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GALLAMINE TRIETHIODIDE (FLAXEDIL) AND CAT RETINAL GANGLION CELL RESPONSES

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SUMMARY

1. Repeated flashes of diffuse light were presented to the cat's eye over long periods of time (hours) while the mixed ganglion cell response was recorded from single axons in the optic tract. This was done for cats receiving gallamine triethiodide intravenously and for cats who did not receive the drug.

2. All responses were transformed into instantaneous pulse density tracings. Such tracings from control and gallamine cells were compared to observe possible effects of gallamine: (*a*) the shape (time course) did not change during gallamine administration; (*b*) maximum firing frequency, total number of spikes and latency was measured for both the on- and the off-component on the pulse density tracings and plotted versus time. Statistical methods failed to reveal any difference between the manner in which these response features of control cells and of gallamine cells varied with time.

INTRODUCTION

Since retinal sensitivity changes rapidly with distance within the receptive field of many visual neurones, reliable eye immobilization is of prime importance in any quantitative study of responses elicited by localized stimuli (Cleland & Enroth-Cugell, 1966; Rodieck, Pettigrew, Bishop & Nikara, 1967; Chow & Lindsley, 1968). Some investigators (Rodieck *et al.* 1967; Stone & Fabian, 1968) have achieved the desired immobilization using low gallamine (triethiodide) doses in conjunction with D-tubocurarine and/or other measures such as mechanical support of the eye or cervical sympathectomy. Others (Cleland & Enroth-Cugell, 1966, 1968; Campbell, Cleland, Cooper & Enroth-Cugell, 1968) have pre-

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ferred gallamine in high dose rates. It has been reported that systemically administered gallamine, even in low dose rates, prolongs the after-discharges recorded from the cerebral cortex (Halpern & Black, 1967), alters the discharge of cuneate hair cells (Galindo, Krnjević & Schwartz, 1968) and results in increased polysynaptic ventral root responses (de Jong, Robles & Morikawa, 1968). Such findings raise the question whether intravenously administered gallamine possibly affects the response mode of, for example, cat retinal ganglion cells with centre-surround organization. The gallamine could affect *both* response mechanisms or one of them selectively; or it could conceivably alter the manner in which the two mechanisms interact. In fact, H. B. Barlow (personal communication, 1968) has posed the very justifiable question whether some of the behaviour observed by Cleland & Enroth-Cugell (1968) in on-centre cells may not, at least in part, be due to the very high gallamine dose rates used by these authors. The goal of the present investigation was to search for possible effects of intravenously administered gallamine upon the functional properties of the receptive field of the ganglion cell.

METHODS

The experimental design was to present repeated whole-field flashes to the cat's eye while recording from a single ganglion cell axon for as long as it could be isolated. This was done for cats which received gallamine as well as for cats which did not receive gallamine. The ganglion cell responses were later compared to observe possible effects of gallamine.

General anaesthesia, preceded by intramusular atropine (0·2–0·4 mg) was initiated with diethyl ether and thiamylal sodium (Cleland & Enroth-Cugell, 1968). This was followed by an intravenous loading dose of urethane (not exceeding 35 mg/kg). Light anaesthesia was then maintained with urethane at 7–15 mg/kg.hr.

The cat, whose pupils were maximally dilated with atropine, looked straight at a uniform source S_1 (Fig. 1) consisting of a set of fluorescent tubes behind opal glass (Cleland & Enroth-Cugell, 1968). The diameter of S_1 subtended 12 degrees at the cat's eye and its luminance varied with time in a square-wave fashion. The modulation depth was 90 % except in a few experiments where it was 100 %. S_1 was superimposed upon a similar but larger source, S_2 (17 degrees), which remained unmodulated. Neutral density filters were used for course luminance attenuation of the two sources; cross-polaroids calibrated in density units (with the mirror M in place) for fine continuous attenuation. To obtain a uniform retinal illumination the light from both sources fell upon a pair of diffusing 'contact lenses' which extended beyond the cornea covering most of the scleral surface. They were made by softening two ping pong ball fragments in boiling water and compressing them in a mould whose radius of curvature was nearly equal to that of the cat cornea. The diffuse light emitted from the concave (corneal) surface of these 'lenses' was measured with a Salford Instrument Photometer while holding the 'lenses' in the same position as during an experiment. When S_1 alone was on (unattenuated), the luminance of the *corneal* lens surface, measured at the maximum of the square wave, was 0·13 mlambert. It will be referred to as the *stimulus luminance*. With S_2 alone the (unattenuated) luminance of the corneal surface of the 'lenses' was 0·9 mlamberts; this is the *background luminance*. The sum of these two, the *total luminance*, was modulated at a

