POPULATION STUDY OF HORIZONTAL CELLS IN LIVE CARP RETINAS USING MICROINJECTIONS OF HORSERADISH PEROXIDASE

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Abstract—In order to randomly sample horizontal cells (HCs) of carp retina, horseradish peoxidase (HRP) was delivered into axon terminals by means of vitreally inserted micropipettes in live fish. Survival time was extended to several hours to permit label diffusion into HC somata. A function measuring the relative complexity of cell contours was defined in order to help classify HCs according to their morphological types. The histogram of measurement from 313 HCs showed three modes fitting normal distributions. The numbers of cells in each group were 267, 36 and 10, respectively, representing a relative population of 85.3% H1s, 11.5% H2s and 3.2% H3s. It is suggested that cone HC somata are extended in a monolayer constituted by predominant type-H1 with interspersed groups of both type-H2 and type-H3 cells.

Teleost   Carp   Retina   Horizontal cells   Horseradish peroxidase   Microinjections

INTRODUCTION

Golgi studies have determined the existence of three morphological classes of cone-driven horizontal cells (HCs) in the cyprinid retina: H1, H2 and H3 (Stell, 1975; Weiler, 1978). They are mainly characterized by the sizes of their soma and the richness and extensions of their dendritic arborizations. They all exhibit fine, often varicose axons that expand into long tubular terminals, which make chemical synaptic contacts in the inner plexiform layer, mostly with bipolar and interplexiform cells (Marshak & Dowling, 1987). Intracellular recordings show three types of response functions to spectral stimuli: mono-, bi- and triphasic (Tomita, 1965). Recording and marking of carp HCs with fluorescent dyes (Mitarai, Asano & Miyake, 1974; Weiler, 1978; Negishi, Teranishi & Kato, 1985) and electron microscopy of functionally identified HCs (Djamgoz & Downing, 1988; Downing & Djamgoz, 1989) have confirmed a relationship between morphology and response, which was originally inferred from the connectivity between cones and HCs (Stell & Lightfoot, 1975). This relationship assigns monophasic responses to type-H1, biphasic responses to type-H2, and triphasic responses to type-H3 cells. Although in other species the HC soma is electrically isolated from its axon terminal (Nelson, Lützow, Kolb & Gouras, 1975; Ohtsuka, 1983), they are coupled in the carp (Weiler & Zettler, 1979; Kouyama & Watanabe, 1986; Yagi, 1986; Yagi & Kaneko, 1988). An intermediate, rod-dependent HC (type-H4) has also been described both as axonless (Kaneko & Yamada, 1972; Stell, 1975; Weiler, 1978; Teranishi, Negishi & Kato, 1984) and axon-bearing (Tsukamoto, Yamada & Kaneko, 1987).

Intracellular injections of Lucifer Yellow show that dye-permeable junctions connect adjacent HC somata of the same morphological type, so that the types appear to constitute segregated functional syncytia (Kaneko & Stuart, 1984). Such junctions are modulated by light and dark adaptation, and by dopamine and its agonists (Teranishi et al., 1984; Mangel & Dowling, 1985, 1987; Yang, Torqvist & Dowling, 1988a, b; Torqvist, Yang & Dowling, 1988). Similar connections have also been found between the axons and terminals of external HCs (Kouyama & Watanabe, 1986).

Still lacking is an evaluation of the extent of the functional syncytia mediated by gap-junctions, linked to the relative number of cells of each morphological type. In this respect, available data stem only from studies on electrical responses (Tomita, 1965; Negishi, Teranishi & Kato, 1985). These provide a raw estimate,

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considering the bias introduced by the selectivity of cell impaling. The purpose of the present study is to determine the contributions of the three morphological types to the population of cone-driven HCs of the carp retina, by means of intracellular injections of horseradish peroxidase (HRP) delivered into axon terminals. This way, a nearly random labelling of the three cell-types may be obtained.

HRP is a medium-weight protein whose diffusion speed within the cell may be slower than that of fluorescent dyes, but it is a sensitive tracer that does not require massive deliveries to yield dense and finely detailed cell labelling. However, external HCs of the carp retina are rather large cells whose volume is mainly distributed between the soma and the expanded terminal; these are connected by a fine fiber that extends for several hundred micrometers and limits the speed of transfer of the label from one compartment to the other. Consequently, the time period required to evenly fill the whole cell often surpasses the useful life of the isolated organ preparation (Djamgoz & Downing, 1988). In order to overcome such limitations, a technique of intracellular recording in the intact retina of live carp (Gutiérrez, Neely & Salinas, 1983) has been adapted to deliver HRP into HCs, under conditions that favour axon-terminals as microinjection targets. The preservation thereafter of the fish in good physiological condition permits an extended survival period, so as to ensure the diffusion of the injected tracer. Double marks, spillage and bursted targets, which often accompany HRP injections into somata, are thus less likely to occur with this method, yielding cleaner images of the labelled somata.

MATERIAL AND METHODS

Carp weighing 450–900 g were paralyzed with d-tubocurarine (2 mg/kg, i.m.) and placed in a specially designed tray supporting them horizontally on a side with an eye exposed. Aerated water fed through a mouth supporting tube flowed freely out of the opercula and compensated for the suspended natural respiration. Once the fish was stably mounted, proparacaine hydrochloride was applied on the cornea; a few minutes later it was partially cut around the limbus and the flap folded back over the eyeball. The lens was gently removed through the pupil with fine forceps, taking care not to injure the iris; the operation should be bloodless. The

vitreous was left intact for the protection of the retina and a drop of Ringer solution was applied to prevent desiccation of its surface. The nonreactive iris allowed the easy insertion through the pupil of the micropipette and a slender light duct also carrying the reference electrode.

