Variability in responses of retinal ganglion cells

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Changes in the maintained discharge of retinal ganglion cells have been modeled by addition of noise to a rate-setting signal, whereas the responses of cortical cells have been reported to indicate a nonlinear relationship between noise and signal. To determine whether this represents a difference between retinal cells and cortical cells, the variance of light-evoked responses of ganglion cells in the retinas of goldfish was compared with the magnitude of the responses. Variance increased for higher firing rates. These results are discussed in terms of mechanisms by which light-evoked signals might interact with the variability of maintained discharges.

INTRODUCTION

Various lines of evidence have indicated that the variance of the maintained discharges of retinal ganglion cells may be produced by a source of noise to which is added a bias that adjusts the mean firing rate.1,2 An apparently contradictory result has been obtained from experiments with the cortex; there, the variance of responses to repeated stimuli is proportional to the magnitude of the responses,3 implying a nonlinear relationship between noise and signal. Tolhurst4 suggested that this may represent an intrinsic difference between retinal and cortical neurons with respect to the ways in which the variance depends on the mean firing rate.

We note, however, that the results indicating an independence of variance and rate reported in the retina and those implying a dependence of variance and rate in the cortex differ in another important way: the rate-independent results apply to differences in maintained discharges in the presence of long-lasting (adaptive) illuminations, while the rate-dependent results in the cortex describe responses to changing visual stimuli. Moreover, the retinal results are based on the statistics of the intervals between successive impulses, while the cortical results (where sustained firing is rarely observed) are based on the numbers of spikes in the responses in successive trials.

To determine whether these are differences between cortical and retinal cells or are differences between maintained discharges and responses (or simply differences in methodology), we have analyzed data from retinal cells in a paradigm analogous to that used for data from cortical cells. We report that retinal cells also exhibit rate-dependent noise in active responses, and we suggest that this raises questions about the way in which response mechanisms interact with those mechanisms responsible for maintained discharges.

METHODS

Extracellular recordings were made from ganglion cells in the isolated retinas of goldfish. Most of the data were culled from experiments performed for other purposes; in addition, we undertook new experiments (nine cells in three retinas) that provide data specifically for a comparison with the cortical recordings.

The physiological preparation was essentially the same for all the experiments. Goldfish (Carassius auratus), 8–15 cm in length, were dark adapted for at least 1 h before dissection. Fish were rapidly pithed, and a retina was removed from each and placed in the experimental chamber with a flow of pure, moist oxygen.5,6 Action potentials were recorded with a platinum–iridium microelectrode, and their times of occurrence (to the nearest millisecond) were stored for later analysis.

Stimuli derived from a three-beam optical system were shone onto the retina from below and centered on the microelectrode tip. Stimulus size, shape, position, wavelength, and irradiance were separately controlled; electromagnetic shutters in each beam permitted their presentation under computer control. Stimulus irradiances ranged from \(10^{11}\) to \(1.6 \times 10^{13}\) quanta cm\(^{-2}\) sec\(^{-1}\).

The previously available data, obtained by various experimenters in this laboratory, were drawn from 100 experiments on 70 cells. There were 35 experiments in which a single several-second flash of light was delivered repeatedly,7 35 experiments in which two different stimuli (differing in spatial or chromatic attributes) were delivered alternately to the ganglion-cell receptive field,8 16 experiments in which a multibladed windmill-like stimulus was presented alternately stationary or rotating, and 14 experiments in which three or four identical 1-sec flashes were presented at various timings relative to a longer conditioning flash, with the sequence repeated at least 20 times.

In the experiments performed specifically for this study, a sequence of four stimuli was presented, with the sequence repeated for at least 20 cycles. The four stimuli were identical (0.8 mm at the retina) circular spots, of the same wavelength (usually 710 nm), but with different retinal irradiances chosen to give four different magnitudes of response. Each flash lasted 1 sec, with at least 7 sec between flashes (see insets in Fig. 1). Each beam of the optical system provided one flash, with the fourth stimulus being the simultaneous presentation of the spots from all three beams.

