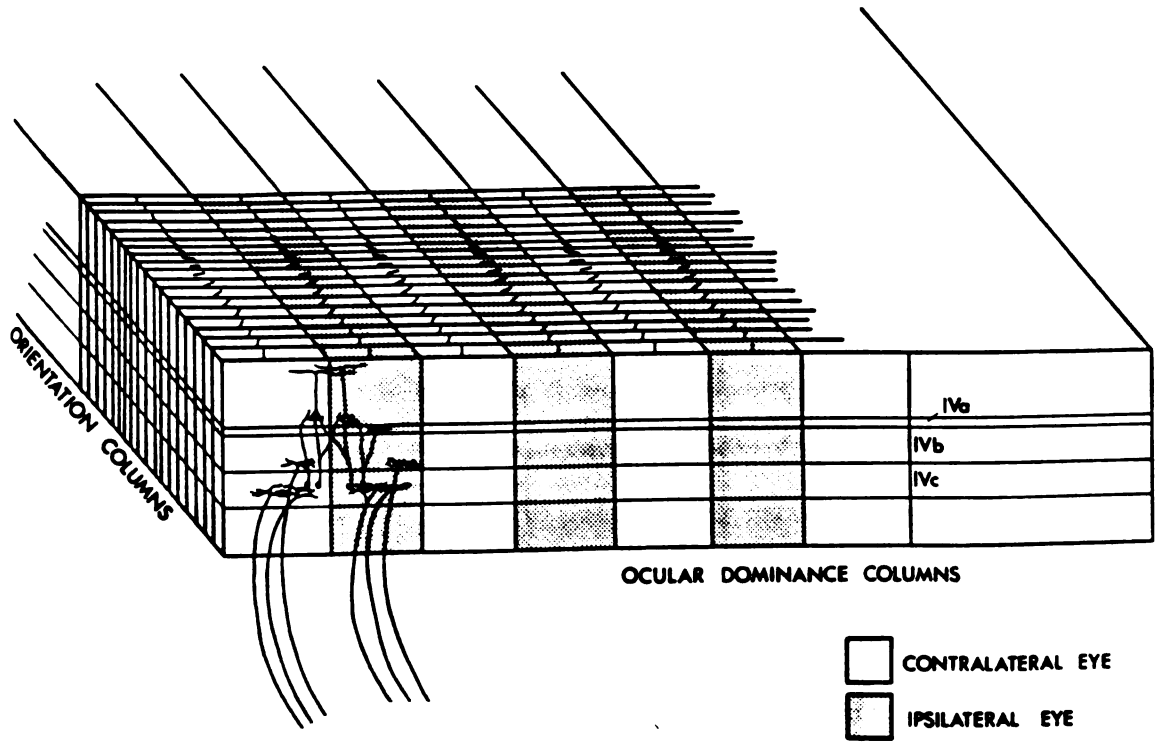

Figure 2
Organization of primary visual cortex into ocular dominance columns. Orientation columns, which run perpendicular to the ocular dominance columns, and cortical layers are also shown. From Hubel and Wiesel (10).

evident in young animals, during the "critical period" of maximal developmental plasticity (11, 12). The mechanisms of developmental plasticity likely operate in the adult brain as well (13, 14), but large shifts in ocular dominance do not occur once the critical period is past.

Normal four week-old kittens do not have fully developed ocular dominance columns. Rather, most cells in primary visual cortex are driven binocularly, with a slight bias towards the contralateral eye (11). If one eye is occluded, however, there is a rapid and reversible shift in dominance towards the open eye, i.e., the most active afferent inputs (11, 15). A similar process probably occurs in humans with deprivation (and some forms of strabismic) amblyopia, where the input from the normally functioning but relatively deprived non-dominant eye is suppressed. Patching the dominant eye intermittently can reverse the process, but only up to a few years of age, when the existing ocular dominance pattern is "cemented" in place (for review see ref. 16).

Using this visual deprivation model, researchers have revealed many of the rules governing synaptic plasticity in visual cortex. These are summarized in figure 3. Through manipulation of the activities of the afferents and of the cortical cells, these studies showed that ocular dominance plasticity depends on competition between the afferents carrying information from both eyes. Note that suppression of both pre- and postsynaptic cortical activity with the sodium channel-blocker tetrodotoxin (TTX) prevents any shift in ocular dominance with monocular deprivation, while selective postsynaptic blockade with muscimol reverses the direction of the ocular dominance shift caused by monocular deprivation. These experiments with muscimol are described in more detail below.

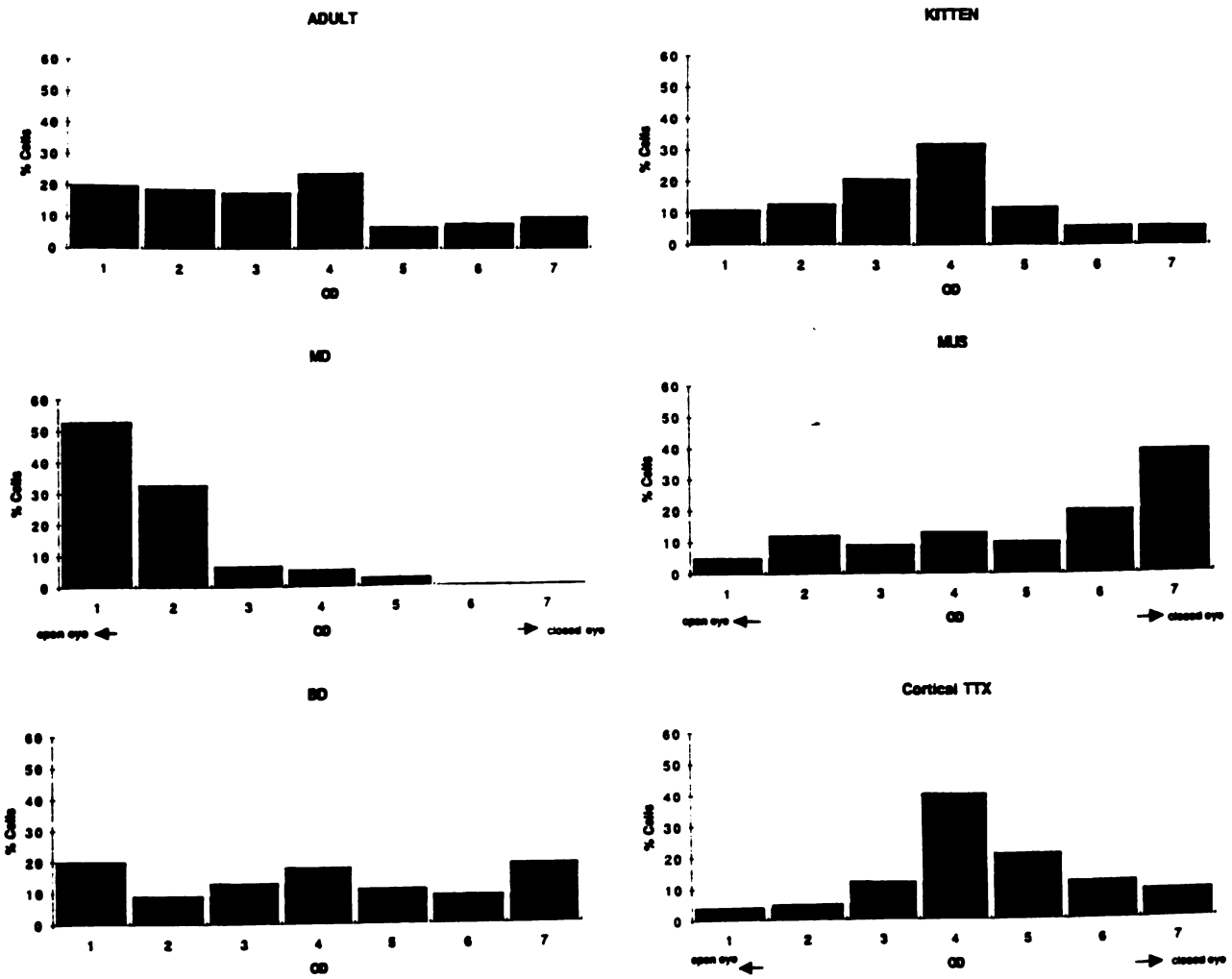

Figure 3
Summary diagram of previous experiments, revealing several rules governing ocular dominance plasticity. Ocular dominance (OD) groups are fully explained in the "Methods" section of the text. Group 1 is driven exclusively by the contralateral eye, group 7 by the ipsilateral eye (i.e., with respect to the cortex being recorded from), and group 4 cells are driven equally by both eyes.

In the normal adult visual cortex (ADULT), most neurons are driven binocularly, with slightly more input from the contralateral eye (17). Normal kittens who have not been visually deprived (KITTEN) display a similar pattern, although they have fewer monocularly-driven cells (17). If the ipsilateral eye is sutured shut for 7-10 days (MD), there is a shift in ocular dominance towards the open eye (from 3-APS control data). In contrast, if the monocular deprivation is combined with muscimol infusion (MUS), the shift towards the open eye is, in the area of impulse blockade, replaced by a shift towards the closed eye (18). Compare the effects of binocular visual deprivation (BD), which does not result in an ocular dominance shift (17), and intracortical tetrodotoxin with monocular deprivation (TTX), whose blockade of both pre- and postsynaptic activity prevents the ocular dominance shift caused by monocular deprivation entirely. After Shatz (6).

