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ACTION AND LOCALIZATION OF γ-AMINOBUTYRIC ACID IN THE CAT RETINA

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SUMMARY

1. The effects of iontophoretically applied GABA (γ-aminobutyric acid) and bicuculline on retinal ganglion cells were studied in the optically intact eye of the anaesthetized cat.

2. GABA suppressed both the spontaneous activity and light-evoked discharge of all retinal ganglion cells, regardless of their type and regardless of the visual stimulus used.

3. Bicuculline antagonized the action of iontophoretically applied GABA. Bicuculline enhanced the spontaneous activity of on-centre cells, but suppressed the spontaneous activity of most off-centre cells. The light-evoked response of on-centre cells was increased by bicuculline. A more complicated picture emerged for off-centre cells. Weak light responses were suppressed by bicuculline, but during strong light responses the initial transient phase of the response was dramatically enhanced.

4. Amacrine cells of the inner nuclear layer and displaced amacrine cells of the ganglion cell layer were labelled, using glutamic acid decarboxylase (GAD) immunohistochemistry and [^{3}H]muscimol uptake. GAD-positive dendrites were found throughout the inner plexiform layer and no sign of dendritic stratification was detected.

INTRODUCTION

Responses of cat retinal ganglion cells can be modulated by the inhibitory transmitter γ-aminobutyric acid (GABA) and its antagonist bicuculline. However, the published results are conflicting because whilst some studies have used *in vivo* systemic application (Kirby & Enroth-Cugell, 1976; Kirby & Schweitzer-Tong, 1981), others have recorded from the isolated eyecup applying the drugs to the bathing medium (Saito, 1981), while still others applied GABA iontophoretically (Straschill & Perwein, 1969; Ikeda & Sheardown, 1983).

Straschill & Perwein (1969), found all ganglion cells to be suppressed by iontophoretic

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application of GABA. This is in contrast to the results of Ikeda & Sheardown (1983) who found that only on-centre cells are modulated by GABA and its antagonists, irrespective of the 'sustained(X)' or 'transient(Y)' type of the cell. A difference between the X and the Y system with respect to GABA and its antagonists has been described after systemic application (Kirby & Enroth-Cugell, 1976; Kirby, 1979). Recording from the isolated eyecup preparation, Saito (1981) found no influence of GABA antagonists on on-centre X cells, whereas on-centre Y cells were affected; with off-centre cells, administration of bicuculline influenced both X and Y cells.

Therefore, it seemed an open question whether GABA and its antagonists affect all ganglion cells, or whether some specific action exists with respect to the on–off or X–Y dichotomies. In the present study of GABA action on retinal ganglion cells, we decided against the use of systemic drug application or recordings from an isolated eyecup coupled with bath application, since these techniques seemed too generalized. We also did not use intracellular recordings (which would permit measurement of membrane polarity and resistance) since long-lasting recordings in the cat have proved exceedingly difficult. Therefore, GABA and its antagonist bicuculline were applied iontophoretically in the intact eye by means of multibarrelled electrodes, thus permitting stable, long-term recordings and drug application directly onto the recorded cell. To obtain some additional information about which retinal structures GABA might be influencing, immunocytochemistry was performed using an antiserum directed against the GABA-synthesizing enzyme L-glutamic acid decarboxylase (GAD). This enzyme is considered a reliable marker for GABAergic neurones (Roberts, 1978; Oertel, Schmechel, Tappaz & Kopin, 1981*a*). In addition, GABA was localized in the retina by [^{3}H]muscimol uptake. Because on- and off-centre ganglion cells have their dendrites in different sublayers of the inner plexiform layer (Nelson, Famiglietti & Kolb, 1978; Peichl & Wässle, 1981), specific action of GABA in the inner plexiform layer would be indicated by uneven labelling with GAD antibodies.

METHODS

Preparation

Experiments were performed on adult cats weighing 2–4·5 kg. Anaesthesia was induced with halothane (2–4%) in a 2:1 mixture of nitrous oxide and carbogen. The nitrous oxide was later supplemented with pentobarbitone (1 mg/kg.h) in the infusion solution and halothane was then omitted. The level of anaesthesia was checked by monitoring the e.e.g. and the diameter of the pupil of the unoperated eye. The animal was paralysed and the parameters of artificial respiration adjusted to produce an end-tidal CO_2 of 4%. The body temperature was maintained at 38 °C.

The cat was placed in a stereotaxic head-holder. The left eye was sewn to a ring at the limbus. A guard needle of 2 mm diameter was inserted into the vitreous through which the recording electrode was advanced on to the retina. Detailed descriptions of the technique are given elsewhere (Peichl & Wässle, 1979; Bolz, Rosner & Wässle, 1982).

Electrode construction, recording, and iontophoresis

Seven-barrelled glass tubing (outer diameter 1·2 mm) was used to make a special tungsten-in-glass micro-electrode (Levick, 1972; Thier & Alder, 1984). The micropipettes were drawn on a standard puller to a tip diameter of 4–5 μm. An electrolytically sharpened tungsten wire was pushed through the central barrel until it protruded 12–15 μm from the tip. The wire at the other end was glued to the glass with DeKhotinsky cement. The surrounding barrels, each with a fused filament, were filled with drug solutions using a Hamilton syringe. Platinum–iridium wires were inserted and the barrels were sealed with DeKhotinsky cement.

Such electrodes record spikes from cells with all the receptive field properties considered below; but regardless of visual properties, recordings could also usually be attributed to a presumed axonal (fibre), somatic, or dendritic origin. Axonal recordings were obtained when the electrode just touched the retina and were characterized by large spikes with a biphasic wave form of short duration; additionally, if the recording position was back-projected onto the tangent screen with the aid of a fundus camera, the electrode was always shown to have a spatial position outside the centre of the receptive field of the unit; such recordings were insensitive to iontophoretic drug application. These recordings were usually avoided by placing electrodes in the raphe region temporal to the central area where the density of fibres is low (Peichl & Wässle, 1981). Somatic recordings were characterized by spikes of slightly longer duration with a biphasic wave form, often with evidence of an initial segment–somatic dendritic (i.s.–s.d.) break. With such recordings, back-projection always revealed a congruence between the receptive field centre and the electrode position. Such somatic recordings had both desired and undesired features. On the one hand, they could often be maintained for long time periods with a high signal-to-noise ratio, and usually showed a reasonable sensitivity to iontophoretic drug application. On the other hand, some somatic recordings were permanently altered by iontophoretic drug application in a way comparable to the effects Kuffler (1953) described when the electrode exerted pressure onto the recorded ganglion cell; others lacked sufficient sensitivity to iontophoretic drugs. When the electrode was progressively inserted deeper into the retina, neural activity was first dominated by on-, and subsequently by off-activity corresponding to the dendritic lamination pattern of the inner plexiform layer of the cat. Isolated dendritic spikes from either lamina were generally small sized and had a more nearly monophasic wave form lacking an i.s.–s.d. break, and back-projection always showed the electrode to be within the receptive field centre (although not always exactly in the centre). Although such recordings were sometimes troubled by signal-to-noise problems, they proved most sensitive to iontophoretic drug application. Since the influence of iontophoresis did not seem to qualitatively differ, the present results comprise a combination of somatic and dendritic recordings.

A number of preliminary experiments rapidly established the general iontophoretic properties of our seven-barrelled electrodes. We sometimes found coupling between channels if adjacent barrels were filled. For this reason, we never filled more than three channels, and there was always an empty barrel separating filled channels. To further ensure that results could not reflect such coupling, we also collected some data with electrodes in which only one or two barrels were filled. This was particularly important in the case of bicuculline application, which often had an influence upon off-centre cells similar to that of GABA. In some experiments, one barrel was filled with saline and used to test possible electrotonic effects. These showed that currents up to 120 nA in either direction did not influence ganglion cell firing, and therefore a current balance through such a saline barrel was used only in some of the earlier experiments. All drugs were iontophoresed by means of a Neurophore BH-2 current source. The resistance of the drug-filled barrels varied between 20 and 60 MΩ depending on the drug and the tip size.

In preliminary experiments we also determined the optimum composition of the solutions of GABA, glycine and related substances to be iontophoresed. In these, GABA, glycine and taurine were used in 0·1–1 M concentration, bicuculline methobromide in 1–5 mM concentration and strychnine in 0·5–5 mM concentration. For all drugs but strychnine, the exact concentration had little qualitative influence. Holding currents exceeding −40 nA were sometimes necessary with higher concentrations; backing currents less than −15 nA usually proved sufficient with lower concentrations. For strychnine, however, concentrations less than 1 mM produced relatively little effect, while concentrations exceeding 5 mM produced an almost uncontrollable, 'all-or-none' effect. Also, we found that although bicuculline and picrotoxin produced similar results to bicuculline methobromide, the latter tended to act more quickly. Finally, we initially made up some of our solutions in distilled water, others in physiological saline and no qualitative difference was observed. One might argue that for solutions in water, the majority of current is carried by hydrogen ions which could produce most of the observed effects. This possibility was ruled out by a number of control experiments. For example, in one case, we filled one barrel of an electrode with bicuculline methobromide made up in saline and filled a second barrel with the same drug made up in water. No difference in the action of the two solutions was observed in nine different cells.

Based on such preliminary experiments, we used the following drug solutions in obtaining all the illustrated data. 2-amino-4-phosphonobutyrate (APB) (Calbiochem) was made up in a 0·1 M solution and adjusted to pH 7 with NaOH. All other substances were made up in physiological saline

(165 mM-NaCl) and adjusted with HCl. These were GABA (Sigma), 0·1 M at pH 3; *n*-methylbicuculline bromide (Cambridge Research Biochemical), 2·5 mM at pH 3; strychnine (Serva), 2·5 mM at pH 3; glycine (Sigma), 0·1 M at pH 3; and taurine (Sigma), 0·5 M at pH 5·4.

