Response Dynamics and Receptive-Field Organization of Catfish Ganglion Cells

HIROKO M. SAKAI* and KEN-ICHI NAKA*:

From the *Department of Ophthalmology and *Departments of Ophthalmology and Physiology and Biophysics, New York University Medical Center, New York, New York 10016

ABSTRACT Responses from catfish retinal ganglion cells were evoked by a spot or an annulus of light and were analyzed by a procedure identical to the one used previously to study catfish amacrine cells (Sakai H. M., and K.-I. Naka, 1992. Journal of Neurophysiology. 67:430–442.).

In two-input white-noise experiments, a response evoked by simultaneous stimulation of the center and surround was decomposed into the components generated by the center and surround through a process of cross-correlation. The center and surround responses were also decomposed into their linear and nonlinear components so that the response dynamics of the linear and nonlinear components could be measured.

We found that the concentric organization of the receptive field was determined by linear components, i.e., the first-order kernels generated by the center and surround were of opposite polarity. Both the center and surround generated second-order kernels with similar signatures, i.e., the second-order components formed a monotonic receptive field. The peak response time of the first- and second-order kernels from the surround was longer by ~20 ms than that of the center.

Except for the DC potential present in the intracellular responses, almost identical first- and second-order kernels for the center and surround were obtained from both the intracellular response and spike discharges. Thus, information on concentric organization of a receptive field is translated into spike discharges with little loss of information.

A train of spike discharges carries, simultaneously, at least four kinds of information: two linear and two nonlinear components, which originate in the receptive field center and the surround. A spike train is not a simple signaling device but is a carrier of complex and multiple signals. Victor, J. D., and R. M. Shapley (1979. Journal of General Physiology. 74:671–687.) discovered similarly that, in the cat retina, static second-order nonlinearity is encoded into spike trains.

Results obtained in this study support the thesis that signals generated by the preganglionic cells are translated into spike discharges without major modification and that those signals can be recovered from the spike trains (Sakuranaga, M., Y. Ando, and K.-I. Naka. 1987. Journal of General Physiology. 90:229–259.; Korenberg, Address correspondence to Ken Naka, NYU Medical Center, 550 First Avenue, PHL-821, New York, NY 10016.
Ganglion cells are the output neurons in the retina and spike discharges generated in these cells are the only means by which information about the visual world is transmitted to the brain. Thus, spike discharges embody the end results of the complex signal processing in the retina. The morphology and physiology of ganglion cells have been the subjects of a large number of studies. Receptive-field characteristics have also been studied extensively. A review of recent results relating to the receptive-field structure of the cat and the monkey is available (Kaplan, 1991). However, not much is known about the way in which the ganglion cells' response is related to the response from the preganglionic cells or about the kinds of signals that are generated in the ganglion cells and are translated into spike discharges to be sent to the brain (Morgan, 1992). To examine these two issues, we evoked responses from ganglion cells, both intracellular responses and spike discharges, by using a set of stimuli identical to those used in a recent study of amacrine cells of the catfish. The responses, both intracellular responses and spike discharges, were analyzed by the same procedure as that used to analyze the responses from the amacrine cells (Sakai and Naka, 1992). Analysis of spike discharges is based on an observation made by Korenberg, Sakai, and Naka, (1989), namely, that the process of spike generation in the catfish retinal ganglion cell is nonlinear, but can be assumed to be approximately static. Thus, the process leading to the generation of spike discharges can be represented by a Wiener cascade, a dynamic linear element followed by a static nonlinearity (Hunter and Korenberg, 1986). With this simplifying assumption, the kernels derived from a ganglion cell's spike discharges can roughly be equated with those derived from the cell's analogue response, the postsynaptic potentials and, by extension, to the processes in the preganglionic cells.

With respect to retinas of the higher vertebrate, the dynamics of cat X and Y cells (Victor, 1987, 1988) and of monkey LGN cells (Kaplan and Shapley, 1986; Bendarete, Kaplan, and Knight, 1992) have been extensively studied by use of sum of sinusoid or sinusoidally modulated gratings. In the case of retinas of lower vertebrates, very little is known about the dynamics of ganglion cell responses, with the exception of the results reported by Schellart and Spekreijse (1972). In this paper, which is a sequel to a similar study on amacrine cells (Sakai and Naka, 1992), we will describe how the receptive-field organization is related to the linear and nonlinear components of the response from catfish ganglion cells.

Three main conclusions will be drawn from our results, as follows: (a) the receptive-field organization, as well as the response dynamics of ganglion cells, is similar to that of amacrine cells. (b) The linear components carry signals about the concentric receptive-field organization and the second-order nonlinear components carry signals about changes around a mean luminance. The nonlinear components form a monotonic receptive field and signal changes occurring anywhere in the field. (c) A spike train is a carrier of multiple signals and the commonly used measures such
as the average (poststimulus time histograms) or instantaneous firing frequencies are of very limited value (McClurkin, Optican, Richmond, and Gawne, 1991).

MATERIALS AND METHODS

All experiments were performed on eye-cup preparations of the channel catfish, *Ictalurus punctatus*. Methods and experimental conditions were identical to those described previously by Sakai and Naka (1992). Ganglion cells were identified on the basis of their responses to a flash of light and some cells were morphologically identified by injection of dye. We have previously made several functional and morphological studies of catfish ganglion cells (Marmarelis and Naka, 1973; Naka and Carraway, 1975; Davis and Naka, 1980; Sakuranaga, Ando, and Naka, 1987; Sakai and Naka, 1987a, 1988a, b). The data presented here are cross-referenced to those in our earlier studies. In addition to intracellular recordings, extracellular spike discharges were recorded in this study. We used platinum-coated tungsten electrodes to register spike discharges and a DAM 50 amplifier (WPI, Sarasota, FL) as an input stage.