depth which depended upon the degree to which the (modulated) stimulus and/or the (unmodulated) background luminances were attenuated. During an experiment one fixed setting of the attenuators of sources S_1 and S_2, as well as one fixed frequency of source S_1 was used. This resulted in a total luminance whose frequency, maximum luminance and depth of modulation were unchanging. A total luminance having these constant properties will be called a *constant stimulus*.

In an effort to maintain optimal conditions throughout the experiments mean arterial pressure, electrocardiogram and temperature were continuously monitored and fluid balance was maintained. In some animals, Rheomacrodex (Dextran 40,

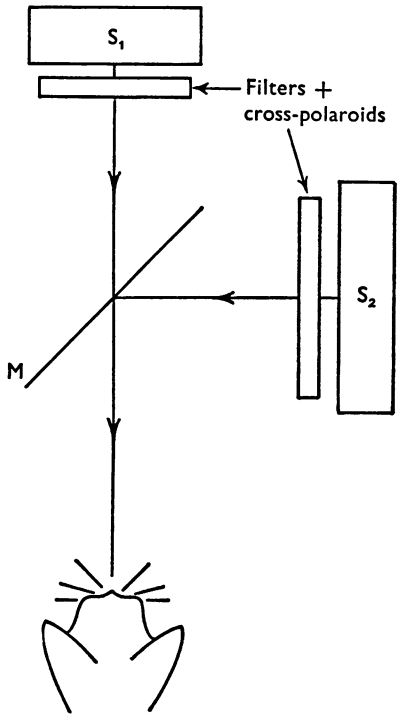

Fig. 1. Schematic drawing of stimulator.

Pharmacia) was used to assure that the mean blood pressure did not fall below 100 mm Hg. At 20–25 strokes/min the tidal volume was adjusted to give the required ventilation (Kleinman & Radford ventilation chart, Harvard Apparatus Co., U.S.A.). As lung atelectasis may cause inadequate oxygenation in spite of appropriate ventilation (e.g. Mead & Collier, 1959), the lungs were passively hyperinflated one to several times per hour.

Retinal ganglion cell mean discharge rate and threshold can also serve as sensitive indicators of the general state of the animal. In healthy animals the mean firing rate of a ganglion cell, whose receptive field is exposed to a constant stimulus, stays remarkably constant for long periods of time. A mean rate which, under these stimulating conditions, decreases steadily with time and/or becomes rhythmic suggests a poor general state of the preparation (Barlow & Levick, 1969; Cleland & Enroth-Cugell, 1970). The mean discharge was therefore continuously monitored on a chart recorder.

From previous experiments (Cleland & Enroth-Cugell, 1968) we knew the range of luminance required, in cats in good condition, to elicit a barely audible response (i.e. *spike frequency fluctuation in synchrony with the stimulus*) to a full-field stimulus modulated at 4 Hz and applied against a steady background attenuated by 5 log. units. The encounter of ganglion cells for which this *threshold* luminance was above the usual range or rose during the experiment served as a warning that the cat's general condition was unsatisfactory. Only if one can be confident that the functional properties of the retina do not change because of deterioration with time, can one hope to assess possible effects of gallamine.

