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THE STRUCTURE OF THE TERMINAL ARBORIZATIONS OF PHYSIOLOGICALLY IDENTIFIED RETINAL GANGLION CELL Y AXONS IN THE KITTEN

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SUMMARY

1. Retinal ganglion cell (r.g.c.) axons ($n = 17$) in the optic tract of 4–5-week-old kittens and adult cats ($n = 4$, this study, $n = 27$ from other reports) were studied both physiologically and morphologically. Axons were initially classified during extracellular recording with a battery of physiological tests that included Fourier analysis of the response to a sinusoidally counterphased sine-wave grating. Y axons had a significant second harmonic response component (> twice the fundamental) present independent of the spatial phase position of the grating. These axons were then recorded from intracellularly and subsequently filled ionophoretically with horseradish peroxidase (HRP). The HRP filled the axons' terminal arborizations in the dorsal lateral geniculate nucleus (l.g.n.). The innervation pattern and structure of the terminal arborizations of the kitten r.g.c. Y axons were compared to those of the adult.

2. The kitten Y axons innervated the l.g.n. in a pattern similar to that of the adult (individual branches from a single axon always innervated lamina A *or* A_1 and may also have innervated lamina C, the medial interlaminar nucleus (m.i.n.) and/or sent branches that coursed medial to the l.g.n.). Fourteen of seventeen of these Y axons in the kitten innervated either of the A-laminae heavily (> 200 terminal boutons per axon). The remaining three r.g.c. Y axons in the kitten had only small arborizations within lamina A (< fifty terminal boutons per axon) but heavily innervated lamina C.

3. The structure of the terminal boutons on the kitten r.g.c. Y axons was highly variable when compared to axons of adult cats. Some of the boutons were spherical or crenulated as in the adult. Many others had filopodia and growth cone-like terminals with fine extensions. This variable maturation of terminal boutons was seen both between axons and on individual axons.

4. The number of boutons on the kitten r.g.c. Y axons in the A-laminae was

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significantly less than that of adult Y axons. The mean numbers of boutons per axon were 476 and 1553 in the kittens and adult cats, respectively ($P < 0.001$, Mann–Whitney U test).

5. The width of the terminal arborization of individual Y axons in the A-laminae of the kittens was considerably smaller than in adult cats (mean widths of the terminal arborizations are 192 and 293 μm in the kittens and adult cats, respectively). However, when these values were normalized for the growth of the l.g.n., the relative growth of the terminal arborizations was less (52 % increase in absolute width *vs.* 15 % increase relative to the growth of the l.g.n.).

6. A comparison was made between the post-natal structural development of physiologically classified r.g.c. Y axons and l.g.n. Y cells. Both populations had reached a similar stage of structural maturity by 4–5 post-natal weeks (61–65 % of final size obtained).

7. The apparent increase in the number of Y cells in the l.g.n. during post-natal development and the susceptibility of the Y pathway to disruption by visual deprivation are considered with respect to the above findings.

INTRODUCTION

A major issue in neurobiology concerns the ontogeny of neural circuits. Particularly, it is of interest to learn whether the afferents to a nucleus in the central nervous system initially produce an excess innervation (Lichtman & Purves, 1980; Innocenti, 1981; Van Essen, 1982; Perry, Henderson & Linden, 1983; Rakic & Riley, 1983) with a subsequent retraction of connexions or if an expansion of innervation occurs during development. The retinal innervation of the brain is a particularly useful model for describing these developmental processes. In the cat, the retinal input reaches the dorsal lateral geniculate nucleus (l.g.n.) of the thalamus at the embryonic age of 32 days (Shatz, 1983). A process of sorting of inputs from the contralateral and ipsilateral eyes to the l.g.n. is virtually complete by post-natal day 2 (Shatz, 1983). However, considerable structural development of individual terminal boutons on the retinal axons within the kitten l.g.n. continues through 8 weeks of age (Mason, 1982*a*, *b*). In addition, the density of synaptic profiles (both of retinal and extraretinal origin) within the kitten l.g.n. changes significantly during this same period of post-natal development (Cragg, 1975; Winfield, Hiorns & Powell, 1980; Winfield & Powell, 1980). In these earlier studies the structural development of the synaptic profiles in the l.g.n. (particularly the terminals of retinal axons) were described. However, the structure of the entire terminal field of single retinal axons in the l.g.n. of the neonate has not been studied. In order to understand the developmental process(es) of this pathway both types of information are needed. These processes may differ between the functional subgroups of retinal axons (W, X and Y axons; see Sherman & Spear, 1982 for review of the properties of W, X and Y cells) which innervate the l.g.n. Demonstration of such differences requires a structural characterization of physiologically identified retinal ganglion cell (r.g.c.) axons in the kitten.

In the present study, we describe the structure of one of these functional types, the r.g.c. Y axons in 4–5-week-old kittens. This sample ($n = 17$) is compared to published results of similarly classified axons from adult cats (Bowling & Michael,

1980; $n = 9$; Sur & Sherman, 1982; $n = 12$; Bowling & Michael, 1984; $n = 15$ and to four Y axons obtained in our laboratory from adult control animals). These data were obtained using the method of intracellular injection of horseradish peroxidase (HRP) into individual, physiologically characterized cells (i.e. optic tract axons). The HRP moves anterogradely, filling the terminal arborization of the r.g.c. axon in the l.g.n. The structure, distribution, number and extent of the terminal boutons of r.g.c. Y axons are compared between kittens and adult cats.

Kittens between 4 and 5 weeks of age were chosen because the optical media are clear (Thorn, Gollender & Erickson, 1976; Bonds & Freeman, 1978; Freeman & Lai, 1978; Freeman, Wong & Zezula, 1978), and the synaptic organization of the l.g.n. is changing during this period (Cragg, 1975; Winfield *et al.* 1980; Winfield & Powell, 1980). The development of Y axons was considered for two reasons: (1) the electrophysiological identification of this cell type in the neonate is straightforward. One of the defining tests for this physiological class (*non-linear* spatial summation within the receptive field; Enroth-Cugell & Robson, 1966; Hochstein & Shapley, 1976*a*, *b*) results in a response similar to that seen in the adult. However, the opposite result (*linear* spatial summation within the receptive field) may be equivocal in the neonate (Hochstein & Shapley, 1976*b*, Daniels, Pettigrew & Norman, 1978; Mangel, Wilson & Sherman, 1983) and (2) the Y cell pathway has been reported to be particularly sensitive to an abnormal visual environment during post-natal development (Sherman, Hoffmann & Stone, 1972; Friedlander, Stanford & Sherman, 1982; Sur, Humphrey & Sherman, 1982; Friedlander & Stanford, 1984). Therefore, a description of the normal ontogenetic sequence for this pathway will add to our understanding of its sensitivity to environmental factors. Preliminary results of these findings have appeared (Friedlander, Martin & Vahle-Hinz, 1983).

METHODS

Surgery

Eight kittens (age 28–37 days) were reared in our breeding colony. Weights at experimental age were 360–510 g. Animals were given a dose of 0·1–0·2 mg atropine/kg subcutaneously, to prevent excessive respiratory secretion. Anaesthesia was initially induced with 1·0–1·5 % halothane (Abbott) in a 1:1 mixture of N_2O:O_2. Surgical procedures were carried out on a temperature-controlled blanket. The femoral vein was immediately cannulated and the halothane was discontinued (time of halothane administration = 4–14 min). We found that halothane administration in kittens of this size for periods > 30 min with concentrations $\geqslant 1{\cdot}0\,\%$ would result in severe hypotension. Anaesthesia for further surgical procedures was effected with Nembutal (Abbott) administered intravenously in a 10 mg/ml solution diluted in warmed physiological saline. The Nembutal was administered as needed during surgery (range of doses administered = 25–50 mg/kg). The femoral artery was then cannulated (the cannula was manoeuvred into the abdominal aorta) and connected to a Statham blood pressure transducer. A continuous record of blood pressure and heart rate was obtained using a Grass Physiograph. Mean blood pressure was kept between 70 and 110 mmHg. If blood pressure dropped below 70 mmHg for > 5 min, the experiment was terminated. The bladder was voided by compression periodically throughout the experiment. After tracheal cannulation, the animal was placed in a stereotaxic frame, paralysed by intravenous administration of an initial dose of 40 mg gallamine triethiodide/kg (Flaxedil, Davis & Geck) and mechanically ventilated. The inspired gases were changed to a 70:30 mixture of N_2O:O_2 and were continued in this fashion for the remainder of the experiment. The entire procedure was completed within 40 min of inducing anaesthesia. Flaxedil administration was continued throughout the experiment with an infusion pump at a rate of 12 mg/kg . h in a 5 % lactated Ringer solution. The scalp incision and craniotomy were completed in another 30 min. Additional Nembutal was administered throughout

the surgical procedures. The continuous monitoring of blood pressure and heart rate while periodically testing with foot pad compression ensured that the animals were well anaesthetized. Expired P_{CO_2} was monitored with a Beckman LB-II CO_2 monitor and kept between 3·7 and 4·3 %. All wound margins were infiltrated with the long-lasting, oil-based local anaesthetic, procaine base and butamben (Anduracaine-Reid Provident). Heart rate was kept between 210 and 250 beats/min. If the animal showed any signs of stress throughout the remainder of the recording experiment (blood pressure or heart rate increase) the 70:30 mixture of $N_2O:O_2$ was supplemented with additional halothane (0·4 %) given for 2–5 min. Electrophysiological data were not collected for at least 30 min after such supplements.