The two-channel optical stimulator had two red-light-emitting diodes (LEDs); one channel produced a spot that was 0.2, 1.2, or 2.5 mm in diameter, and the other channel produced an annulus with an internal diameter of 1.5 or 3 mm. The outer diameter of these annuli was 5 mm in all experiments. We selected a combination of a spot and an annulus with diameters such that the responses from a receptive-field center and surround were optimally segregated. The LEDs had a peak wavelength of 660 nm (H-3000; Stanley, Tokyo, Japan). The output of LEDs was monitored by a photodiode S1406 (Hamamatsu Electric, Tokyo, Japan). The intensity of the stimulus was calibrated with a quantum sensor (Li-19C; Li-Cor, Lincoln, NE). Light stimuli were attenuated by a series of neutral density filters. The unattenuated illumination of the unattenuated light measured at the retinal surface was $6 \times 10^7$ photon $\mu m^{-2} s^{-1}$. White-noise signals were obtained from a noise generator (1360 Burst Random Noise Generator; NF Electric Instruments, Tokyo, Japan). Cellular signal and output of the photodiodes were initially stored on digital audio tapes with a data recorder (RD-101T, TEAC, Tokyo, Japan).

Stimulus Paradigm

Fig. 1 is a graphical summary of the experimental procedure. The inputs were a spot and a concentric annulus of light. In most of the experiments, white-noise signals had a flat power spectrum from near DC to 50 Hz. We performed one- and two-input experiments: in the two-input experiments, both the spot and the annulus were modulated by two independent white-noise signals and in the one-input experiment one of the two inputs was white-noise-modulated whereas the other input was kept at a steady luminance that corresponded to the mean of the white-noise modulation; the steady illumination is equivalent to an extremely small modulation. In some two-input experiments, the depth of modulation was reduced so that the two inputs generated a comparable response. The depths of modulation were defined as:

$$C_R = (L_{\text{max}} - L_{\text{min}})/(L_{\text{max}} + L_{\text{min}})$$

where $L_{\text{max}}$ is $-3\sigma$ and $L_{\text{max}}$ is $+3\sigma$ of the Gaussian distribution of input white-noise signal. This is the Rayleigh contrast (Shapley and Enroth-Cugell, 1984). The range of modulation used was between 30 and 80%. We found that the relative depths of modulation of the two white-noise inputs were important factors in the generation of well defined first- and second-order kernels. In the catfish, unlike in the cat and monkey (Shapley and Victor, 1978), the depth of modulation controlled only the sensitivity, i.e. the amplitude of kernels, but not the response dynamics, i.e., the waveform of kernels (Sakai, Wang, and Naka, 1995). The dynamics of the modulation response was invariant of the depth of modulation.
Spike Analysis

An intracellular response from a ganglion cell is composed of a postsynaptic potential (PSP), which is an analogue process, and spike discharges, namely, a point process. An analogue process is capable of carrying far larger amounts of information than a point process of limited frequency band, i.e., <500 Hz in the case of spike discharges, in which only the timing of an event carries information. Moreover, it is generally believed that there is noise, namely a jitter, in the timing of each spike discharge. Jitter is thought to limit the amount of information carried by a spike train. In the present study, each spike discharge triggered a unitary pulse of 2 ms in duration. Cross-correlation was performed between the input white-noise signals and the trigger-generated pulses (Fig. 1). Korenberg et al. (1989) showed that the spike generation by the ganglion cells is approximately static, i.e., spike generation does not depend upon the recent past of the cell’s PSP but only depends, to a good approximation, on the value of the PSP at the instant the spike is generated. The process leading to the generation of a ganglion cell’s PSP can be described by a cascade, a Wiener structure, in which a dynamic linear filter is followed by a static (i.e., no memory) nonlinearity (Hunter and Korenberg, 1986). If the spike generation is nonlinear but static, two static nonlinearities, one for the PSP and other for the spike generation, no matter how complex they are, can be recombined into a single static nonlinearity. Thus, the overall neuron network receiving the light input and producing spike discharges can be represented by a Wiener structure. The static nonlinearity in the spike-generation process affects only the proportionality constant that scales the Wiener kernel of a given order. Further experimental and theoretical justification for this spike analysis can be found elsewhere (Korenberg et al., 1989).
Computation of Kernels

Both the PSP and spike discharges, transformed into unitary pulses, were analyzed by a software system, Spatio-temporal Analysis Routines (STAR), that was run on a combination of a microVAX 3600 computer (Digital Equipment, Maynard, MA) and an AP5000 array processor (Floating Point Systems, Portland, OR). Large spike components in intracellular recordings were removed by the process described by Sakuranaga et al. (1987). Time resolution of the analysis was 2 ms for both PSPs and spike discharges. STAR was developed by Dr. M. Sakuranaga at the National Institute for Basic Biology (Okazaki, Japan). Computational algorithms can be found in Sakuranaga and Naka (1985).

Definitions of Kernels

In visual physiology, in which a nonzero mean luminance is modulated by a white noise, the amplitude as well as waveform of kernels is a function of the luminance and, therefore, is a measure of a cell's incremental (or decremental) sensitivity at the mean luminance (Sakuranaga et al., 1987). From a system in which the output is spike discharges, namely, a point process, a kernel can also be derived by a process of reverse correlation, as originally proposed by de Boer and Kuypers (1968). Schellart and Spekreijse (1972) obtained a first-order kernel by trigger correlation from spike trains in the goldfish retina. Meister, Pine, and Baylor (1994) also used a similar method to measure the first-order kernels. The kernel obtained by the reverse correlation method is interpreted as the waveform of the input that is optimal for triggering a spike discharge. Although dual interpretation of a first-order kernel is possible, one for the reverse correlation and the other for forward correlation, the two kernels obtained by forward and reverse correlation are identical in their waveforms but time runs in the opposite direction. As in the past, we measured the transport delay by the peak response time (PTR) of the first-order kernels. Unlike the latency, measurement of PTR was straightforward.

A second-order kernel represents the degree of deviation from the linear superposition of the response evoked by two points in the stimulus. The second-order kernel also has a dual interpretation, as discussed above for the first-order kernel. The value on the diagonal at which X equals Y shows the deviation from the linearity when the amplitude of the stimulus is changed. We show the diagonal section of the kernels in Figs. 3, 4, 6, and 7. A more rigorous definition of kernels as a measure of incremental sensitivity can be found elsewhere (Sakuranaga et al., 1987).