Experimental protocol. After isolation of an optic tract fibre, the above test of threshold at low background luminance was performed. The centre type of the unit was then determined by switching Source S_1, at threshold luminance, to 0·4 Hz and averaging 60 responses 'on-line'. The response ('on' or 'off'), which under these stimulus and background conditions is of centre type (e.g. Wiesel, 1960), was then noted. Next, stimuli at 0·4 Hz and of various luminances were applied against different background levels until that background–stimulus combination was found, which yielded on- and off-components as large and also as equal in magnitude as possible (usually 0·13–0·013 m lambert for the background). A stimulus of that luminance, of constant modulation frequency and depth (0·4 Hz, 90 %) was then applied against the one constant background until the unit was lost. The ganglion cell spikes were recorded continuously on magnetic tape. The longest recording time for one cell was $2\frac{1}{2}$ hr, the shortest $\frac{3}{4}$ hr. A total of 11 units (four on- and seven off-centre cells) in seven cats were held long enough to perform the type of experiment outlined above. Five of these served as *control cells*, as *no* gallamine was given. From the remaining six cells data were obtained during administration of gallamine at rates from 8 to 50 mg/kg.hr. These cells are referred to as *gallamine cells*. For two of them the response was first recorded for a short period without gallamine. In the other cases recording started at a rate of 8 mg/kg.hr which was then stepped up to 25 and finally 50 mg/kg.hr. Just before each increase in dose rate, one single priming dose of 20–30 mg was given. The duration of the 50 mg/kg.hr dose rate varied as follows among the six gallamine cells: $1\frac{1}{2}$ hr; 1 hr 10 min; 1 hr (two cells); $\frac{1}{2}$ hr and the sixth unit was lost before the 50 mg level was reached.

For analysis of the results the responses to groups of sixty individual stimulus cycles were averaged to yield pulse density tracings as described in Cleland & Enroth-Cugell (1968). The *groups* were spaced at 5–10 min intervals. Three features of the on- and the off-response were measured on the pulse density tracings of all cells: (1) peak firing frequency, (2) total number of spikes, and (3) latency. How these measurements were performed is detailed in Fig. 2 and its legend.

RESULTS

Under most stimulus conditions retinal ganglion cell responses to diffuse light reflect the mutually antagonistic contributions of both centre and surround response mechanisms, i.e. the responses are mixed. The characteristics (e.g. amplitude and time course) of the response of the *ganglion cell* depends both upon the magnitude of the signal that it receives from

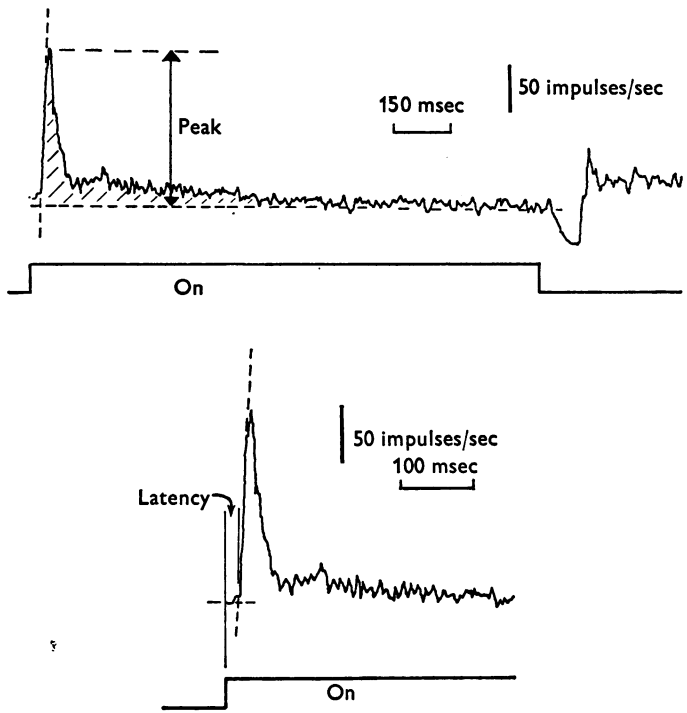

Fig. 2. In each pair of the pulse density is indicated by the upper tracing, the stimulus time course by the lower one. Vertical axis: impulses/sec. Horizontal axis: time. Upper half: the total number of spikes comprising the on- and the off-response was obtained by measuring with a planimeter (Keuffel & Esser No. 4236) the area bounded by the pulse density tracing and a horizontal line drawn at the level of firing during the terminal portion of the appropriate stimulus half-cycle (i.e. in this case at the level that prevailed just before the cessation of the stimulus). The peak firing rate was measured as the vertical distance between the horizontal line mentioned above and a second horizontal line through the response peak. The precision of the planimetric measurements was $\pm 2\%$. Lower half: the interrupted lines indicate; (1) the firing level immediately before the fast upstroke of the response peak and (2) the slope of that upstroke. The distance between the intersection between these two lines and the onset of the stimulus yielded the latency. The accuracy of the measurement was ± 2 msec.