Optical stimulation, ganglion cell classification and data analysis

The cornea of the left (operated) eye was protected by an opaque contact lens; the transparent round centre of 4·5 mm diameter served as an artificial pupil. Atropine eye-drops were employed to dilate the pupil and paralyse accommodation. The refraction of the eye was measured with an eye refractometer (Jena optics) and if necessary corrected by spectacle lenses for the screen distance

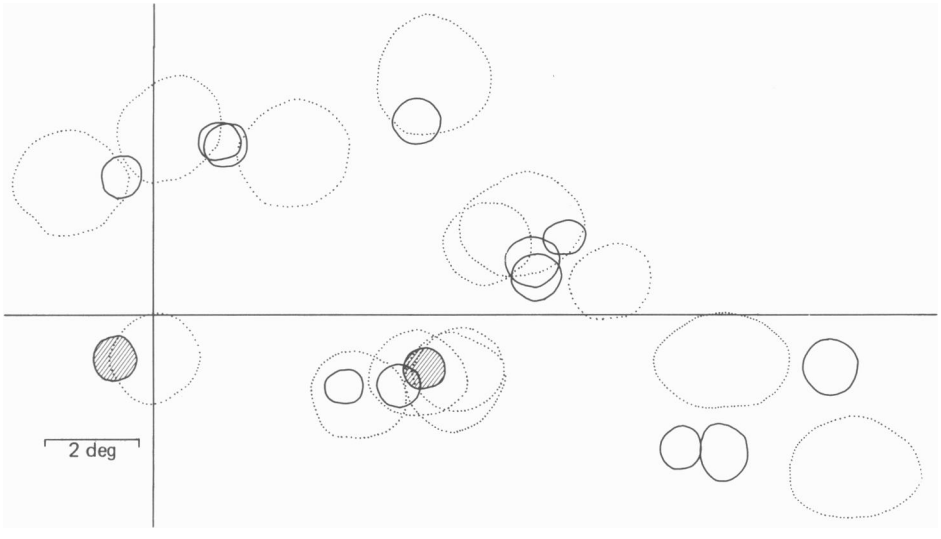

Fig. 1. Receptive field centres of all ganglion cells recorded in a typical cat as plotted on the tangent screen. The estimated position of the area centralis (the intersection of the horizontal and vertical straight lines) was determined by means of back-projection with a fundus camera. Units classified as brisk-transient(Y) are indicated with dotted lines while those classified as brisk-sustained(X) are indicated by continuous lines. Notice that the receptive field centres vary from approximately 1 to 20 deg from the estimated area centralis centre and that brisk-sustained(X) cells always have much smaller receptive field centres than brisk-transient(Y) cells. Responses from the left and right brisk-sustained(X) units indicated by hatching are presented in Fig. 3*C* and *D*, respectively.

of 172 cm. The estimated position of the central area was back-projected onto the screen with a fundus camera (Zeiss). After 2–3 days of experimentation, the cornea sometimes became cloudy, in which case the corneal epithelium was gently brushed away to restore transparency. The optical quality of the eye was continuously assessed ophthalmoscopically to ensure that the internal media remained clear.

Stimuli were provided by a two-channel projection system, each channel stemming from separate tungsten halogen sources. Stimulus size and shape, luminance, and intensity were controlled by means of apertures or other targets, shutters, and neutral density filters. Both channels provided sharply focused images on a tangent screen. In addition, flood lights usually provided a diffuse background over the entire tangent screen.

The receptive field centres of ganglion cells were mapped on a tangent screen with a background illumination of about 5 cd/m^2 using flashing light spots with a luminance of about 30 cd/m^2 and a diameter about 1/5 the expected receptive field centre size. As described by Peichl & Wässle (1979), brisk-transient(Y) cells have a receptive field centre diameter about three times larger than

brisk-sustained(X) cells. As shown in Fig. 1, which is a plot of the centre of all the receptive fields analysed in detail in one cat, such a procedure generally provides a clear-cut distinction between these two cell types. Nevertheless, we used several additional tests described in detail by Cleland & Levick (1974*a*, *b*) to further ensure the validity of our classification. These include a consideration of the response to standing contrast, the periphery effect, and sensitivity to size and speed of disk targets. Cells which could not be unambiguously classified as brisk-transient(Y) or brisk-sustained(X) are excluded from the following report. As exemplified by the post-stimulus time histograms presented in Figs. 5 and 7, responses from these cells are typical of others so classified in the literature. Fig. 1 also shows that the retinal locations for our recording electrodes varied from within 2 deg to greater than 15 deg distance from the presumed centre of the area centralis. We found no qualitative differences in pharmacological action that related to these different retinal locations. All cells reported here were from the central 20 deg of retina.

In the course of the present series of studies, responses were evoked by a wide variety of stimuli varied in luminance between 0·00003 and 400 cd/m^2 of a wide variety of shapes under many different prevailing levels of adaptation. For mesopic stimulation, the spot was 3–35 cd/m^2 against a background of 3–5 cd/m^2, or for photopic stimulation, the spot was 330 cd/m^2 against a background of 90 cd/m^2. The light spot was flashed on and off at 512 ms intervals. Responses to sixteen or thirty-two presentations of the stimulus were averaged by the computer as post-stimulus time histograms (p.s.t.h.s) with a bin width of 16 ms. The integrated spike count over single stimulus cycles (1024 ms) was continuously recorded for analysing the time course of drug action.

All of the present results were obtained from nine cats in which seventy cells were analysed in detail (thirty on-centre cells, forty off-centre cells; twenty-seven brisk-sustained cells, forty-three brisk-transient cells).

Immunocytochemistry

A well-described and characterized antiserum to GAD was generously supplied to us by Dr W. H. Oertel (Oertel *et al.* 1981*a*, *b*) for immunocytochemistry. Briefly, adult cats were deeply anaesthetized with barbiturates and their eyes removed. In two cats, colchicine (10 and 100 μg in 50 μl saline) was injected intravitreally 24 h before enucleation. The anterior segment was cut away and the eyes were immersion fixed for 1–2 h at room temperature with 4% paraformaldehyde, 0·1 M-DL-lysine HCl and 0·01 M-sodium periodate in 0·1 M-phosphate buffer (PB) pH 7·4 (McLean & Nakane, 1974). After cryoprotection with PB containing 30% sucrose, 10–15 μm sections of the retina were cut with a cryostat and picked up on gelatine-coated slides, dried at room temperature and stored at −20 °C until staining by either an immunofluorescence or a peroxidase–antiperoxidase procedure. The sections were washed in PB and then incubated for 12–24 h at 4 °C in GAD antiserum diluted to 1:500 or 1:1000 with 0·3% Triton X-100 in PB. For the immunofluorescence, the sections were then washed in PB, incubated in rabbit antisheep IgG conjugated to fluorescein isothiocyanate at 1:100, washed in PB and coverslipped in carbonate-buffered glycerine. For the peroxidase–antiperoxidase procedure, the sections were washed in PB, incubated in rabbit antisheep IgG at 1:20, then washed in PB, incubated in sheep peroxidase–antiperoxidase at 1:50 and washed again in PB. The retinal sections were then incubated with 3'3'-diaminobenzidine HCl (35–70 mg/100 ml) for 5 min after which 0·01% H_2O_2 was added and the sections were incubated for an additional 10 min. The sections were then washed, dehydrated and coverslipped. Specificity was assessed by substituting normal sheep serum in place of the primary antiserum.

For autoradiography, the retinae were dissected immediately after enucleation in a Krebs bicarbonate medium equilibrated with a 95% O_2 and 5% CO_2 mixture (Iversen, Mitchell & Srinivasan, 1971). Pieces of the retinae were incubated at 37 °C for 15 or 45 min in the Krebs medium containing 10^{-7} M- or 10^{-6} M-[^{3}H]GABA (specific activity 74·5 Ci/mmol) or [^{3}H]muscimol (specific activity 29·4 Ci/mmol). The samples were then fixed in 1% paraformaldehyde and 1·25% glutaraldehyde in PB, osmicated, dehydrated and embedded in Epon. 1 μm sections were cut, dipped in Kodak NTB-2 nuclear track emulsion and exposed at 4 °C for 1–6 weeks. They were then developed in Kodak D-19, stained with Toluidine Blue, differentiated in alcohol and coverslipped.

RESULTS

Maintained discharge

Ganglion cells of the cat retina exhibit a spontaneous discharge when the eye is facing an uniformly illuminated field. The discharge rate differs from unit to unit and is only weakly correlated with the level of background illumination (Barlow & Levick, 1969). The maintained discharge rates found in our experiments at background levels

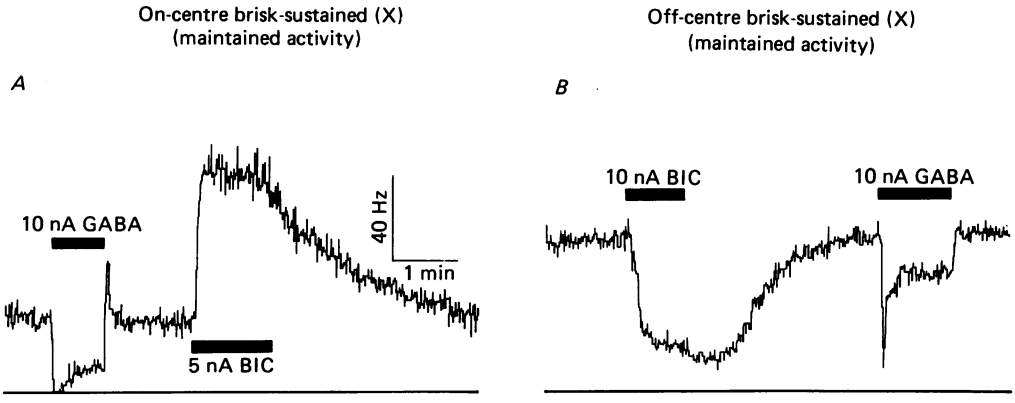

Fig. 2. The effects of GABA and bicuculline (BIC) on the spontaneous activity of an on-centre brisk-sustained(X) cell (in *A*) and an off-centre brisk-sustained(X) cell (in *B*). The continuous record indicates the number of spikes counted in a 1024 ms interval (continuous trace): the bottom horizontal line indicates zero counts. The filled bars show the time intervals of drug application. The strength of iontophoretic current (in nA) and the applied drug are indicated. The background illumination was 5 cd/m^2.

of 5 cd/m^2 were as high as 60 Hz. Fig. 2 presents continuous integrated spike records of the maintained activity in an on-centre brisk-sustained(X) and an off-centre brisk-sustained(X) cell (Fig. 2*A* and *B*, respectively). The cells were from subsequent recordings in the same retinal area with the same electrode and had overlapping receptive field centres. Exogenous GABA application inhibited both cells and this inhibition typically had a biphasic time course which consisted of an initial pronounced transient and a subsequent sustained component. GABA application suppressed the discharge rate of all ganglion cells encountered independent of type and stimulus used.

