ON and OFF Channels of the Frog Optic Tectum Revealed by Current Source Density Analysis

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Nakagawa, Hideki and Nobuyoshi Matsumoto. ON and OFF channels of the frog optic tectum revealed by current source density analysis. J. Neurophysiol. 80: 1886–1899, 1998. The spatiotemporal patterns of excitatory synaptic activity in response to diffuse light ON and OFF stimuli were examined by means of current source density (CSD) analysis. The qualitative and quantitative analyses obtained from 24 depth profiles for each stimulus revealed obviously different distributions of synaptic activity in the laminar structure. Two or three dominant current sinks I, II, and III were evoked in response to diffuse light ON stimulation. Sink I was observed at the bottom of the retinorecipient layer. Both sinks II and III, showing an identical spatial pattern, were observed just above sink I. On the other hand, diffuse light OFF stimulation elicited up to six current sinks IV, V, VI, VII, VIII, and IX. Sink IV was observed at the bottom of the retinorecipient layer. Sink V was observed in the most superficial layer. Both sinks VI and VIII were located between the two preceding sinks. Finally, sinks VII and IX occurred below the retinorecipient layer. Five electrically evoked current sinks A, B, C, D, and E, characterized in our previous study, were also recognized in the present quantitative analysis. A statistical analysis revealed that, in visually evoked responses, statistical differences in the spatial distribution were not present between sinks I and IV, and sinks II and VIII (P < 0.05). The analysis also showed that, in electrically evoked responses, only a pair of sinks C and E exhibit virtually identical spatial distribution (P < 0.05). Based on well-known properties of the retinal ganglion cells, possible neuronal mechanisms underlying each of current sinks in the ON and OFF channels and their functional meanings were considered. Sink I reflects the excitatory monosynaptic activity derived from R3 retinal ganglion cells. Sink IV reflects the excitatory monosynaptic activity derived from both R3 and R4 cells. Sinks V, VI, VII, and IX may be composed of successive polysynaptic excitatory potentials derived from convergence of inputs from both R3 and R4 cells. We concluded that the early four sinks play in particular an important role in eliciting avoidance behavior. On the other hand, sinks II, III, and VIII reflect excitatory synaptic activities derived from ON-OFF retinal fibers of another type having slow conduction velocity. These late current sinks were suggested to mediate prey catching and its facilitation.

INTRODUCTION

Physiological, anatomic, and behavioral studies have shown that different features or cues in a visual stimulus are transmitted through segregated pathways and processed through a series of hierarchically organized visual cortical areas. Thus it is well-known that individual cortical areas have different functions in the analysis of visual information (Van Essen and Gallant 1994). On the other hand, how visual information is processed and how functional properties of the cortical neurons are established in individual cortical areas, even in the primary visual cortex, still remain to be demonstrated.

A powerful approach to address these fundamental questions is the analysis of the spatiotemporal features of synaptic currents by using a current source density (CSD) analysis (Freeman and Nicholson 1975; Nicholson and Freeman 1975). In mammals, especially cat and monkey, combined with nonselective electrical stimulation of the optic nerve or optic tract, this method has revealed the principal neuronal organization related to visual information processing (Freeman and Singer 1983; Mitzdorf and Singer 1977–1980). Moreover, in some cases, the neuronal activity in response to visual stimuli has also been examined by this method (Givre et al. 1995; Mitzdorf 1985, 1986). These comprehensive analyses raise an important question: how are the neuronal responses to different features in a visual stimulus represented in the distribution of current sinks and sources.

In the cat and the monkey, typical retinal ganglion cells have concentric ON and OFF receptive fields. In the monkey retina, they can be grouped in two main categories, M and P (Kaplan and Shapley 1986; Shapley and Perry 1986). In the cat, on the other hand, they can be divided into X, Y, and W groups (Enroth-Cugell and Robson 1966; Wässle et al. 1981). These classifications were based on both anatomic and physiological criteria. The latter involves the size of the receptive field and the sensitivity to moving stimuli. Thus, in these animals, it is impossible to activate the specific input channels by means of visual stimuli that elicit synchronous activation of homogeneous neuronal populations in two dimensions. This is one of the prerequisites for applicability of one-dimensional CSD analysis. It is the reason why the input-specific synaptic activity was deduced mainly by conduction velocity of the primary afferents and the latency of the current sinks in the previous CSD studies.