Optics

The kittens' pupils were dilated with 1 % atropine sulphate (Alcon), the nicitating membranes were retracted with 10 % phenylephrine hydrochloride (Neo-Synephrine, Winthrop) and the eyes were flushed with ophthalmic antibiotic (Neosporin, Burroughs Wellcome). The kittens were fitted with plano contact lenses (base curvature range = 5·00–6·25 mm). The appropriate lenses were fitted to make the animals' retinas conjugate with the stimulus display positioned at a viewing distance of either 171 or 57 cm. If additional optical correction was needed (after being determined by streak retinoscopy) appropriate spectacle lenses were placed in front of the eyes.

Electrodes

Bipolar electrodes were used for electrical stimulation of the optic chiasm. These electrodes were made of lacquer-coated tungsten wires insulated to within 0·5 mm of their tips. The stimulating electrodes were positioned at Horsley–Clark stereotaxic coordinates, anterior 9·0 mm, lateral 1·0 mm. The optimum electrode depth was determined by lowering the electrode while recording the evoked potential in response to 2·0 Hz stroboscopic illumination of the eyes. The electrodes were then cemented to the skull with dental cement. Recording micropipettes (tip diameter = 0·2–0·5 μm) were filled with a solution of 0·2 M-KCl, 0·05 M-Tris (Sigma), 4·0 % HRP (Sigma VI) at a pH of 7·0. The micropipettes were bevelled to final impedance of 90–140 MΩ at 200 Hz.

Stimulus display and cell classification

Receptive fields of retinal ganglion cell axons were initially plotted with flashing spots on a tangent screen at a viewing distance of 171 cm. Subsequent testing was done with a Tektronix model 608 (phosphor P 31) display monitor positioned at a viewing distance of 57 cm (screen area = 10 deg × 10 deg). Flashing spots or sinusoidally counterphased spatially sinuosoidal vertical gratings were generated on the display monitor by a Picasso Image Generator (Innisfree, U.S.A.). Retinal ganglion cell axons were tested for several properties. These included: (1) ocular dominance, (2) receptive field centre position, size and contrast sign (measured qualitatively by listening through an audio monitor to the vigour of the axon's response to manually controlled light flashes of 2 s duration), (3) presence of an inhibitory surround region (evaluated by comparing the axon's response to a spot of appropriate contrast that just filled the receptive field centre with an equal luminance spot which was 5 times as large as the centre, (4) linearity of spatial summation to a counterphased vertical sinusoidal grating (mean luminance = 32 cd/m^2, counterphase rate = 1–2 Hz, spatial frequency appropriately high to reveal the second harmonic of the cell's response to the counterphased grating; responses were considered non-linear if a Fourier analysis of the axon's modulated response had a second harmonic/fundamental ratio > 2·0), (5) responsiveness to a rapidly moving (> 200 deg/s) large (12 deg × 12 deg) target of contrast opposite to that of the receptive field centre sign (Y axons typically respond reliably with an increased firing rate each time this stimulus is moved through its receptive field) and (6) latency to suprathreshold electrical stimulation of the optic chiasm at 2 Hz (measured from the onset of the stimulus artifact to the beginning of the action potential).

HRP injection and tissue processing

After the axon had been characterized as an r.g.c. Y axon, it was penetrated with the micropipette. The intra-axonal recording condition was indicated by a large drop in the d.c. level to between −30 and −68 mV (see records in Fig. 1). The axons' receptive field properties were always rechecked while recording intracellularly. Intracellular recordings from 10 to 15 min were not uncommon. The micropipette was considerably less likely to come out of the axon than was

the case when recording from neuronal somata in the kitten l.g.n. The axon was ionophoretically filled with HRP by passing depolarizing current pulses of 2·0–8·0 nA (200 ms duration) from 3 to 15 min. The micropipette was then withdrawn and a new penetration was made. Only r.g.c. axons that were impaled in the optic tract and well filled with HRP were included in this study since penetration of retinal axons within the l.g.n. laminae may occur past axonal branch points and result in incomplete filling with HRP. Only two axons were injected in each optic tract with different ocular dominance in order to reliably identify the HRP-injected axon from which the electrophysiological data were obtained. The animals were over-anaesthetized with Nembutal from 3 to 20 h after the last axon was injected and perfused transcardially with heparinized saline followed by a modified Karnovsky's fixative (2·5% gluteraldehyde, 1·0% paraformaldehyde in phosphate buffer). The l.g.n. was subsequently blocked in the coronal plane. Sections were cut on a Vibratome at 100 μm and reacted for HRP activity with the cobalt intensification method of the 3–3′-diaminobenzidine method (Adams, 1977). Sections were dehydrated and cleared in an ascending series of alcohols and xylenes, respectively. Filled axons were drawn at 500 × (with a 50 × oil immersion objective). After drawings were completed, the tissue was rehydrated and counter-stained with cresyl violet so that the laminar boundaries within the l.g.n. could be drawn relative to the terminal arborizations of the r.g.c. axons.

RESULTS

Twenty-one r.g.c. Y axons were successfully characterized (both physiologically and morphologically) in this study (see Table 1 for a summary of the properties of these axons). Four of these twenty-one Y axons were from adult cats (ages 10–13 months). These adult Y axons served as controls (in addition to the published results of Bowling & Michael, 1980, 1984 and Sur & Sherman, 1982). The remaining seventeen Y axons were recovered from kittens (ages 28–37 days). Fourteen of these seventeen axons heavily innervated one of the A-laminae and the extent of this innervation was compared to the adult material. An additional three Y axons in the kittens innervated lamina C heavily but had only a single or few branches to lamina A which ended in small clusters of boutons. These axons are described but they are not included in the quantitative comparisons to the adult material. All of these axons in the kittens responded in a similar fashion to adult Y axons when a sinusoidally counterphased sine-wave grating pattern was placed in their receptive fields. In response to this stimulus, adult Y axons show non-linear spatial summation within their receptive field. This is manifested by a response to each half-cycle of the grating modulation (frequency-doubled response). One example of this type of response is shown in Fig. 1 for a Y axon from a 30-day-old kitten. The axon's response to the grating at an initial phase position of 0 deg is shown in Fig. 1*A*. The grating was counterphased at a temporal frequency of 2·0 Hz. Note the strong frequency-doubled response of the axon in the mean firing rate histogram. The axon's response was Fourier analysed to assess the contribution of the second harmonic component. The results of this analysis are illustrated in Fig. 1*B*. There was a strong contribution of the second harmonic to the response (ratio of second harmonic to fundamental component = 12·2). Note that all of the additional even harmonics were large in amplitude, characteristic of a Y cell's non-linear response. A 90 deg phase shift in the spatial position of the grating elicited the response shown in Fig. 1*C*. The frequency-doubled response was still apparent at this position. The Fourier analysis (Fig. 1*D*) of this response also had a strong second harmonic component (second harmonic to fundamental ratio = 0·94). The insets in Fig. 1*B* and *D* are samples of the intra-axonal recordings