Bicuculline influenced the activity of all ganglion cells in a less stereotyped manner. As illustrated in Fig. 2*A*, the spontaneous activity in all on-centre cells was increased by bicuculline, but as exemplified by the record in Fig. 2*B*, bicuculline decreased the spontaneous activity of most off-centre cells. The action of bicuculline generally reversed within about a minute of terminating its application.

Bicuculline proved to be an effective antagonist of GABA in the cat retina. In the experiment outlined in Fig. 3, we pulsed GABA in 25 s cycles in which GABA was ejected with positive current for 10 s, then held with a negative current for 15 s. In Fig. 3*A*, cyclic ejection of GABA was continued before, during, and after the

presentation of bicuculline. Ejection of GABA suppressed the maintained discharge of the on-centre, brisk-transient(Y) cell; application of bicuculline increased the discharge rate of the cell and immediately blocked the modulation of the discharge by GABA. One might argue that blocking occurs only because the discharge rate of the cell is increased by bicuculline and is hence unspecific. Fig. 3*B* shows this is not

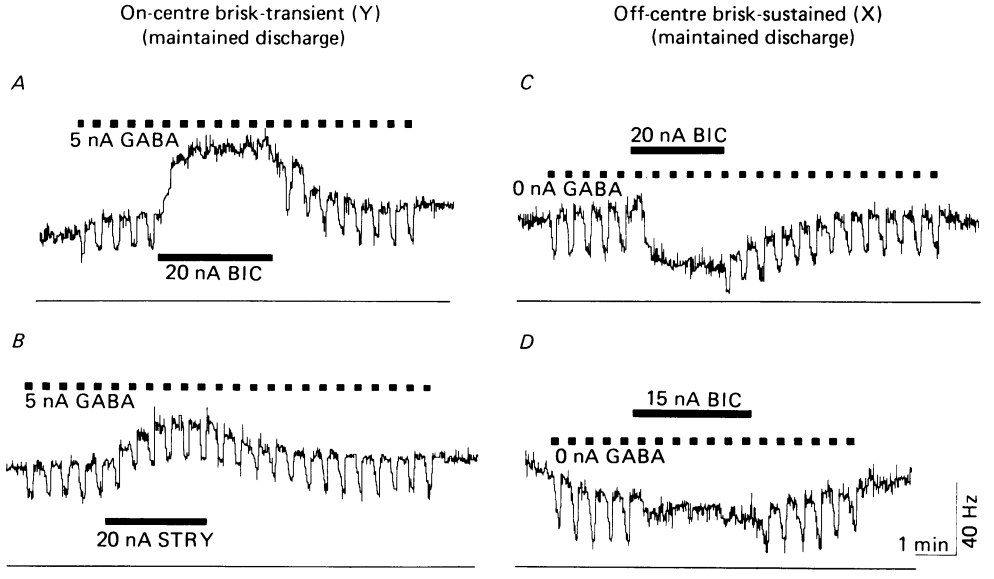

Fig. 3. The influence of GABA, bicuculline (BIC), and strychnine (STRY) upon the maintained discharge of three ganglion cells adapted to a background of 3 cd/m². The left tracings (*A* and *B*) are from the same on-centre brisk-transient(Y) cell and the right tracings (*C* and *D*) are from two off-centre brisk-sustained(X) cells. The number of spikes counted in 1024 ms intervals is recorded by the continuous trace: the bottom horizontal line indicates zero counts. The holding current for all drugs was approximately −10 nA and GABA was presented in 25 s cycles. At the time indicated by the short bars, GABA was ejected for 10 s with +5 nA (left tracings), or by withdrawing the holding current (i.e. 0 nA ejection current, right tracings). GABA ejection produces a marked inhibition of activity in the initial control records (left of each tracing). The time period of presentation and ejection currents of both bicuculline (in *A*, *C* and *D*) and strychnine (in *B*) are indicated by the labelled long bars. Although both antagonists increase spike activity of the on-centre brisk-transient(Y) cell in *A* and *B*, only bicuculline blocks the GABA-elicited inhibition. The receptive field centres of the two cells in *C* and *D* are represented, respectively, by the left and right plots indicated by the hatching in Fig. 1. Notice that one cell was from the central area and one at 6 deg eccentricity.

the case. Application of strychnine also increased the firing rate of the on-centre cell but did not block GABA action: the modulation of the response by GABA was increased and not reduced. This result makes three points: first, increasing the discharge rate does not block GABA; secondly, strychnine does not block GABA action; and thirdly, bicuculline can block exogenous GABA.

Fig. 3*C* presents the results of similar experiments in an off-centre brisk-sustained(X) cell. Notice that bicuculline decreased over-all activity and blocked the influence of

GABA. One might be tempted to attribute the bicuculline-related loss of GABA suppression of Fig. 3*C* to an over-all reduction in cell firing. This explanation is unlikely since GABA became effective immediately after the removal of bicuculline although the discharge of the cell remained low for about 1 min. Furthermore, in the off-centre, brisk-sustained(X) cell illustrated in Fig. 3*D* (the over-all activity of which

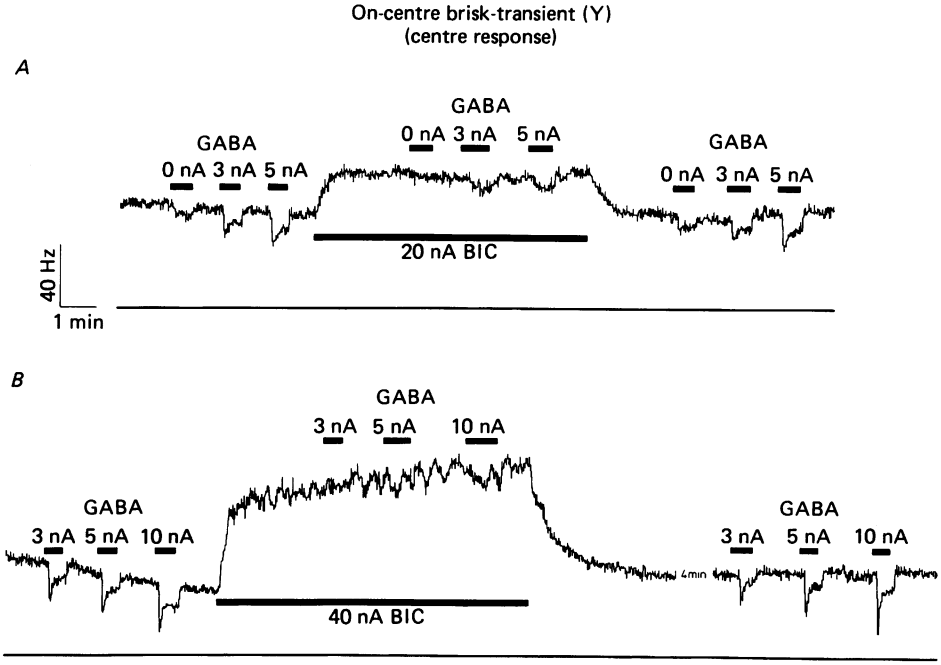

Fig. 4. Incomplete blockage of GABAergic inhibition by bicuculline (BIC). These continuous records of integrated spike activity were obtained from separate on-centre brisk-transient(Y) cells the receptive field centres of which were stimulated by an optimum sized spot with a diameter of about 2 deg. At the indicated time periods, the GABA holding current was changed to the ejection current specified over the short horizontal bars. This was done under initial control conditions, during bicuculline ejection (time period indicated by long horizontal bars), and after bicuculline ejection was terminated. In *A*, the GABA and bicuculline holding currents were −8 and −5 nA respectively and the eye was stimulated under mesopic conditions (5 cd/m^2 light spot against a background of 3 cd/m^2). In *B*, the GABA and bicuculline holding currents were both −7 nA and the eye was stimulated under photopic conditions (320 cd/m^2 spot against a background of 75 cd/m^2).

was not changed much by bicuculline) bicuculline still blocked the influence of exogenous GABA. Results from many such experiments show that bicuculline always suppresses the inhibition resulting from exogenous GABA application, regardless of whether neural activity is light-evoked or spontaneous.

As is the case in the visual cortex of the cat (e.g. Sillito, 1975, Fig. 1*d*), iontophoretically applied bicuculline could not always *completely* block exogenous GABA, particularly if GABA were applied for durations exceeding 15 s. An example of incomplete blockage is shown in Fig. 4*A*, a continuous integrated spike record from

an on-centre brisk-transient(Y) cell. At the indicated time periods, the GABA holding current of −12 nA was removed and replaced by ejection currents of either 0, +3 or +5 nA. Notice that before bicuculline application, the inhibitory influence of GABA upon spike activity increased with the positive value of the ejection current and the influence of GABA consisted of an initial transient and a subsequent sustained component. Bicuculline application increased the over-all activity of the cell, completely suppressed the influence of 0 nA of GABA, and greatly attenuated, but did not completely abolish, the influence of +3 and +5 nA of GABA. Similar results from another on-centre brisk-transient(Y) cell are shown in Fig. 4*B* in which we used higher GABA ejection currents. It is possible that in the absence of bicuculline, the change from a transient to a sustained phase of GABAergic inhibition represents desensitization as has been suggested previously for many other neurones including those in mudpuppy retina (Miller, Frumkes, Slaughter & Dacheux, 1981*a*, *b*). However, in the presence of bicuculline, notice that the remaining GABA influence was sustained. This might reflect the competitive nature of the block of the GABA receptor by bicuculline. The 'sustained' GABA influence may represent some 'breakthrough' in the blockade.