In contrast to cats and monkeys, the retinal ganglion cells in the frog have more elaborated receptive fields. They are generally classified into four main categories (R1, R2, R3, and R4) and an additional slow ON-OFF class, based on their characteristic response patterns to specific features of visual stimuli. Both R1 and R2 ganglion cells give a sustained discharge when a moving target is suddenly stopped within the receptive field but no discharge to diffuse light ON and OFF stimuli. R3 and slow ON-OFF ganglion cells give a phasic discharge to diffuse light ON and OFF stimuli and do not discharge to a stationary target. Finally, R4 ganglion cells respond to diffuse light OFF stimuli with a long-lasting firing (Lettvin et al. 1959; Maturana et al. 1960; Witparr and Keurs 1975). This shows that diffuse light ON stimuli elicits...
only R3 and slow ON-OFF specific neuronal activity, whereas diffuse light OFF stimuli elicits a combination of activity derived from R3, R4, and slow ON-OFF cells.

We have shown in the frog tectum that CSD analysis is a very powerful tool to analyze not only visual input pathways but also the flow of visual information processing including polysynaptic activity within and between tectal layers (Nakagawa et al. 1997). Therefore the CSD analysis of tectal field potentials to diffuse light ON and OFF stimuli can demonstrate the spatiotemporal pattern of synaptic activity derived from only R3, R4, and slow ON-OFF ganglion cells. In our previous work using electrical stimuli of the optic tract, we found five prominent current sinks (A, B, C, D, and E) in the optic tectum (Nakagawa et al. 1997). Comparing the results from the previous and the present experiments, we now consider which current sinks observed in the previous work are derived from these types of ganglion cells.

One purpose of this study is to examine whether, in the same tectal area, different visual stimuli will elicit different spatiotemporal patterns of synaptic activity. Another purpose is to obtain fundamental building blocks for interpretation of the response patterns to more complicated natural visual stimuli. Finally, based on the present results, the neuronal mechanisms underlying visually guided prey-catching and avoidance behavior are proposed in terms of activity of the tectal neuronal populations.

**Methods**

**Animals**

Adult frogs, *Rana catesbeiana*, of either sex, measuring 11–13 cm in length, were obtained commercially and kept in laboratory tanks before use. The animals were fed on chicken liver twice a week.

**Preparations**

Experimental animals were anesthetized with ether, immobilized by injecting suxamethonium chloride (Succin, Yamanouchi; 0.02 mg/g body wt) subcutaneously and then fixed on a metal frame. After infiltration of local anesthetic (Xylocaine, Fujisawa) on the skin of the head, the left optic tectum was exposed by carefully removing a piece of bone with a drill and by retracting the dura mater and the arachnoid with fine scissors and forceps. The left, nonstimulated eye was covered by an opaque occluder. A ground lead was attached to the skin of the animal, and the recording electrode was connected and OFF stimuli were obtained. The recording electrode was inserted orthogonal to the tectal surface where large visual responses were obtained. The recording electrode was connected to a high-input impedance d.c. amplifier (MEZ-7200, Nihon-Kohden), and the amplified signals were fed to a computer display (FlexScan 76F, NANAO) together with field potentials for averaging analysis.

**Recording of evoked potentials**

Twenty-four penetrations were made in the optic tectum in 16 frogs. Evoked potentials in the optic tectum were recorded with microtip electrodes filled with 2 M NaCl. To avoid closely approaching single-unit somata, the pipette tips were broken to ~10 μm diam under a microscope. In later experiments, the pipette tips were bevelled for easier penetration with an EG-40 beveler (Narishige). The electrode resistance was ~500 kΩ. The recording electrode was inserted orthogonal to the tectal surface where large visual responses were obtained. The recording electrode was connected to a high-input impedance d.c. amplifier (MEZ-7200, Nihon-Kohden), and the amplified signals were fed through a band-pass filter from 3 Hz to 3 kHz. In 10 penetrations, to remove nonvisual spontaneous deflection of the recording trace, field potentials were recorded by differential mode. In this case, one recording electrode was placed in the cerebrospinal fluid around the tectum, and the other one was in the tectum. This difference in methodology did not affect the results. Laminar profiles of field potentials were obtained by recording potentials every 20 μm, to a depth of 800 μm from the tectal surface.