In 1984, Carew *et al,* (19) published the results of their investigation of the role of the postsynaptic cell in synaptic plasticity. They used electrical hyperpolarization to inhibit postsynaptic action potentials in the sea slug *Aplysia californica* during classical conditioning of the withdrawal reflex. They found that, while Hebb's postulate requires coincident pre- and postsynaptic action potentials to strengthen a synapse, in *Aplysia* plasticity is possible without postsynaptic action potentials. That is, only presynaptic factors determined the change in synaptic strength.

Reither and Stryker tested the role of the postsynaptic neuron in the kitten monocular deprivation model (18). They used an implanted osmotic mini-pump to infuse muscimol (MUS), a $GABA_A$ (γ-amino-butyric acid) agonist, into primary visual cortex in a manner similar to the TTX experiments mentioned previously. GABA is found in kitten visual cortex (20), where it is the main inhibitory neurotransmitter (21, 22). While GABA binds both pre- and postsynaptically (23), the $GABA_A$.receptor subtype has been found only postsynaptically in mammalian brain (24, 23), and muscimol binds selectively to the $GABA_A$ receptor in cat visual cortex (25). Thus, muscimol should be a selective inhibitor of postsynaptic cell activity in kitten visual cortex.

By combining muscimol infusion with monocular deprivation, Reiter and Stryker found that, in the absence of postsynaptic action potentials, plasticity (i.e., a shift in ocular dominance) occurred, but towards the closed eye. Thus, just changing the postsynaptic activity had made the same afferent activity cause plasticity in the opposite direction. They concluded that postsynaptic cell function is crucial to normal plasticity in area 17, and that the direction of that plasticity is a function of the postsynaptic membrane

conductance. Postsynaptic action potentials are not necessary for plasticity *per se* -- a departure from Hebb's postulate.

This shift towards the less-active afferent inputs can be explained by a modification of Hebb's rule: in the area of muscimol infusion, the less-active presynaptic cell's activity (reflecting input from the occluded eye) correlated better with the less-active (inhibited) postsynaptic cell's activity (18). And local responses, without postsynaptic action potentials, are sufficient to generate plasticity. Stent's rule could also explain the reverse-directed plasticity, if failure of the most-active inputs (from the open eye) to trigger postsynaptic action potentials causes their synapses to weaken.

Another interpretation of Reiter and Stryker's results is possible, however. The GABA receptor system is far more complex than suggested by the usual "A" and "B" subtype designations (26, 27). Muscimol may well bind to a subtype specifically related to plasticity, or may bind to other, as yet uncharacterized, receptors. Either way, the reverse-directed plasticity seen with muscimol may not be the result of any Hebb-type interaction, but may be a specific result of the muscimol infusion unrelated to normal processes of synaptic plasticity.

We therefore repeated the muscimol experiments using different (i.e., non-muscimol) GABA-A agonists, to check whether the reverse-directed plasticity was a specific to the muscimol treatment. These experiments were completed from 1989 to 1991.

Agonist selection

Unfortunately, the relative potencies of many GABA agonists are not known *in vivo* in kitten visual cortex. The available potencies, based on *in vitro* peripheral receptor-binding assays and on studies examining GABA-induced behaviors (mediated by the basal ganglia) in rats, do not agree (28,

29). Nor are peripheral nervous system and basal ganglia receptors likely to be identical to those found in visual cortex.

Furthermore, any agonist used must be potent enough in isotonic solution to block activity to a useful distance from the infusion cannula, and must be stable in solution at body temperature for up to two weeks.

Based on these criteria, we settled on the agonists 3-aminopropane-sulfonic acid (3-APS) and isoguvacine (IGV) (fig. 4) as stable, selective postsynaptic $GABA_A$ agonists for our experiments(23, 32-33; also, personal communication with P. Krogsgaard-Larsen, 21 October 1991).

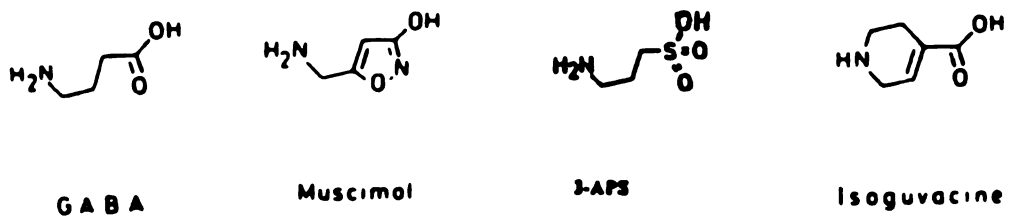

Figure 4
Structures of GABA and the GABA agonists muscimol, 3-APS, and isoguvacine (29, 31).

MATERIALS AND METHODS

Subjects

The subjects of the experiments were 9 kittens, ages 28-31 days, which were bred in an isolated colony. Experimental procedures were approved by the UCSF Committee on Animal Research.

Pump implantation

Surgical procedures were similar to those used by Reiter and Stryker (18, 34). Each kitten was anesthetized with 1-1.5% halothane in 2:1 nitrous oxide:oxygen mixture and placed in a stereotaxic frame. Anesthesia depth was judged by respiratory rate, continuous ECG rate, and withdrawl reflex to footpad pinch. Using aseptic technique, a 30-gauge stainless steel cannula was implanted at Horsley-Clarke coordinates A/P 0.0, lateral 2.0, depth 2.0 from the dural surface (all dimensions in millimeters). Animals received bilateral cannulae for redundancy in case of pump system failure (e.g., blood clot clogging cannula), and for testing of various agonist concentrations. The steel cannulae were connected via vinyl tubing (Bolab V/3) to osmotic minipumps (Alza 2002, Alza Corp., Palo Alto, CA), which infuse a volume of 237 microliters at a rate of 0.5 microliter/hour over 14 days.

Minipumps were filled with sterile agonist in isotonic aqueous solution with NaCl; see table 1 for concentrations used in each animal. 3-APS was obtained from Tocris Neuramin, Ltd. (Essex, England), isoguvacine from Research Biochemicals, Inc. (Natick, MA).

Table 1

Animal	Agonist	Concen. (mM)	Cortex recorded	MD	Age at implant (days)	Days before MD	Days before recording	Expt. time (hrs.)
687*	3-APS	100	Right	Left (CON)	28	2	7	33
689	3-APS	200	Left	Right (CON)	29	2	8	39
691	3-APS	200	Left	Right (CON)	28	3	7	39
692	3-APS	200	Left	Left (IPSI)	31	2	8	38
701	3-APS	200	Right	Left (CON)	28	2	8	35
737*	IGV	100	Left	Right (CON)	31	4	6	53
738	IGV	100	Left	Right (CON)	30	3	6	38
739	IGV	100	Left	Left (IPSI)	30	5	6	40
746	IGV	100	Left	Right (IPSI)	30	4	6	60

Summary of experimental method for each animal. All recording from experimental areas was performed using the "blind" method described in the text, except from the animals marked with asterisks ("*"). "MD" is monocular deprivation. "Expt. time" is the total time for microelectrode recording, including surgical preparation.

Monocular deprivation

Two to five days were allowed for the area of neural blockade to stabilize before eyelid suture (18). The eye to be sutured was randomly selected (q.v. table 1), and was closed using several silk sutures under halothane/nitrous oxide anesthesia as above. Topical lidocaine and antibiotic ointments were applied to the sutured eye.

Microelectrode recording

After seven days of monocular deprivation, which allowed the ocular dominance shift to occur (18), the animal was prepared for single-cell recording. Anesthesia was induced with inhaled anesthesia as above, and the ECG and temperature were monitored continuously. Temperature was maintained by a feedback-controlled heating pad set to 38 degrees C. Atropine and dexamethasone were given every 12 hours.

The femoral vein was cannulated, and anesthesia maintained thereafter by thiopental sodium infusion (10 mg/kg loading followed by 2-4 mg/kg-h maintainance) and 3:1 nitrous oxide:oxygen inhalation. Anesthesia was titrated by heart rate, withdrawl reflex (before paralysis), and EEG (placed before paralysis).

The trachea was cannulated, and the kitten was placed in the stereotaxic apparatus. Once the skull was exposed and the EEG electrode was in place, the animal was paralyzed with gallamine, 0.1mg/kg-hr plus an initial bolus of one hour's dose. End-tidal CO_2 was maintained at 3.6-4.2% to minimize cerebral edema. We then opened a craniotomy (12X12mm), built a well of dental cement to hold oil or agar during recording, and opened the dura. Contact lenses maintained focus of the eyes and prevented drying of the cornea.

Single-unit recordings were made using laquer-coated tungsten microelectrodes (35) positioned by a stepping-motor microdrive. Penetrations

were vertical with respect to the stereotaxic system. After verifying that the electrode was fuctioning normally by a penetration far anterior to the cannula, the area of total activity blockade surrounding the cannula was mapped out. Figure 5 is a schematic of the recording setup.

The area of **total blockade** was defined by penetrations to 2 mm deep without visually evoked action potentials. Some injury discharges were present. The block boundary was defined as the first sign of visual evoked potentials moving anteriorly away from the cannula. An area of partial blockade, with partially inhibited visual responses, extended several millimeters beyond the area of total blockade. It was not studied further.