For the analyses, responses were defined as the number of action potentials in a sampling period (0.25, 0.5, or 1 sec) immediately after each stimulus transition (onset or offset).
Fig. 1. Variance of the firing rate versus the mean firing rate, for maintained discharges and responses to repeated 1-sec flashes. Data were analyzed for sampling of periods of 1 sec (solid squares, fitted with solid line), 0.5 sec (solid triangles, fitted with dashed line), and 0.25 sec (stars, fitted with dotted-dashed line). Maintained discharges were taken from the second preceding the first flash in each cycle. The mean rate and the variance of the rate were computed from the firing in the corresponding sampling periods of all the cycles analyzed. In the examples shown in Fig. 1 (the insets of which show the averaged responses to 12 cycles of flashes), there are four onset responses, four offset responses, and the maintained discharge for each of the three sampling periods (0.25, 0.5, and 1 sec), a total of 27 means and variances from each data set. The previously available data were similarly analyzed, but only 1- and 0.5-sec periods were used.

If the cell were changing in sensitivity or responsivity (owing to adaptation, deterioration, or a cyclic phenomenon perhaps related to that discussed by Tolhurst\textsuperscript{3,4}), there would be an increase in the variance that should be evident in the responses. To avoid such nonstationarity, the block of cycles with the most-stable firing was selected for analysis. The analysis of only one block of cycles was reported for each cell; when other similarly stable blocks were available from the same cell, the results of those analyses were always qualitatively similar.

\section*{RESULTS}

The variance of the rate was compared with the mean rate for 109 cells. In 84 of the 89 cells for which a stimulus caused an increase in firing above the maintained rate, there was also an increase in the variance of the responses. The few exceptions were cases in which the variance would not be expected to change significantly because the change in the rate was either quite small or so large that the responses approached saturation.

Since most of the ganglion cells had a moderate maintained discharge, it was also possible to observe responses in which the firing declined from the maintained level. For about half the cells, if the firing increased at the onset of the stimulus, it declined at the offset, or vice versa. Stimulus-evoked declines in the firing rate were not reported for experiments in cortical cells, where the maintained discharge is low or nonexistent.\textsuperscript{4}

In 40 of 54 cells, declines in the rate were associated with decreases in the variance. In eight cases, however, declines in the rate were accompanied by increases in the variance. This was probably due to nonstationarity in the data. Indeed, analyses of fewer cycles of firing of those cells that showed an increase in variance with a decline in firing often showed the variance decreasing during declines in firing. Note that, whereas taking fewer cycles often reversed the increases in the variance with declines in the rate, it never abolished the increases in the variance that accompanied increases in the rate.

For a more-direct comparison with the studies of the firing of cortical neurons, we plotted the variance as a function of the firing rate on double logarithmic axes similar to those presented previously.\textsuperscript{3,4} Two plots typical of those we obtained are shown in Fig. 1. Note that we represent responses as the rate of firing action potentials, rather than as the number of action potentials in a sampling period. Conversion from the former to the latter would amount to a uniform translation of all the points for a given sampling period. Thus the functions on the double logarithmic plots are unaffected by the method of representation used.
A plot was made for each of the 32 previously recorded cells that provided suitable data (multiple single-second flashes in a sequence) and for each of the nine cells in the new experiments designed to provide a range of response amplitudes. Each point represents either a response to a particular stimulus or the maintained discharge. Principal component lines were fitted to the data for each sampling duration, and the slopes were tabulated. The correlation coefficient served as an index of the appropriateness of a straight-line fit. We note, however, that there was no consistent relationship between the correlation coefficients and the slopes of all 123 principal component lines.

As noted above, the vast majority of cells showed an increase in the variance with the firing rate. If we assume a power function relating the variance to the mean, we may characterize the nature of the relationship from the slope of the straight line on the double logarithmic plot. In 14 cases, more than one subset of the data was used to generate plots; there were minimal substantive differences among the plots derived from any cell. In these cases, we retained only the plot with the highest overall correlation coefficients because it provided the most reliable estimates of the slopes. Note, however, that the same data were used for all three sampling periods. For the statistical comparisons, we considered plots from only the 30 cells that provided at least one fit with a correlation coefficient greater than 0.70 (i.e., those for which a regression line accounts for at least 50% of the variance).