**CSD method and data processing**

Ten or 20 consecutive recordings at each recording site were averaged. From the set of 41 field potentials per trajectory, CSD depth profiles were computed with a UNIX computer (TITAN 1300 MP). We applied the computational algorithm described by Mitzdorf and Singer (1978). It is based on the assumption that the extracellular space has the properties of an Ohmic conductor, and the electrical field is quasi-static. Because, in the optic tectum, the field potentials show distinct changes only normal to the layers (Freeman et al. 1980), one-dimensional (z-axis) CSD depth profiles were computed by assuming that the tissue conductivity is constant (Freeman et al. 1980; Mitzdorf and Singer 1980; Nicholson 1973; Rappelsberger et al. 1981; Vanegas et al. 1979). In our previous study, we showed that the assumption of constant conductivity is valid in the frog optic tectum (Nakagawa et al. 1997).

CSD profiles can be calculated according to the following formula

\[
\frac{\partial^2 \Phi}{\partial z^2} = \Phi(z + n\Delta z) - 2\Phi(z) + \Phi(z - n\Delta z) \frac{1}{(n\Delta z)^2}
\]

where \( \Phi \) is the extracellular field potential, \( z \) is the coordinate perpendicular to the layers, \( \Delta z \) is the sampling interval (20 μm in the present study), and \( n \Delta z \) is the differentiation grid. Systematic variation of the numerical differentiation grid used to compute the CSD according to the above formula indicated that a grid of 80 μm provided the best compromise between spatial resolution and signal-to-noise ratio. We used an extrapolation method to obtain a full description of the CSD distribution (Vaknin et al. 1988). In the following CSD profiles, current sinks are indicated by upward deflections (filled) and current source by downward deflections (opened).

To quantitatively evaluate the spatiotemporal pattern of current sink and source obtained from more than one penetration, each CSD depth profile was subjected to the following analysis. 1) The onset of the first current sink in a CSD depth profile was defined as time = 0. 2) The largest amplitude sink in the depth profile was detected. 3) Sink amplitude was sampled at 1-ms intervals. These values were compared with a given threshold level (i.e., 15% of the largest amplitude sink). The occurrence of the sink was represented as the number of 1-ms bins across all CSDs in each depth exceeding 15% of maximum sink amplitude in the temporal window under consideration. Thus the result was presented as a function of time and depth (Fig. 1). 4) Procedures 2) and 3) were also applied to current sources. 5) The results obtained from all
penetrations were collected and displayed as three-dimensional surface plots. Histograms of the occurrence of detected sinks and sources at the specific time window were used for a statistical analysis, the Lapage test, a combination of Wilcoxon rank sum test, and Ansari-Bradley test ($P < 0.05$). In the following text and tables, "N" is the sum of the occurrences in the histogram that represents the spatial distribution of a specific sink or source. The spatial distribution is demonstrated as mean ± SD of the histogram.

RESULTS

Twenty-four field potential and CSD depth profiles for each of diffuse light ON and OFF stimulations were obtained.

FIG. 2. A: field potentials evoked by diffuse light ON stimuli at successive depths in steps of 20 μm in the frog optic tectum. Each sweep is an average of 20 responses. B: current source densities (CSDs) evoked by diffuse light ON stimuli in the optic tectum, calculated from the field potentials of A. Here, and in Fig. 5B, for each individual trace, an upward deflection (filled) means sink and a downward deflection (open) means source. The former reflects excitatory postsynaptic activity, whereas the latter reflects passive current leaving the cell at proximal and distal membrane sites. The bottom CSD profile is the sum of all CSD traces from the tectal surface to 800 μm. The conductivity is assumed constant and is ignored in the calibrations. Three current sinks, I, II, and III are located exclusively in the retinorecipient layer. Numerals on the left show distance from the tectal surface in micrometers. Arrows show the onset of visual stimulation. Calibration: vertical, 0.5 mV; horizontal, 20 ms (in A), vertical, 250 mV/mm²; horizontal, 20 ms (in B).
Although the evoked potentials to diffuse light stimuli were much less stable than those to electrical stimuli of the optic tract (Nakagawa et al. 1997), the shapes of these field potentials were similar to those described in previous studies using the toad (Gernert and Ewert 1995; Schwippert et al. 1996). These field potentials were subjected to one-dimensional CSD computation to reveal the spatiotemporal distribution of synaptic activity underlying each of diffuse light ON and OFF responses.