Silicone oil (Accumetric Inc., Elizabethtown, KY) and agar were applied to maintain stability and to prevent drying of the exposed cortex. Receptive fields were plotted using a hand-held shuttered lamp on a screen in front of the animal.

Once the area of blockade was mapped, the tubing to the cannula was cut and the block allowed to wear off. For the last 2 isoguvacine animals, the cannula was cut at the start of the preparatory surgery on the first day of recording, due to the length of time required for the block to wear off.

Approximately thirty "control" units were recorded anterior to the blocked region, in normally responsive cortex. This data confirmed the effectiveness of the monocular deprivation, and provided a basis for evaluating any shift seen with GABA agonist infusion.

Ten to 15 units were recorded per penetration, and electrolytic lesions were made to allow later histologic identification of each electrode track. Each

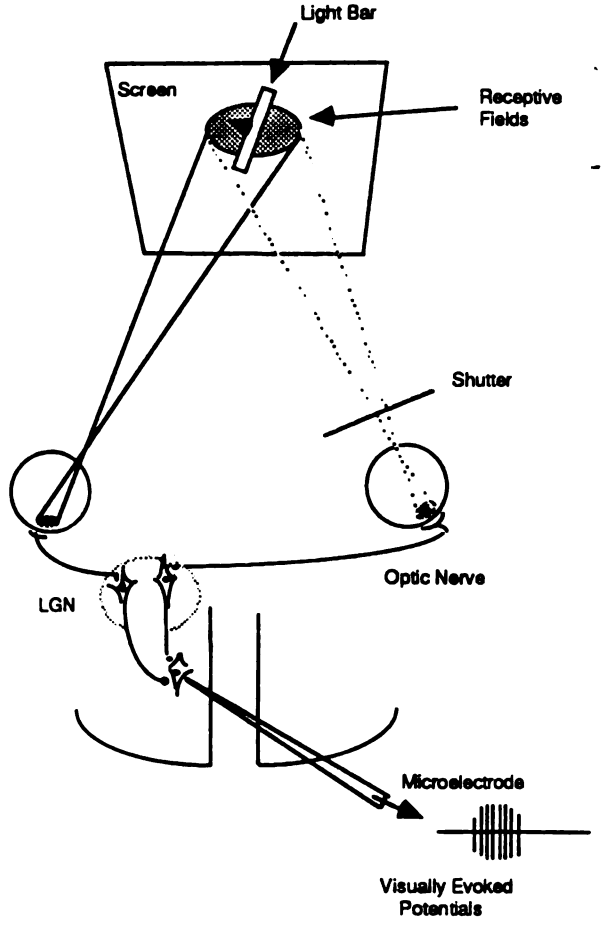

Figure 5
Setup for microelectrode recording from visual cortex. The cat sits facing a white screen, onto which is projected a light bar of optimal length and orientation. When the bar crosses the receptive field of the non-shuttered eye, the extracellular microelectrode picks up the resulting train of evoked action potentials from a single cortical cell. This spike train is displayed on an oscilloscope, and is also played through an audio amplifier. (After Bishop, ref. 36)

cell's optimal orientation and direction preference were recorded, as were responsiveness, habituation, and afferent responsiveness. Ocular dominances were recorded using a seven-point scale devised by Hubel and Wiesel (37):

1 Exclusively contralateral
2 Contralateral eye much more effective than ipsilateral
3 Contralateral eye slightly more effective than ipsilateral
4 No obvious difference in the two eyes' effects
5 Ipsilateral eye slightly more effective
6 Ipsilateral eye much more effective
7 Exclusively ipsilateral

The blockade was allowed to wear off to within 0.5 mm of the cannula, while the animal remained anesthetized (20-45 hrs.), before recordings were made within the area of the blockade ("**experimental**" data). The time course of the experiments is given in table 1; the numbers of units recorded in each animal are given in table 2.

All animals except 687 (3-APS) and 737 (isoguvacine) were studied using a "blind" procedure, where the investigator making the recordings in the previously blocked cortex had no knowledge of which eye had been sutured shut since both the animal's eyes were surgically altered to appear identical. He also was not allowed to see the control data from the unblocked area.

A contralateral bias index (CBI) was calculated for each penetration, and for various summary data (see below). The index is calculated by the formula:

$$CBI = 100 \times [(\mathbf{1}-7) + ((2/3) \times (\mathbf{2} - 6)) + ((1/3) \times (\mathbf{3} - 5)) + n] / (2 \times n),$$

where the boldface numbers **1** to **7** are the number of units in each OD group, and n is the total number of visually responsive units (34).

To create figures 8-10, the CBI was adjusted for those animals receiving contralateral monocular deprivation to allow them to be plotted on the same scale as the animals receiving ipsilateral monocular deprivation:

$$CBI_{contra} = (100 - CBI) + 10$$

The adjustment of adding "10" assumes that the *shift* in ocular dominance from an original value of 55, the average value for normal kittens (17), is more reflective of the ocular dominance shift due to the synaptic plasticity process than is the final value of the CBI. Without this adjustment, for example, a +10 point shift in the CBI for an ipsilaterally deprived animal would give a final CBI of 65, while in a contralaterally deprived animal the final CBI would be 45 + 10, or only 55.

To compare the distribution of ocular dominances, a *monocularity index* (MI) was also calculated by the formula

$$MI = \{(1 + 7) + [2/3 \times (2 + 6)] + [1/3 \times (3 + 5)]\} / n$$

Where the symbols used have the same meaning as for the CBI, above (17).

Ocular dominance histograms were plotted for both control and experimental data.. The Mann-Whitney U statistic was calculated to compare experimental with normal CBI's and MI's, using Statview II statistics software (Abacus Concepts, Inc., Berkeley, CA).

Histology

At the end of each experiment, the animal was deeply anesthetized with thiopental until it was bradycardic. It was then exsanguinated by transcardial infusion of isotonic phosphate-buffered saline, and perfused with 10% formalin in buffered saline. The brains were removed intact and immersion fixed, then imbedded in gelatin-albumin-glutaraldehyde and cut on the vibratome in 50 μm sections. Nissl-stained sections were examined to identify the lesions and electrode tracks, which allowed identification of all penetrations in all animals, except for 2 of the blocked tracks in animal 739, whose cortex was damaged during recording, making layer assignment impossible.

RESULTS

All areas of recording had normal, vigorous afferent activity. Furthermore, the Nissl-stained sections showed no histologic abnormalities in the areas of recording (except animal 739, as described above). Two hundred ninety-five data units were recorded in 28 electrode penetrations (150 3-APS and 145 isoguvacine); 275 control units were recorded in 10 penetrations. Blockade sizes were measured as described above; results are shown in table 2. Times necessary for each block to wear off sufficiently for experimental data recording are also shown in the table, and ranged from 20-24 hrs. for 3-APS to 30-45 hrs. for isoguvacine . Blocks were of similar size to those obtained with muscimol: 1.5-2.5 mm for 3-APS and 2.2-4.0 mm for isoguvacine , versus 1.0-3.5 mm for muscimol. Interestingly, the cortex near the cannula was paler than the more distant cortex. This blanching gradually disappeared after the cannula tubing was cut, and is thought to be due to local vascular auto-regulation causing vasoconstriction in the area of decreased neural activity.

Ocular dominance histograms are provided for individual kittens from the 3-APS and isoguvacine groups, and for summary data for each drug for blocked and unblocked areas (figs. 6-7). All data are plotted as if the ipsilateral eye was deprived. The control data from each animal showed a strong ocular dominance shift towards the open eye, confirming the effectiveness of the monocular deprivation procedures (i.e., no small openings developed in the eyelid suture line, etc.). Contralateral bias indices for the control and experimental areas in each animal are summarized in table 2.

Experimental areas, unlike the control areas, showed no consistent shift towards the open eye. These results are similar to those seen in non-

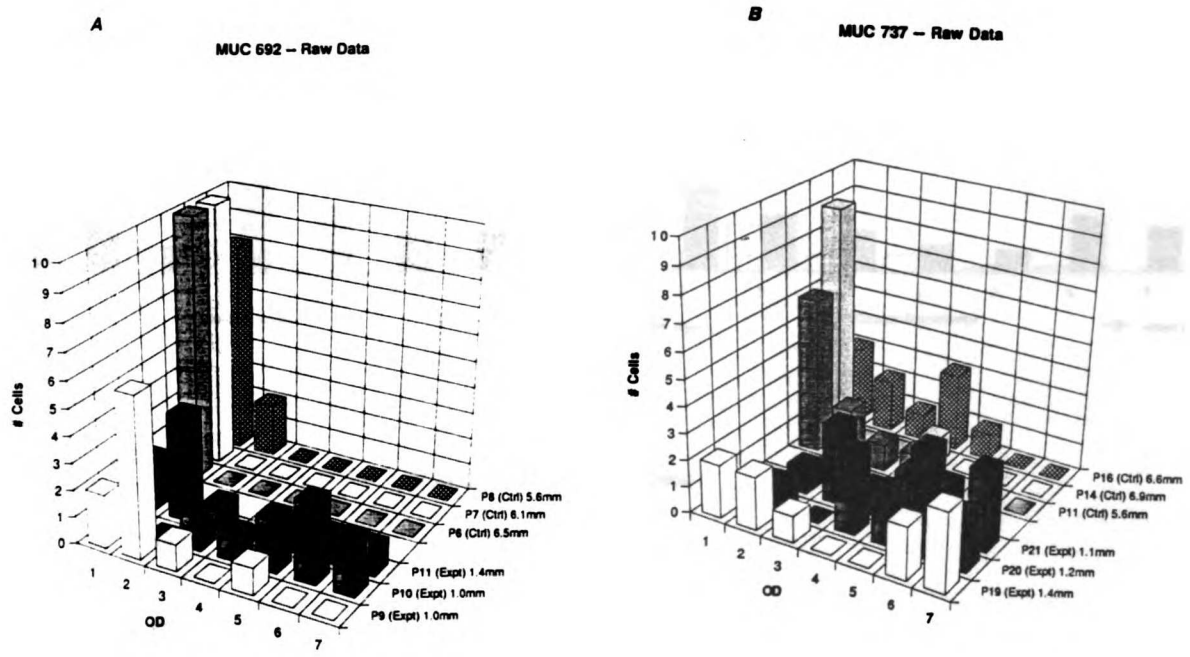

Figure 6

Examples of ocular dominance histograms for one animal from each agonist group. Plotted as though all animals received ipsilateral monocular deprivation (i.e., OD group 1 is driven by the open eye).