TABLE 1. Receptive field properties and projection pattern of r.g.c. axons

Unit number	Age (days)	Spatial summation	Latency to optic chiasm stimulation (ms)	Receptive field centre sign	Receptive field size (deg)	Receptive field eccentricity (deg)	Innervation				Illustrated in text Figures
							A-laminae	C-laminae	M.i.n.	Other	
Kittens											
1	30	N.l.	0·7	On	4·5	X = 8·0 Y = +1·5	A	+	+	–	Figs. 2, 5*A*, Pl. 1*B*
2	29	N.l.	0·8	Off	3·2	X = 4·5 Y = −0·5	A_1	−*	–	+	Figs. 3, 5*B*
3	31	N.l.	0·6	On	6·5	X = 31·0 Y = −5·4	A	–	+	–	Figs. 4*A*, 5*C*, Pl. 1*C*
4	31	N.l.	0·6	Off	1·8	X = 1·2 Y = +0·5	A†	+	+	–	Figs. 4*B*, 5*D*, Pl. 1*D*
5	37	N.l.	0·6	Off	3·6	X = 6·0 Y = +1·2	A	+	+	+	–
6	35	N.l.	0·7	Off	2·5	X = 3·5 Y = −0·4	A_1	–	+	+	–
7	35	N.l.	1·0	On	5·9	X = 29·5 Y = −4·2	A_1	–	–	–	–
8	34	N.l.	0·6	Off	4·7	X = 21·0 Y = +2·7	A	+	–	–	–
9	34	N.l.	0·8	On	3·0	X = 11·4 Y = +9·5	A	+	+	–	–
10	34	N.l.	0·9	On	5·0	X = 35·2 Y = −1·4	A_1	–	+	+	–

11	33	N.l.	0·7	Off	4·2	X = 6·2 Y = −0·2	A_1	−	+	−	−
12	33	N.l.	0·6	On	2·6	X = 3·1 Y = +2·2	A_1	−	−	+	−
13	33	N.l.	0·8	On	2·9	X = 5·1 Y = +4·5	A	−	+	−	−
14	33	N.l.	1·1	Off	2·8	X = 1·9 Y = −6·0	A	+	−	+	−
15	28	N.l.	0·8	On	3·5	X = 14·8 Y = +0·7	A†	+	+	−	−
16	30	N.l.	0·7	Off	2·8	X = 7·4 Y = +2·2	A†	+	+	−	−
17	28	N.l.	0·7	Off	2·4	X = 3·1 Y = −0·2	A_1	−	+	+	−
Adults											
18	310	N.l.	0·6	Off	1·3	X = 4·0 Y = +0·4	A	+	+	+	Pl. 1 *A*
19	331	N.l.	0·5	Off	1·0	X = 6·0 Y = −1·2	A_1	−	+	−	−
20	352	N.l.	0·7	On	1·8	X = 10·1 Y = +1·1	A	+	+	−	−
21	360	N.l.	0·8	Off	0·9	X = 2·0 Y = −0·5	A	+	−	+	−

* = small branch to C.
† = restricted branches to A (< 100 boutons).
N.l. = non-linear spatial summation.

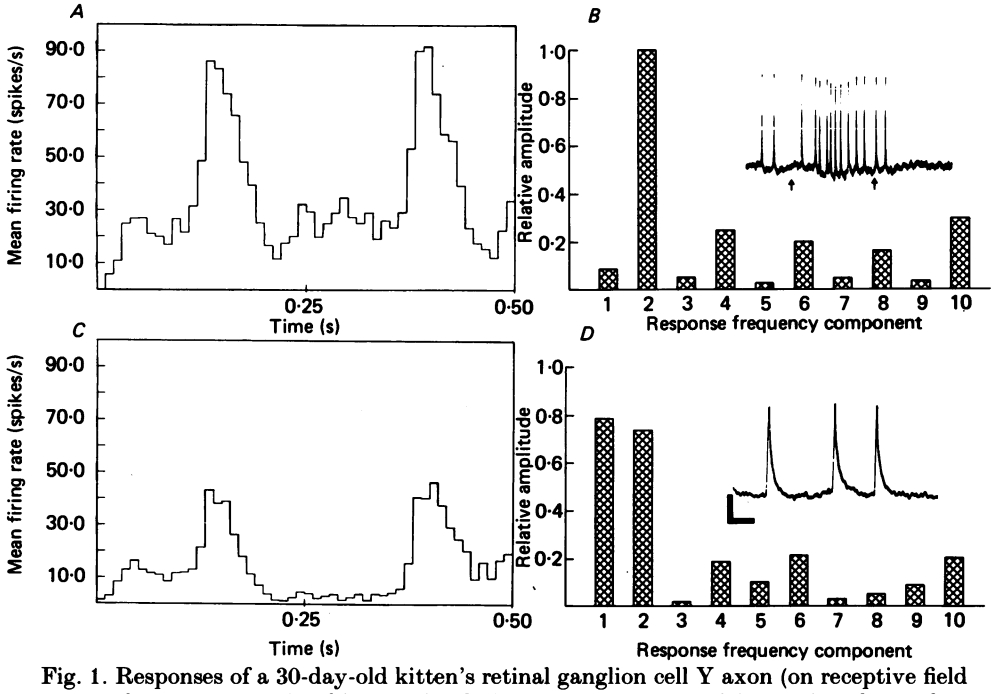

Fig. 1. Responses of a 30-day-old kitten's retinal ganglion cell Y axon (on receptive field centre, latency to optic chiasm stimulation = 0·7 ms, eccentricity, azimuth = 8 deg, elevation = 1·5 deg, receptive field centre size = 4·5 deg) to visual stimulation. *A*, mean firing rate histogram (bin width = 10 ms, 100 trials) of Y axon in response to sinusoidally counterphased vertical sine-wave grating. Mean luminance = 32 cd/m², temporal modulation rate = 2·0 Hz, spatial frequency of grating = 1·6 cycles/deg and grating contrast = 0·4. Note response doubling (two firing frequency peaks) to each cycle of the stimulus counterphase. *B*, Fourier analysis of response shown in *A* and inset of intracellular recording from Y axon (see below). The response amplitude of the fundamental component (same as stimulus frequency) and subsequent nine harmonics are normalized to the peak response component of the axon (second harmonic). Note that all additional even harmonics have large amplitude. *C* and *D* are histograms and Fourier analysis as in *A* and *B*, respectively. However, the sinusoidal grating was shifted (with digital control) to a phase position 90 deg past the position used in response *A*. All other parameters are identical to those used in *A* and *B*. Note that while the second harmonic response is still large, the fundamental response of the axon is more prominent at this phase position. The insets in *B* and *D* illustrate the intracellular recordings obtained from this Y axon. The scale bar under the lower trace indicates 10 mV for both traces and 100 ms and 2 ms in the upper and lower panels, respectively. Note the characteristic rapid rise and monophasic decay of the intra-axonally recorded action potentials. Arrows in upper trace indicate presentation of a light spot to the axon's receptive field centre. Lower trace illustrates three of these same action potentials at a faster sweep rate.

from this kitten Y axon. The upper trace is the axon's response to illumination of its receptive field centre (arrows indicate stimulus onset and offset). The lower trace shows three of these same action potentials at a faster sweep speed. Note the characteristic rapid upstroke and monophasic wave form of the intracellularly recorded action potential.