Activity pattern of the optic tectum in response to diffuse light ON stimulation

For diffuse light ON stimulation, the CSD pattern varied only slightly in 24 different penetrations. Each of the 24 sets of CSDs showed the identical basic spatiotemporal pattern with two or three dominant current sinks being located in the retinorecipient layer. The typical CSD depth profile is shown in Fig. 2B, which was calculated from the evoked potentials represented in Fig. 2A. We first describe this CSD profile qualitatively and then note the results from the quantitative analysis of 24 CSD profiles.

The earliest sink (I in Fig. 2B) occurred at a depth corresponding to the deepest part of the retinorecipient layer. This sink has a short duration, with corresponding sources located above and below it. The second sink (II in Fig. 2B) occurred just above sink I, but was less coherent than sink I. The sink drew its current exclusively from a source that was located below. In Fig. 2B, sink II was followed by a distinctive current sink III (III). The duration and spatial distribution of sink III were identical to those of sink II. The spatial extent of the corresponding source was also identical in both current sinks.

In the quantitative analysis, sink I can be recognized easily as the highest peak I in Fig. 3. Although the onset latency of sink I varied considerably (range ~50–120 ms) depending on the animal, the duration was more constant (23.2 ± 3.9 ms, mean ± SD, n = 22). At 10 ms, this sink reached a peak amplitude at a depth of 320 ± 70 μm (N = 566, I in Fig. 4A), with a vertical extent ranging from 60 to 100 μm in almost every CSD depth profile (23/24). At the same time, a bimodal distribution of source corresponding to sink I can be recognized (I′ in Fig. 4B, Table 1).

Sink II was observed clearly in 20 experiments. Sink II occurred as a second peak following sink I by ~40 ms (II in Fig. 3). The height of this peak was lower than that corresponding to sink I described above, and moreover, the duration was much longer than that of sink I. These properties reflect the following observations about sink II in each penetration. 1) The amplitude of sink II was smaller than that of sink I. In four penetrations, sink II was not observed. 2) The onset latency of sink II and the delay from the onset of sink I varied considerably, depending on the animal. The former ranged from ~90 to 200 ms, and the latter ranged from ~40 to 110 ms, respectively. 3) In contrast with sink I, the duration of sink II was also variable (39.3 ± 13.4 ms, n = 18). At 75 ms, the maximum amplitude of this sink occurred at a depth of 220 ± 50 μm (N = 185, II in Fig. 4A), with a vertical extent ranging from 60 to 100 μm in almost every profile (19/20). Simultaneously, the bimodal distribution of the source corresponding to sink II can be recognized (II′ in Fig. 4B, Table 1).

Sink III was observed in only five penetrations. Because of the small number of observations, sink III must contribute minimally, if at all, to the second peak plotted in Figs. 3 and 4.

Activity pattern of the optic tectum in response to diffuse light OFF stimulation

In contrast to diffuse light ON stimuli, diffuse light OFF stimuli elicited a much more variable spatiotemporal pattern of CSDs. Each of the 24 CSD profiles showed similar spatiotemporal patterns, with up to 4 current sinks in the retinorecipient layer and 1 or 2 sinks below that layer. A typical CSD...
A: histograms of occurrence of diffuse light ON-evoked current sinks in successive time every 5 ms. Two peaks are labeled according to the corresponding sinks. The 1st labeled at 10 ms represents sink I. The 2nd labeled at 75 ms represents sink II.

B: histograms of occurrence of diffuse light ON-evoked current sources in successive time every 5 ms. Peaks are labeled according to the corresponding sources. I' labeled at 10 ms represent sources above and below sink I. II' labeled at 75 ms represent those corresponding to sink II.

The depth profile is shown in Fig. 5B, which was calculated from the evoked potentials demonstrated in Fig. 5A. Again, we first discuss this CSD profile qualitatively.

The depth of the earliest prominent sink (IV in Fig. 5B) almost overlapped the depth of sink I (see Activity pattern of the optic tectum in response to diffuse light ON stimulation). This sink lasted a very short time. The corresponding sources were located above and below the respective sink. The second and third sinks (V and VI in Fig. 5B, respectively) occurred at a depth corresponding to the intermediate part of layer 9. Both sinks lasted slightly longer than sink IV. The source corresponding to sink V occurred above the respective sink, whereas that for sink VI occurred below. The fourth sink had a small amplitude (VII in Fig. 5B) and occurred below the retinorecipient layer, especially layer 7. This sink was much less coherent than sink IV. Its corresponding small sources were observed above and below.