Although bicuculline did not always completely block GABA action, we found it extremely specific for GABA. Whether bicuculline caused an increase or decrease in spike activity, it never influenced the inhibitory action of glycine or taurine (Bolz, Thier, Voigt & Wässle, 1985).

Centre response

On-centre cells are excited when a light spot is projected into their receptive field centre and are shortly suppressed when the spot is switched off. In Fig. 5*A*, post-stimulus time histograms (p.s.t.h.s) from a typical on-centre brisk-sustained(X) cell are shown. A short transient peak at light on was followed by a sustained raised firing rate. At light off the cell returned, after a short post-excitatory inhibition, to its maintained firing level. When GABA was applied to the cell with a 10 nA ejecting current, all three phases of the p.s.t.h. were equally suppressed: the peak at light on, the sustained response and the discharge in the light-off phase. Application of bicuculline with a 5 nA current raised the total response of the cell from 44 to 108 Hz. Again all three phases of the p.s.t.h. were raised. It is interesting that the suppression at light off was still very strong.

In Fig. 5*B*, p.s.t.h.s from a typical on-centre brisk-transient(Y) cell are shown. A very sharp transient peak was followed by a short sustained component which disappeared during the light-on cycle. In the light-off phase only a few spikes were found. Application of GABA with a 20 nA current resulted in a reduction of the total discharge and both the peak and the sustained phase were reduced. During bicuculline ejection, the total discharge was raised and all three phases of the p.s.t.h. show higher discharge rates. The p.s.t.h. under bicuculline looks more sustained and comparable to the brisk-sustained(X) cell (Fig. 5*A*). The suppression at light off was not cancelled by bicuculline.

Fig. 5*C* demonstrates that bicuculline antagonized the action of iontophoretically applied GABA during centre stimulation. The trace of the continuous recording on the left shows that the action of periodically applied GABA (5 nA current) was

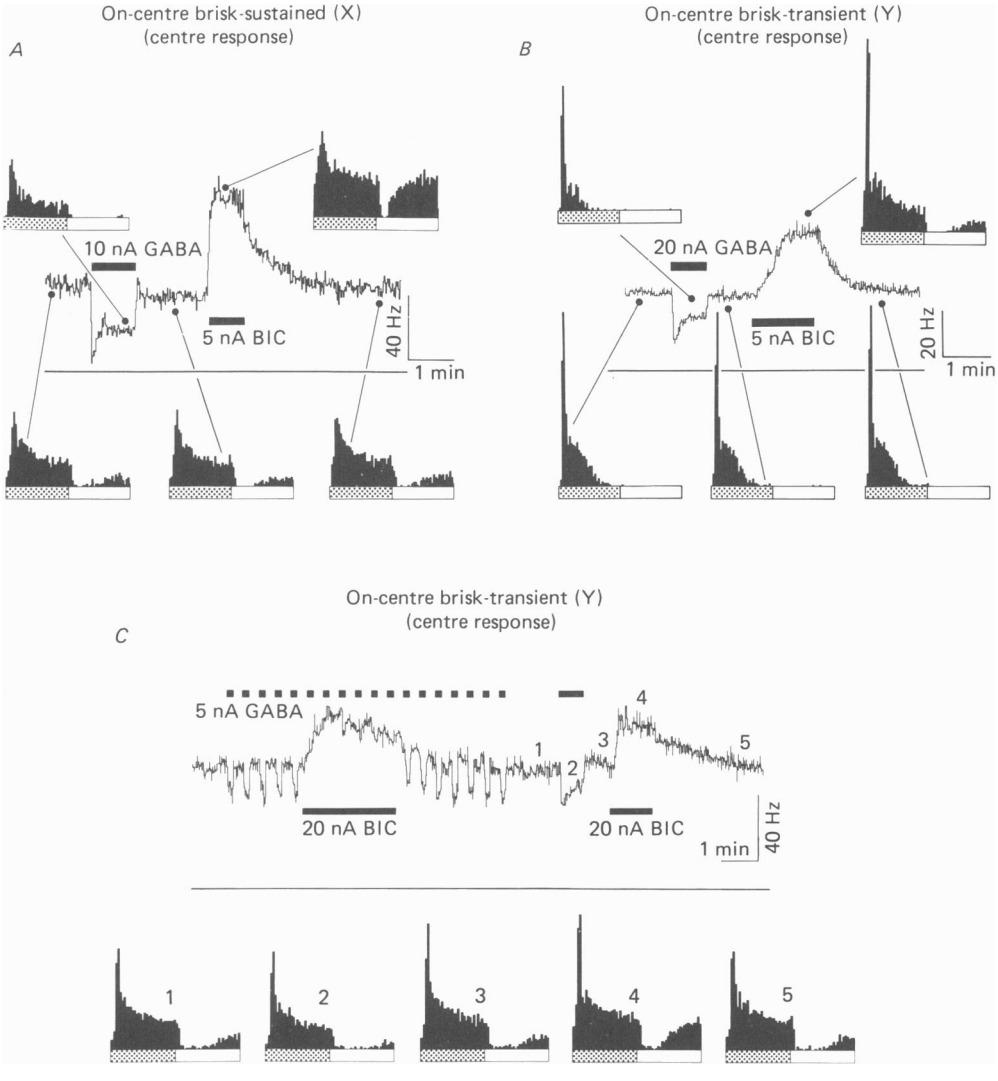

Fig. 5. Responses from an on-centre brisk-sustained(X) cell in *A* and an on-centre brisk-transient(Y) cell in *B* to the illumination of their receptive field centre. The diagram for each cell consists of a continuous record of the total discharge during light stimulation counted in 1024 ms intervals, and of selected p.s.t.h.s. The horizontal line is the zero count base line. The pointers show during which part of the continuous record the corresponding p.s.t.h.s were measured. The light trace is indicated by the boxes below each p.s.t.h. (hatched box, 512 ms light on; open box, 512 ms light off). The vertical height of the box serves as the scale for the p.s.t.h.s and represents 40 spikes/s. The bin width was 15 ms. In this and the following illustrations, p.s.t.h.s below the continuous record represent pre- and post-drug responses; p.s.t.h.s above the continuous record show responses influenced by the drugs. The brisk-sustained(X) cell in *A* is the same cell as in Fig. 2*A*, receptive field centre (r.f.c.) diameter 1·2 deg, spot size 1 deg, eccentricity 16 deg. The brisk-transient(Y) cell was from 10 deg eccentricity, r.f.c. diameter 2·4 deg, spot size 2 deg. The background luminance was 5 cd/m^2, the spot luminance was 3 cd/m^2 and is superimposed on this 5 cd/m^2 background (5+3 cd/m^2). *C*, evidence that bicuculline can block the GABA influence during centre stimulation. The continuous record shows the response of

blocked by bicuculline applied with a 20 nA current. On the right, the actions of GABA (5 nA) and bicuculline (20 nA) are shown separately. Histograms taken during those measurements again show a decrease or increase of all three phases.

For a sample of twenty-three on-centre cells, Fig. 6*A* is a scatter diagram illustrating the influence of bicuculline on the response in the light-on and light-off phases. The abscissa gives the increase of the total firing rate during light on, the ordinate respresents the change in total firing during light off. If both phases had been influenced identically by bicuculline, all points should have coincided with the 45 deg line. The data show a large amount of the variability in the cell responses and only half of the points are found in the vicinity of that line. Note that more points are below this line suggesting that for many cells, the response during light on is raised more by bicuculline than the response during the light-off phase.

The influence of GABA and bicuculline on the centre response of off-centre cells is more interesting. Off-centre cells are suppressed when a light spot is projected into their receptive field centre and are excited when the spot is switched off. Fig. 7*A* shows p.s.t.h.s and average discharge rates from a typical off-centre brisk-sustained(X) cell. The cell was stimulated with a relatively dim light spot providing only moderate contrast, and the bottom histograms show only a weak modulation with the light cycle. When GABA was ejected, all three phases of the histogram (the transient peak at light off, the sustained firing at light off, and the lower firing rate during light on) were reduced. During bicuculline application, the total discharge of the cell was greatly reduced and this is comparable to the reduction found in the maintained discharge of the same cell in Fig. 2*B*. Analysis of the histogram indicates a differential action of bicuculline on the components: the transient peak has not changed much, but the sustained discharge at light off and the discharge at light on are reduced.

A comparable action of GABA and bicuculline can also be observed in an off-centre brisk transient(Y) cell (Fig. 7*B*). The transient peak increased during bicuculline application, whereas the sustained component and the total discharge decreased. For the same unit the influence of the drugs was also measured during photopic stimulation (Fig. 8). Fig. 8*A* shows a continuous record during photopic centre stimulation. The periodic suppression caused by the pulsed application of GABA can be blocked by ejection of bicuculline. Histograms during photopic stimulation (background 90 cd/m^2, stimulus 420 cd/m^2) are shown in Fig. 8*B*. The pre-drug histogram is more phasic than the comparable histograms during mesopic stimulation (Fig. 7*B*, bottom row), but application of GABA again suppressed the cell. Fig. 8*C* shows histograms from the same cell at higher contrast (background 25 cd/m^2, stimulus 320 cd/m^2). Bicuculline reduced the sustained component of the histogram and raised the transient peak; the total discharge was reduced from 41 to 34 Hz.

The records of the off-centre brisk-transient(Y) cell in Figs. 7*B* and 8 show that GABA suppressed the cell under both mesopic and photopic centre stimulation.

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an on-centre brisk-transient(Y) cell during centre stimulation. The periodic suppression by GABA (pause 15 s, −10 nA holding current; eject 10 s, +5 nA current) is effectively blocked by 20 nA bicuculline. The right part of the continuous record shows the application of 5 nA GABA and 20 nA bicuculline independently. As indicated by the numbers 1–5, p.s.t.h.s were measured and are shown below the continuous record (spot size 1·9 deg, r.f.c. diameter 2 deg, spot luminance 5+3 cd/m^2, eccentricity 5·5 deg.).