each of the response mechanisms and upon the manner in which the two mechanisms interact. The magnitude of the signal generated by the individual response *mechanisms* is a function of: (i) the properties of the mechanism in question and (ii) the spatial and temporal distribution of the light which falls upon the receptors belonging to the mechanism. As a

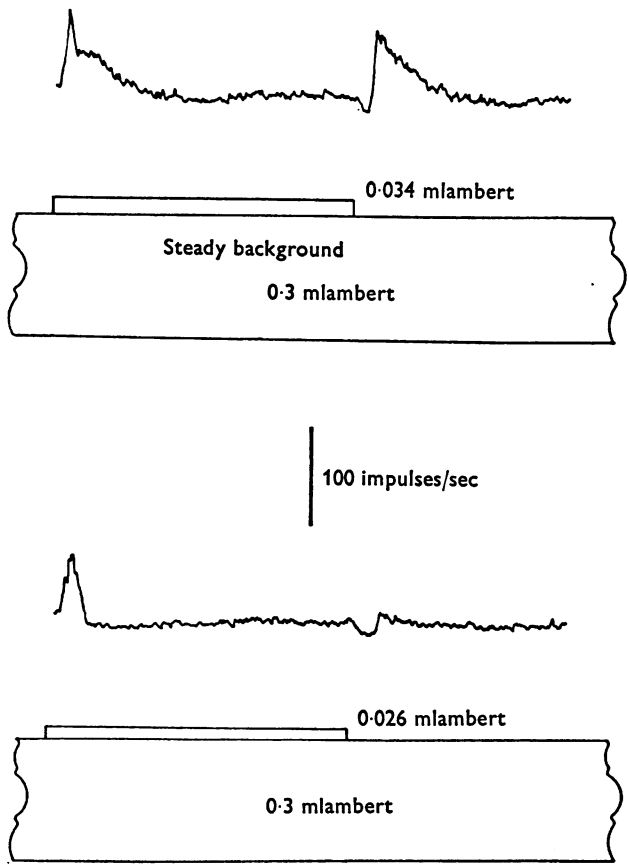

Fig. 3. Mixed response from on-centre cell to exemplify change in response shape and magnitude as stimulus luminance changes by 0·1 log. unit. (0·034 mlambert corresponds to neutral density 0·6, 0·026 mlambert to neutral density 0·7). The background luminance was 0·3 mlambert (neutral density 0·5) in both upper and lower record.

demonstration that the mixed responses in our particular preparation and stimulus situation do in fact vary in magnitude and/or time course (shape) to a measurable degree as the functional properties of the receptive field change, we intentionally varied the centre-surround balance in a few preliminary experiments. An easy way to do this with diffuse retinal illumina-

tion is to maintain a steady background luminance while varying the mean luminance of the square-wave stimulus. As expected quite modest increases or decreases in stimulus luminance changed the responses noticeably. Fig. 3 is from such an experiment and shows how in this case a 0·1 log. unit decrease in stimulus luminance altered both the amplitude and the shape of the on- and the off-component. It would thus seem reasonable to expect that, if indeed intravenous gallamine affects the properties of the receptive field of retinal ganglion cells, this could be detected by careful observation of both shape and magnitude of mixed responses to diffuse illumination.