A: Animal number MUC 692, who received 3-APS. Each "P#" represents an electrode penetration. Control (Ctrl) and experimental (Expt) penetrations are indicated, along with each penetration's distance from the drug infusion cannula.

B: Animal number MUC 737, who received isoguvacine. Conventions as for figure 6A, above.

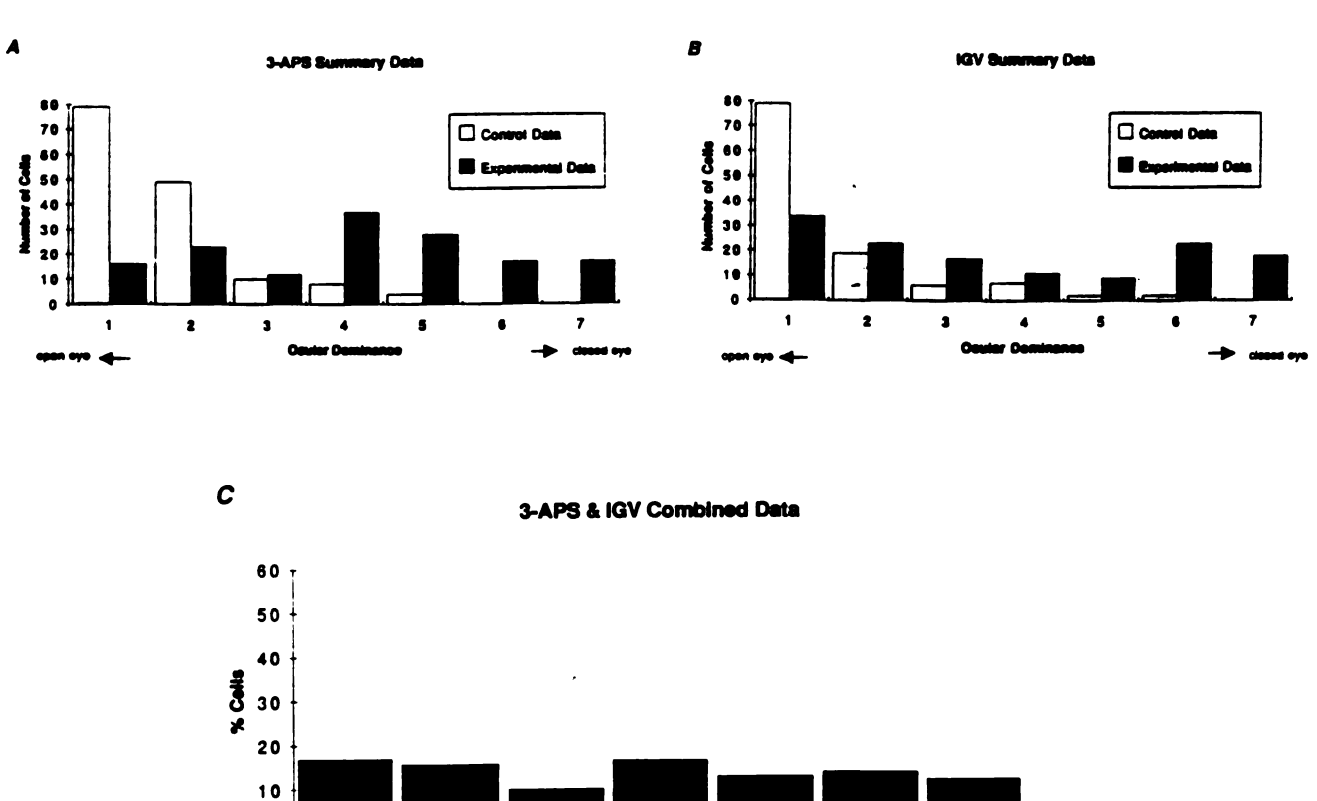

Figure 7

Ocular dominance histograms for aggregate data. Plotted as though all animals received ipsilateral monocular deprivation.
A: Summary data for all animals receiving 3-APS plotted together.
B: Summary data for all animals receiving IGV plotted together.
C: Aggregate data for all 3-APS and isoguvacine (IGV) animals, plotted together on the same scale used in fig. 3 to allow comparisons.

Table 2

Animal	Agonist	Concen. (mM)	Time for block to wear off (hrs)	Size of Total Block (mm.)	Control units	Expt. units	CBI ctrl/expt
687	3-APS	100	20	1.5-2.4	30	30	93.9/52.8**
689	3-APS	200	22	1.5-2.3	30	30	97.9/60.6**
691	3-APS	200	25	2.0-2.5	30	30	89.4/57.2**
692	3-APS	200	24	2.0-2.5	30	30	98.9/59.4
701	3-APS	200	23	2.0-2.6	30	30	99.4/56.1**
All 3-APS					**150**	**150**	
737	IGV	100	36	2.2-2.7	30	34	82.2/47.3**
738	IGV	100	30	2.5-3.0	34	44	91.9/64.4**
739	IGV	100	30*	2.5-3.0	34	34	95.6/62.6
746	IGV	100	45*	4.1-4.7	27	33	97.5/63.1
All IGV					**125**	**145**	

* Animals 739 and 746 had their agonist infusion cannulae cut at the start of the surgical preparation for microelectrode recording.
** CBI's for animals with contralateral monocular deprivation were adjusted by the method described in the text.

Summary of experimental method and results for each animal.

Table 3

	CBI		MI	
Agonist	**Normal**	**TTX**	**Normal**	**TTX**
3-APS	0.46	0.17	0.27	0.46
IGV	0.52	0.14	0.06	0.03
3-APS+IGV	0.49	0.10	0.07	0.10

p-Values for the Mann-Whitney U test comparing the distributions of CBIs and MIs for the agonist groups and for data in the literature (from ref. 17). "Normal" is kittens with normal visual experience, "TTX" is intracortical TTX infusion plus monocular deprivation.

monocularly-deprived animals, and in animals who received TTX blockade of all cortical activity (pre- and postsynaptic).

The Mann-Whitney U statistic was calculated to compare the difference between the CBI's and MI's of 6 non-deprived normal kittens, 6 kittens which received intracortical TTX with monocular deprivation (data from ref. 17, q.v. fig. 3), and the kittens that received 3-APS and isoguvacine . For this test, the null hypothesis is that both comparison groups come from the same distribution. The CBI and MI data are shown in fig. 8, and the resulting p values are shown in table 3. Thus, in contrast to the findings with muscimol, no significant shift in the CBI from non-visually-deprived kittens of similar age was seen using either 3-APS or isoguvacine . The only significant difference for the MI distributions is for the isoguvacine vs. TTX groups (i.e., there is a 3% chance that their MI's come from the same distribution). Compare the ocular dominance histograms in fig. 3 to see the shapes of the distributions.

Since we encountered a wide range of blockade sizes, we analyzed CBI's as a function of distance to the block boundary for each experimental penetration in each animal (fig. 9). CBI does not seem to be a function of position -- nor, presumably, agonist concentration -- within the totally blocked area.