Classification of Cells

We used three parameters to characterize responses and, hence, the ganglion cells that generate these responses. These parameters were (a) the polarity of responses evoked by a pulsatile input; (b) the polarity of the first-order kernels; and (c) the signature of the second-order kernel. Based on the first two parameters, cells were classified into three types, on-center (GA), off-center (GB), and on-off (GC) cell. The third parameter indicated the origin of the second-order nonlinearity.

RESULTS

Intracellular Responses

Fig. 2 shows the responses from a GA and a GB cell produced by a pulsatile stimulus, either in the form of a spot or an annulus of light, in the presence of steady illumination by an annulus or a spot. The organization of the center and surround of the cells' receptive fields is clearly seen from the polarity of the responses. We next
combined two stimuli and modulated the responses of the two cells, Fig. 2, A and B, by two independent white-noise signals (Fig. 1 A). For each cell, four kernels were obtained, two first-order kernels and two second-order kernels generated by the spot and annular inputs. These kernels are shown for the GA cell (Fig. 3) and for the GB cell (Fig. 4). In both figures, A shows four first-order kernels. One pair, shown by solid lines, was obtained by stimulation of the center and surround of the cell's receptive field by a spot or an annulus (one-input experiment). The other pair, shown by dashed lines, was obtained by simultaneous stimulation of the center and surround by two independent white-noise stimuli (two-input experiment). We note that: (a) the first-order kernels produced by a spot or annular stimulation are opposite in their polarity in both one- and two-input experiments; (b) the PRT of the center kernel, whether it was hyperpolarizing or depolarizing, was shorter by ~20 ms than of the surround kernel; and (c) in the two-input experiments, the amplitude of the surround kernels was somewhat smaller than those of the surround kernels obtained in the one-input experiments. The first observation shows that the concentric receptive-field organization was carried by the first-order component, as was evident from the response to a pulsatile input shown in Fig. 2. The second observation shows that the surround response had a longer latency than the center response. The third observation shows that there was cross-talk or interference between the center and surround; modulation of the center reduced the sensitivity of the surround. Here we recall that the kernel is a measure of incremental sensitivity (Sakuranaga et al., 1987). We found that the center's response was more robust than the surround's response; the center kernels were less prone to be influenced by the presence of annular modulation. When full-field illumination was used, the resulting
response had polarity similar to that produced by stimulation of the center of the cell’s receptive field. This similarity may be partly due to the fact that the activity of the center depressed the sensitivity of the surround (see Fig. 5).

In both Figs. 3 and 4, C and D show the second-order kernels that were generated by a spot (center) and an annular (surround) stimulation and were computed from postsynaptic potentials. The kernels from the GA cell had structures characteristic of the kernels from some NA amacrine cells (Fig. 3, C and D) irrespective of whether they were generated by a spot or an annular stimulus. The initial hyperpolarizing valley is one of the characteristics of kernels from NA cells (Sakai and Naka, 1987a, 1992). The second-order kernels from the GB cells had structures similar to that of
kernels from the NB amacrine cells, independent of their generation by a spot or an annular stimulus. The initial depolarizing peak is one of the characteristics of the NB cell's second-order kernel (Sakai and Naka, 1987a, 1992). In both cells, the surround kernels had appreciably longer PRT. Figs. 3 B and 4 B show the sections of these second-order kernels made through their diagonals: the sections are the side views of the three-dimensional kernels cut through the diagonal line. In the two figures, the

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{example.png}
\caption{First-order kernels (A), second-order kernels (C and D), and diagonal sections of the second-order kernels (B) computed from post-synaptic potentials of a GB cell. (A) shows four kernels, the pair in solid lines was derived from two one-input experiments, whereas the pair in dashed lines was derived from a single two-input experiment. Kernels were opposite in terms of polarity in the two experiments; the kernels generated by the spot input were hyperpolarizing and the kernels generated by the annular input were depolarizing. The PRT of the annular kernels was longer than that of the spot kernel by 18 ms. (C and D) Second-order kernels generated by the spot and annular input. Sections of the two kernels along the diagonal line are shown in B, in which the solid line is for the spot and the dashed line is for the annular kernel. The two sections were superimposed by shifting the annular kernel by 18 ms. This superposition shows that the spot and annulus generated almost identical second-order components, the only difference being in the kernels' PRTs. The length of the abscissa is 25 x 10^{-6} mV \mu m^{-2} photon^{-1} for A, and 20 x 10^{-12} mV \mu m^{-4} photon^{-2} for B. The diagonal section of the surround kernel was normalized to match that of the center kernel.}
\end{figure}

The observations made so far lead to two conclusions: (a) the major part of the concentric receptive-field organization is carried by the DC (zeroth-order) and linear (first-order) components; and (b) the second-order components do not show a concentric receptive-field organization. The second-order components, as judged
from the signature of the second-order kernels, are similar, irrespective of whether they are generated by a spot or an annular stimulus. The major difference between second-order kernels generated by the receptive-field center and surround was the PRT. Similar conclusions were reported for the receptive-field organization of catfish N amacrine cells (Sakai and Naka, 1992).

We recorded data from 117 cells (37 GA and 80 GB cells). Of these cells, 75 cells produced N-type second-order kernels, 15 cells produced C-type second-order kernels, and 16 cells produced kernels that did not correspond to either type of kernel. 11 cells did not produce any well-defined second-order kernels. In those cells that generated well-defined second-order kernels in one- or two-input experiments, the polarity of the first-order kernels was always opposite whereas the second-order kernels were similar in their signature.

**Figure 5.** Spike discharges from a GA cell (A1–A3) and a GB cell (B1–B3) shown as post-stimulus time histograms. (A1 and B1) Responses produced by the simultaneous presentation of the spot and the annular stimulus. (A2 and B2) Responses produced by the pulsatile spot input in the presence of steady annular stimulation. (A3 and B3) Responses produced by the pulsatile annular input in the presence of steady spot illumination. The most obvious nonlinearity in the response evoked by the pulsatile input is rectification because spike discharges have no negative values. The transient off-response of the GB cell indicates another kind of nonlinearity.