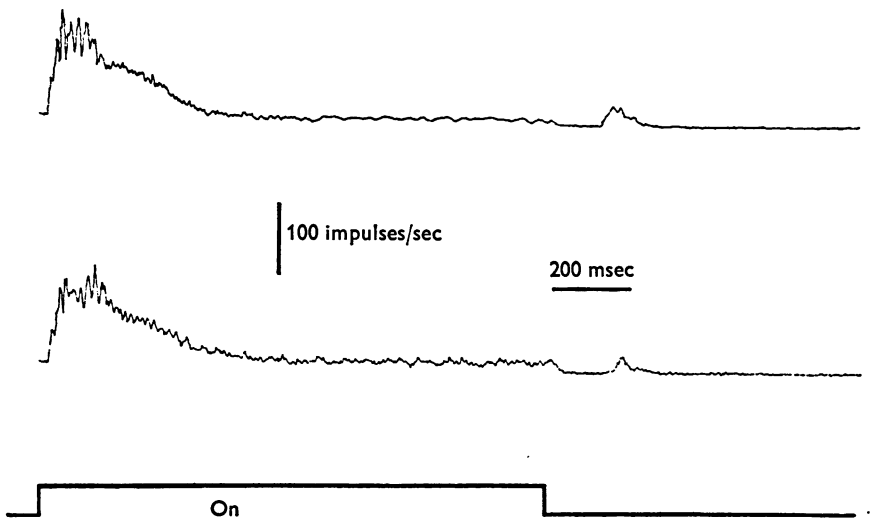

Fig. 4. Upper pulse density tracing: response obtained early in the experiment from an on-centre cell while no gallamine was given. Lower tracing obtained 2 hr 20 min later from the same cell. Gallamine (50 mg/kg.hr) had then been administered for 1 hr 10 min, preceded by lower dose rates. Note that the general response shape is unaltered although in this particular case, the off-component is somewhat smaller in the lower tracing.

Gallamine does not alter the response shape. For each individual control and gallamine cell pulse density tracings obtained early in the experiment were superimposed upon others obtained later during the same experiment. Although small differences between individual average responses were observed, no consistent change in shape of the on- or the off-component could be demonstrated in any of the cells. This is exemplified in Fig. 4 where the upper response is from the beginning of the experiment while no gallamine was given. The lower response was elicited after 2 hr 20 min of gallamine administration; for the last 1 hr 10 min the dose rate was 50 mg/kg.hr. (As described in methods, background and stimulus

luminances were chosen so that the on- and the off-components were as equal in magnitude as possible. The difference was unusually large for this cell). The failure to reveal changes in receptive field properties during gallamine administration by superimposing response tracings does not necessarily mean that such changes are not detectable by quantitative measurements of certain response features. We chose to measure two aspects of the response magnitude for the on- and the off-component; peak firing frequency and the total number of spikes. In addition the latency of both components was measured (see Methods).

Gallamine does not alter response magnitude or latency. The peak firing frequency, the total number of spikes and the latency were plotted versus time for all eleven cells. Inspection of these plots revealed no tendency toward curvature or cyclic variation (see Fig. 5 for examples). For this reason straight (regression) lines were fitted to all plots by the method of least squares. The average number of points upon which each regression line was based was 14 (range 9–20) and each point represents a measurement upon the *average* response to 60 stimulus cycles. In this comparison the actual response or latency magnitude is of little interest; it is determined by the properties of the individual cell and the experimenter's choice of background and stimulus luminances. What one wants to establish is whether or not the *manner* in which response magnitude and latency vary with time when gallamine *is* given differs from the manner in which they vary in the *absence* of gallamine. Hence, the regression *slopes* constitute the appropriate measure for a comparison between gallamine and control cell behaviour. The means of the slopes for the peak firing frequency, the total number of spikes and the latency from both cell categories appear in Table 1. In the Table, those response components to which the *central* response mechanism contributes *excitatory* inputs (on-component for on-CENTRE cells, off-component for off-CENTRE cells) were grouped together under one heading: *centre-excited component.* Similarly, the response components to which the *surround* response mechanism contributes *excitatory* inputs (off-component in on-CENTRE cells, on-component in off-CENTRE cells) were grouped under one heading: *surround-excited component.* It was felt that this would facilitate the detection of any shift in the centre-surround balance if indeed gallamine preferentially affects one of the response mechanisms. The unpaired t test, using pooled variance, established that the probability that the regression slopes (three for each response component of any individual cell) for the gallamine cells and the corresponding regression slopes of the control cells, belong to a population with the same mean is 0·2 or more. In other words, differences between the manner in which control cell characteristics and gallamine cell characteristics change with time can be explained by random variation.