Ocular dominance (38) and GABA receptor distribution (25) may vary in each cortical layer, so we examined the CBI for the cells in each layer (fig. 10). Cells at the inter-laminar borders were not included in this analysis. Otherwise, all units from experimental penetrations were included, except for 2 of 3 tracks in animal 739, whose cortex was damaged during recording (sub-pial hemorrhage and resulting edema). While we did not see an obvious

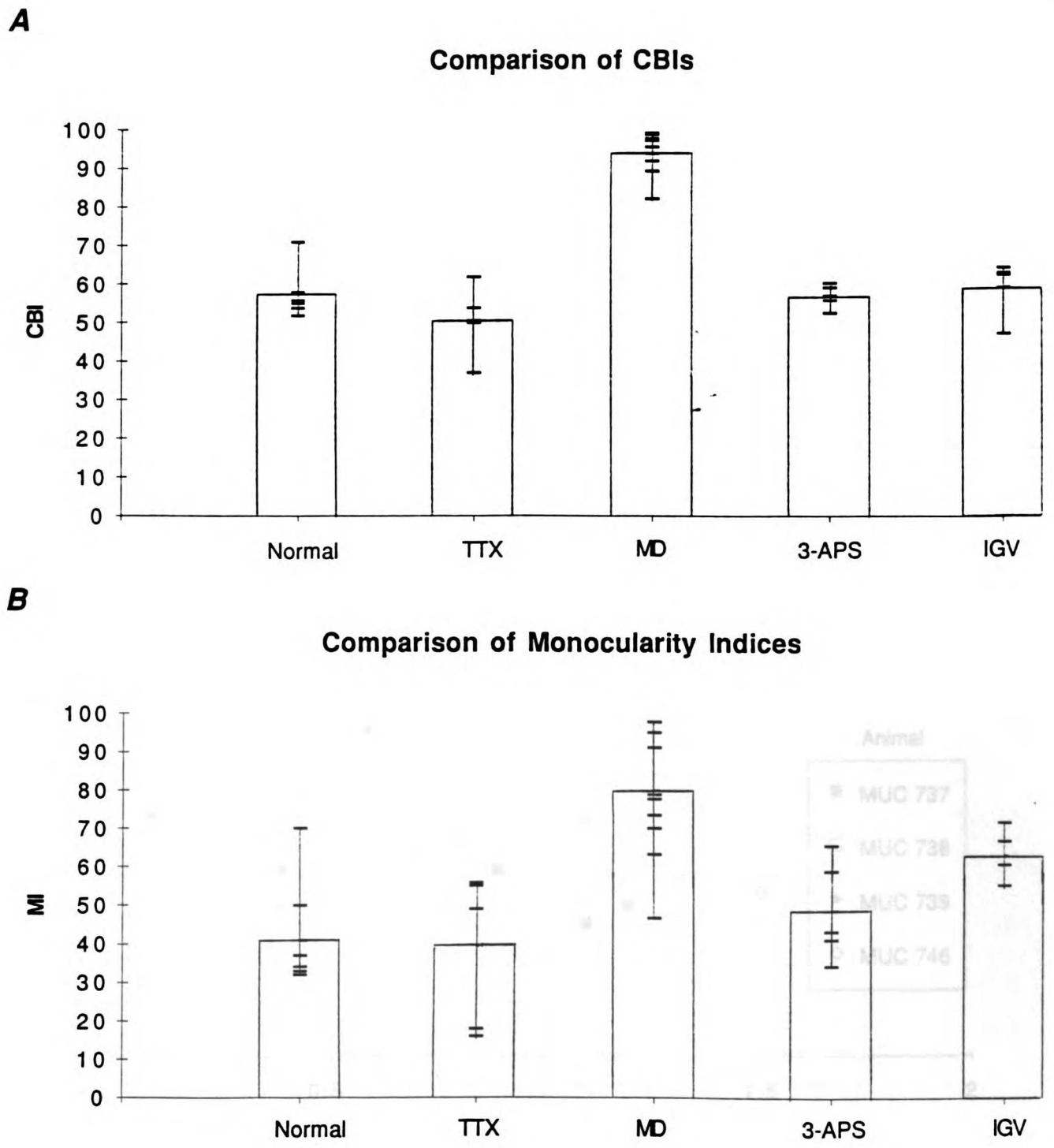

Figure 8
Comparison between indices describing the ocular dominance distributions of kittens with normal visual experience (Normal), intracortical TTX with monocular deprivation (TTX), monocular deprivation only (MD), and 3-APS or isoguvacine (IGV) plus monocualr deprivation. Groups are the same as in fig. 3. Each horizontal tick mark represents a single kitten.
A: Contralateral bias indices for the above groups. Columns indicate group means.
B: Monocularity indices for the above groups. Columns indicate MIs for aggregate data for each group.

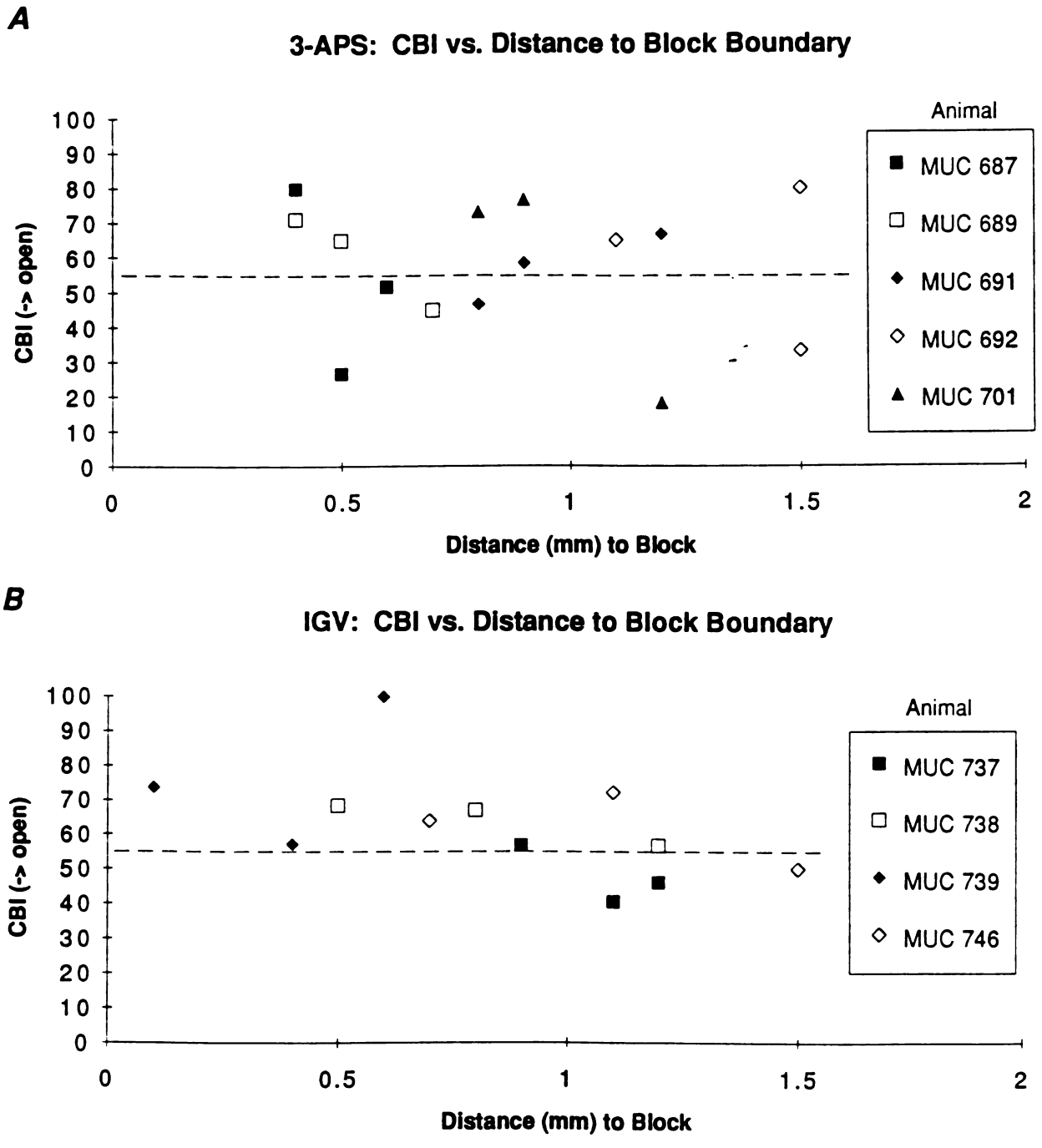

Figure 9
Contralateral bias index (CBI) as a function of approximate distance to the boundary of the total blockade. Each experimental penetration for each animal is plotted. Since animals are plotted as if they all received ipsilateral MD, the expected CBI for all animals is approximately 55 (34).
A: 3-APS group.
B: Isoguvacine group.

relationship between cortical layer and CBI, layers V and VI were under-represented as a result of making our electrode penetrations down the medial bank of area 17 (figs. 1, 11).

Histologic sections within the area of blockade appeared normal, with well-defined layers (fig. 12). Kitten 739's cortex was damaged during recording, preventing reconstruction of 2 experimental tracks. Otherwise, all other tracks were located by identification of tracks and/or electrolytic lesions.