**Spike Discharges**

Fig. 5 shows spike discharges, as post-stimulus time histograms, from a GA (Fig. 5 A) and a GB (Fig. 5 B) cell. The center-surround receptive-field organization is indicated either by an increase or a decrease in the frequency of spike discharges. We note that the responses to full-field illumination (Fig. 5 A1 and B1) were similar to the responses produced by spot illumination (Figs. 5, A2 and B2). The activity of receptive-field center depressed the response from the field's surround. We replaced the pulsatile inputs with modulation of the mean luminance by a white-noise signal. Cross-correlation was made between the input and resulting spike discharges, transformed into unitary pulses. Figs. 6 and 7 show the results of white-noise analysis performed on spike trains from a GA and a GB cell. In Figs. 6 A and 7 A, four first-order kernels are shown. The pair drawn with solid lines corresponds to the one-input experiments in which one input, either a spot or an annular input, was white-noised-modulated while the other input was held at a steady mean luminance; the other pair drawn with dashed lines corresponds to the two-input experiments in
which both the spot and the annular input were modulated by two independent white-noise signals. In both types of experiment, we observed that the kernels evoked by the spot and annular inputs were opposite in polarity, a result that demonstrated the concentric organization of the receptive field. For example, stimulation of the

![Figure 6](image)

**Figure 6.** First-order kernels (A), second-order kernels (C and D) and diagonal sections of second-order kernels (B) computed from spike discharges of a GA cell. (A) shows four kernels, the pair in solid lines was derived from two one-input experiments whereas the pair in dashed lines was derived from a single two-input experiment. Kernels were opposite in their polarity in the two experiments; the polarity of the kernels generated by the spot input was positive (an increase in the spike discharges) and that of the kernels generated by the annular input was negative (a decrease in spike discharges). The annular kernel in the two-input experiment was smaller in amplitude than the similar kernel in the one-input experiment. The PRT of the annular kernels was longer than that of the spot kernel by 20 ms. (C and D) Second-order kernels generated by the spot and annular inputs. The two kernels are similar in terms of their four-eye signature, an indication that the second-order nonlinearity originated in the C amacrine cells. Sections of the two kernels along the diagonal line are shown in B, in which the solid line is for the spot and the dashed line is for the annular kernel. The two sections were superimposed by shifting the annular kernel by 20 ms. This superposition shows that the spot and annulus generated almost identical second-order components, the only difference being in the kernels' PRTs. The length of the abscissa is $10^{-7}$ spikes $\mu$m$^{-2}$ photon$^{-1}$ s$^{-1}$ for A and $3 \times 10^{-14}$ spikes $\mu$m$^{-2}$ photon$^{-2}$ s$^{-2}$ for B. The diagonal section of the surround kernel was normalized to match that of the center kernel.

center either increased (in the GA cell in Fig. 6) or decreased (in the GB cell in Fig. 7) the discharges. We also observed that, in the two-input experiments, the kernels were often but not always smaller than they were in one-input experiments. In the case of the spike discharges, the center kernels were more robust as in the case of the
intracellular responses (Figs. 3 and 4). Figs. 6, C and D, and Figs. 7, C and D show second-order kernels generated by the spot and annular inputs. The kernels have similar signatures. In the case of the GA cell (Fig. 6), the kernels have a four-eye structure that is characteristic of the second-order kernels generated by the C amacrine cells (Sakai and Naka, 1987a, 1992). The kernels from the GB cell (Fig. 7) have a signature similar to that obtained from the NB amacrine cells. Thus, in both

![Figure 7](image)

**Figure 7.** First-order kernels (A), second-order kernels (C and D), and diagonal sections of the second-order kernels (B) computed from spike discharges of a GB cell. (A) shows four kernels, the pair in solid lines was derived from two one-input experiments whereas the pair in dashed lines was from a single two-input experiment. Kernels were opposite in terms of polarity in the two experiments; the kernels generated by the spot input were negative (a decrease in spike discharges) and the kernels generated by the annular input were positive (an increase in the spike discharges). The PRT of the annular kernels was longer than that of the spot kernel by 20 ms. (C and D) Second-order kernels generated by the spot and annular inputs. The structures of the two kernels are similar, with an initial depolarizing peak and elongation of the valley and hills whose axes are orthogonal to the diagonal line. Sections of the two kernels along the diagonal line are shown in B, in which the solid line represents the spot and the dashed line represents the annular kernel. The two sections were superimposed by shifting the annular kernel by 20 ms. The length of the abscissa is $2 \times 10^{-7}$ spikes $\mu$m$^{-2}$ photon$^{-1}$ s$^{-1}$ for A and $6 \times 10^{-14}$ spikes $\mu$m$^{-4}$ photon$^{-2}$ s$^{-2}$ for B. The diagonal section of the surround kernel was normalized to match that of the center kernel.

the GA and GB cells, two second-order kernels generated by the spot and annular stimulus are similar in their signature. The PRTs of these kernels differ, however. To clarify these differences we generated diagonal sections of the second-order kernels (Figs. 6 B and 7 B). The diagonal sections of the annular kernels are shown in dashed lines and are displaced laterally (along the time axis) by 20 ms. Two sections, one of the spot and the other of the annular second-order kernel, are superimposed on one
another for both the GA (Fig. 6 C) and the GB (Fig. 7 C) cell. We conclude that the
concentric organization of the receptive field was encoded, as in the case of PSPs, by
the polarity of the first-order components and that the second-order components
lacked such an organization. The waveforms of kernels shown in Figs. 3 and 4 (from
intracellular recordings) and in Figs. 6 and 7 (from spike discharges) are strikingly
similar. In our previous studies, we concluded that the analogue processes were
translated into point processes without any loss of information, so far as the first- and
second-order components of receptive fields were concerned (Sakuranaga et al.,
1987; Korenberg et al., 1989). The present study shows that receptive-field organiza-
tion is also translated, within the second-order approximation, into point processes,
spike discharges, with little loss of information.