The depth of the fifth sink (VIII in Fig. 5B) overlapped those of sinks II and III (see Activity pattern of the optic tectum in response to diffuse light ON stimulation). This sink was also less coherent than sink IV. The corresponding sources were located above and below the respective sink. A final long-lasting sink (IX in Fig. 5B) was observed below the retinorecipient layer. It occurred at a depth that corresponds to the depth of sink VII. This sink was much less coherent than any preceding sinks. The corresponding small sources were observed above and below, the one above being much more prominent.

The results from the quantitative analysis of 24 CSD profiles are as follows. Sink IV is the highest peak in Fig. 6 (IV). As with sink I, the duration of sink IV was quite constant (18.8 ± 2.8 ms, mean ± SD, n = 24), although the onset latency varied considerably depending on the animal (range: ~30–80 ms). At 5 ms, sink IV reached a peak amplitude at a depth of 330 ± 70 µm (N = 538, IV in Fig. 7A), with a vertical extent ranging from 60 to 100 µm in 19 of 24 CSD depth profiles. Simultaneously, the bimodal distribution of the source corresponding to sink IV can be recognized (IV' in Fig. 7B, Table 2).

In many cases, it was very difficult to segregate current sinks V and VI. Therefore, usually, it was not feasible to characterize the spatiotemporal properties of the two sinks precisely in each penetration. However, quantitative analysis revealed two obvious peaks corresponding to sinks V and VI, respectively. Sink V occurred as a second peak in the most superficial layer, following that of sink IV by ~10 ms.
FIG. 5. A: field potentials evoked by diffuse light OFF stimuli at successive depths in steps of 20 μm in the frog optic tectum. Each sweep is an average of 20 responses. Recordings were obtained from the same preparation as in Fig. 2. B: CSDs evoked by diffuse light OFF stimuli in the optic tectum, calculated from the field potentials of A. Four current sinks IV, V, VI, and VIII are located in the retinorecipient layer, whereas 2 sinks VII and IX are below the retinorecipient layer. In A and B, arrows indicate the onset of visual stimulation. Numerals on the left show distance from the tectal surface in micrometers. Calibration: vertical, 0.5 mV; horizontal, 20 ms (in A); vertical, 250 mV/mm²; horizontal, 20 ms (in B).

(V in Fig. 6). Sink VI occurred as a third peak between the two previous prominent peaks described above. The peak followed that of sink V by 5 ms (VI in Fig. 6). The height of the two peaks was slightly lower than, and the durations slightly longer than, that of the peak representing sink IV. Although it was very difficult to characterize these current sinks in each penetration, the results from the quantitative analysis revealed the following properties. 1) Their amplitudes were usually smaller than that of sink IV. Alternatively, they could not be elicited in some penetrations. 2) Their durations were slightly longer than sink IV. 3) Their onset latencies and delay from the onset of sink IV were rather constant. Thus these responses were relatively stable. At 20 ms, sink V reached a peak amplitude at a depth of 170 ±...
110 μm (N = 704, V in Fig. 7A). On the other hand, at 30 ms, the maximum amplitude of sink VI occurred at a depth of 250 ± 110 μm (N = 514, VI in Fig. 7A). The bimodal distribution of current sources corresponding to sinks V and VI could be recognized (V′, VI′ in Fig. 7B, Table 2). Although the amplitude was usually very small, sink VII was observed in all penetrations. Sink VII occurred as a fourth, very small and broad peak following sink IV by ~30 ms (VII in Fig. 6). This peak reflects the following observations about sink VII in each penetration. 1) The amplitude of sink VII was much smaller than those of sinks IV, V, and VI in the retinorecipient layer. 2) The onset latency of sink VII varied considerably depending on the animal (range ~60–120 ms). On the other hand, the delay from the onset of sink IV was rather stable (35.3 ± 9.2 ms, mean ± SD, n = 24). 3) The duration of sink VII appeared to be quite variable and ranged from ~30 to 70 ms. At 45 ms, the maximum amplitude of this sink occurred at a depth of 350 ± 100 μm (N = 289, VII in Fig. 7A), with a vertical extent ranging from 60 to 160 μm in different penetrations. Simultaneously, although their amplitudes were very small, the bimodal distributions of sources corresponding to sink VII were recognized (VII′ in Fig. 7B, Table 2).