Micropipettes were drawn from 1.0 mm o.d. WPI capillaries which, unlike other glass tubing tried, gave consistently dependable results. They were filled with 2 M KCl and tested; acceptable electrodes measured 30–70 MΩ. The KCl electrolyte was replaced by 4% HRP (Sigma type VI) in a 0.2 M KCl solution buffered to pH 7.6 with Tris (0.05 M). They were kept overnight in a cold cabinet and tested immediately prior to use. Clogged and noisy micropipettes could be usually cleared by passing pulsing current through them until their impedance stabilized. Normal values ranged from 90 to 180 MΩ.

The micropipette was introduced into the vitreous under visual guidance with the mechanical positioner supporting the probe. After a few minutes in the dark, the electrode was slowly advanced by means of the hydraulic driver, while the stimulus, consisting of alternating 960 msec pulses of green (520 nm) and red (650 nm) light, was continuously delivered through the optic-fiber duct. This simple sequence permitted the immediate recognition of the target. As required, stimulus wavelengths and intensities could be easily shifted in order to better identify the impaled cell.

Contact with the retina was signaled by a baseline jolt and a slight increase of background noise. At this point, the presence of residual movements of the fish could be detected, in which case the experiment was momentarily suspended until they subsided; occasionally, the animal was administered a supplementary dose of curare. Once a cell was impaled, it was observed under continuous stimulation for about a minute. Responses were monitored on an oscilloscope and recorded both on paper for immediate reference, and digitally on videotape for later analysis. Iontophoretic delivery of the enzyme proceeded if the cell was considered viable; only units showing stable responses with a dark membrane potential of at