Spike discharges evoked by pulsatile inputs given in the dark show a clear
rectification nonlinearity because there are no negative discharges (Fig. 5). If there is
a strong rectifying nonlinearity, there would be a large diagonal positive peak in
second-order kernels. The second-order kernels shown in Figs. 6 and 7 lack such a
large positive peak that indicates the presence of a rectifying nonlinearity. The
intracellular response and spike discharges produce similar second-order kernels as
shown in Figs. 3, 4, 6, and 7. It seems, therefore, that the strong rectifying
nonlinearity in the discharges evoked by the pulsatile stimulus seen in Fig. 5 is absent
or is much less strong in the discharges evoked by a modulation of a mean
luminance. Victor and Shapley (1979) made a similar observation in the cat retina.

Of 236 cells analyzed in terms of spike trains, a total of 131'cells produced N-type
second-order kernels, 58 cells produced C-type kernels, 14 cells produced kernels
that could not be classified as either C- or N-type kernels, and 33 cells did not
produce any well defined kernels. The average difference between the PRT of the
center and surround kernels was 21.1 ms ± 7.3 ms for the cells with an N-type
kernel, 21.0 ± 4.8 ms for the cells with a C-type kernel, and 21.0 ± 4.8 ms for the
remaining cells. In our previous paper, the difference between PRTs for the center
and surround kernels of the N amacrine cells was given as 21.0 ± 8.8 ms (n = 57).
Thus, the transport delay found in the surround of ganglion cells was similar to that
found in the N amacrine cells (Sakai and Naka, 1991).

Stability of Receptive-Field Organization

So far, we have shown that a single spike train can be decomposed into two linear
components that represent the concentric nature of the organization of the cell's
receptive field. This decomposition can be performed with spike trains produced by
stimuli that encompass a large range of mean luminance. Two examples are
presented in Fig. 8, which shows results from spike trains recorded from a GA (Fig.
8, A1 and A2) and a GB (Fig. 8, B1 and B2) cell. The mean luminance was varied over
a range of 5 log units for the GA cell and a range of 4 log units for the GB cell. Mean
luminance was controlled by interposing neutral-density filters between the light
source and the preparation. In these experiments, a pair of kernels was derived from
a single spike train at each mean luminance, and kernels were computed from the
unattenuated input signal and the resulting spike discharges. The kernels represent,
therefore, the contrast sensitivity of the receptive-field center and surround at each
luminance level (Naka, Chan, and Yasui, 1979). These kernels can be converted into
incremental sensitivity by multiplying their amplitude by a factor that corresponds to the attenuation at each neutral-density filter. Although the mean luminance was varied over a wide range, the kernel amplitude, contrast sensitivity, did not change much. The most extreme case was seen with the kernels for the GA cell's annular response (Fig. 8, A2); the amplitude of the kernel generated by the dimmest mean luminance, the kernel marked "4," was only one third of the amplitude of the kernel generated by the brightest mean luminance, the kernel marked "1." The difference in the mean luminance was 3 log units. As the mean luminance was decreased, the

Figure 8. First-order kernels from two-input experiments performed at five (in A1 and A2 for a GA cell) and four (in B1 and B2 for a GB cell) mean levels of luminance. These kernels were computed from spike trains. This figure shows that, over a large range of mean luminance, a single train of spike discharges carries simultaneously the signals from both the receptive-field center and the surround. Amplitudes of kernels plotted here on the contrast-sensitivity scale do not differ much in spite of the large changes in mean luminance. The Weber-Fechner relationship holds for both the center and the surround. The dynamics of the linear components change as the mean luminance changes. The changes are not consistent for each set of kernels. The numbers below indicate the extent of attenuation of light in log units. The abscissa for A1 and B1 is $20 \times 10^{-6} \text{spikes/um}^2 \text{photon}^{-1} \text{s}^{-1}$, and the abscissa for A2 and B2 is $12 \times 10^{-6} \text{spikes/um}^2 \text{photon}^{-1} \text{s}^{-1}$.

The kernels became slower and the PRT became longer. The extent of these changes differed from cell to cell as seen from the difference in the waveforms of two sets of kernels from two cells in Fig. 8. These findings show that the concentric organization the receptive field is retained over a wide range of mean luminance and that the contrast sensitivity of both the center and annular region is Weber-Fechner-like. Similar observations were made on the cat ganglion cells (Enroth-Cugell and Lennie, 1975).

The signature of the second-order kernels computed from a ganglion cell also
remained fairly constant over a large range of mean luminance. Examples from an intracellular recording are shown in Fig. 9, in which four second-order kernels for the receptive-field center, obtained at four mean luminance levels, namely, 0 to -3 log units, are shown. The kernels all have a four-eye signature. Note that to facilitate comparison of kernels obtained at four mean luminance, the kernels are plotted on different time axes; the kernel shown in A has time axes of 0.12 s whereas the kernel shown in D has a time axes of 0.3 s. Generation of a four-eye kernel can be modeled by a cascade, the Wiener structure (Fig. 17 in Sakai and Naka, 1987b). In this structure, the output of a dynamic linear filter is modified by a static nonlinear process. In the catfish retina, the process is equivalent to squaring. The fact that kernel signatures, although on different time scales, remained similar over a wide range of mean luminance shows that the changes in luminance had the greatest effect on the dynamics of the linear filter and not on the static nonlinear operation. The changes in the dynamics of the linear filter can probably be traced to the receptors (Naka, Itoh, and Chappell, 1987). Although the kernels shown here were computed from intracellular responses to obtain MSEs, a similar series of kernels could be obtained from a spike train and also from the surround of a receptive field. The linear and nonlinear components for the center and surround are encoded into a ganglion-cell response or into a single spike train over a large range of mean luminance.