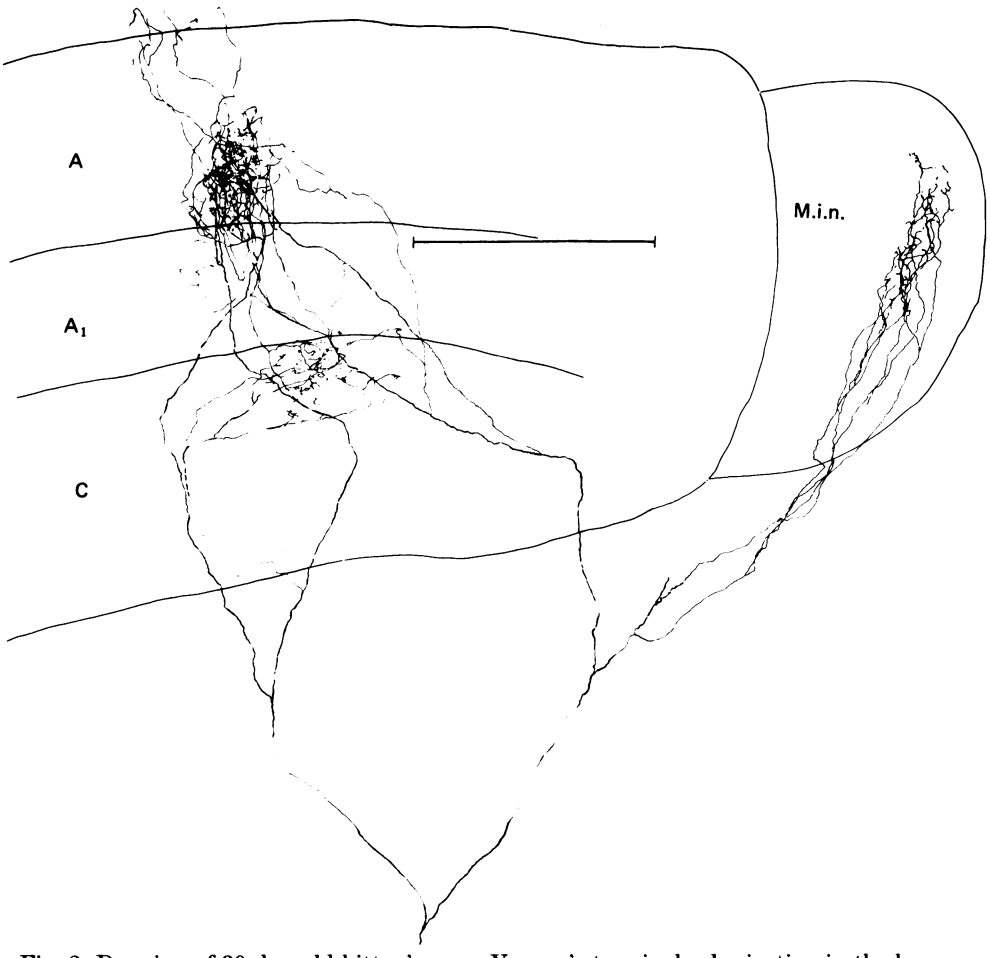

Fig. 2. Drawing of 30-day-old kitten's r.g.c. Y axon's terminal arborization in the l.g.n. This is the same axon from which the physiological records in Fig. 1 were obtained. Scale bar = 500 μm. The receptive field of this axon was from the right eye contralateral to the l.g.n. which it innervates. Abbreviations: A = lamina A, A_1 = lamina A_1, C = C complex, m.i.n. = medial interlaminar nucleus. This drawing as well as those in Figs. 3 and 4 were reconstructed from serial coronal sections that were drawn with a 50× oil immersion objective.

Fig. 2 is a drawing of the kitten r.g.c. Y axon from which the physiological records in Fig. 1 were obtained. The receptive field properties of this axon are listed in Table 1 and described in the Figure legend. This axon was from an r.g.c. located in the contralateral eye. Note the multiple branches and subsequent convergence of these branches to three major sites of termination within the l.g.n.; lamina A, lamina C and the medial interlaminar nucleus (m.i.n.). This pattern of innervation of the l.g.n. is also characteristic of adult r.g.c. Y axons (Bowling & Michael, 1980, 1984; Sur & Sherman, 1982; and our control data). It is at these terminal arborization sites that the axon branches give rise to terminal boutons which are thought to be presynaptic

to l.g.n. cell dendrites in adult cats (Guillery, 1969; Mason & Robson, 1979; Robson & Mason, 1979).

On some kitten axons, swellings with filopodia and growth cone-like appendages were observed (see Pl. 1). These profiles have not been seen in adult material (Bowling

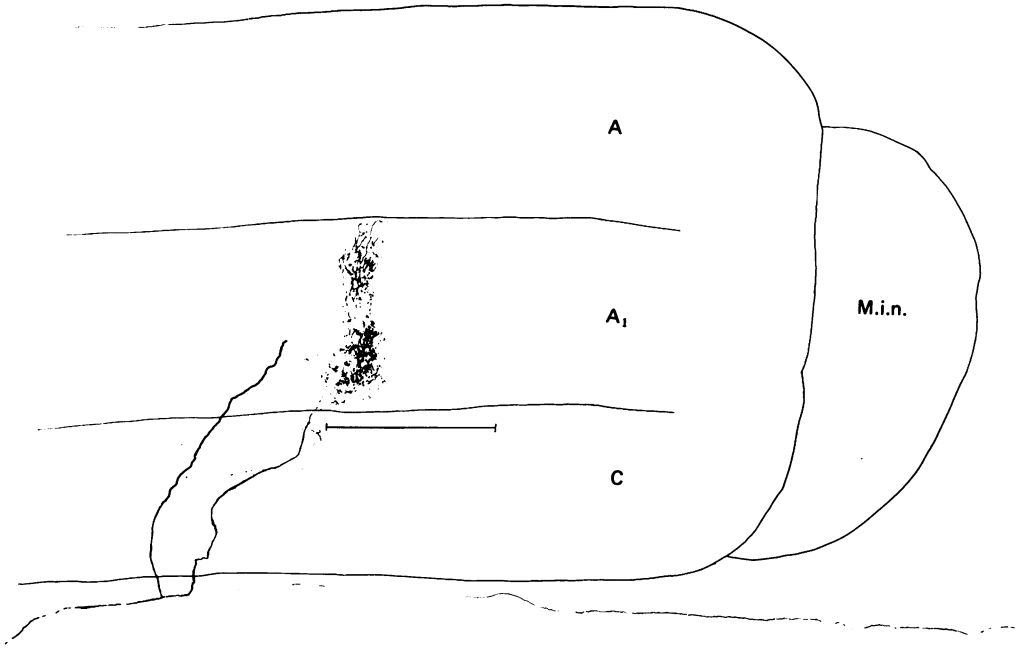

Fig. 3. Drawing of the terminal arborization of an r.g.c. Y axon from a 29-day-old kitten. This axon had an off-centre receptive field, a response latency to stimulation of the optic chiasm of 0·8 ms and a 3·2 deg diameter receptive field centre located at an eccentricity of azimuth = 4·5 deg; elevation = −0·5 deg. It responded to grating counterphase in a non-linear fashion similar to that shown for the Y axon's records in Fig. 1. The receptive field of this axon was from the left eye ipsilateral to the l.g.n. which it innervates. The axon's membrane was penetrated by the micropipette in the optic tract lateral and ventral to the first bifurcation point. Note that the axon innervates lamina A_1 heavily with a broader base of the terminal arborization (compared to the apex). This pattern has been reported for off-centre Y r.g.c. axons in adult cats by Bowling & Michael (1984). A small branch of the axon courses in the C complex. Another branch was traced medial and caudal to the l.g.n. Abbreviations are as in Fig. 2. Scale bar = 500 μm.

& Michael, 1980, 1984; Sur & Sherman, 1982). There is evidence that in the kitten these are bouton-like structures that form synapses (Mason, 1982*b*) and we have counted them as such for the purposes of analysis. The distributions of these terminal boutons for the Y axon illustrated in Fig. 2 and subsequent Figures are considered further below and illustrated in Fig. 5.

The off-centre Y axon illustrated in Fig. 3 was recovered from a 29-day-old kitten. The r.g.c. of origin for this axon was in the ipsilateral eye. This axon's innervation pattern in the l.g.n. has a characteristic convergence of many branches into a well defined terminal zone (in this case in l.g.n. lamina A_1). A small branch issues a few terminal boutons in the C complex. However, most of the boutons are located in

laminae A_1. This axon does not innervate the m.i.n. but does have a single branch that courses medial and caudal to the m.i.n. which probably innervates the pretectum or superior colliculus. The terminal arborization of this axon spans the entire lamina A_1 (as did four of the seventeen kitten r.g.c. Y axons) but stops abruptly at the laminar borders. There is a distinct pyramidal shape to the terminal arborization when viewed in the coronal plane. Five of the nine axons with off-centre receptive fields have similarly shaped terminal arborizations. This same feature has been observed for off-centre r.g.c. Y axons in the adult cat when viewed in the saggital plane (Bowling & Michael, 1980, 1984).

Examples of two other types of innervation patterns seen for Y axons in the kitten l.g.n. are illustrated in Fig. 4. Both of these examples were obtained from 31-day-old kittens. The axon illustrated in Fig. 4*A* innervates lamina A and the m.i.n. only. The parent axon bifurcates as it enters the C complex from the optic tract with a single branch projecting to lamina A where it then divides into multiple segments. The same pattern is true for the branch that innervates the m.i.n. The very dark staining of the axon illustrated in Fig. 4*A* makes it unlikely that other branches were missed due to poor filling with HRP. The terminal arborization of this axon occupies 40 % of the dorsoventral axis of lamina A. Thirteen of the seventeen axons in the kitten are similar in extent (spanning from 40 to 80 % of the dorsoventral axis of either of the A-laminae). This axon does not innervate the C laminae or other brain-stem sites. Another type of innervation pattern seen in kittens is illustrated in Fig. 4*B*. Three of the seventeen kitten Y axons have this pattern of innervation with only a few branches in lamina A that end in large swellings with a few boutons. In normal adult cats (Bowling & Michael, 1984, $n = 15$; Sur & Sherman, 1982, $n = 12$; Friedlander *et al.*, this report, $n = 4$) r.g.c. Y axons always have their major projection to one of the A-laminae. This earlier innervation of lamina C than of lamina A for a subpopulation of r.g.c. Y axons in the neonate is considered with respect to the effects of visual deprivation in the Discussion.