In general, the slopes of the lines relating log variance to log mean rate were steepest for the 1-sec sampling periods and shallowest for the 0.25-sec periods [note especially Fig. 1(a)]. Paired t tests comparing the logarithms of the slopes for 1-sec periods with those for 0.5-sec periods and comparing the results for 0.5-sec periods with those for 0.25-sec periods showed that these differences were statistically significant for 1 sec versus 0.5 sec, \( t = 3.69;\) for 0.5 sec versus 0.25 sec, \( t = 3.91;\) both with 29 degrees of freedom; \( p < 0.001.\) The mean logarithms (plus or minus the standard deviation) of the slopes for the 1-, 0.5-, and 0.25-sec periods were, respectively, \(-0.03 \pm 0.28,\) \(-0.11 \pm 0.25,\) and \(-0.18 \pm 0.21.\) Thus the geometric mean slopes were 0.93, 0.78, and 0.66. The logarithms of the slopes were used in order to compensate for the skewed distributions of the slopes indicated in the histogram shown in Fig. 2.

Figure 2 shows that the slopes tended to cluster around the range 0.5–0.7, with most slopes greater than 0.5 [e.g., Figs. 1(b)]. Nevertheless, all three mean logarithms of slopes reported above differed significantly from –0.30 (the logarithm of 0.5): even for the 0.25-sec periods, \( t = 3.20 \) and \( p < 0.005.\) The mean logarithms of slopes for the 0.25- and 0.5-sec periods also differed from 0.00, the logarithm of 1.00 (\( t_{0.25} = -4.69,\) \( p < 0.001; t_{0.50} = 2.42,\) \( p < 0.05),\) but that for 1 sec did not (\( t_1 = -0.55).\) A slope of 1.00 would indicate that the variance of the rate was proportional to the rate.

As was noted above, when more than one experiment was completed on a single cell, the slopes of the lines from the various experiments were markedly similar. This implies that the outcome was a property of the cell, not of the particular experiment. We do not have sufficient data for a strict statistical analysis, but we discern no tendency for the slope to correlate with center response types (on center, off center, or on–off). There was, however, a tendency for the seven cells classified as X-like to have steeper slopes than the 11 classified as not-X-like.9 Only the difference in the logarithms of the slopes for the 0.25-sec periods proved statistically significant (\( t = 2.27,\) \( p < 0.05;\) 16 degrees of freedom).

**DISCUSSION**

Our main finding is that, as in the cortex, the variance of ganglion cell responses increases with the firing rate. We thus affirm a nonlinear relationship between the response and the variance for retinal ganglion cells, a result similar to that reported for cat cortex and monkey cortex.4

Van Dijk and Ringo11 recently published results for essentially the experiment that we report here, but with diametrically opposed results. They found no dependence of the variability of firing on the mean rate. We believe that there are two reasons for this discrepancy, both of which may be attributable to their use of a high-bicarbonate-CO_2 superfuse. First, their firing rates were generally low. The mean maintained discharges of the four cells shown in their figures were barely over 6 impulses/sec; the largest response shown to lights that we estimate were comparable with our maximum was only 40 impulses/sec. Low firing rates provide a limited range over which to demonstrate the dependency of variability on the rate. Second, the high variability of their maintained discharge strongly suggests bursty firing, which is characteristic of the normal physiological state. The large aberrant variability would easily mask small changes in variability owing to the modest changes in the mean rate. We therefore believe that the dependency of the variability of firing on the mean rate that we report is correct and that Van Dijk and Ringo did not observe it because of the state of the preparation.

We have shown that goldfish retinal ganglion cells, as do cat cortical cells, exhibit a nonlinear relationship between the variance and the firing rate. It is unfortunate that we do not have comparable data from cat ganglion cells, for it is possible that goldfish retinal cells may have cortexlike properties that are not common to mammalian retinal cells. There are data from two cat ganglion cells recorded by Barlow et al.11 that show an increase in the variance with the mean firing rate in response to stimulation. However,
those data were obtained with low stimulus levels, several orders of magnitude less than those used in the present study and the studies of cat cortical cells. The variability in response to extremely weak stimuli is mainly due to quantal fluctuations.