![Figure 9. Stability of second-order nonlinearity over a large range of mean luminance. Second-order kernels were computed from post-synaptic potentials of a ganglion cell, obtained at four mean luminance. All four kernels have four-eye structures although the details differ from kernel to kernel. MSEs for the linear and second-order models are 81 and 62% for the 0 log record, 76 and 56% for the 1 log record, 74 and 64% for the 2 log record, and 92 and 83% for the 3 log record. Kernels were generated by a spot input in the presence of steady annular illumination. Note that the time scale differs between kernels.](image-url)
DISCUSSION

The ganglion cells represent the output stage of the retinal neuron network. The spike discharges generated in the cells are, therefore, the end results of complex processing of signals within the retina, and they are the only means by which the retina communicates with the brain. Because of their importance, the responses of the ganglion cells, and far more often, the spike discharges, have been the focus of numerous studies. Earlier reports on this subject were extensively reviewed by Shapley and Enroth-Cugell (1984) and newer results were reviewed by Kaplan (1991). Our present study is unique, we believe, because we have decomposed a cell's response, both the intracellular response and spike discharges, into two components, one generated by the center and the other by the surround, and we have further decomposed the center and surround components into their linear and nonlinear components. These components were compared to similar components from amacrine cells that had been described in a previous report (Sakai and Naka, 1992).

The Receptive Field of Ganglion Cells

In past studies of the catfish's ganglion cells we found that the kernels, computed either from intracellular responses or from spike discharges evoked by full-field illumination, were very similar to the kernels obtained from preganglionic cells (Sakai and Naka, 1987a). We also found that the center-surround organization of catfish N (sustained) amacrine cells was encoded by the linear component, whereas the nonlinear component had a monotonic field (Sakai and Naka, 1992). We have now shown in the present report that the center-surround organization of the ganglion cell is mostly encoded by the linear component, whereas the center and surround generate similar second-order components. There is a problem associated with the identification of the origin of the linear component of the ganglion cell's response that forms the concentric field. The bipolar cells and the linear component from the N amacrine cells both have concentric organization. We do not know the relative contributions of the bipolar and N amacrine cells to the ganglion cell's response. Davis and Naka (1980) found that the sizes of receptive fields of ganglion cells varied considerably from cell to cell; some had a small field, similar in size to the field of bipolar cells, whereas others had a large field, similar to that of N amacrine cells. The cells for which data are shown in Figs. 3, 4, and 7 had N-type second-order kernels. It is, therefore, reasonable to suppose that these cells were receiving some of the linear inputs from N amacrine cells. It is also possible that the cell for which data are shown in Fig. 6 and which produced a four-eye kernel was receiving a nonlinear input from C amacrine cells. In this cell, it is not likely that the cell's linear component was derived from bipolar cells. This is because the linear component in the C cell is usually small and noisy (Sakai and Naka, 1987a, 1992). Obviously, one future problem is to find the relationship between the nature and magnitude of second-order components of a ganglion cell and the cell's spatial organization. Use of a sinusoidal grating (in the space domain) or spatio-temporal white-noise in combination with identification of nonlinear components should yield important results. Here we have tacitly assumed that both linear and nonlinear components generated in N
amacrine cells were transmitted simultaneously to the ganglion cells. This assumption is supported by the finding from a current injection study which demonstrated that transmission of signals between amacrine and ganglion cell was linear, rapid, and bidirectional (Sakai and Naka, 1990).

The fact that the nonlinear components have a monotonic receptive field is consistent with our hypothesis that the C amacrine cell, which forms a monotonic field (Davis and Naka, 1980) and which forms a space (Naka and Christensen, 1981), is the source of primordial (second-order static) nonlinearity, which is transmitted to the ganglion cells either directly (to the cells with the four-eye nonlinearity) or through some transformation via N amacrine cells (to the cells with N-type nonlinearity). The monotonic receptive-field organization of C (on-off) amacrine cell has been well documented (Kaneko and Hashimoto, 1969; Sakai and Naka, 1992). In the past, we have identified the rapid transmission that is capable of transmitting nonlinearity with little modification, and a filter that transforms C-type nonlinearity into N-type nonlinearity was identified (Sakai and Naka, 1988a,b, 1990).

**Spike Analysis**

We have recovered, from the spike trains, first- and second-order kernels for the receptive-field center and surround. These kernels are very similar to those computed from the intracellular response (Sakai and Naka, 1987a,b). This similarity confirms our previous conclusions that the process of spike generation is approximately static and that intracellular potential (PSP) and spike discharges carry similar information, i.e., within a second-order approximation, there is no loss of information when the PSP is translated into spike discharges (Korenberg et al., 1989). It will be interesting to determine whether these conclusions can be applied to the ganglion cells of other species or even to the nervous system in general. The first- and second-order kernels recovered from the frog (Sakuranaga et al., 1987) and rabbit (Mangel, Sakai, and Naka, manuscript in preparation) are almost identical to those from the catfish retina. It is likely that spike trains from these species can be decomposed into center and surround components.

We have shown here that a second-order correlation recovered well defined second-order kernels from a spike train for the receptive-field center and surround over a large range of mean luminance (Fig. 9). Thus, information is carried by the relative timing of two spike discharges and such timing can only be revealed by second-order cross-correlation. Conversely, a spike can be generated by the nonlinear interaction between two points in the past stimulus. In the visual system, this type of information coding has only been noted by Victor and Shapley (1979) and ourselves. In the histogram approach used widely, information on the relative timing of two spike discharges, that generate the second-order kernel, is lost. Average or instantaneous firing frequencies provide only a very limited fraction of the information carried by a spike train. McClurkin et al. (1991) observed that neurons in the visual areas simultaneously carried multiple, stimulus-related messages by utilizing multiplexed temporal codes. This is exactly the case with the spike discharges from catfish ganglion cells.
Comparison with Results Obtained from Other Retinas

In the case of the higher vertebrates, spike trains from ganglion cells have been intensively analyzed (Kaplan, 1991). The idea of nonlinear subunits in the Y cells of the cat is well established (Victor and Shapley, 1979). The nonlinear subunits in the cat are similar to the C amacrine cells in the catfish in two respects: (a) it is a static nonlinearity that can be approximated by a rectifier or a squaring device; (b) the cell that generates such nonlinearity lacks a concentric field organization; and (c) the nonlinearity can be recovered from spike trains. In this context, two comments can be made; (a) The recovery of nonlinearity shows that the relative timing of each spike in a train is important and that the nonlinearity is transformed into a spike train without any major modification. (b) In the cat and the catfish, the generation of spike discharges is highly nonlinear but approximately static. The nonlinearities differ in so far as, in the cat, it is the subunits that generate nonlinearity whereas, in the catfish, it is the C amacrine cells that form a space (Naka and Christensen, 1981) and are extensively tracer coupled (Sakai and Naka, unpublished observation).