The boutons on the branches of the r.g.c. Y axons are distinct swellings in the adult. They occur in both *en passant* and at terminal positions. The structure of these boutons on adult and kitten r.g.c. Y axons are compared in Pl. 1. Plate 1*A* illustrates an example of this structure on an adult r.g.c. Y axon that innervates lamina A. Note the characteristic spherical or crenulated shapes of the boutons with sizes that range from 0·5 to 4 μm. For purposes of comparison, photomicrographs of boutons on kitten r.g.c. Y axons (also terminating in the A-laminae) are shown in Pl. 1*B*, *C* and *D*. These examples are taken from the axons illustrated in Figs. 2, 4*A* and 4*B*, respectively. Plate 1*B* illustrates part of the terminal arborization from the axon shown in Fig. 2. Note the greater variety of bouton shapes that occur in the neonate than in the adult. Plate 1*C* is taken from the axon illustrated in Fig. 4*A*. There are many long, stalked terminal appendages (arrows) and divergent multilobed endings that are similar in appearance to growth cones (Jackowski & Lieberman, 1979). These structures have not been reported to occur in the adult (Sur & Sherman, 1982; Bowling & Michael, 1984). Plate 1*D* is taken from the axon illustrated in Fig. 4*B* which has only a restricted innervation of lamina A. These darkly stained branches end in distinct clusters which contain filopodia and spike-like extensions. No such endings were observed on this same axon's terminal arborization in lamina C.

The distribution of the boutons in the A-laminae for the r.g.c. Y axons from kittens

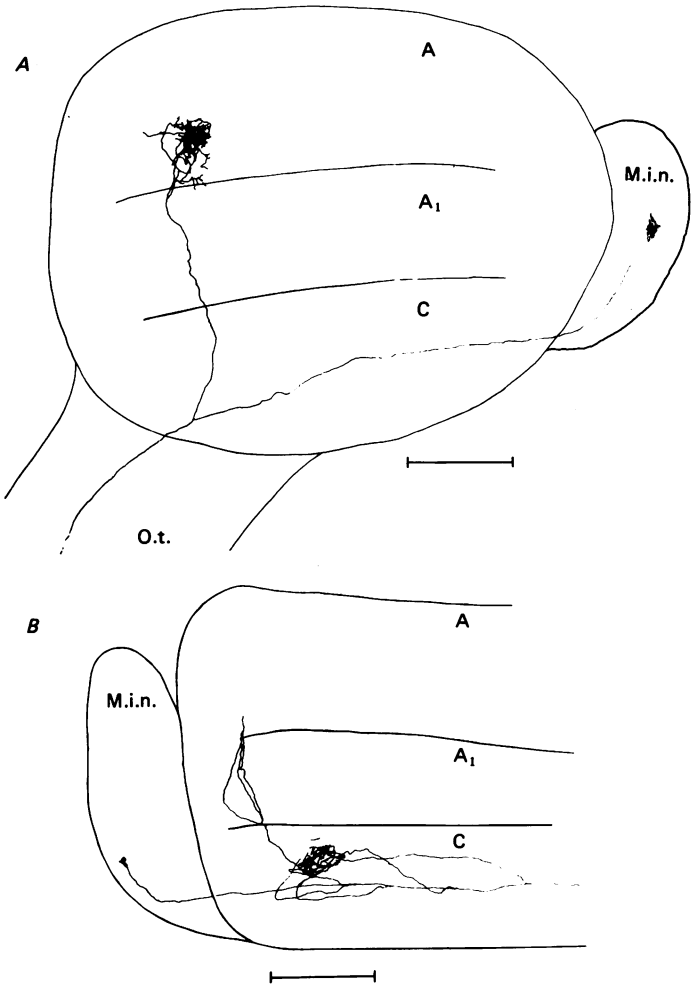

Fig. 4. Drawings of two r.g.c. Y axons from 31-day-old kittens that have patterns of l.g.n. innervation that differ from the axons illustrated in Figs. 2 and 3. Scale bars = 500 μm. *A*, this Y axon had an on-centre receptive field with a centre size of 6·5 deg located at an eccentricity of azimuth = 31·0 deg and elevation = 5·4 deg. It responded to the counterphased grating (spatial frequency = 1·4 cycles/deg, contrast = 0·4, mean luminance = 32 cd/m^2, counterphase rate = 2·0 Hz) with a non-linear response (second harmonic/fundamental response ratio = 1·34 at 0 deg spatial phase position of the grating). Latency to electrical stimulation of the optic chiasm was 0·6 ms. The axons' receptive field was from the right eye contralateral to the l.g.n. that it innervates. Note that a single branch innervates lamina A and the m.i.n. with no branches to the C complex. O.t. = optic tract. All other abbreviations are as in the previous Figures. *B*, this Y axon had an off-centre receptive field with a centre size of 1·8 deg located at an eccentricity of azimuth = 1·2 deg, elevation = +0·5 deg. Its response to counterphase of a grating (spatial frequency = 2·6 cycles/deg, contrast = 0·4, mean luminance = 32 cd/m^2, counterphase rate = 1·0 Hz) also showed non-linear spatial summation (second harmonic/fundamental response ratio = 2·35 at 0 deg spatial phase position of the grating). Its latency to electrical stimulation of the optic chiasm was 0·6 ms. This axon's receptive field was from the left eye contralateral to the l.g.n. which it innervates. The axon was impaled in the optic tract before the initial bifurcation. Note the heavy innervation of the C complex and branch to the m.i.n. However, only a small innervation of lamina A was observed.