Fig. 5 shows all the regression lines and the data points from which they were computed for one gallamine and one control cell. These were the cells that we held for the longest period of time ($2\frac{1}{2}$ hr). The five first average responses (points) for the gallamine cell were obtained before gallamine infusion was initiated. The gently sloping regression lines and all the mean values seen in this Figure are typical of those encountered in other galla-

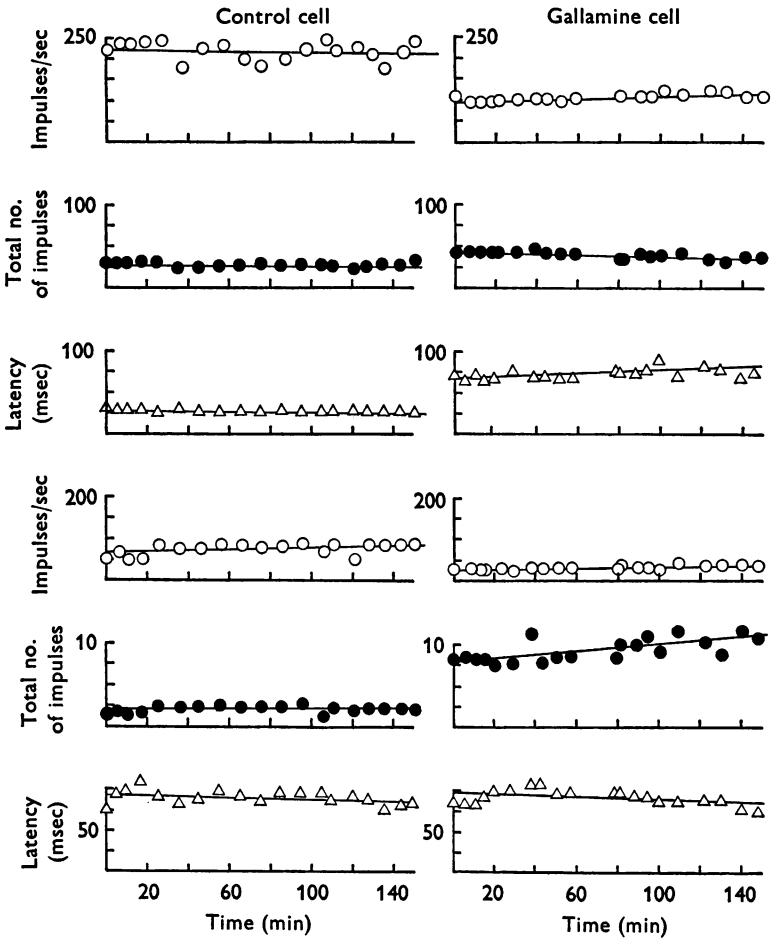

Fig. 5. Comparison between one control cell (left) and one gallamine cell (right), both of off-centre type. Vertical axis in upper three plots for each cell refer to (1) peak firing frequency, (2) total number of spikes and (3) latency of the centre-excited response component (in this case the off-response); in lower three plots to the corresponding quantities for the surround excited (on) component. The horizontal axis is time in all plots. For individual points the time is the midpoint of each group of sixty individual stimulus cycles. Note that the vertical scales are not the same for the corresponding quantities for centre- and surround-excited components.

mine and control cells. The regression slope for the total number of spikes contained in the surround-excited response component of the gallamine cell is the steepest in its category.

The behaviour of the regression slopes for *individual* cells (not included in Table 1) differed somewhat among gallamine and control cells. Among the total of thirty-six regression slopes computed for the six gallamine cells, twelve were significantly different from zero (seven at the 1 % level, five at the 5 % level). For the five control cells the regression slopes totalled 27, not 30, because the surround-excited component of one cell was contaminated by another unit so that response averaging became unreliable. Of these twenty-seven slopes four were significantly different from zero (at the 1 % level). The reason that a larger proportion of the individual slopes among the gallamine cells than among the control cells differed from zero is probably that, for any one cell, the total time during which responses were collected tended to be longer for the gallamine cells (average for the six cells: 124 min) than for the control cells (average for the five cells: 96 min).

Stability of control cell response. It was our desire to learn about possible effects of systemic gallamine administration upon retinal ganglion cell behaviour that prompted these experiments. In order to judge whether or not the manner in which retinal ganglion cells respond to a certain stimulus is altered during gallamine infusion, one must know how the discharge pattern varies with time as ganglion cells respond to the same type of a constant stimulus, in the same type of preparation, but in the absence of gallamine. This need for control cells yielded quantitative information regarding the long-term stability of retinal ganglion cell responses which in itself is useful. Very little information of this kind has been reported before.