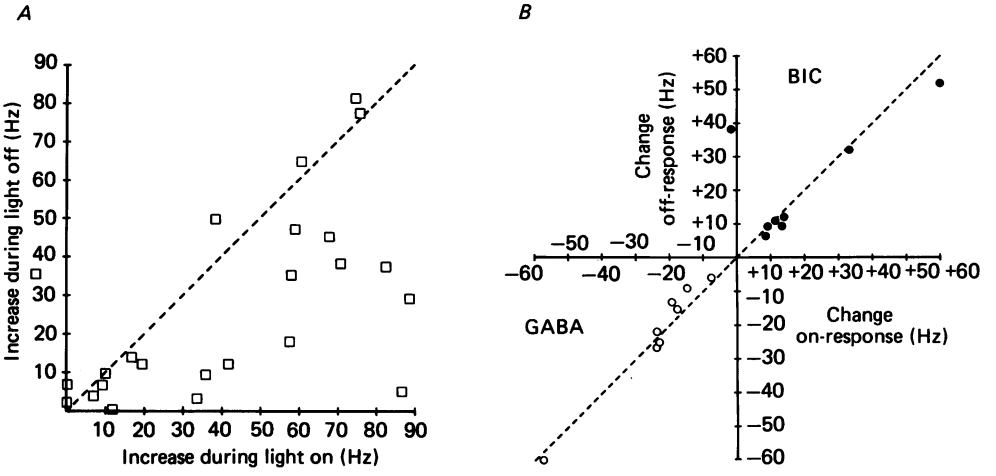

Fig. 6. *A*, scatter diagram indicating the increase of on-centre ganglion cell firing produced by bicuculline application. For centre stimulation (512 ms on; 512 ms off) the increase of firing caused by bicuculline during the light-on phase (abscissa) is plotted against the increase during the light-off phase (ordinate). *B*, scatter diagram from centre/surround balanced stimulation to analyse the effect of GABA and bicuculline on on-centre ganglion cells. Stimuli (either ganzfeld or annuli) were chosen, which elicit balanced centre and surround components, i.e. about the same number of spikes in the on- and in the off-phase of the p.s.t.h. The abscissa of the scatter diagram shows how the on-phase of the response was lowered or raised by GABA or bicuculline. The ordinate shows the corresponding change during the off-phase. In most histograms the on-(centre) and off-(surround) phase are equally raised or suppressed and the corresponding points of the scatter diagram coincide with the 45 deg line.

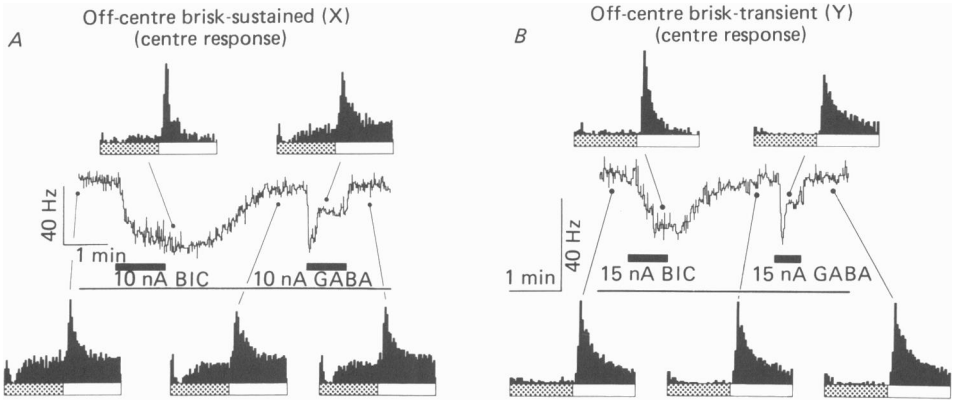

Fig. 7. *A*, responses from an off-centre brisk-sustained(X) cell to centre stimulation during GABA and bicuculline (BIC) application. Conventions as in Fig. 5. The cell was stimulated with a light spot of 1 deg diameter and 5+5 cd/m² luminance. The receptive field centre was 1·2 deg, the eccentricity of the cell was 16 deg. *B*, responses from an off-centre brisk-transient(Y) cell to centre stimulation during GABA and bicuculline application. Conventions as in Fig. 5. The cell was stimulated with a light spot of 1·9 deg diameter and 5+3 cd/m² luminance. The receptive field centre was 2 deg, the eccentricity of the cell was 4·5 deg.

The differential action of bicuculline is analysed in Fig. 9. GABA suppressed both the total discharge and the different components of the histograms at all stimulus intensities used. Bicuculline application shows a differential action depending on the light contrast: at low contrast (Fig. 9*A*) the total discharge was reduced, at medium

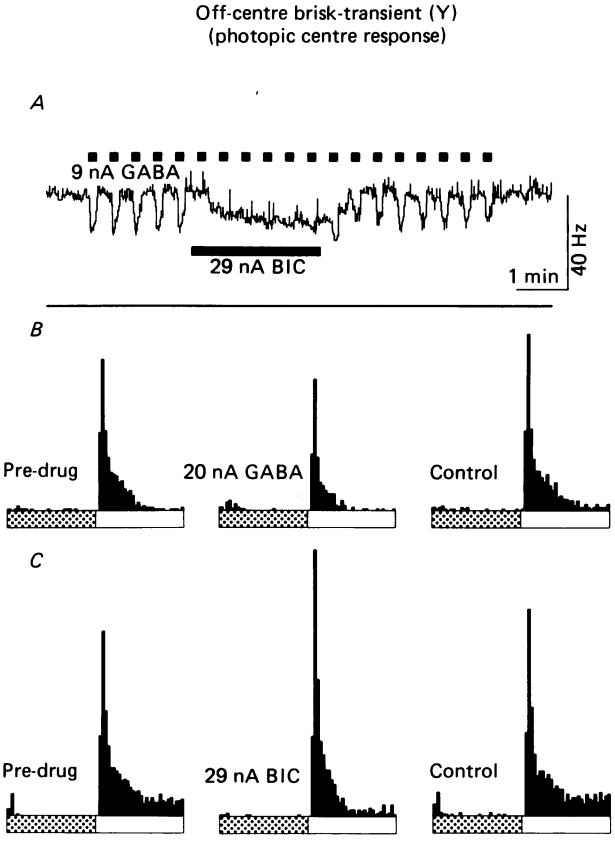

Fig. 8. Responses of the same off-centre brisk-transient(Y) cell as in Fig. 7*B* to photopic centre stimulation. *A*, continuous record during periodic GABA application (pause 15 s, −10 nA holding current; eject 10 s, +9 nA current). Application of 29 nA bicuculline blocked the periodic GABA suppression (light spot 90+420 cd/m^2). *B*, histograms of the cell during photopic stimulation (light intensity as in *A*). Application of 20 nA GABA suppressed the light response of this off-centre cell. *C*, histograms of the cell during photopic stimulation (spot intensity 25+320 cd/m^2). Application of bicuculline suppressed the sustained response, but enhanced the transient peak. Other conventions as in Fig. 5.

contrast (Fig. 9*B*) no change was observed and at high contrast (Fig. 9*C*) the total discharge was increased during iontophoresis of the drug. The corresponding p.s.t.h.s (Fig. 9, right column) show the basis for this change in total discharge. At low intensities (upper row) the histogram is only weakly modulated around the maintained discharge. During bicuculline this maintained firing level was reduced. The small transient peak stayed much the same. At the medium light intensity (middle row)

the p.s.t.h. shows a clear transient peak followed by a small sustained discharge. During bicuculline application, the peak showed a remarkable increase but the sustained component was suppressed. The two opposite effects balanced each other and the total discharge did not change. With similar moderate contrast stimuli, this finding was obtained in response to centre stimuli in all off-centre cells (thirteen

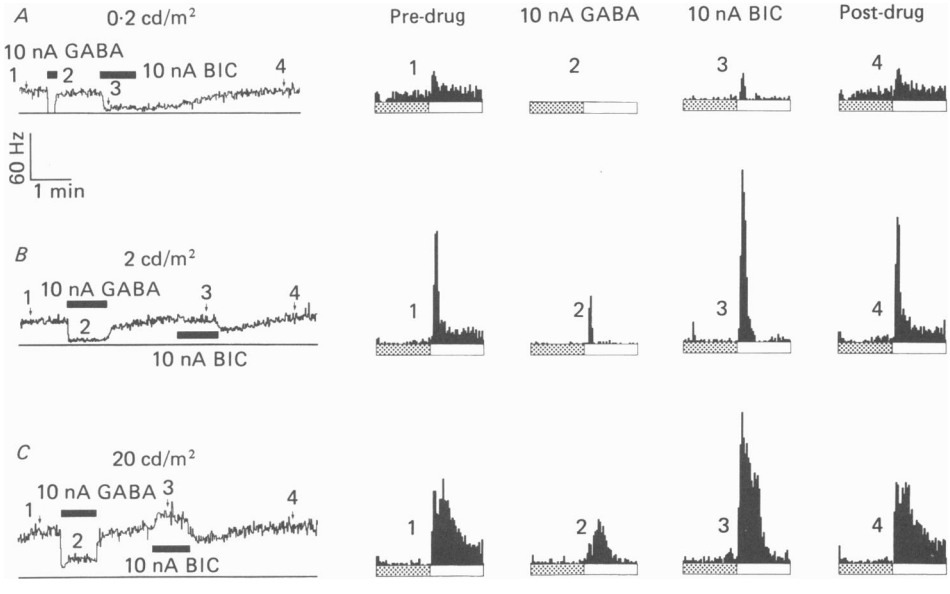

Fig. 9. Responses from an off-centre brisk-transient(Y) cell to centre stimulation during GABA and bicuculline application. *A*, *B* and *C* show the responses of the cell at different stimulus intensities. At the left, the effect of GABA and bicuculline on the total discharge are compared. The histograms at the right were taken before, during and after GABA or bicuculline application. The numbers refer to the time during the continuous record, when the histograms were measured. Other conventions as in Fig. 5. Eccentricity 18 deg, r.f.c. diameter 2 deg, spot size 1·8 deg, background 2 cd/m². The spot luminance in *A* was 2+0·2; in *B*, 2+2; in *C*, 2+20 cd/m².

brisk-sustained(X) and twenty-two brisk-transient(Y)) that we tested. When the stimulus intensity was increased the response became dominated by the transient peak and during bicuculline application, both the transient peak and the total response increased. In eleven other brisk-transient(Y) cells and in eight brisk-sustained(X) cells, we similarly attempted to reverse the bicuculline effects by increasing stimulus intensity. With the exception of two brisk-transient(Y) cells, this was always possible in the off-centre cells tested here. Notice that bicuculline reduced over-all activity in off-centre cells when the receptive field centre was stimulated under photopic (Fig. 8*C*) as well as mesopic (Fig. 7*B*) conditions, if the stimulus contrast was comparable.