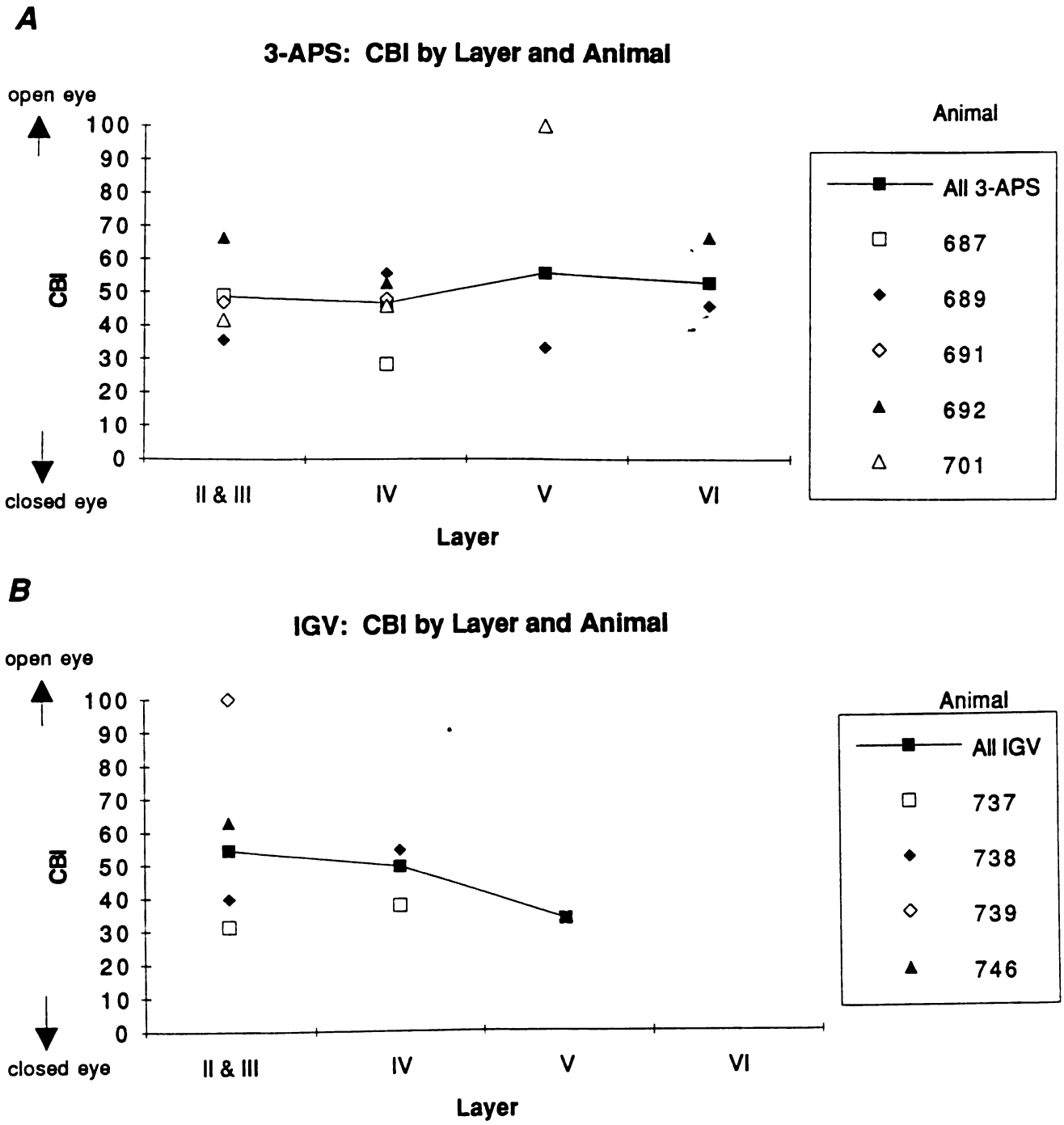

Figure 10
Contralateral bias index is plotted for cells in each cortical layer from all experimental penetrations in each animal.
A: Animals receiving 3-APS.
B: Animals receiving isoguvacine.

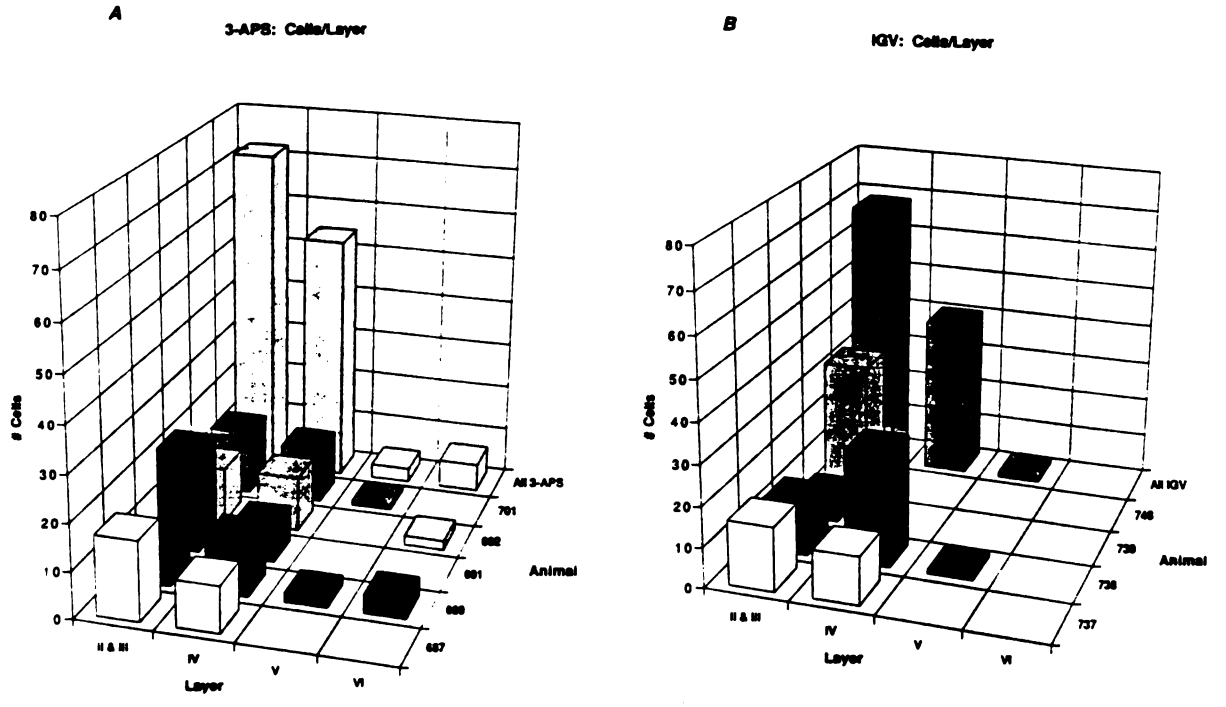

Figure 11
Number of cells, from experimental penetrations, in each layer is shown for each animal, and for all animals receiving each agonist. Note that relatively few cells were recorded in the deeper layers (V and VI).
A: Animals receiving 3-APS.
B: Animals receiving isoguvacine.

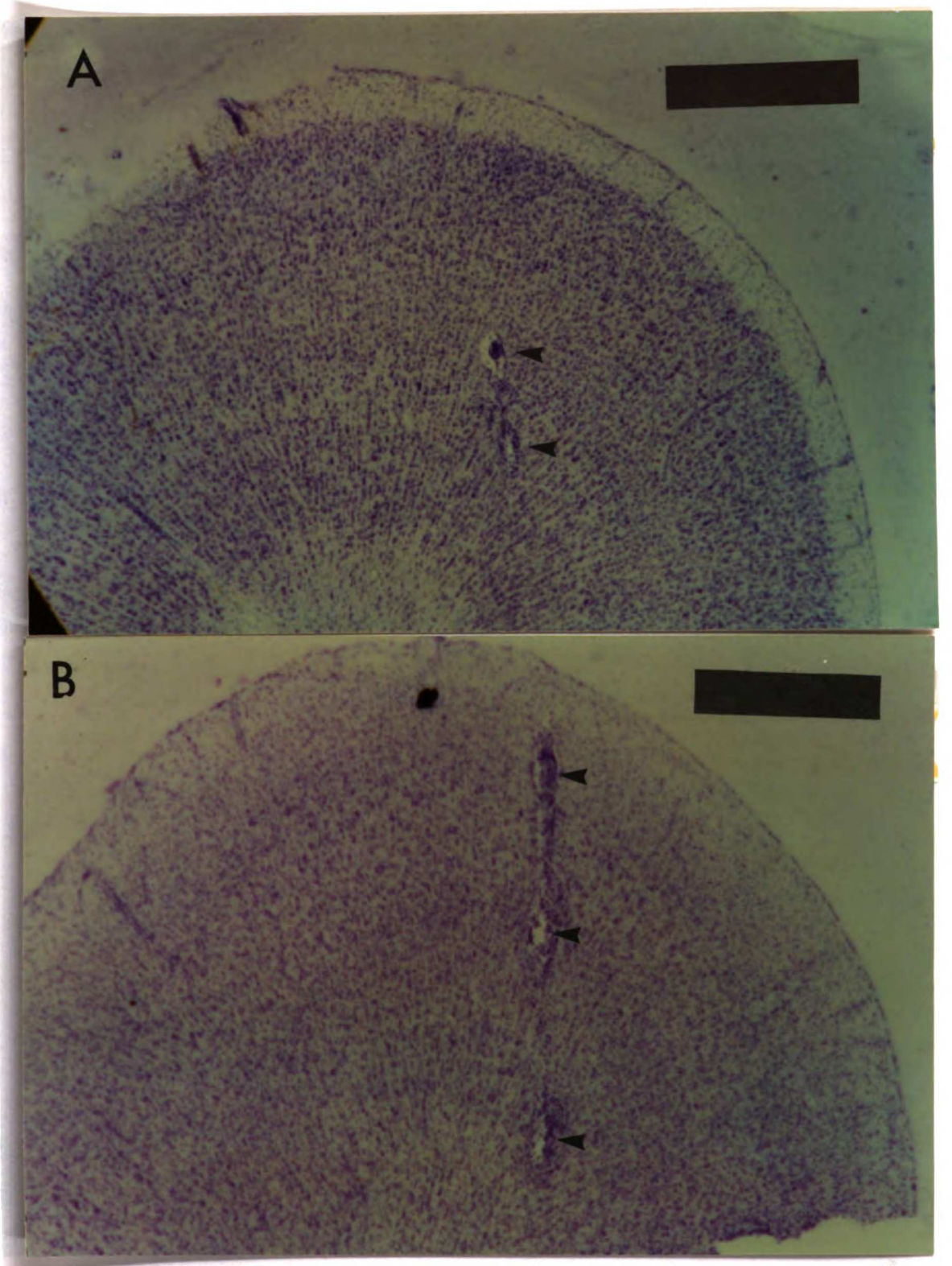

Figure 12
Nissl-stained sections of primary visual cortex, showing control and experimental electrode penetrations. The spacing and number of the electrolytic lesions in each track allow identification of the specific penetration, and also allow us to assign an actual depth to each cell recorded. Lesions are indicated by arrows. Scale bar=1mm.
A: 3-APS control penetration from animal MUC 692. Layers and lesions are clearly visible.
B: 3-APS experimental penetration from animal MUC 692. Track is 2.0 mm from the cannula. The actual path of the electrode is visible.