Pöppel and Eckerhorn (1981) observed the first-order kernels of opposing polarity from the center and surround of the concentric field in the cat retina. Pöppel and Eckerhorn (1981) noted that the latency of the surround response was longer than that of the center response by ~4–8 ms and that the surround response had a larger high frequency component.

Implications

The observations that we have made here have several implications with respect to the way we view the function of the neuron network in the retina.

(a) In the past, several attempts were made to define a receptive field in a joint domain of time and space. Receptive-field profiles were obtained by a first-order correlation between one-dimensional spatial white noise (Davis and Naka, 1980; Powers and Arnett, 1981) or a spatio-temporal white noise and the resulting spike discharges (Hida and Naka, 1982; Mizuno, Imai, Tsukada, Hida, and Naka, 1985; Reid and Shapley, 1992; Meister et al., 1994). These results could not have been obtained if the main features of field organization were not encoded by the linear component. Conversely, the results of the present study provide evidence in support for the use of a spatio-temporal input and first-order cross-correlation to delineate the spatial organization of a receptive field.

(b) All ganglion cells, with a few exceptions, produce a well-defined first-order kernel and the linear component of the intracellular response ranges from 20 to 80% in terms of MSE (Sakai and Naka, 1987a). Thus, even in the case of ganglion cells that receive nonlinear inputs from the C amacrine cells, linear components, probably from bipolar cells, are added to the nonlinearity. From an analysis of frequencies and the complexity hierarchy of neurons in the catfish retina, Korenberg, Sakai, and Naka (manuscript in preparation) made a similar observation. They found that the response from amacrine cells is very complex and contained the highest frequency component whereas the response from ganglion cells is less complex and its frequency component is lower. Korenberg et al. (1989) have attributed these observations to the fact that, in the ganglion cells, signals from a bipolar cell (which
produces a less complex and slower response) are added to the signals from amacrine cells. The fact that signals from bipolar and amacrine cells are combined in the ganglion cells puzzles us because the second-order nonlinearity is established in the C amacrine cells (such cells have only a very small linear component) from the linear input from bipolar cells.

(c) In this series of experiments, two linear components and two nonlinear components were measured from a single spike train. A spike train carries, therefore, multiple signals. We, the experimenters, know the characteristics of the two inputs and are able to decompose the spike trains, through the process of cross-correlation, into four components. For the catfish, however, the multiple signals must be decoded on line without reference to the input signals. In the natural habitat, visual input to the catfish retina is much more complex than, for example, white-noise modulation of a spot or an annulus of light. Fibers of the catfish optic nerve must carry very complex signals and the decoding of such signals must require a complex system, although Bialek and Rieke (1992) have proposed simple decoding algorithms. Recent results from multiple units recording also show that subtle timing between spike discharges carry information (Meister et al., 1994). As indicated by Bialek, Rieke, DeRuyter, van Steveninck, and Warland (1991), the real difficulty lies in the fact that we do not know how neurons process signals in time and space. The process of decomposing a response into a linear and a nonlinear component or into a center and surround component is artificial and it is possible that neurons in the catfish process signals in a manner that is incomprehensible to us. Our methodology has enabled us to identify the kind of information carried by a spike train, but the knowledge that we have obtained to date fails us when we try to understand the way in which the neuronal network functions (Sakai and Naka, 1992). More than 60 yr have passed since spike discharges were shown to carry sensory information to the brain (Adrian, 1931). Results of recent studies including this study, however, show that we still do not understand exactly how sensory information is encoded into a spike train (McClurkin et al., 1991; Bialek et al., 1991; Bialek and Rieke, 1992).

We thank Dr. Susan Stone for valuable comments on the manuscript and Hildred Machuca for editing the manuscript.

This research was supported by NEI grants EY07738 and EY08848, by NSF grants DIR871841 and BNS 891993, and by a grant from Research to Prevent Blindness to the Department of Ophthalmology, New York University Medical Center. K.I. Naka thanks Research to Prevent Blindness for his Jules and Doris Stein Professorship.

Original version received 17 August 1994 and accepted version received 18 January 1995.

REFERENCES

Adrian, E. D. 1931. Croonian Lecture. The messages in sensory nerve fibres and their interpretation. Proceedings of Royal Society of London B. 109:1-18.

Bernadete, E. A., E. Kaplan, and B. W. Knight. 1992. Contrast gain control in the primate retina. Visual Neuroscience. 8:483-486.

de Boer, E., and P. Kuyper. 1968. Triggered correlation. IEEE Transactions on Biomedical Engineering. 15:169-179.
Bialek, W., and F. Rieke. 1992. Reliability and information transmission in spiking neurons. *Trends in Neuroscience.* 15:428–434.

Bialek, W., F. Rieke, R. R. DeRuyter van Steveninck, and D. Warland. 1991. Reading a neural code. *Science.* 262:1854–1857.

Davis, G. W., and K.-I. Naka. 1980. Spatial organizations of catfish retinal neurons. 1. Single- and random-bar stimulation. *Journal of Neurophysiology.* 43:807–831.

Enroth-Cugell, C., and P. Lennie. 1975. The control of retinal ganglion cell discharge by receptive field surrounds. *Journal of Physiology.* 247:551–578.