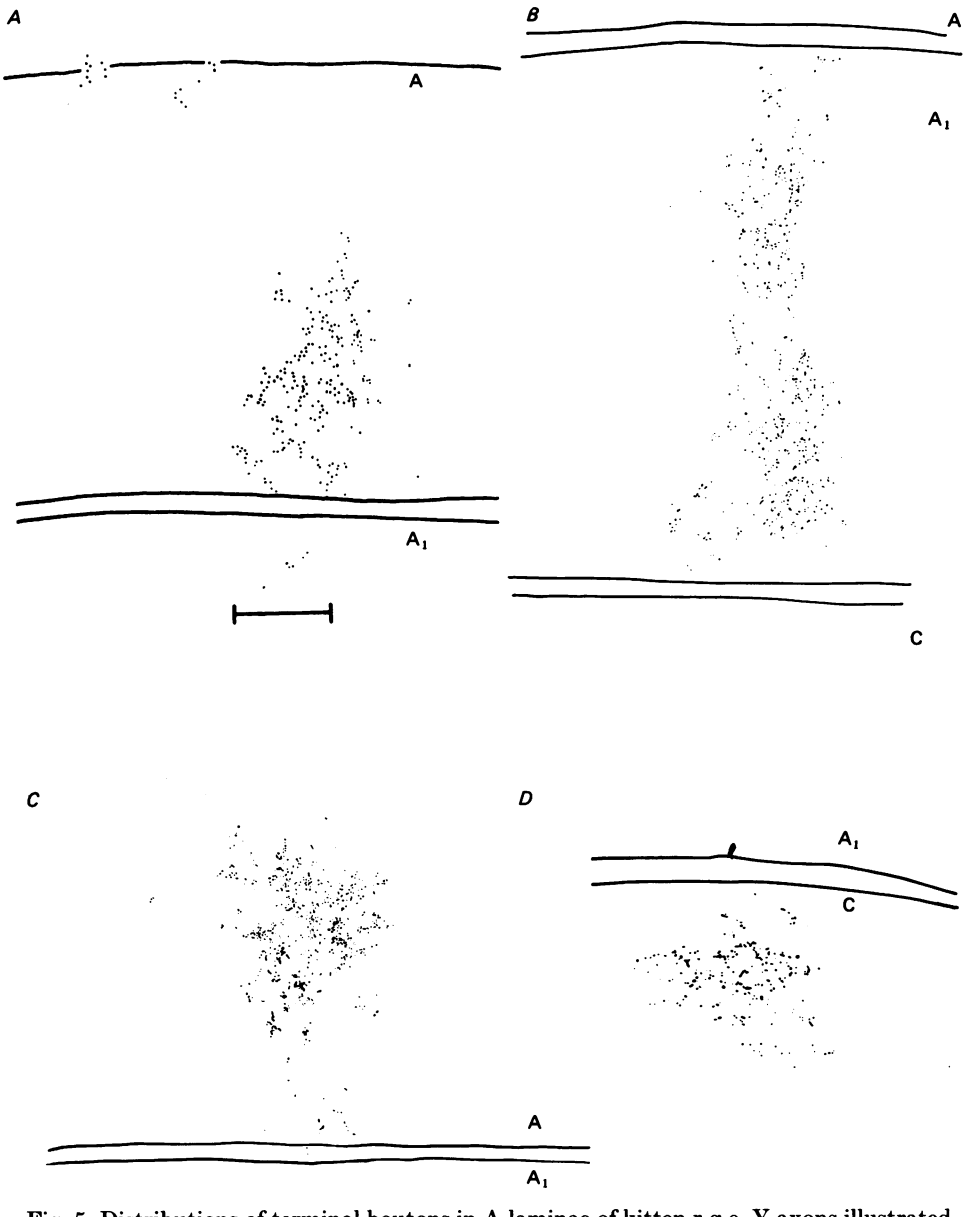

Fig. 5. Distributions of terminal boutons in A-laminae of kitten r.g.c. Y axons illustrated in Fig. 2(*A*), Fig. 3(*B*), Fig. 4*A*(*C*) and boutons in the lamina C from kitten r.g.c. Y axon illustrated in Fig. 4*B*(*D*). Parallel lines represent laminar boundaries and enclosed interlaminar zones. Scale bar = 100 μm.

illustrated in Figs. 2, 3 and 4*A* and in lamina C for the axon shown in Fig. 4*B* are illustrated in Fig. 5. The axon from Fig. 2 restricts most of its boutons to lower lamina A. However, note that a small number of boutons overlap into A_1 and a few branches with boutons wander to the top of lamina A as well (Fig. 5*A*). The terminal arborization of the axon from Fig. 3 (bouton distribution shown in Fig. 5*B*) is similar

to the adult pattern reported by Bowling & Michael (1984). The boutons are broadly distributed at the base, narrowing at the apex when viewed in the mediolateral plane. Fig. 5*C* and *D* show the bouton distribution for the axons illustrated in Fig. 4*A* and *B*, respectively. Distinct clustering of the boutons is apparent in Fig. 5*C*.

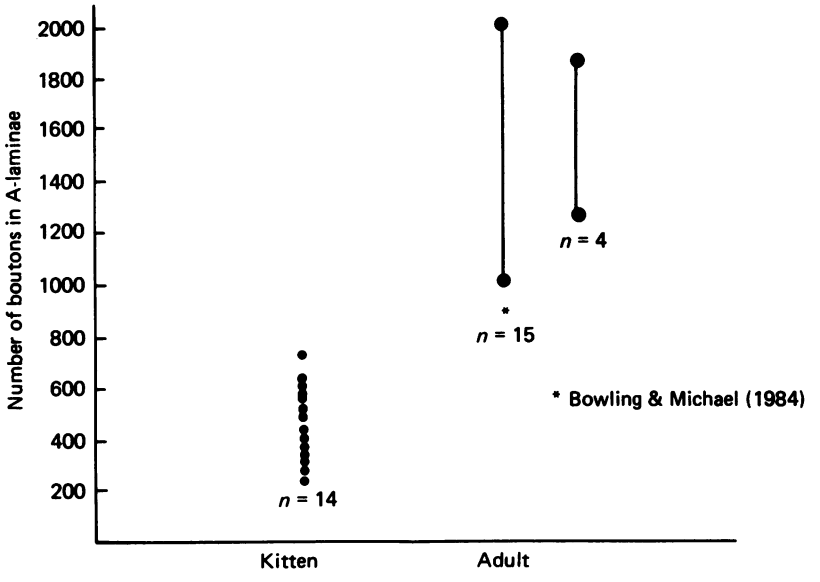

Fig. 6. Plot of numbers of boutons in A-laminae on individual r.g.c. Y axon terminal arborizations in 4–5-week-old kittens (n = 14, three axons with only a single branch to lamina A are not included in the analysis) compared to Y axons in adult cats (n = 15, data of Bowling & Michael (1984) and n = 4, control data from this study). No kitten r.g.c. Y axon had as many boutons as any adult Y axon. These populations differ significantly ($P < 0{\cdot}001$) on a Mann–Whitney U test. Mean number of boutons on kitten Y axons = 476, range 226–740. Mean number of boutons on adult Y axons (Bowling & Michael, 1980, 1984) = 1500, range = 1000–2000. Mean number of boutons on adult Y axons (our control data) = 1553, range 1280–1900.

The number of boutons on the r.g.c. Y-axon terminal arborization within the A-laminae of adult cat l.g.n. range from 1000 to 2000 per axon (Bowling & Michael, 1980, 1984). Our adult control data (1280–1900 boutons per axon) are in agreement with these values. We made similar counts for the kitten axons and obtained the result illustrated in Fig. 6. In all fourteen cases, the kitten Y-axon terminal arborizations in the A-laminae have fewer boutons than the adult Y axons (kitten mean = 476 boutons per axon, range = 226–740 boutons per axon; adult range (Bowling & Michael, 1980, 1984) = 1000–2000 boutons per axon, adult mean (Friedlander *et al.*, this study) = 1553 boutons per axon, adult range = 1280–1900 boutons per axon, $P < 0{\cdot}001$, Mann–Whitney U test). The width of the terminal arborizations within the A-laminae in the mediolateral dimension was also measured for the kitten Y axons and compared to the terminal widths of adult Y axons. This result is illustrated in Fig. 7. The upper panel compares the raw data between kittens and adult material (three studies). The mean widths are indicated by arrows on the ordinate. Note that

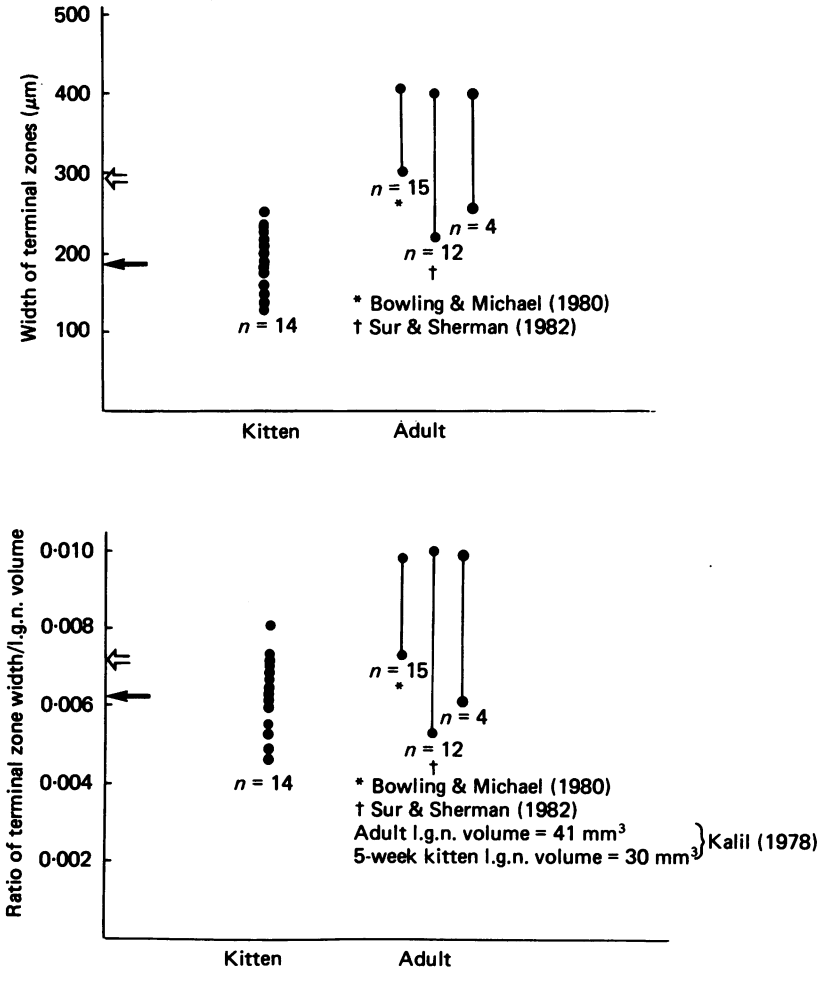

Fig. 7. Upper panel, plot of width of terminal arborizations (measured at widest point) in A-laminae for kitten r.g.c. Y axons (n = 14) compared to three samples of r.g.c. Y axons from adult cats (Bowling & Michael (1980) n = 15, Sur & Sherman (1982) n = 12 and Friedlander *et al.*, this study, n = 4). The kitten Y axons have a mean maximum width of their terminal fields = 192 μm, range 131–252 μm. Adult ranges = 300–400 μm (Bowling & Michael, 1980), 220–410 μm (Sur & Sherman, 1982) and 258–412 μm (this study). The mean width of the kitten population (192 μm) is indicated by the filled arrow on the ordinate and the mean width of the adult population (reported by Sur & Sherman (1982) = 293 μm) is indicated by the open arrow. Lower panel, the width of the terminal arborizations of the kitten r.g.c. Y axons (n = 14) are normalized on the ordinate for the volume of the l.g.n. at 5 weeks of age (Kalil, 1978). The normalized data for the kitten Y axons are compared to those from Y axons from adult cats from two other reports (n = 15 and n = 12) and from this report (n = 4). The means of the distributions (0·0062 in the kitten, filled arrow and 0·0071 in the adult, open arrow) are indicated on the ordinate. Note that the relative increase in width of r.g.c. Y axon terminal arborization within the A-laminae (15%) is less than the increase in absolute size (52%).