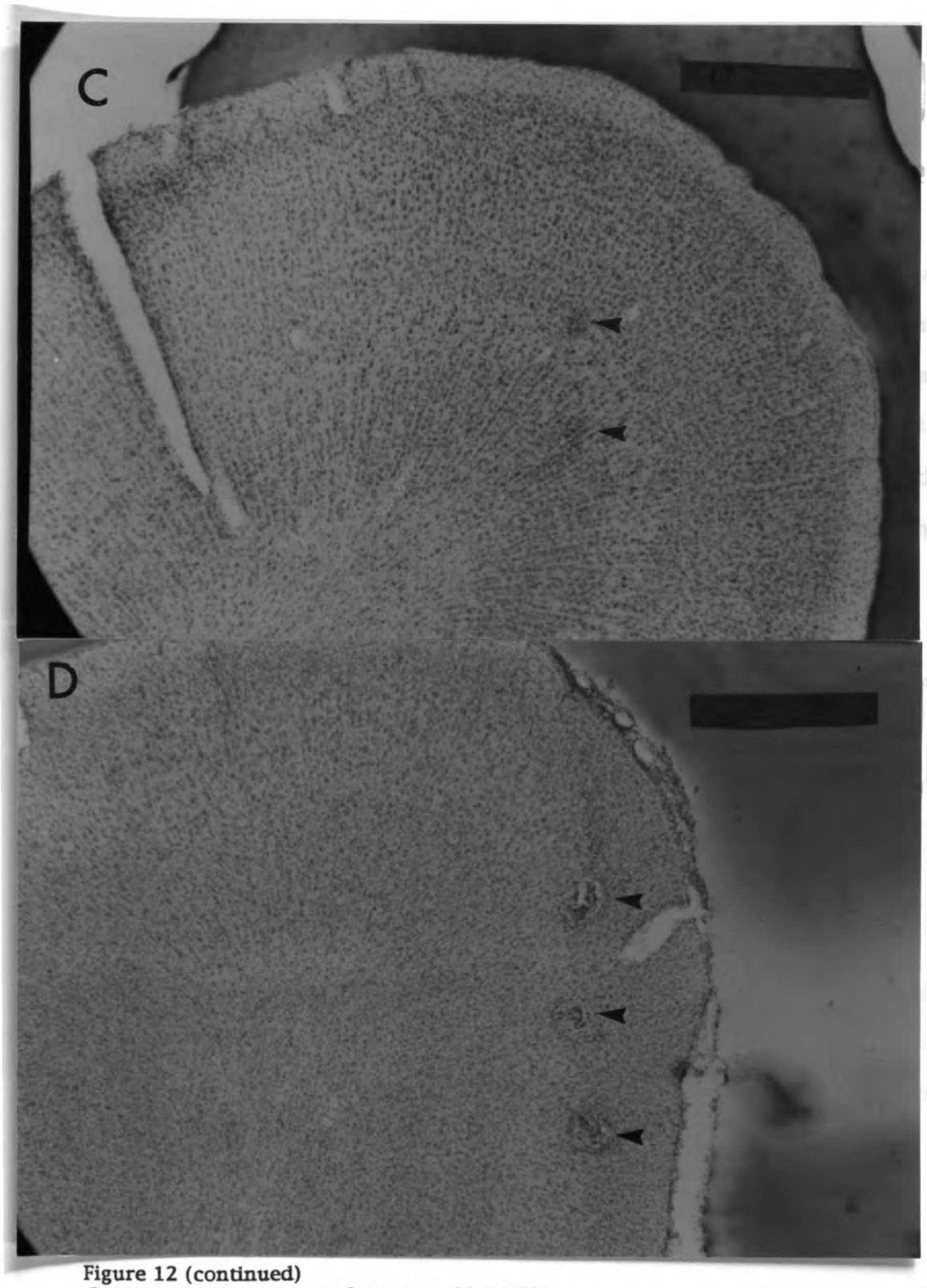

Figure 12 (continued)
C: IGV control penetration from animal MUC 738.
D: **IGV experimental penetration from animal MUC 746. Track is 6.1 mm from the cannula. The track runs down the medial bank of area 17, within layers II and III, near the border with layer IV.**

DISCUSSION

These results, like those found using muscimol, suggest that spike activity of the postsynaptic cell is essential for normal ocular dominance plasticity. The cortex treated with 3-APS and isoguvacine , while apparently receiving the same afferent input as the untreated cortex, did not show a shift in ocular dominance towards the open eye. Unlike the muscimol experiments, however, no shift towards the less-active (closed eye's) inputs was observed.

There are several possible explanations for the differences between the results seen with muscimol and those seen using 3-APS and isoguvacine. The phenomenon of reverse-directed plasticity may be unrelated to normal developmental processes, and is an aberration caused by some effect of the muscimol treatment. Under this hypothesis, spike activity of the postsynaptic cell would be necessary for any ocular dominance plasticity, in either direction.

Alternatively, reverse-directed plasticity may represent an aspect of normal cortical development that was not revealed by the 3-APS and isoguvacine treatments. Postsynaptic spike activity may be necessary for normal ocular dominance plasticity, but not for the phenomenon of reverse-directed plasticity.

I will consider both of these possibilities, and will then discuss future experiments that might clarify the issue.

Muscimol-specific effects

The simplest explanation for the observed differences in muscimol's effects on the ocular dominance shift caused by monocular deprivation is that muscimol may have unique effects on the developing visual cortex.

Muscimol may have effects on primary visual cortex other than selective inhibition of postsynaptic cells. For example, muscimol binds both pre- and postsynaptically in the frog peripheral nervous system (39); and, although it has not yet been found to do so in mammalian central nervous system, the possibility cannot be ruled out. Even though afferent electrical activity seemed normal during the muscimol experiments (18), we cannot be sure that neurotransmitter release was entirely so (q.v. below, "Inhibition of afferents").

Alternatively, muscimol may bind to receptors, other than the $GABA_A$ receptor, that have not yet been characterized. These receptors may, in turn, trigger the reverse-directed plasticity. Or, muscimol may bind to a subgroup of the $GABA_A$ receptors different from that bound by 3-APS and isoguvacine .

In the past few years, investigators have discovered that the GABA receptor system is far more complex than the "A" and "B" subtypes would suggest. Molecular techniques have revealed substantial diversity in $GABA_A$ receptor subtypes, which are assembled from different combinations of subunits (fig. 13)(30, 26). These subunits have several ligand binding sites, and are subject to allosteric effects (41). And, different GABA agonists may have different allosteric effects on the receptor (31), and even the same agonist may have different effects on different $GABA_A$ receptor subtypes (41). Muscimol, for example, is known to bind with different affinities to various subtypes of the $GABA_A$ receptor in different areas of rat brain; it also binds differently from other GABA agonists in given areas (41). Such effects could explain the different results found by using the various agonists.

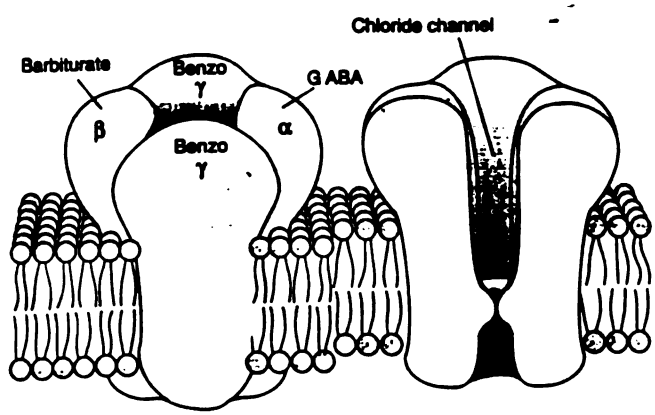

Figure 13
$GABA_A$ receptor, showing the hetero-oligomeric assembly from 4 subunits. The receptor incorporates binding sites for GABA, as well as barbiturates and benzodiazepines. All the subunits contribute to form the chloride ion channel (from ref. 41).