Surround stimulation

Retinal ganglion cells have antagonistic receptive field surrounds which respond when stimulated at light off for on-centre cells and at light on for off-centre cells. It

was found that GABA and bicuculline influenced the surround responses in the same way as the centre responses. On-centre cell surround responses were suppressed by GABA and enhanced by the application of bicuculline. For off-centre cells, the situation is again more complicated and Fig. 10 shows this for the same brisk-transient(Y) cell described in Fig. 9. The histograms of the bottom trace show that

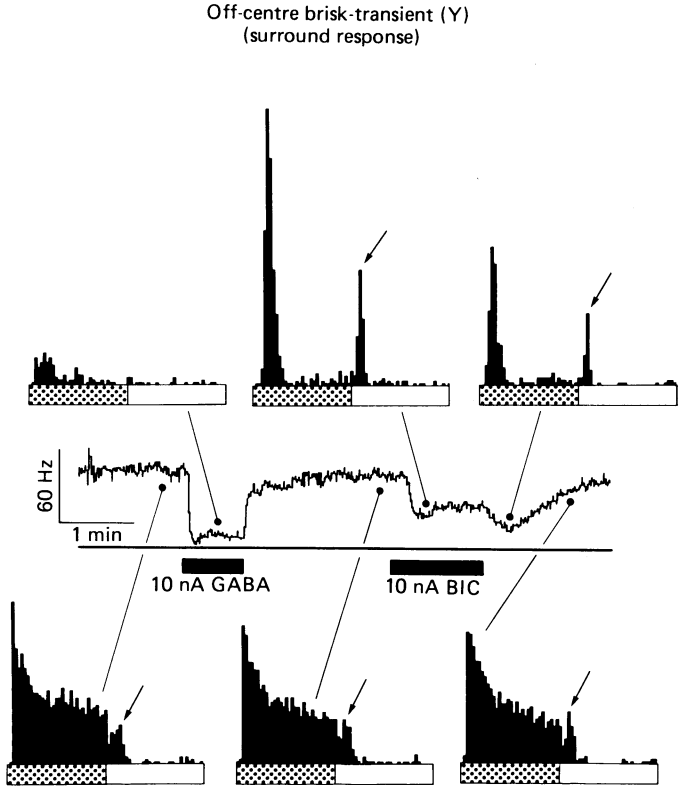

Fig. 10. Responses from an off-centre brisk-transient(Y) cell to surround stimulation during GABA and bicuculline application. Same cell as in Fig. 9, conventions as in Fig. 5. The small arrows point to the weak centre component in the histograms. The receptive field centre was covered by a steady adapting spot of 8 cd/m^2. The background was 2 cd/m^2. The cell was stimulated with an annulus (inner diameter 2·4 deg, outer diameter 17 deg) of 2+20 cd/m^2.

one can elicit a strong phasic/tonic surround response during the light-on phase from the unit. The arrows in the histograms point to a very small centre component in the response, which might be caused by stray light falling onto the centre. During GABA application the cell was strongly suppressed, both in the total response and in the histogram. Ejection of bicuculline reduced the total response of the cell. However, in the histogram once again a differential effect is observed: the sustained components are completely suppressed, the transient components are greatly enhanced. Both the peak at light on (surround response) and the peak at light off (centre response) show a sharp increase. Therefore the surround response is influenced qualitatively by GABA and bicuculline in the same sense as the centre response.

Centre and surround stimulation

Kirby & Enroth-Cugell (1976) reported that GABA antagonists change the balance of Y-cell receptive fields causing the centre response to become more dominant. In the course of the present study, we occasionally found data which apparently supported this claim, as exemplified by the off-centre brisk-transient(Y) cell shown in Fig. 10. Note that bicuculline changed the tonic on-surround dominated response (lower row of histograms) into a phasic on–off response (the two histograms in the upper right).

In order to find out whether the relative influences of GABA and bicuculline on the centre and surround response of ganglion cells are quantitatively different, light stimuli were chosen which elicited balanced centre and surround components in the p.s.t.h.s. The stimuli were either whole-field illumination or annuli of light, covering parts of the centre and the surround. Fig. 11 shows the responses of an on-centre brisk-sustained cell to stimulation with an annulus. The histograms in the bottom row exhibit a transient peak at light on, followed by an increasing sustained component. The response at light off consisted of a more sustained discharge attributable to surround stimulation. When bicuculline was ejected, both the response at light on and the response at light off increased. The two inhibitory troughs at light on and off were still present and were apparently unblocked by bicuculline. Application of GABA reduced all components of the histogram with the exception of the transient peak at light on.

From Fig. 11, no difference is apparent between the actions of GABA or bicuculline with respect to centre and surround stimulation. This is shown more quantitatively in the scatter diagram of Fig. 6*B* which compares the total discharges of eight on-centre brisk-sustained(X) cells during centre/surround stimulation. The upper right quadrant indicates the increase of discharges during bicuculline ejection, the lower left quadrant shows for the same cells the decrease during GABA application. With one exception all points are close to the 45 deg line and therefore the centre component and the surround component were influenced by the two drugs equally.

Are there changes in the receptive field centre dimensions during GABA and bicuculline application?

Kirby & Schweitzer-Tong (1981) reported that intravenous injection of GABA antagonists changes the receptive field centre size in Y cells; in an earlier study, it was also reported that X-cell responses remain virtually unaffected by GABA antagonists (Kirby & Enroth-Cugell, 1976). In the present study, we measured area–response curves of the receptive field centre mechanism for two brisk-sustained(X) cells. For each spot size a complete cycle as illustrated in Fig. 5*A* was necessary and the measurement of one area–response curve took more than 2 h. This explains the small sample. Fig. 12*B* shows the area–response curves of the on-centre brisk-sustained(X) cell. During GABA ejection the cell response was reduced, while during bicuculline ejection it was increased without any change of the form of the area–response curve. The peak in all three curves is found at about 1·2 deg which corresponds to the centre summating area. Thus, there is no change in the receptive field centre size of this unit by GABA and bicuculline.

The area–response curves of an off-centre brisk-sustained(X) cell are shown in Fig. 12*A*. During GABA ejection, the curve was merely shifted to a lower value of response without changing its general shape or the peak. During bicuculline ejection the reversal from increased to decreased firing can be observed. For small spot sizes the cell was not driven very strongly and bicuculline suppressed the total discharge. For spot sizes congruent to the receptive field centre, a strong response was

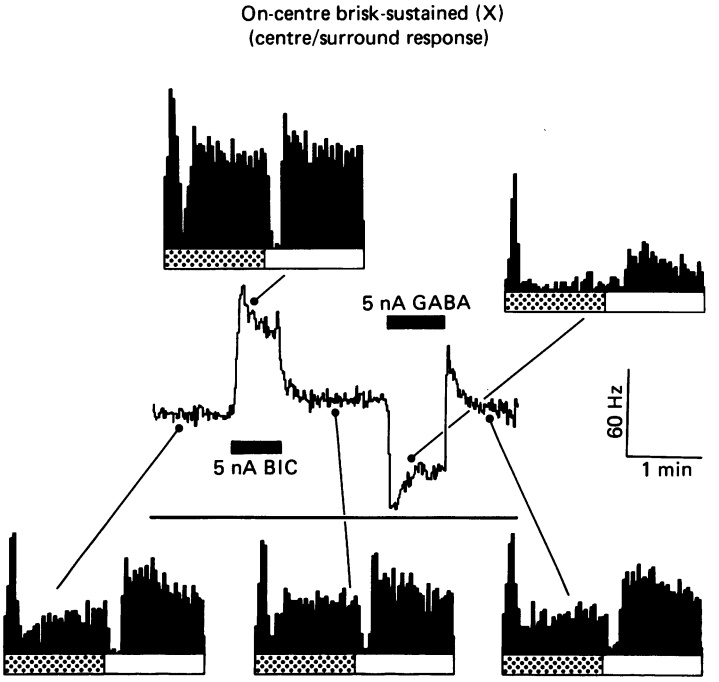

Fig. 11. Responses from an on-centre brisk-sustained(X) cell to centre/surround balanced stimulation during GABA and bicuculline application. Conventions as in Fig. 5. The centre was covered by a steady adapting spot of 6·5 cd/m². The background was 4 cd/m². The light stimulus was a ganzfeld of 4 + 2 cd/m². R.f.c. diameter 1·3 deg, eccentricity 17 deg.

elicited, which was augmented during bicuculline application. For large spot sizes, where the cell response had decreased because of surround inhibition, bicuculline exhibited an over-all suppressive effect. Although the area–response curve with bicuculline is steeper, the peak location at about 0·7 deg has not changed. Hence, the receptive field centre diameter of this off-centre brisk-sustained(X) cell has not changed.

Histochemical localization of GABA in the cat retina

The iontophoresis experiments described so far allow no conclusion as to the sites of action of GABA in the cat retina.

In previous attempts to visualize GABAergic neurones in the mammalian retina, the uptake of [^{3}H]GABA was studied by autoradiography (Ehinger & Falck, 1971; Bruun & Ehinger, 1974; Marshall & Voaden, 1975; Brandon, Lam & Wu, 1979; Nakamura, McGuire & Sterling, 1980). We found that [^{3}H]GABA was accumulated in

certain neurones, but in addition it also was taken up by Müller (glia) cells. Plate 1 *A* shows the heavy labelling of Müller cell bodies in the inner nuclear layer and also of Müller cell pedicles at the optic nerve fibre layer. But some cells of the amacrine layer and the inner plexiform layer have also been heavily labelled. Thus, the uptake of GABA is not restricted to cells thought to utilize GABA as a neurotransmitter.