least 25 mV were selected. The tracer-injecting current consisted of 2.5–5.0 min trains of 100 msec, 3.0–6.0 nA depolarizing pulses at a rate of 5/sec, delivered through the input bridge of a Model 707 W.P.I. Microprobe System, which permitted the uninterrupted control of cell responses. Charge transfer varied from 180 to 960 nC.
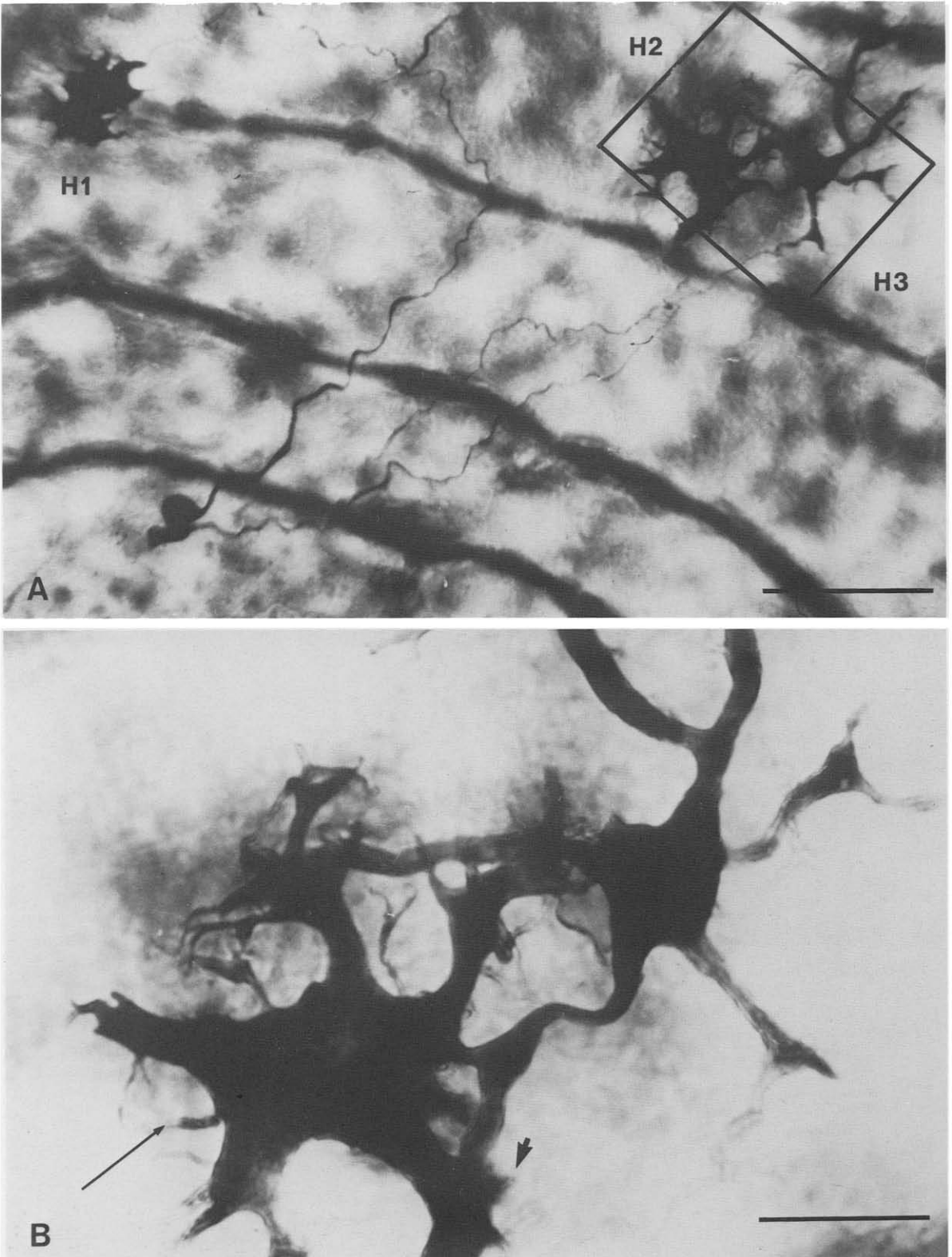
Fig. 1. (A) Three HCs of different morphological types (H1, H2, H3) were labelled by a single HRP microinjection delivered into crossing axon terminals (arrow). At least one of the enlarged terminals seems to have been obliterated in the process. The three cells are on the same focal plane. (B) Enlarged micrograph of the H2- and H3-type cells shown in (A) (rectangle), extending their dendrites along similar courses (heavy arrow) or just across a soma (thin arrow). Nevertheless, there is no overlapping of somata. Scale bar: (A) 50 μm; (B) 20 μm.
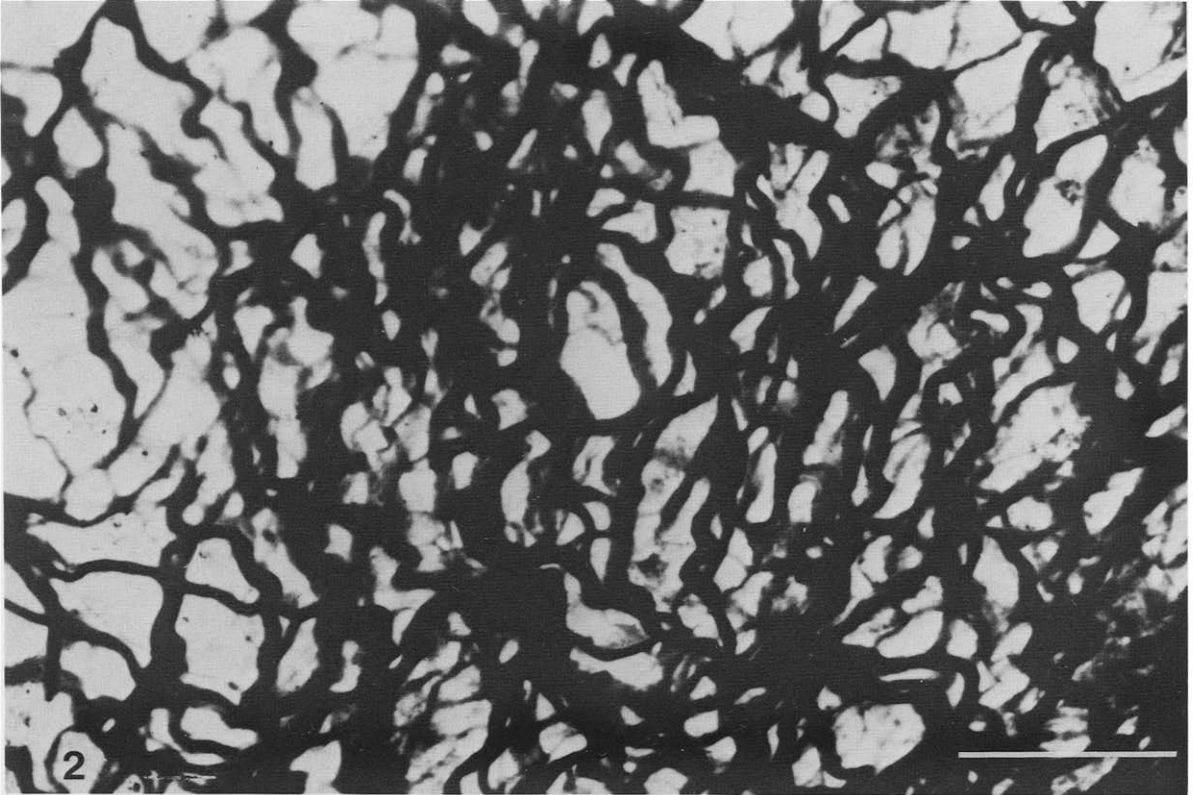
Fig. 2. Unusual view of the mesh of HC fibers and axon terminals in carp retina, which illustrates the density of HC targets facing a vitreally inserted microelectrode. It was obtained during preliminary trials made to ascertain the optimal conditions for these experiments. Survival time was extended 23 hr after multiple and massive HRP deliveries into both luminosity and chromaticity units. The presence of membrane debris (out of focus) indicates the occurrence of extensive cellular damage, which may explain the spread of the label. Scale bar: 50 μm.
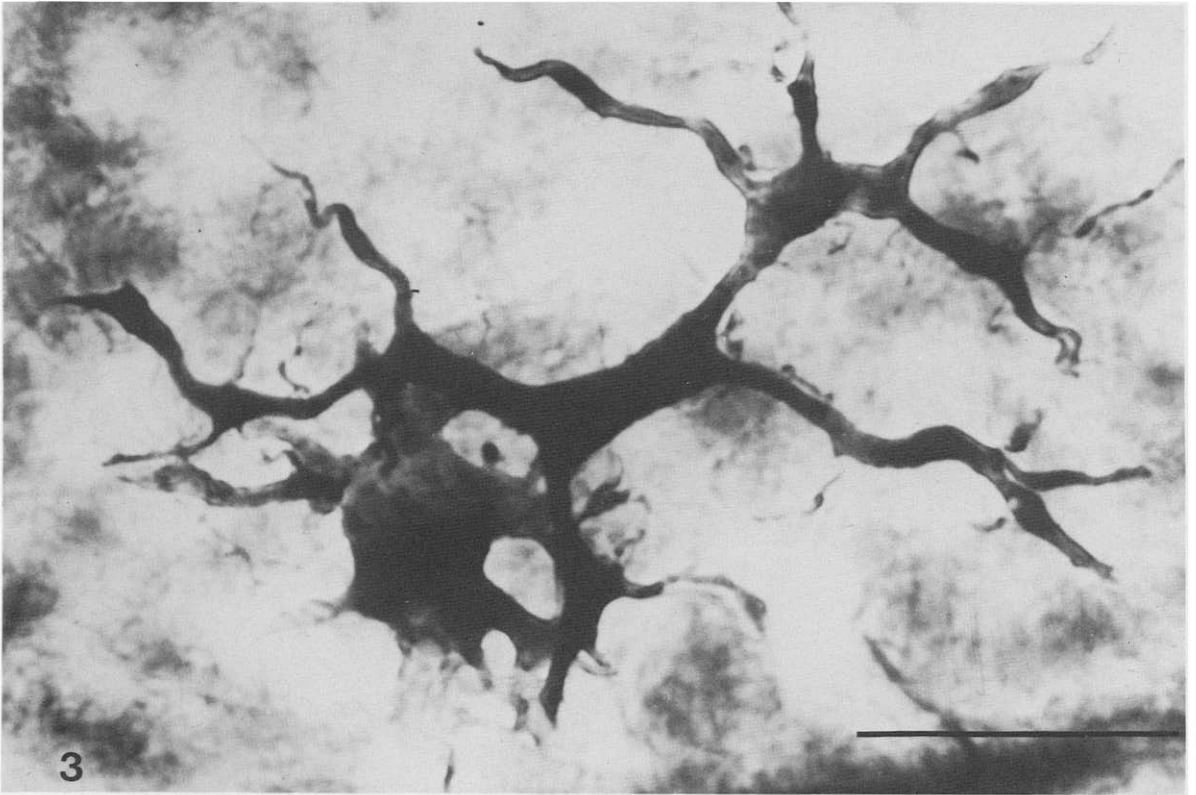
Fig. 3. Juxtaposed HCs obtained after 24 hr of survival time from two different HRP injections delivered nearly 2 mm apart in the anterior–dorsal retina. An H1-type cell (left) is lodged between two prolongations of an H3-type cell (right). Their dendrites intercross, but both somata are on the same focal plane. Scale bar: 50 μm.