the extent of the kitten Y-axon arborizations is narrower than in the adults (kitten mean = 192 μm, range = 131–252 μm), adult mean (study of Sur & Sherman, 1982) = 293 μm, range = 220–410 μm, adult mean (Friedlander *et al.*, this study) = 312 μm, range = 258–412 μm, adult mean (Bowling & Michael, 1984) = 375 μm,

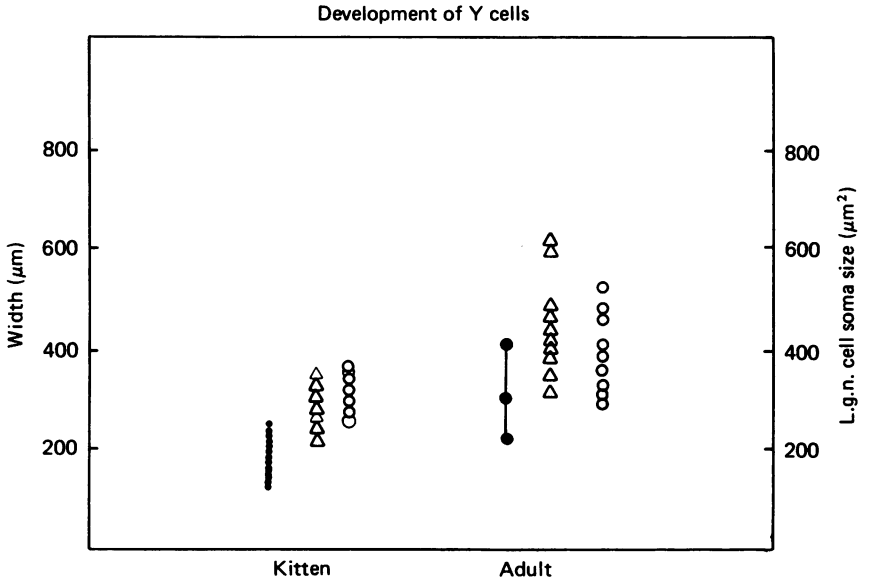

Fig. 8. Comparison of post-natal structural development of the terminal arborizations of r.g.c. Y axons (in the A-laminae) to the soma–dendritic maturation of Y cells in the A-laminae of the l.g.n. The left ordinate is the scale for the width of the r.g.c. Y axons' terminal arborization and of the dendritic tree of l.g.n. Y cells. The right ordinate is the scale for the area of the somata of the l.g.n. Y cells. These values are compared for kittens (age 4–5 post-natal weeks) and adult cats. Soma and dendritic measurements were made on two samples of l.g.n. Y cells obtained in this laboratory (kitten, Friedlander, 1982 and subsequent studies; adult cats, Friedlander *et al.* 1981) and additional cells obtained since completion of those studies. The 'growth ratios' are the ratios of the mean value in the adult/the mean value of the 4–5-week-old kitten for the three parameters listed. These values express the proportion of the adult size obtained for r.g.c. Y axons and l.g.n. Y cells by 4–5 post-natal weeks of age. ●, retinal axon terminal zone width; △, l.g.n. cell dendritic width; ○, l.g.n. cell soma size. Growth ratios: ●, 0·63; △, 0·61; ○, 0·65.

range = 300–400 μm. There is no overlap between the widths of the Y-axon terminal arborizations of the kittens and those of the adult cats (our control data and those of Bowling & Michael, 1980, 1984). Three of the kitten axons have widths that overlap with the adult axons in the report of Sur & Sherman (1982). When these data are normalized for the increase in volume of the l.g.n. between kittens of five post-natal weeks of age and adult cats (Kalil, 1978), the relative increase in width of the r.g.c. Y-axon arborizations is considerably less (15 % relative increase *vs.* 52 % absolute increase in width). This *relative* increase (15 %) in area occupied by individual r.g.c. Y axons is the same when the values are normalized for growth of the A-laminae, alone (mean kitten A-laminae volume = 22·3 mm^3, mean adult A-laminae volume = 31·1 mm^2; our calculations).

The increased width of the Y-axon's terminal arborization is almost paralleled by growth of the target nucleus (l.g.n.; see Fig. 7). However, these axons are increasing their relative innervation of the A-laminae by 15%. To what degree is this growth paralleled by the development of the l.g.n. Y cells? In Fig. 8, we make this comparison. We analysed a sample of seven l.g.n. Y cells from kittens of the same age used in the r.g.c. axon study (4–5 post-natal weeks). These cells were both physiologically and morphologically characterized using methods similar to those described in this study (see Friedlander, 1982). The relative development of two morphological features between these kitten l.g.n. Y cells and a sample of l.g.n. Y cells obtained from adult cats (Friedlander, Lin, Stanford & Sherman, 1981) is compared in Fig. 8. Both samples were obtained from the A-laminae of the l.g.n. The width of the dendritic tree (in the mediolateral dimension) for l.g.n. Y cells is 61% (growth ratio indicated in Fig. 8) of the adult value by this age. The size of the somata of these same l.g.n. Y cells is 65% of adult values. The r.g.c. Y axons are 63% of their adult size (width of their terminal arborization in the A-laminae in the same mediolateral dimension) at this same age. These data suggest that neither the l.g.n. Y cells nor their innervating r.g.c. Y axons have a particular lead in structural development at an age of 4–5 post-natal weeks.

DISCUSSION

We have demonstrated that in 4–5-week-old kittens, many r.g.c. axons can be physiologically classified as Y cells. Their responses to counterphase of a grating (brisk response with non-linear spatial summation) and to electrical stimulation of optic chiasm (short latency responses = 0·6–1·1 ms) are adult-like at this age. However, their receptive field centre sizes are larger than those of the adult Y axons at a particular eccentricity (as has been reported; Rusoff & Dubin, 1977; Hamasaki & Sutija, 1979). While the innervation pattern of the Y axons in the kitten l.g.n. is similar to that seen in the adult cat, the distribution and structure of the terminal arborizations are immature in the kittens.

Although Shatz (1983) reported that by birth the segregation of retinal axons in the kitten l.g.n. was adult-like, our results indicate that after this prenatal sorting of retinal axons, a considerable period of growth and reorganization occurs in the Y pathway. The appendages on the r.g.c. Y axons have features in common with growth cones and filopodia in active stages of development as reported by other investigators both *in vitro* (see Letourneau, 1982 for review) and *in situ* (Mason, 1982*a*; Raper, Bastiani & Goodman, 1983; Mason & Gregory, 1984). The range of morphological diversity of these boutons (even on an individual Y axon) suggests a differential rate of development both within and between axons of the same functional class. It is not certain if these immature boutons form functional synapses with l.g.n. cells since we have not examined this material with the electron microscope. However, Mason (1982*b*) reported that similar immature terminal structures on 5-week-old kitten optic tract axons do make synaptic contact with l.g.n. cell dendrites.