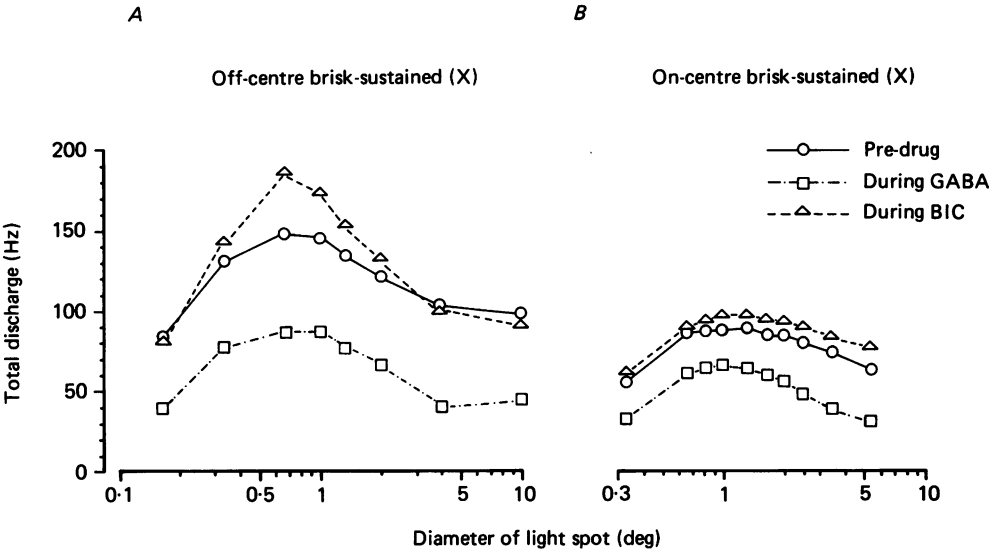

Fig. 12. Area–response curves of an off-centre (*A*) and an on-centre (*B*) brisk-sustained(X) cell. Light spots of increasing diameter were projected into the r.f.c. and the total discharge during light on (in *B*) or during light off (in *A*) was measured. The abscissa give the diameter of the light spots, on a logarithmic scale, while the ordinate gives the discharges of the cell. *A*, background 4 cd/m², spot luminance 4+20 cd/m², eccentricity 6 deg, r.f.c. ∅ 0·7 deg. *B*, background 4 cd/m², spot luminance 4+4 cm/m², r.f.c. ∅ 1·2 deg, eccentricity 12 deg.

The presence of the GABA-synthesizing enzyme GAD is considered to be a reliable marker for GABAergic neurones (see Roberts, 1978). Therefore, an antiserum directed to GAD (Oertel *et al.* 1981*a*, *b*) was used for immunocytochemistry to visualize GAD-positive structures in the cat retina. The results obtained with the GAD antibody are compared with the uptake of the GABA analogue [³H]muscimol.

GAD immunoreactivity was present in somata of the inner nuclear layer (i.n.l.) and the ganglion cell layer (g.c.l.), Pl. 1*C* and *D* shows that labelled cells were located at the inner margin of the i.n.l., i.e. in the position of the amacrine cells. The labelled somata in the g.c.l. most probably represent displaced amacrine cells, because no labelled ganglion cell axons in the optic nerve fibre layer could be detected. Up to 30 % of labelled somata were found to be displaced amacrine cells. Furthermore, the inner plexiform layer (i.p.l.) was completely stained without evidence for any lamination. No reliable GAD immunoreactivity could be observed in the outer plexiform layer (o.p.l.). However, sometimes, processes originating from labelled cell bodies of the i.n.l. and extending towards the o.p.l. could be detected. Colchicine

administration did not alter the staining, and in control sections incubated with normal serum no staining was present.

In contrast to the [^{3}H]GABA uptake (Pl. 1*A*), [^{3}H]muscimol is taken up only by neurones in the cat retina (Pourcho, 1981; Pourcho & Goebel, 1983; Nakamura *et al.* 1980). Plate 1*B* shows that the accumulation of silver grains after [^{3}H]muscimol uptake is found over cells of the i.n.l. and g.c.l. in the same position as those cells labelled by the GAD antibody. As with GAD staining, the i.p.l. is labelled to its full extent and only occasionally silver grains are observed in the o.p.l. Similar results with [^{3}H]muscimol were described previously (Pourcho, 1981; Pourcho & Goebel, 1983).

GAD immunoreactivity and [^{3}H]muscimol uptake suggest that GABA is present in amacrine cells and displaced amacrine cells, which have dendritic ramifications within the whole inner plexiform layer.

DISCUSSION

Conclusions from GABA localization

There is strong evidence that several morphological types of amacrine cell and also an interplexiform cell of the cat retina are GABAergic (Pourcho & Goebel, 1983; Freed, Nakamura & Sterling, 1983). Assuming that morphologically distinct neurones have also differing functions, a diversity of physiological action of GABA can be expected. The contrasting effects of GABA on the different ganglion cell classes described in the present paper might be caused by those amacrines. On the other hand, the GAD immunocytochemistry, described in the Results section, provides evidence that the processes of these amacrine cells fill the whole i.p.l. Therefore, there is a good chance that dendrites of all ganglion cell classes, which branch in different laminae of the i.p.l., receive synaptic input from GABAergic amacrines. Off-centre ganglion cells have their dendritic processes close to the inner nuclear layer (Nelson *et al.* 1978; Peichl & Wässle, 1981). If off-centre cells, as suggested by Ikeda & Sheardown (1983), do not receive GABAergic input, this lamina should be less densely labelled with GAD then the lamina where on-centre cells have their dendritic branches, but no such differences can be observed in Pl. 1*C*. In the pigeon retina, peptidergic amacrine cells show a very specific pattern of lamination (Brecha, 1983; Karten & Brecha, 1983); in the chick and rabbit retina the cholinergic system forms two narrow bands (Baughman & Bader, 1977; Masland & Mills, 1979) and in rat GAD labelling is laminated in the i.p.l. (Vaughn, Famiglietti, Barber, Saito, Roberts & Ribak, 1981; Famiglietti & Vaughn, 1981). Such lamination could be the basis of an exclusive drug action on certain ganglion cell classes (Ehinger, 1982; Daw, Ariel & Caldwell, 1982).

The physiological results presented in this paper show that GABA and bicuculline act on all ganglion cell classes investigated: on- and off-centre brisk-sustained(X) and brisk-transient(Y) cells. A few sluggish concentric units (Cleland & Levick, 1974*a*, *b*) were recorded as well and GABA also had a suppressive effect, but they were not investigated systemically. The dichotomy of GABA action observed in the present paper was between on-centre and off-centre cells and not between brisk-sustained(X) and brisk-transient(Y) cells.

In previous investigations using serotonin (Thier & Wässle, 1984) and APB (Bolz,

Wässle & Thier, 1984), a comparable dichotomy between on- and off-centre cells was observed. Thus, the two systems seem to be pharmacologically fundamentally different. No qualitative difference between brisk-sustained(X) and brisk-transient(Y) cells has so far been observed, but iontophoretic application with brisk-sustained (X) cells is always more potent than with brisk-transient(Y). The receptive field diameter of brisk-transient(Y) cells is three times larger than that of brisk-sustained cells, hence this differing 'effectiveness' of the iontophoresis is mainly a problem of diffusion of the drug.

Comparison with previous results

Straschill & Perwein (1969) applied GABA iontophoretically onto cat retinal ganglion cells. They found all cells to be suppresed by GABA. Unfortunately, in their experiments the cornea and lens of the eye were dissected so they could only apply diffuse light stimuli, which caused on–off discharges from all ganglion cells. Our finding that both on-centre and off-centre cells are suppressed by GABA agrees with their statement. Ikeda & Sheardown (1983) recently published a very elaborate study of GABA and bicuculline iontophoresis in the cat retina. They found that only on-centre ganglion cells were modulated by GABA/bicuculline and that off-centre cells were unaffected. This result is in contrast to our findings and therefore we carefully checked the parameters which might have caused these differences. Raising the background light to the higher illuminance that they used (see Figs. 7*B* and 8), and using dark spot stimulation, did not abolish the influence of GABA on the off cells we studied. Changing the anaesthesia from nitrous oxide + 1 mg/kg . h pentobarbitone (Nembutal) to nitrous oxide + 0·2 % halothane did not abolish the GABA and bicuculline action on off-centre cells. This was tested because it is known that pentobarbitone might alter GABA action (Alger & Nicoll, 1982), and Ikeda & Sheardown (1983) used 0·1–0·2 % halothane. The only remaining difference, is the use of pipettes for recording by Ikeda & Sheardown in contrast to the tungsten-in-glass micro-electrode employed by us. It is known (Peichl & Wässle, 1983) that tungsten-in-glass electrodes can record from dendrites of the i.p.l. and this was found to be an advantage for iontophoresis, because retinal ganglion cells receive synaptic inputs mainly on their dendrites (Kolb, 1979; Sterling, 1983) with only a few synapses on the soma. This does not mean that receptors for GABA are only found at synaptic sites, they might also be present at the soma. However, if one wants to block internal GABA by iontophoretic application of bicuculline, administration of the drug close to the synapses seems to be preferable. Pipettes of 2–4 μm tip diameter as used by Ikeda & Sheardown probably record mostly from the soma. It is possible that the receptors on dendrites and soma elicit different effects. Many of our recordings were also from cell bodies, but we never observed qualitatively different effects although we did observe weaker drug actions.