After completing the microinjections, the cornea was sutured and the fish was allowed to survive for periods of up to 24 hr.

Dark adapted eyes were enucleated under MS-222 anaesthesia, which was not used in previous steps as it is known to block photopigment regeneration (Hoffman & Basinger, 1977). The retinas were excised and fixed for an hour in a cold solution of 1% paraformaldehyde and 2% glutaraldehyde in phosphate buffer, pH 7.3. Finally, they were developed with the DAB method, flat-mounted, dehydrated, cleared, impregnated with resin, and glass coverslipped. Observations were made on these wholemounted retinas, with the vitreal side up.

These procedures were performed on 184 retinas from 122 live carp. Only 93 retinas showed satisfactorily marked cells with clear signals that the microinjections were delivered into axon terminals.

It was determined that quantitative criteria rather than subjective judgements should guide the morphological classification of labelled HCs. This posed some peculiar requirements, so that an *ad hoc* procedure was adopted. The expression $PA^{-1/2}$, involving the perimeter P and the enclosed area A of cell profiles drawn in a *camera lucida*, was found to closely follow increasingly convoluted cell contours independently of their size. It was measured by means of a digitizer (Houston Instruments, Mod. 8024) using ARC/INFO software (ESRI, Redlands, California, U.S.A.). To get an estimation of the standard error, the whole procedure from drawing to digitizing was repeated five times on two widely different cells. Standard errors turned out to be closely proportional to the average values, suggesting the function should rather be made a logarithmic one. This way, the *eidometric index* $I_E = \ln(PA^{-1/2}) - 1.266$ was defined, the constant making the index equal to zero for a circle. The use of Napierian logarithms permitted to spread measured values within a more comfortable range.

RESULTS

Unlike fast diffusing Lucifer Yellow, HRP slowly travelled along axons; with time, it reached the most distant parts of the injected cell, labelling its processes with minute detail. Preliminary experiments showed that the minimum time required to permit an adequate transfer of the tracer from the injected compartment of the cell to the other was extremely variable, depending on their relative sizes, on the length and thinness of the connecting fiber and on the amount of injected label. In fact, of 43 HRP marks recoverd from both isolated and *in situ* injected retinas after survival periods up to 52 min, only one displayed a whole type-H1 cell extending for about 260 μm (not shown). The rest exhibited either somata or enlarged axon terminals with variable lengths of attached fibers, as an indication that the migration of the enzyme was interrupted by the fixation of the retinas. It could be established that the minimum time required to consistently label a whole HC was 4 hr. Accordingly, survival times in these experiments were extended beyond 4 hr, most of them ranging from 12 to 24 hr.

A common occurrence was the labelling of more than one cell with a single injection. In a few instances, it could be related to accidental displacements of the micropipette tip while injecting, signaled by shifts of the responses being recorded. In most cases, though, it was an unexpected result in an otherwise neat process. The extent of this effect varied from a faint partial dyeing of a second cell to the full labelling of three or more HCs, often of a different morphological type. The examples shown in Fig. 1A were obtained from a retina *in situ* after a survival time of 21 hr. They are fair representatives of the three morphological types of cone-HCs of the cyprinid retina, as revealed by the Golgi method (Stell, 1975; Weiler, 1978).