Hida, E., and K.-I. Naka. 1982. Spatio-temporal visual receptive fields as revealed by Spatio-temporal random noise. *Z. Naturforsch.* 37c:1048–1049.

Hunter, I. W., and M. J. Korenberg. 1986. The identification of nonlinear biological systems: Wiener and Hammerstein cascade models. *Biological Cybernetics.* 55:135–144.

Kaneko, A., and H. Hashimoto. 1969. Electrophysiological study of single neurons in the inner nuclear layer of the carp retina. *Vision Research.* 9:37–55.

Kaplan, E. 1991. The receptive field structure of retinal ganglion cells in cat and monkey. In *The Neural Basis of Visual Function.* A. Leventhal, editor. Macmillan Publishing Co., London, 10–40.

Kaplan, E., and R. M. Shapley. 1986. The primate retina contains two types of ganglion cells, with high and low contrast sensitivity. *Proceedings of the National Academy of Sciences, USA.* 85:2755–2757.

Korenberg, M. J., H. M. Sakai, and K.-I. Naka. 1989. Dissection of the neuron network in the catfish inner retina. III. Interpretation of spike kernels. *Journal of Neurophysiology.* 61:1110–1120.

McClurkin, J. W., L. M. Optican, B. J. Richmond, and T. J. Gawne. 1991. Concurrent processing and complexity of temporally encoded neuronal messages in visual perception. *Science.* 253:675–677.

Marmarelis, P. Z., and K.-I. Naka. 1973. Nonlinear analysis and synthesis of receptive-field responses in the catfish retina. I. Horizontal cell to ganglion cell chain. *Journal of Neurophysiology.* 36:605–618.

Meister, M., J. Pine, and D. A. Baylor. 1994. Multi-neuronal signals from the retina: acquisition and analysis. *Journal of Neuroscience Methods.* 51:95–106.

Mizuno, M., S. Imai, M. Tsukada, E. Hida, and K.-I. Naka. 1985. A micro-computer system for spatio-temporal visual receptive field analysis. *IEEE Transactions on Biomedical Engineering.* 32:56–59.

Morgan, I. G. 1992. What do amacrine cells do? *Progress in Retinal Research.* 11:193–214.

Naka, K.-I., and N. R. G. Carraway. 1975. Morphological and functional identifications of catfish retinal neurons. I. Classical morphology. *Journal of Neurophysiology.* 38:53–71.

Naka, K.-I., R. Y. Chan, and S. Yasui. 1979. Adaptation in catfish retina. *Journal of Neurophysiology.* 42:441–454.

Naka, K.-I., and B. N. Christensen. 1981. Direct electrical connections between transient amacrine cells in the catfish retina. *Science.* 214:462–464.

Naka, K.-I., M.-A. Itoh, and R. L. Chappell. 1987. Dynamics of turtle cones. *Journal of General Physiology.* 89:521–537.

Pöppel, B., and R. Eckerhorn. 1981. Dynamic aspect of cat retinal ganglion cell's centre and surround mechanisms: a white-noise analysis. *Vision Research.* 21:1693–1696.

Powers, R. L., and D. W. Arnett. 1981. Spatio-temporal cross-correlation analysis of catfish retinal neurons. *Biological Cybernetics.* 41:179–196.

Reid, R. C., and R. M. Shapley. 1992. Spatial structure of cone inputs to receptive fields in primate lateral geniculate nucleus. *Nature.* 356:716–718.

Sakai, H. M., and K.-I. Naka. 1987a. Signal transmission in the catfish retina. IV. Transmission to ganglion cells. *Journal of Neurophysiology.* 58:1307–1328.

Sakai, H. M., and K.-I. Naka. 1987b. Signal transmission in the catfish retina. V. Sensitivity and circuit. *Journal of Neurophysiology.* 58:1329–1350.
Sakai, H. M., and K.-I. Naka. 1988a. Dissection of the neuron network in the catfish inner retina. 1. Transmission to ganglion cells. *Journal of Neurophysiology.* 60:1549–1567.

Sakai, H. M., and K.-I. Naka. 1988b. Dissection of the neuron network in the catfish inner retina. II. Interactions between ganglion cells. *Journal of Neurophysiology.* 60:1568–1583.

Sakai, H. M., and K.-I. Naka. 1990. Dissection of the neuron network in the catfish inner retina. IV. Bidirectional interactions between amacrine and ganglion cells. *Journal of Neurophysiology.* 63:105–119.

Sakai, H. M., and K.-I. Naka. 1992. Response dynamics and receptive-field organization of catfish amacrine cells. *Journal of Neurophysiology.* 67:430–442.

Sakai, H. M., J. L. Wang, and K.-I. Naka. 1995. Contrast gain control in the lower vertebrate retinas. *Journal of General Physiology.* 105:815–835.

Sakuranaga, M., Y. Ando, and K.-I. Naka. 1987. Dynamics of ganglion cell response in the catfish and frog retina. *Journal of General Physiology.* 90:229–259.

Sakuranaga, M., and K.-I. Naka. 1985. Signal transmission in the catfish retina. 1. Transmission in the outer retina. *Journal of Neurophysiology.* 53:373–389.

Schellart, N. A. M., and J. Spekreijse. 1972. Dynamic characteristics of retinal ganglion cell response in goldfish. *Journal of General Physiology.* 59:1–21.

Shapley, R. M., and C. Enroth-Cugell. 1984. Visual adaptation and retinal gain controls. *Progress in Retinal Research.* 3:263–343.

Shapley, R. M., and J. D. Victor. 1978. The effect contrast on the transfer properties of cat retinal ganglion cells. *Journal of Physiology.* 285:275–298.

Victor, J. D. 1987. The dynamics of the cat retinal X cell centre. *Journal of Physiology.* 386:219–246.

Victor, J. D. 1988. The dynamics of the cat retinal Y cell subunit. *Journal of Physiology.* 405:289–320.

Victor, J. D., and R. M. Shapley. 1979. The nonlinear pathway of Y ganglion cells in the cat retina. *Journal of General Physiology.* 74:671–687.