The number of boutons on the terminal arborizations of the r.g.c. Y axons within the A-laminae increases threefold from 4 to 5 post-natal weeks to adulthood. This result is not likely to be an artifact of poor HRP filling of branches of the axons in

the kittens. Examination of the most peripheral branches of these axons in the kittens reveals dark staining and well defined terminal structure (Pl. 1). Also, the Y axon's structure is characterized by an initial divergence of the parent axon into several branches that then converge into the terminal zone. The peripheral branches (that would set the limits for the terminal field width measurements) are not as distant from the parent axons as those branches that innervate the middle of the field. The increase in the numbers of boutons on r.g.c. Y axons in the l.g.n. is occurring as the total number of synaptic profiles in the l.g.n. is increasing (Cragg, 1975; Kalil & Scott, 1979). However, much of this increase is due to the addition of terminals that form symmetric synapses (Winfield *et al.* 1980; Winfield & Powell, 1980) which are non-retinal in origin (Guillery, 1969; Hendrickson, 1969; Famiglietti, 1970) most likely originating from l.g.n. interneurones (Famiglietti, 1970) or cells of the perigeniculate nucleus (Ide, 1982). Winfield *et al.* (1980) estimated that the mean number of retinal synapses per synaptic glomerulus declines between 3 and 8 post-natal weeks. However, they were not able to estimate the absolute number of retinal synaptic contacts in the l.g.n. of the neonate *vs.* the adult. Future studies which include electron microscopical analysis of the terminal boutons of individually labelled r.g.c. axons may address this issue by comparing the numbers of synaptic contacts per bouton in neonates and adult animals.

The functional significance of the post-natal expansion of the Y axons' terminal fields in the A-laminae of the l.g.n. is not clear. However, we suggest that this result in addition to the increase in bouton number may account for the increased encounter of l.g.n. Y cells with age (Daniels *et al.* 1978; Mangel *et al.* 1983). In their study, Mangel *et al.* (1983) suggest that the increase is due to the late development of the retinal substrate that underlies the characteristic Y-cell response (non-linear spatial summation within the receptive field). Therefore, presumptive l.g.n. Y cells in the neonate are misclassified as X cells (since they would respond in a linear fashion to the test for spatial summation). We propose that the post-natal increase in l.g.n. Y cells to their adult proportion (33–50 %; Friedlander *et al.* 1981; Friedlander & Stanford, 1984) may also be due to a larger fraction of l.g.n. cells receiving retinal Y input and/or an increased Y input to other l.g.n. cells. The expansion of the Y axons' terminal arborization and the increase in numbers of boutons support this hypothesis. We are currently testing this hypothesis by comparing the distribution of retinal inputs onto l.g.n. cells in kittens that receive Y input (J. Somogyi, M. J. Friedlander & K. A. C. Martin, personal communication) to adult Y cells (Wilson, Friedlander & Sherman, 1984).

In addition to describing the normal ontogeny of the retinogeniculate Y system, our results may also be useful to explain the susceptibility of the Y-cell pathway to factors in the visual environment. Kittens reared in darkness (Kratz, Sherman & Kalil, 1979) or with monocular eyelid suture (Wiesel & Hubel, 1963; Sherman *et al.* 1972; Friedlander & Stanford, 1984) develop abnormalities in the retinogeniculate pathway, particularly in Y cells. These effects of monocular eyelid suture include (but are not limited to) a reduced cell size in the l.g.n. (Wiesel & Hubel, 1963; Guillery & Stelzner, 1970; Garey, Fisken & Powell, 1973; Hickey, 1980; Kalil, 1980) particularly of the large (Y) cells (Friedlander *et al.* 1981); a reduced proportion of recorded Y cells (Sherman *et al.* 1972; Friedlander & Stanford, 1984); altered

structure/function relationships for l.g.n. cells (Friedlander *et al.* 1982) and absence of innervation of the A-laminae by some r.g.c. Y axons (Sur *et al.* 1982). This last result is similar to our findings in the normal kittens which had a poorly developed innervation of the A-lamina for three of ten Y axons from the contralateral eye (see Table 1, Fig. 4*B* and Pl. 1*D*) although their input to lamina C was mature. For the above reasons, we suggest that monocular deprivation may cause an arrest of development of the terminal arborizations of r.g.c. Y axons. This would explain the reduced numbers of l.g.n. Y cells encountered in visually deprived cats.

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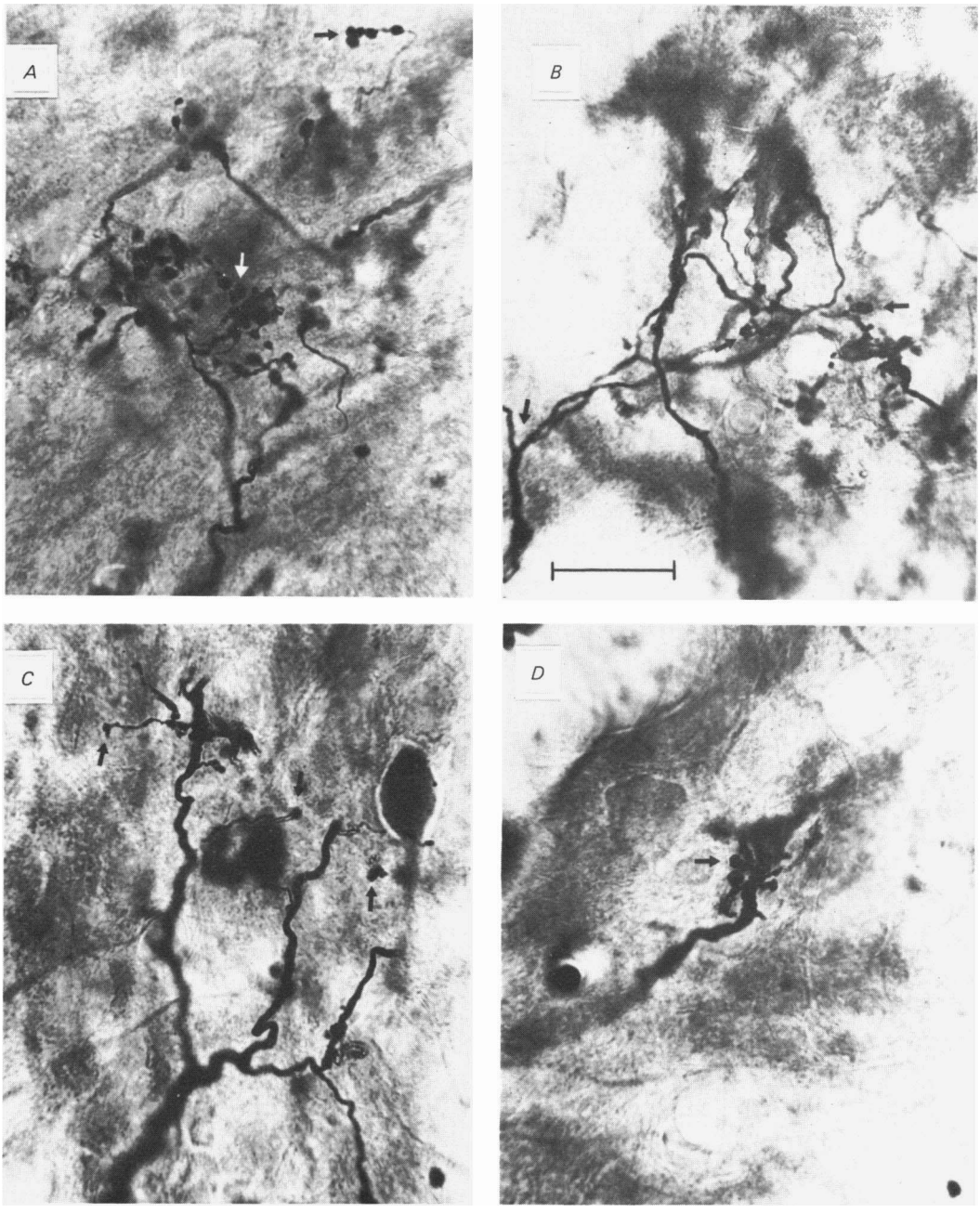
A
B
C
D

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EXPLANATION OF PLATE

Photomicrographs of boutons on terminal arborizations within the A-laminae of r.g.c. Y axons from a normal adult cat (*A*) and from 4–5-week-old-kittens (*B–D*). This Figure illustrates the range of morphological variety of terminals and boutons found on the kitten Y axons compared to those of the adult. Scale bar = 20 μm in all photomicrographs. Photomicrographs were taken with a 100 × oil immersion objective and a Kodak Writter 48A blue filter to enhance contrast. *A* is taken from the terminal arborization of a normal adult r.g.c. Y axon. *B* is from the terminal arborization of the kitten axon illustrated in Fig. 2. *C* is from the A-lamina terminal arborization of the kitten axon illustrated in Fig. 4*A*. *D* is from the A-lamina branches of the kitten axon illustrated in Fig. 4*B*. The arrows point to boutons. Note the regular spherical shape of the boutons on the adult Y axon (*A*). Similar boutons are found on kitten Y axons (*B*), however a greater degree of heterogeneity of these structures also occurs in the kitten (*C* and *D*).