Microinjections were mostly delivered into axon terminals, which the vitreal insertion of the micropipette made the first HC targets for impalement. Exceptions were easily spotted and discarded. Identification of injection marks was unequivocal and made easier by the high incidence of multiple cell labellings favoured by the density of intercrossings in the compact mesh of axon terminals (Fig. 2). This effect, which would be undesirable in other circumstances, was used in these experiments to obtain non-selective labelling of several cells with a single microinjection, although at the cost of not being able to consistently relate responses to morphology. Among several hundred labelled cone-HCs many instances could be observed of adjacent somata of different morphological types whose dendrites intercrossed (Figs 1B and 3) but no examples were found of overlapping somata. It is also noteworthy that no rod-driven (type H4)
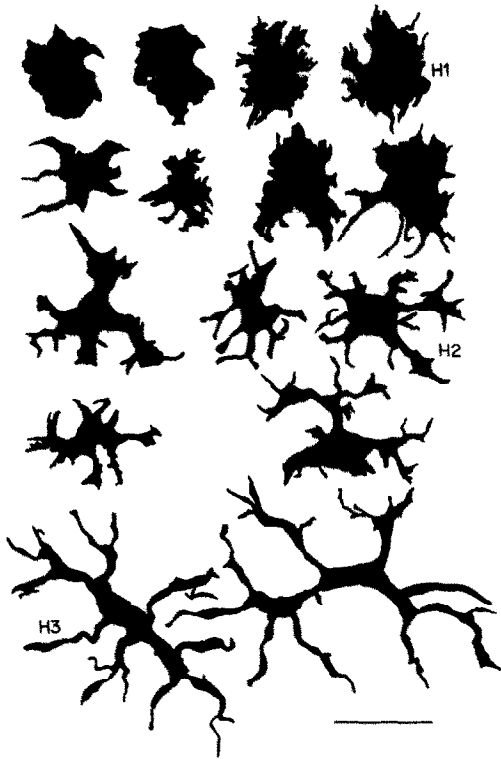
Fig. 4. *Camera lucida* drawings from a sample of 313 HRP-labelled HCs. From left to right and top to bottom, a gradation of increasingly branched HC somata is displayed. Three particular silhouettes resembling Stell's prototypes have been marked H1, H2 and H3. Scale bar: 50 μm.

HCs were ever recovered from microinjections delivered into axon terminals.

Classification of the labelled cells into morphological types required a different approach than visual appraisal, as most profiles displayed features common to H1 and H2, or to H2 and H3 (Fig. 4). The profiles of 313 isolate cell somata selected for their complete and clean outlines were drawn in a *camera lucida* and their I_E measured (see Methods). The axonal cone was included, but the axon itself and minute details such as short appendages and dendritic terminal clusters were omitted.

The histogram of I_E (Fig. 5) displayed the 313 cells in a continuous distribution with three well pronounced modes. Three cells previously identified by their similarity to Stell's prototypes as belonging to morphological types H1, H2 and H3 (Fig. 4), were neatly distributed among the three modes, which could be closely fitted to slightly overlapping Gaussian curves (Fig. 6). The number of cells included in each of the three groups was 267, 36 and 10, respectively, showing relative contributions of 85.3% H1, 11.5% H2 and 3.2% H3 to the HC population (Table 1). Information concerning the areas of their respective dendritic fields is shown in Table 2.
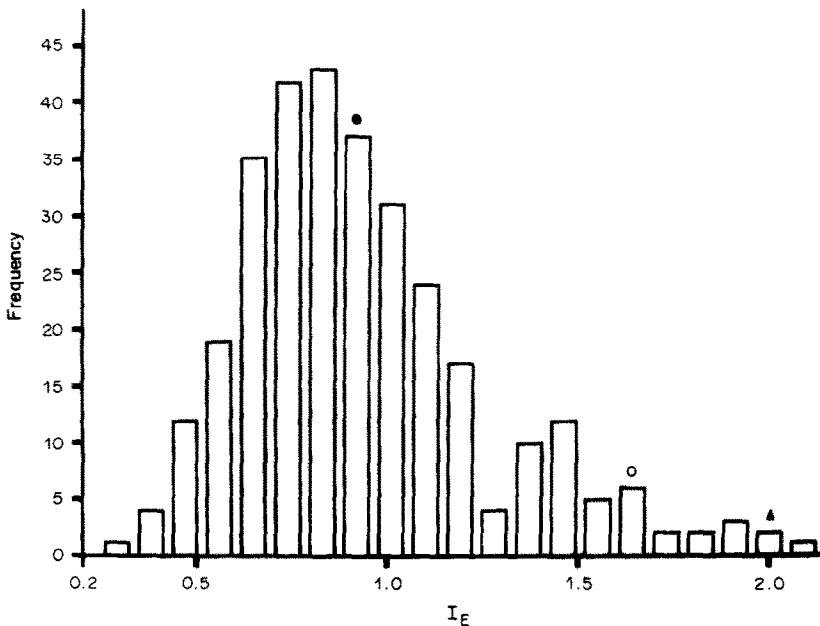