The control cell presented in Fig. 5 is in every respect typical for our observations on stability of the response to a constant stimulus, *provided* the general condition of the cat was good and the unit held for a reasonable length of time. As seen in the Figure, none of the three response features of either component vary much with time. Yet, two of the four control cell slopes which *did* differ from zero are from this very cell; the slope for the peak firing frequency of the surround-excited component (third plot from below) differed from zero at the 5 % level and this amounted to an *hourly* increase of 10·8 spikes/sec at a mean of 69·5 spikes/sec; the mean value for the total number of spikes contained in the surround-excited response component (second plot from below) was 1·94 spikes and it increased by 0·27 spikes/hr, corresponding to a slope which differed from zero at the 1 % level. The regression slope for the latency of that same response component (bottom plot) was not different from zero and translated into

practical terms, the latency decreased by 2·4 msec/hr at a mean value of 85·5 msec.

On an *average* (see Table 1) there was no change with time in any of the three response features that we measured; none of the means for the regression slopes of the control cells were significantly different from zero. We conclude that if care is taken to keep the preparation in good condition, then the stability of the response of cat retinal ganglion cells to a constant stimulus is quite remarkable.

TABLE 1. Comparison of slopes of control and gallamine cells

Response feature as function of time	Control cells mean ± S.E. of regression line slopes	*N*	Gallamine cells mean ± S.E. of regression line slopes	*N*	Probability that difference in means occurs by chance
Peak firing frequency of *centre*-excited response component (spikes/sec per min)	0·116 ± 0·156 ($P > 0·4$)	5	0·134 ± 0·049 ($P < 0·05$)	6	$P > 0·9$
Peak firing frequency of *surround*-excited response component (spikes/sec per min)	0·0786 ± 0·035 ($P > 0·05$)	4	0·011 ± 0·045 ($P > 0·8$)	6	$P > 0·3$
Total number of spikes contained in *centre*-excited response component (spikes/min)	−0·008 ± 0·020 ($P > 0·6$)	5	−0·009 ± 0·012 ($P > 0·4$)	6	$P > 0·3$
Total number of spikes contained in *surround*-excited response component (spikes/min)	0·0011 ± 0·006 ($P > 0·8$)	4	0·0047 ± 0·005 ($P > 0·3$)	6	$P > 0·6$
Latency for *centre*-excited response component (msec/min)	−0·0076 ± 0·048 ($P > 0·8$)	5	0·0042 ± 0·018 ($P > 0·8$)	6	$P > 0·7$
Latency for *surround*-excited response component (msec/min)	−0·020 ± 0·035 ($P > 0·6$)	4	0·0322 ± 0·025 ($P > 0·3$)	6	$P > 0·2$

P-values in parentheses are the probability that the mean differs from zero by chance.

DISCUSSION

Previous reports concerning the effect of gallamine upon central nervous system neurones (Halpern & Black, 1967; Galindo *et al.* 1968; de Jong *et al.* 1968) were not concerned with visual neurones. If this drug affects

some central nervous system neurones, it is reasonable to question whether in the visual system too, neurones alter their response mode during gallamine administration. We were particularly interested in knowing if high dose rates might shift the normal balance between centre and surround inputs to retinal ganglion cells. We could not find any difference between ganglion cell behaviour in the absence of gallamine and during administration of the drug. Hence, it is difficult to believe that gallamine, even at 50 mg/kg.hr, significantly alters the centre-surround balance within the receptive field or retinal functional properties in general. We conclude that it is permissible to use this drug in doses up to 50 mg/kg.hr in quantitative experiments on cat retinal ganglion cells when required for eye immobilization.

Bishop & Rodieck (1965), tested the repeatability of retinal ganglion cell responses to a constant stimulus in animals receiving D-tubocurarine and *small* gallamine doses. They present two post-stimulus histograms (their Fig. 6), obtained several hours apart. The stimulus conditions were the same for both histograms and very similar to ours. We have enlarged these two average responses photographically and measured peak firing frequency and total numbers of spikes in the same manner as for our own responses. According to our measurements one peak firing frequency was 6·7 % larger than the other and the total number of spikes in the response that had the higher peak firing frequency was smaller by 1·7 %. These differences are of the same order as the variation in response magnitude over the same period of time in our experiments using *high* gallamine dose rates.

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