We note that the results for the few goldfish ganglion cells that could be analyzed at different levels of maintained discharge seemed comparable with the results for the cat ganglion cells. We thus observe a rate-independent noise for maintained discharge but a rate-dependent noise in responses in the same preparation.

A nonlinear relationship between noise and signal has been suggested for goldfish ganglion cells. Ginsburg et al. found that the percentage of variance that was cross correlated between neighboring ganglion cells was the same during maintained discharges and light-evoked responses. They explained this phenomenon by a model in which the retinal gain is changed by the photic signal. Levine and Shefner reported that the coefficient of variation (defined as the standard deviation of the intervals between impulses divided by the mean interval) was not correlated with changes in the mean rate caused by spontaneous drifts or responses to stimulus flashes. They interpreted this as a multiplicative effect of the rate-changing signal on the noise. An alternative (which may be equivalent) is a variable-clock time dilation within intervals.

We must consider what kind of nonlinear relationship between noise and firing rate might be invoked. If the rate-setting signal simply multiplies the noise, one would expect the standard deviation of the rate (square root of the variance) to be proportional to the rate. In that case, the slope of the variance versus the rate would be two on the double logarithmic plot. However, the majority of the slopes were less than two. Our shallowest slopes were generally obtained with the shortest-duration sampling periods (0.25 sec), which may represent the purest responses, since the longer sampling periods would also include the response plateau, which is a form of maintained discharge.

It seems puzzling that the slopes obtained with short sampling periods are shallow, but this apparent paradox is easily explained. There are two components of the variance. The one of interest is the variance in the responses. This component, as we have shown, depends on the strength of the response but apparently is not strongly dependent on the duration of the sampling period. This weak dependence on the sampling period might be expected if the response occurred mainly in the first 0.25 sec or if the variance of the response, which is nonstationary, were not dependent on the sampling period in the same way as in a random process. The second component of the variance, which is due to the maintained discharge, is not dependent on the response strength but is inversely proportional to the duration of the sampling period [see Eq. (6) in Ref. 1]. The component that is due to maintained discharge is relatively more important for weak responses, where the variance that is due to the response strength is minimal. Thus the component of the variance that is due to maintained discharge increases the total variance relatively more for weaker responses than for stronger responses and makes the slope of the plot of log variance of firing rate versus log firing rate shallower than it would be if the only source of variance were response dependent. Since the variance of maintained discharge is greatest with short sampling periods, it is the plot for the 0.25-sec sampling period that has the most degraded slope. We thus believe that the slope of plots for 1-sec sampling periods is the best indicator of the relationship between the variance and the response strength; i.e., the variance is proportional to the firing rate. This is the same relationship reported for experiments in cortex, where there is minimal maintained discharge to degrade the slope at any sampling period duration.

We have been assuming that the variance changes as a direct consequence of the rate change. It is possible, however, that the change in variance is secondary to other changes in the retina or the cortex that are initiated by stimulation. Levine and Shefner suggested that there are long-term variabilities associated with ON and OFF systems that operate in the push–pull fashion in goldfish retina. The variabilities of responses may be partially related to the varying influences of the ON and the OFF systems as they are successively engaged to produce the changes in the firing rate. In support of this, we note that in a few cases in which stimulus flashes longer than 1 sec were used, the variance of the responses remained elevated even after the firing had returned to nearly its plateau value. In some cases, the greatest variance (for 1-sec periods of the response) came in the second second of the response, while the greatest elevation of the rate was in the initial second. For these reasons, we feel that the signal related to photic stimulation interacts with maintained discharges in a complicated fashion and that simple measurements of variance are insufficient to unravel the mechanism.

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Note Added in Proof: Data collected by B. G. Cleland, A. W. Freeman, and M. W. Levine at the University of Sydney (personal communication) indicate that a similar relationship may obtain between the variance of rate and the mean firing rate in retinal ganglion cells in the cat. Their data also suggest the same effect for relay cells in the cat's dorsal lateral geniculate nucleus.

REFERENCES


Levine et al.