Fig. 5. Frequency histogram of 313 HCs according to I_E values, showing three well-defined modes around 0.73, 1.36 and 1.90. Symbols placed at 0.85 (●), 1.55 (○) and 1.95 (▲), show the position in the histogram of cells marked in Fig. 4 as H1, H2 and H3, respectively.
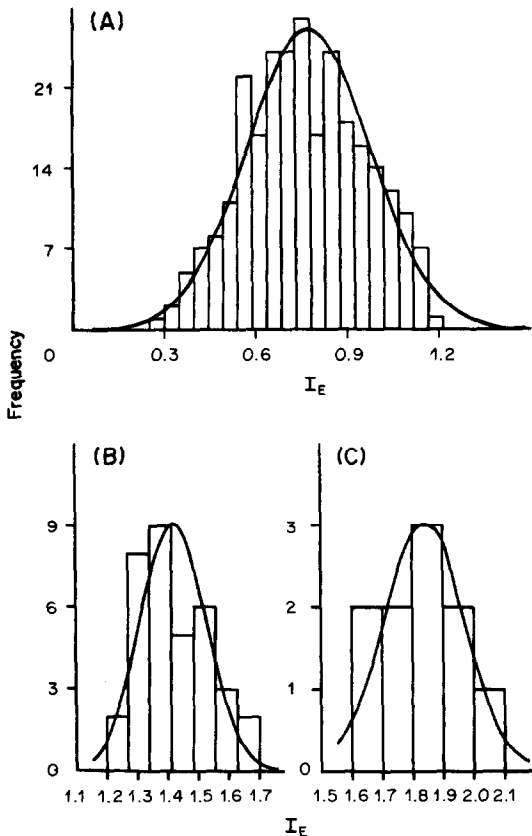
Fig. 6. Gaussian curves fit the three modes of the I_E frequency histogram of Fig. 5. The Kolmogorov–Smirnov test showed each of them to be a good fit: (a) $D_{max} = 0.038$ ($P < 0.05$; $n = 267$), (b) $D_{max} = 0.123$ ($P < 0.05$; $n = 36$), (c) $D_{max} = 0.122$ ($P < 0.05$; $n = 10$).

DISCUSSION

A distinctive feature of HRP labelling is its ability to reveal Golgi-like detail without the unpredictable selectivity of Golgi staining. The success of HRP as a neuronal marker depends on a survival period long enough to permit the complete filling of the cell processes. In the vertebrate retina dimensions involved are generally such, that a satisfactory diffusion of the injected label inside a cell may be attained within the relatively brief periods allowed by the currently used isolated organ preparations (Kretz, Ishida & Stell, 1982; Ohtsuka, 1983;

Odgen, Mascetti & Pierantoni, 1984, 1985; Djamgoz, Downing & Wagner, 1985, 1989; Leeper & Charlton, 1985; Nelson & Kolb, 1985; Saito, Kujiraoka, Yonaha & Chino, 1985; Kouyama & Watanabe, 1986; Ohtsuka & Kouyama, 1986). Concerning the external horizontal cells of the teleost retina, though, our preliminary experiments on both isolated retinas and short term live preparations confirmed that the usually permissible survival periods are not long enough to take full advantage of HRP as a marker, as label delivered into a terminal does not reach the soma, and *vice versa* (Djamgoz & Downing, 1988; Downing & Djamgoz, 1989). Live preparations permit to extend the survival time to obviate this problem and also allow us to explore the retina, select cells, and inject them unhurriedly. Furthermore, a large number of cells may be labelled in a single retina.

Double marks in relation with HRP studies on different types of retinal cells have been repeatedly reported (Leeper & Charlton, 1985; Kraft & Burkhardt, 1986; Djamgoz et al., 1989) and also analyzed with reference to cone labellings in the teleost retina (Kaneko, Nishimura, Tauchi & Shimai, 1981), where double impalements seem to induce the formation of conduit-like structures between both cells. A similar effect may provide an explanation for our multiple cell labels, involving intercrossing axon terminals (Fig. 1A).

The eidometric index was devised to serve as a suitable tool for classifying the HC population after evaluating other possible options; no biological meaning should be attached to it, though. As its use is restricted solely to plane shapes, a first question arises concerning its applicability to horizontal cells. Teleost HCs are markedly flat cells extending nearly parallel to the surface of the retina. Although the index is not invariant under an oblique projection, it tolerates considerable tilts of a cell profile with no significant change. In fact, the usefulness of the eidometric index resides in its sensitivity to the presence of prolongations that increase the

Table 1. Statistical summary of the sample of 313 labelled HCs distributed into three groups: H1, H2 and H3. Data on I_E, perimeter, area and relative area referred to H1 are shown

HC-type	$n = 313$	%	Average I_E	Perimeter (μm)		Area (μm^2)		Relative average area
				Average	SD	Average	SD	
H1	267	85.3	0.78	232.52	78.98	879.43	352.00	1.00
H2	36	11.5	1.42	500.26	103.75	1182.54	396.72	1.35
H3	10	3.2	1.84	912.03	209.18	1675.89	455.99	1.91

SD = standard deviation.

Table 2. Statistical summary of dendritic field areas
(mean ± SD) of the three cone-HC types. Column 3 shows
the relative average dendritic field areas referred to H1. The
coverage (%) of the dendritic field areas of the three HC
populations are shown in column 4. They represent the
product of column 3 and the corresponding frequencies
shown in column 3 of Table 1. These values are related to
the relative extensions of the functional syncytia mediated
by gap-junctions and constituted by each cone-HC type

HC type	Dendritic field area (μm^2)	Relative average area	Average dendritic field area (%)
H1	1708 ± 523	1.00	68.00
H2	2951 ± 602	1.73	15.86
H3	10810 ± 3949	6.33	16.14

perimeter of a contour to a given area, indepen-
dently of the cell size. It should be recalled that
HC prototypes were defined according to such
morphological criteria applied to the projec-
tions of the cells on the plane of the retina
(Stell, 1975). The fact that the index could
discriminate the 313 cells of our sample, and
group them into three Gaussian distributions
containing cell profiles akin to each of Stell's
prototypes, respectively, means that it pointed
to a distinctive feature of carp HCs (Figs 5
and 6). In fact, the number and dendritic
extensions of each HC-type are related to the
number of cones of the classes with which they
connect and the field size of their pedicles
(Stell & Lightfoot, 1975; Downing & Djamgoz,
1989).