Furthermore, the $GABA_A$ receptor subtypes have different laminar and regional distributions in mammalian brain (42, 43) We do not have enough data from layers V and VI to completely rule out a sampling bias obscuring different agonist effects in the deeper layers.

A final explanation for muscimol's effect on plasticity is that muscimol's metabolites may also be active (44), with as-yet-undetermined effects on the plasticity process.

The greatest barrier to assessing differential effects of muscimol is that it is, in the literature, the most-studied specific $GABA_A$ agonist. The various effects of muscimol may also be caused by the other two agonists, but have simply not been observed yet.

Other examples of reverse-directed plasticity

It would seem less likely that the phenomenon of reverse-directed ocular dominance plasticity is merely a side effect of muscimol treatment if there were other examples of reverse-directed plasticity. Two other examples (i.e., not involving muscimol treatment) of an ocular dominance shift towards the less-active inputs are available. Reverse-directed plasticity has been observed with selective pharmacologic blockade of the NMDA receptor by the antagonist 2-amino-5-phosphonovaleric acid (APV) (5). A shift in ocular dominance towards the closed eye was seen in the area closest to the antagonist infusion cannula, while no shift occurred in the area of cortex slightly farther away. The authors proposed that in the area of densest NMDA receptor blockade, and hence greatest postsynaptic inhibition, the depression of synaptic strength produced by presynaptic activity alone was seen; while farther away, the *partial* suppression of postsynaptic activity by a lower concentration of APV disrupted the usual voltage-dependent plasticity mechanism and blocked the ocular dominance shift entirely.

Shulz and Fregnac (14) also induced reverse-directed ocular dominance plasticity by hyperpolarizing, and hence inhibiting, the postsynaptic cell with an extracellular iontophoretic electrode. The fact that the shift in ocular dominance towards the closed eye was observed in two additional, separate models besides the muscimol/monocular deprivation system makes it more likely that the phenomenon really is related to normal processes of synaptic plasticity.

Relative potencies of the GABA agonists, and possible effects

The different relative potencies of the three $GABA_A$ agonists, and hence different intensities of postsynaptic inhibition, may be responsible for their different effects on plasticity. Bear *et al*'s work (5), which found reverse-directed plasticity close to the cannula but nearly no shift slightly farther away, would support this hypothesis. The LTP/LTD experiments discussed in the introduction to this thesis also showed that quantitative changes in the state of the postsynaptic cell -- not involving action potentials -- can result in dramatic changes in the strength of the synapse (4, 6, 8). Thus, even while action potentials are inhibited, differences in the chloride currents induced by the various agonists could conceivably result in different effects on synaptic strength; and the blockade sizes achievable with the agonists and minipumps we used may be too small to allow observation of such concentration gradient effects within the area of blockade. For an example, see figure 14.

Monocular deprivation alters the GABA receptor system

Some of the differences between muscimol, isoguvacine , and 3-APS effects could be accounted for if visual deprivation alters the distribution of GABA receptors, and if the different agonists used have different effects on the receptors.

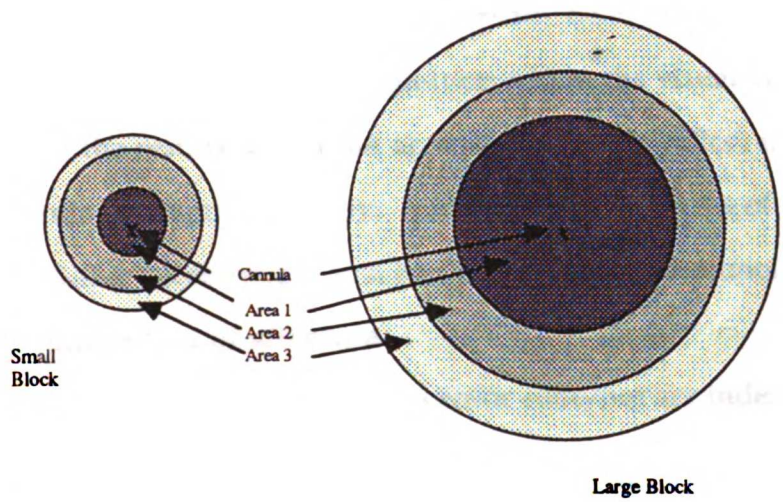

Figure 14
Example of how the effects of different blockade densities might not be observed with a small block area. Assume that the inhibition is inversely proportional to distance from the cannula, and that different plasticity mechanisms are dominant in areas 1,2, and 3. It is technically difficult to record very near the cannula (area 1), and we avoided recording near the total block boundary (area 3). We might therefore have failed to observe the different directions of ocular dominance shift occurring in areas 1 and 3 due to our small block sizes. Larger minipumps that deliver higher infusion rates will give larger areas of blockade and may allow observation of the ocular dominance shifts in areas 1 and 3. (c.f. Bear *et al*, ref. 5.)

Monocular deprivation in adult monkeys has been shown to alter presynaptic GABA production (45), which might lead to changes in postsynaptic GABA receptor quantity and function. The effect of monocular deprivation on GABA production in kittens, however, is not known. While the effects of 3-APS and isoguvacine on GABA release is not known, muscimol does not affect presynaptic GABA release in rat brains; its effect would be added to that of endogenous GABA (46), whose effects might already be altered. If the monocular deprivation process alters inhibition within the visual system, then any or all of the results with GABA agonists might not reflect natural processes governing synaptic plasticity. Additionally, the effects of 3-APS and isoguvacine on GABA release might well be different from muscimol's.

Possible inhibition of afferents

Finally, it is possible that $GABA_A$ receptor subtypes are indeed active presynaptically, as they are in the frog motor system (39), and that afferent activity and/or neurotransmitter release in the blocked regions is affected in a way that was not evident to us during our experiments. We might thus have observed a situation like that caused by TTX infusion, with block of the monocular deprivation-induced shift in ocular dominance (see figs. 3 and 8). The results observed with 3-APS and isoguvacine are indeed similar to those seen in animals that are not visually deprived ("normal"), as well as in animals treated with intracortical TTX infusion before monocular deprivation (34). Except for the comparison between the isoguvacine and the TTX groups' MIs, the ocular dominance distributions of the 3-APS and isoguvacine kittens are not significantly different from those of "normal" and intracortical TTX kittens (table 3).

Our experiments using extracellular single-cell recording would not have distinguished between a presynaptic effect of 3-APS and isoguvacine,

and a blockade of ocular dominance plasticity resulting solely from inhibition of postsynaptic cell activity. A slice preparation, as described below, could resolve this issue.

Significance and proposals for future studies

Our experiments, and the earlier experiments with muscimol, demonstrate the vital role of the postsynaptic cell's activity in ocular dominance plasticity. This result is in contrast to the purely presynaptic mechanism suggested by the studies of *Aplysia* (19).

However, our laboratory's studies using $GABA_A$ agonists do not agree on the role of the postsynaptic cell and the effects of inhibiting its activity. The results obtained with 3-APS and isoguvacine are difficult to explain using the Hebb postulate and related rules. Shulz and Fregnac, who observed reverse-directed plasticity in their system (14), concluded that repeated failure of the postsynaptic cell to fire when stimulated weakened the active afferent synaptic connections -- Stent's rule. Our results with 3-APS and isoguvacine do not support this hypothesis. Nor do they support the extension of Hebb's postulate described in the introduction to this thesis, namely, that the correlation between the less-active inputs and the less-active postsynaptic cells strengthens these synapses in the blocked areas.

We think it likely that both results -- reverse-directed plasticity, and prevention of plasticity -- represent different aspects of the plasticity process, since: (i) we observed blockade of plasticity with two different agents, (ii) reverse-directed plasticity has already been observed in two other studies not involving muscimol, and (iii) Bear and his colleagues (5) found both results at differing levels of the same NMDA antagonist. It may be that, at the level of inhibition obtained using 3-APS and isoguvacine, rules other than Hebb's govern synaptic plasticity; while at the level of inhibition caused by

muscimol, correlation-based plasticity dominates. Clearly, more experiments will be needed to settle the issues raised in this discussion.

The effect of agonist potency on synaptic plasticity will be explored by experiments currently underway in our laboratory with larger minipumps which deliver greater infusion rates. These experiments, with their larger block sizes, should demonstrate different muscimol results over a wider range of effective concentrations, and may show that the relative potencies of the different agonists are responsible for the different effects seen (cf. fig. 14). They will also attempt to correlate the anatomic and physiologic results of muscimol infusion by using histologic techniques to measure the size of the ocular dominance columns.

The problem of determining the agonists' effects on the pre- and postsynaptic cells can be approached in several ways. Cortical slice preparations would allow more controlled experiments under *in vitro* conditions. Alternatively, outside-out patch-clamp studies could be done on pieces of pre- and postsynaptic cell membranes to examine the effects of applying the various agonist agents on the membrane potential. Slice preparations with voltage clamping of whole postsynaptic cells would allow comparison of the postsynaptic currents caused by different GABA agonists binding to the postsynaptic cells.

Inhibition of presynaptic neurotransmitter release by $GABA_A$ agonists could be studied using intracellular postsynaptic recording in cortical slices to examine the postsynaptic currents resulting from afferent (white matter) stimulation with and without agonist present.

In any event, if we can one day unravel the cellular mechanisms governing synaptic plasticity, we may be able to re-induce this plasticity in adults. Such techniques, and others that we can not yet imagine, may hold

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