The results from studies using intravenous injection of GABA and bicuculline indicate a difference between X- and Y-type retinal ganglion cells (Kirby & Enroth-Cugell, 1976; Kirby, 1979; Kirby & Schweitzer-Tong, 1981). X cells seem to be unaffected by GABA and its antagonists, Y cells show a difference in the centre surround organization and in the centre size when bicuculline or picrotoxin are

injected. Clearly, these results contrast with those of the present study showing a strong GABAergic influence on brisk-sustained(X) cells which cannot be explained. The present results also contrast with those of Saito (1981) who applied bicuculline to the bathing medium of the retinal eyecup preparation. He found that bicuculline blocked the centre and surround responses of off-centre X and Y cells; for on-centre cells, bicuculline blocked the surround inhibition of Y cells but not of X cells. In a more recent paper Saito (1983) reported basically the same findings, but if one looks at his Fig. 2, there is clear evidence that bicuculline increased the total firing level of all on-centre cells, irrespective of whether they were X or Y cells. Bicuculline also suppressed the response of all off-centre cells, irrespective of X and Y type (his Fig. 4). Therefore, we would argue that his basic findings agree with the results of the present paper, but his interpretation does not.

Comparison with other species

In some vertebrates like fish, turtle, or salamander, it is possible to study the action of GABA in the isolated retina using intracellular recordings. Although a much clearer understanding of GABA action and localization has emerged, these results cannot directly be transferred to the cat. In fish, for instance, GABA has been localized in horizontal cells (Lam, 1975; Lam, Su, Swain, Marc, Brandom & Wu, 1979) and also its physiological action (Murakami, Shimoda, Nakatani, Miyachi & Watanabe, 1982*a*) in the feed-back from horizontal cells to cones has been measured (Murakami, Shimoda, Nakatani, Miyachi & Watanabe, 1982*b*).

In the mudpuppy, the action of GABA in the i.p.l. has been investigated (Miller, Dacheux & Frumkes, 1977; Miller *et al.* 1981*a*, *b*) and it was shown that GABA causes an increased conduction of membrane channels to chloride in both amacrine and ganglion cells. The overwhelming majority of amacrine and ganglion cells showed high sensitivity to both GABA and glycine, and all light responses of amacrine and ganglion cells were enhanced by bicuculline. There is also evidence that GABA is continuously released and light-evoked inhibitory phenomena are superimposed on a tonic release mechanism. These interactions between the sustained and transient inputs to the ganglion cell have been further elaborated (Belgum, Dvorak & McReynolds, 1982) and it is possible that our results from off-centre cells in the cat can be explained on the basis of the findings in the mudpuppy. In contrast to the situation in the o.p.l., the action of GABA in the i.p.l. may be comparable between the cat and the mudpuppy.

Interpretation of GABA and bicuculline action

The action of GABA and bicuculline on on-centre cells follows the classical scheme of agonist *versus* antagonist. The inhibitory GABA action can be completely counterbalanced by bicuculline. The possibility that different parts of the p.s.t.h. could be specifically modulated by GABA and bicuculline was tested, but no signs of specific action were observed. The on-phase during centre stimulation was raised more by bicuculline application than the off-phase, but during balanced centre/surround stimulation both the centre (on-phase) and the surround component (off-phase) of the response were modulated equally by GABA and bicuculline. In some

cells the peak and transient components of the histogram were less influenced by GABA and bicuculline than the sustained portion.

The action of GABA and bicuculline on off-centre cells is more complicated. Both GABA and bicuculline suppress the maintained firing and the sustained parts of the histogram, which seems to contradict the classical agonist/antagonist scheme. There are several possible explanations for this result. First, off-centre ganglion cells could have two differing GABA receptors: one which hyperpolarizes and one which depolarizes the cell, which might in addition be spatially differently distributed. In hippocampal pyramidal cells, two kinds of GABA receptors have been described, one of which is located at the soma and elicits hyperpolarizing responses and the other at the dendrites which depolarizes the cell during GABA application (Alger & Nicoll, 1982).

Another explanation of the suppressive effects of both GABA and bicuculline could be the involvement of an interneurone in addition to direct GABA receptors on the ganglion cell. This interneurone could inhibit the ganglion cell tonically by a transmitter different from GABA, but it could itself be under GABAergic influence. Application of bicuculline would depolarize this interneurone, which then would increase its inhibitory action on the ganglion cell. The balance of bicuculline action directly on the ganglion cell membrane and on the interneurone would determine the over-all response of the ganglion cell. A third explanation could be a direct suppressive action of bicuculline on the ganglion cell membrane as mentioned above, together with a blockage of GABAergic inhibition. In addition to this modulation of the sustained response, a dramatic increase of the transient components of the histogram was observed when bicuculline was applied. This indicates that off-centre ganglion cells receive a bicuculline-sensitive, phasic GABAergic inhibition, at both light on and at light off. A good candidate for such an action could be on–off amacrine cells (Werblin, 1979; Frumkes, Miller, Slaughter & Dacheux, 1981).

The present paper does not explain the role of GABAergic inhibition in the cat retina. It is mainly a screening type of study demonstrating the nature of the action of GABA on different ganglion cell classes. More information will be gained from experiments where the interactions of spatially separated stimuli or time-shifted stimuli are studied.

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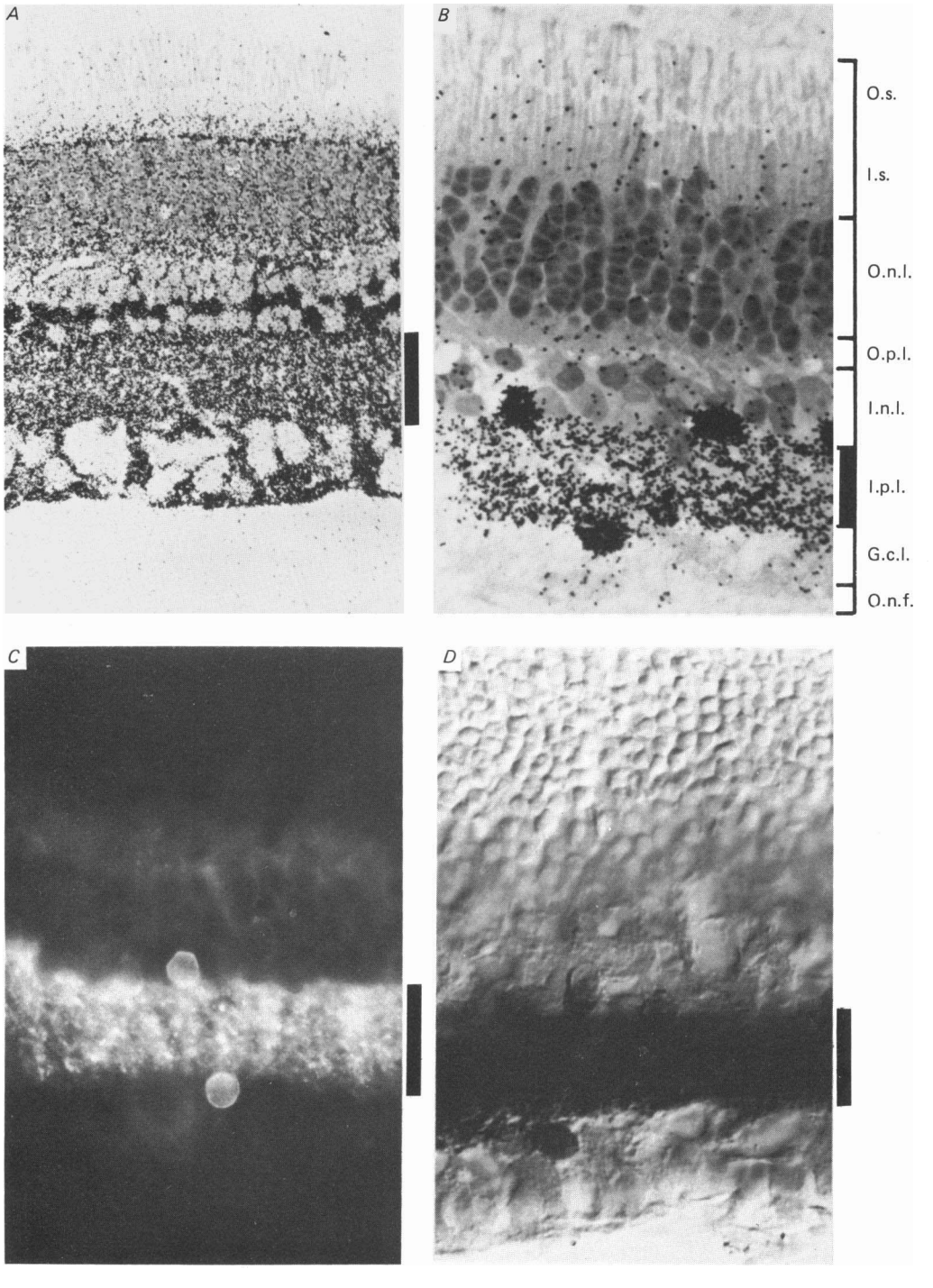
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EXPLANATION OF PLATE

A, vertical section of the cat retina: autoradiograph after [^{3}H]GABA uptake. The heavy label found in the inner plexiform layer, the inner nuclear layer and in the optic nerve fibre layer is mainly from Müller-cell labelling. *B*, vertical section of the cat retina: autoradiograph after [^{3}H]muscimol uptake. Only amacrine cells of the inner nuclear layer and displaced amacrine cells have labelled cell bodies. The inner plexiform layer is also labelled. *C*, vertical section of the cat retina: fluorescence photomicrograph following GAD immunocytochemistry. Cell bodies of amacrine cells, displaced amacrine cells and the inner plexiform layer show bright fluorescence. *D*, vertical section of the cat retina, where GAD-positive structures are labelled by the peroxidase–antiperoxidase technique. The labelling corresponds to the fluorescence micrograph in *C*. The vertical bars in the micrographs indicate the position and thickness of the inner plexiform layer and represent 30 μm. The labelling in *B* has the following conventions: outer segments (o.s.), inner segments (i.s.), outer nuclear layer (o.n.l.), outer plexiform layer (o.p.l.), inner nuclear layer (i.n.l.), inner plexiform layer (i.p.l.), ganglion cell layer (g.c.l.), optic nerve fibre layer (o.n.f.).