Another fundamental question is: how close
is our sample to the global HC population?
Several factors should be considered. From the
initial sample of 379 HCs retrieved from HRP
injections delivered into axon terminals, 66 units
(17.4%) were rejected due to partial maskings
by blood vessels, pigment blots, HRP spillage
and other accidents that blurred cell contours.
There is no reason, though, to assume that the
final selection was significantly biased by this
procedure. In these experiments, microinjec-
tions were delivered into axon terminals, the
foremost HC targets offered to the vitreally-
inserted micropipettes. However, even though
axon terminals are fairly comparable among
HC types, it would be very difficult to estimate
how random the relative target areas are within
the dense mesh of intercrossing axon terminals
(Fig. 2). This point may be further examined by
comparing our results with the distribution of a
large number of recordings from the somata of
HCs responding with monophasic (478 units),
biphasic (123 units), and triphasic (26 units)
potentials reported by Negishi et al. (1985).
These numbers can be manipulated to take

into account the probability of impaling an
average HC of a given type, which depends
both on the relative frequencies of the different
cell types and on the relative effective areas
they offer to micropipette penetration. Our
study shows that the average effective areas of
H1, H2 and H3 cells are in the ratio 1:1.34:1.91
(Table 1). Adjusting the figures from Negishi
et al. (1985) accordingly gives H1, H2 and H3
relative proportions of 82.0%, 15.6% and
2.4% respectively, in close agreement with the
values here obtained (Table 1). These, we pro-
pose, show the most probable relative popu-
lations of the three types of cone-HCs in the
carp retina.

An immediate corollary of these results con-
cerns the architecture of the external HC layer
which contains cone-HC somata. Most prob-
ably, they are disposed as a monolayer, accord-
ing to our own observations of numerous
instances of adjoining cells, frequently of differ-
ent types (Figs 1B and 3), without a single
sample of overlapping somata. Its predominant
components are type H1 cells with a compara-
tively reduced presence of H2 and particularly
of H3 cells. However, these are grouped consti-
tuting segregated syncytia mediated by gap-
junctions (Teranishi et al., 1984; Kaneko &
Stuart, 1984). In order to determine the relative
coverage of each of these groups, their average
dendritic field areas were computed as shown in
Table 2. According to them, the relative num-
bers of both H2 and H3 cells should be at least
four times larger than those here reported in
order to cover the same retinal expanse as H1
cells. Consequently, the external HC layer is
most probably organized as a predominant H1
functional syncytium, with interspersed patches
of similar syncytia of both type-H2 and type-H3
cells.

A final comment refers to type-H4, rod-
related HCs. They have been described both as
axonless (Kaneko & Yamada, 1972; Stell, 1975;
Weiler, 1978; Teranishi et al., 1984) and axon-
bearing (Tsukamoto et al., 1987). The fact that
no type-H4 cells were ever retrieved from about
150 HRP microinjections delivered into axon
terminals indicates that, even if they do have an
axon, most probably they lack an enlarged
terminal.

REFERENCES

Djamgoz, M. & Downing, J. (1988). A horizontal cell selectively contacts blue-sensitive cones in cyprinid fish retina: Intracellular staining with horseradish peroxidase. *Proceedings of the Royal Society of London, B, 235,* 281–287.

Djamgoz, M., Downing, J. & Wagner, H. (1985). The cellular origin of an unusual type of *S*-potential: An intracellular horseradish peroxidase study in a cyprinid fish retina. *Journal of Neurocytology, 14,* 469–489.

Djamgoz, M., Downing, J. & Wagner, H. (1989). Amacrine cells in the retina of a cyprinid fish: Functional chracterization and intracellular labelling with horseradish peroxidase. *Cell and Tissue Research, 256,* 607–622.

Downing, J. & Djamgoz, M. (1989). Quantitative analysis of cone photoreceptor-horizontal cell connectivity patterns in the retina of cyprinid fish: Electron microscopy of functionally identified and HRP-labelled horizontal cells. *Journal of Comparative Neurology, 289,* 537–553.

Gutiérrez, O., Neely, A. & Salinas, R. (1983). The horizontal system of the carp retina. Transients reveal chromatic influences on L-units with participation of interplexiform cells. *Archivos de Biologia y Medicina Experimental, 16,* 271–281.

Hoffman, R. & Basinger, S. (1977). The effect of MS-222 on rhodopsin regeneration in the frog. *Vision Research, 17,* 335–336.

Kaneko, A. & Stuart, A. E. (1984). Coupling between horizontal cells in the carp retina revealed by diffusion of Lucifer Yellow. *Neuroscience Letters, 47,* 1–7.

Kaneko, A. & Yamada, M. (1972). *S*-Potentials in the dark-adapted retina of the carp. *Journal of Physiology, London, 227,* 221–273.

Kaneko, A., Nishimura, Y., Tauchi, M. & Shimai, K. (1981). Morphological observation of retinal cells presumably made syncytial by an electrode penetration. *Journal of Neuroscience Methods, 4,* 299–303.

Kouyama, N. & Watanabe, K. (1986). Gap-junctional contacts of luminosity-type horizontal cells in the carp retina: A novel pathway of signal conduction from the cell body to the axon terminal. *Journal of Comparative Neurology, 249,* 404–410.

Kraft, T. W. & Burkhardt, D. A. (1986). Telodendrites of cone photoreceptors: Structure and probable function. *Journal of Comparative Neurology, 249,* 13–27.

Kretz, R., Ishida, A. T. & Stell, W. K. (1982). Ratfish retina-intracellular recordings and HRP injections in an isolated, superfused all-rod retina. *Vision Research, 22,* 857–861.

Leeper, H. F. & Charlton, J. S. (1985). Response properties of horizontal cells and photoreceptor cells in the retina of the tree squirrel, *Sciurus carolinensis. Journal of Neurophysiology, 54,* 1157–1166.

Mangel, S. & Dowling, J. E. (1985). Responsiveness and receptive field size of carp horizontal cells are reduced by prolonged darkness and dopamine. *Science, 229,* 1107–1109.

Mangel, S. & Dowling, J. E. (1987). The interplexiform horizontal cell system of the fish retina: Effects of dopamine, light, stimulation and time in the dark. *Proceeding of the Royal Society of London, B, 231,* 91–121.

Marshak, D. W. & Dowling, J. E. (1987). Synapses of cone horizontal cell axons in goldfish retina. *Journal of Comparative Neurology, 256,* 430–443.

Mitarai, G., Asano, T. & Miyake, Y. (1974). Identification of five types of *S*-potentials and their corresponding generating sites in the horizontal cells of the carp retina. *Japanese Journal of Ophthalmology, 18,* 161–176.

Negishi, K., Teranishi, T. & Kato, S. (1985). Spatial orientation of horizontal cell axon terminals in the carp retina. *Brain Research, 359,* 104–112.

Nelson, R. & Kolb, H. (1985). A17: A broad-field amacrine cell in the rod system of the cat retina. *Journal of Neurophysiology, 54,* 592–614.

Nelson, R., Lützow, Av., Kolb, H. & Gouras, P. (1975). Horizontal cells of cat retina with independent dendritic systems. *Science, 189,* 137–139.

Ogden, T. E., Mascetti, G. G. & Pierantoni, R. (1984). The internal horizontal cell of the frog. Analysis of receptor input. *Investigative Ophthalmology and Visual Science, 25,* 1382–1394.

Ogden, T. E., Mascetti, G. G. & Pierantoni, R. (1985). The outer horizontal cell of the frog retina: Morphology, receptor input and function. *Investigative Ophthalmology and Visual Science, 26,* 643–656.

Ohtsuka, T. (1983). Axons connecting somata and axon terminals of luminosity-type horizontal cells in the turtle retina: Receptive field studies and intracellular injections of HRP. *Journal of Comparative Neurology, 220,* 191–198.

Ohtsuka, T. & Kouyama, N. (1986). Electron microscopic study of synaptic contacts between photoreceptors and HRP-filled horizontal cells in the turtle retina. *Journal of Comparative Neurology, 250,* 141–156.

Saito, T., Kujiraoka, T., Yonaha, T. & Chino, Y. (1985). Reexamination of photoreceptor-bipolar connectivity patterns in carp retina: HRP-EM and Golgi-EM studies. *Journal of Comparative Neurology, 236,* 141–160.

Stell, W. K. (1975). Horizontal cell axons and axon terminals in goldfish retina. *Journal of Comparative Neurology, 159,* 503–520.

Stell, W. K. & Lightfoot, D. O. (1975). Color-specific interconnections of cones and horizontal cells in the retina of the goldfish. *Journal of Comparative Neurology, 159,* 473–502.

Teranishi, T., Negishi, K. & Kato, S. (1984). Regulatory effect of dopamine on spatial properties of horizontal cells in the carp retina. *Journal of Neuroscience, 4,* 1271–1280.

Tomita, T. (1965). Electrophysiological study of the mechanism subserving color coding in the fish retina. *Cold Spring Harbor Symposia of Quantitative Biology, 30,* 559–566.

Torqvist, K., Yang, X-L. & Dowling, J. E. (1988). Modulation of cone horizontal cells activity in the teleost fish retina—III. Effects of prolonged darkness and dopamine on electrical coupling between horizontal cells. *Journal of Neuroscience, 8,* 2279–2288.

Tsukamoto, Y., Yamada, M. & Kaneko, A. (1987). Morphological and physiological studies of rod-driven horizontal cells with special reference to the question of whether they have axons and axon terminals. *Journal of Comparative Neurology, 255,* 305–316.

Weiler, R. (1978). Horizontal cells of the carp retina: Golgi impregnation and Procion-yellow injection. *Cell and Tissue Research, 195,* 515–526.

Weiler, R. & Zettler, F. (1979). The axon-bearing horizontal cells in the teleost retina are functional as well as structural units. *Vision Research, 19,* 1261–1268.

Yagi, T. (1986). Interaction between the soma and the axon terminal of retinal horizontal cells in *C. carpio. Journal of Physiology, London, 375*, 121–135.

Yagi, T. & Kaneko, A. (1988). The axon terminal of Goldfish retinal horizontal cells: A low membrane conductance measured in solitary preparations and its implication to the signal conduction from the soma. *Journal of Neurophysiology, 59*, 482–494.

Yang, X-L., Torqvist, K. & Dowling, J. E. (1988a). Modu-

lation of cone horizontal cells activity in the teleost fish retina—I. Effects of prolonged darkness and background illumination of light responsiveness. *Journal of Neuroscience, 8*, 2259–2268.

Yang, X-L., Torqvist, K. & Dowling, J. E. (1988b). Modulation of cone horizontal cells activity in the teleost fish retina—II. Role of interplexiform cells and dopamine in regulating light responsiveness. *Journal of Neuroscience, 8*, 2269–2278.