Sink VIII was recognized as the fifth, latest small peak following sink IV by ~60 ms (VIII in Fig. 6). This peak reflected the small amplitude of sink VIII in each penetration. It also reflected the fact that this sink could not be observed in five penetrations. The onset latency of sink VIII and the delay from the onset of sink IV varied depending on the animal. The former ranged from ~90 to 180 ms, and the latter ranged from ~60 to 100 ms, respectively. The duration of this sink was also variable (35.0 ± 10.4 ms, mean ± SD, n = 19). At 85 ms, sink VIII reached a peak amplitude at a depth of 220 ± 60 μm (N = 160, VIII in Fig. 7A), with a vertical extent ranging from 60 to 100 μm in 17 of 19 CSD depth profiles. Simultaneously, the bimodal distributions of sources corresponding to sink VIII were recognized (VIII′ in Fig. 7B, Table 2).

Because of the small amplitude and the small number of observations, sink IX could not be recognized in the quantita-
tive analysis in Figs. 6 and 7. Although it was very difficult to characterize this sink even in each penetration, it was recognizable in 11 depth profiles. The onset latency and the delay from the onset of sink IV both varied considerably. The former ranged from ~100 to 200 ms, whereas the latter ranged from ~60 to 130 ms. The duration of this sink was also variable (66.6 ± 25.0 ms, mean ± SD, n = 11). The vertical extent of sink IX ranged from 60 to 200 μm. The corresponding source below sink IX was not observed in seven penetrations.

Quantitative analysis of five current sinks elicited by electrical stimulation of the optic tract

In our previous report, we characterized five current sinks in response to electrical stimuli of the optic tract. To compare synaptic events in visually and electrically evoked responses, the same quantitative analysis was applied to six CSD depth profiles obtained in the previous study (Nakagawa et al. 1997). The previously characterized five current sinks could be easily recognized in Fig. 8. In the retinoreceptive layer, three major current sinks were observed. Sink A occurred as the earliest small peak (A in Fig. 8). Sink B occurred as a second peak in the most superficial layer. This followed sink A by 2 ms (B in Fig. 8). Sink D occurred as the fourth peak between the two previous peaks described above, and followed that representing sink B by 6 ms (D in Fig. 8). On the other hand, below the retinoreceptive layer, two prominent sinks were observed. Sink C occurred as a third peak that followed that representing sink A by 4 ms (C in Fig. 8). Finally, sink E occurred as a fifth long-lasting peak following that representing sink C by 8 ms (E in Fig. 8).

The histograms in Fig. 9 show spatial distributions of current sinks and sources at the specific times. At 2 ms, the spatial distribution of sink A was 380 ± 50 μm (N = 26, A in Fig. 9A). At 2 ms, the distribution of sink B was 140 ± 40 μm (N = 55, B in Fig. 9A). At 10 ms, that of sink D was 210 ± 70 μm (N = 106, D in Fig. 9A). The distribution of sink C was 460 ± 30 μm (N = 35, C in Fig. 9A) at 8 ms. Finally, at 22 ms, the distribution of sink E was 460 ± 50 μm (N = 51, E in Fig. 9A). Distributions of sources corresponding to the five sinks described above at the specific time are demonstrated in Fig. 9B (A’, B’, C’, D’, and E’) and summarized in Table 3.

DISCUSSION

In the frog optic tectum, the present CSD analysis revealed characteristic spatiotemporal patterns of current sink and source responses to diffuse light stimuli. As in previous studies, all current sinks were assumed to be current derived from excitatory postsynaptic potentials (EPSPs) and to represent excitatory activity of neuronal populations unless otherwise noted (Mitzdorf 1985; Mitzdorf and Singer 1978; Nakagawa et al. 1997).

In many experiments, sinks generated in response to electrical stimuli are preceded by small and short-lasting sinks. The preceding sinks are related to presynaptic activity in the terminals of retinal ganglion cells in the cat (Mitzdorf and Singer 1977, 1978) and in the frog (Freeman 1977; Freeman et al. 1980; Nakagawa et al. 1997). However, in contrast, no current sink was preceded by such a small sink in CSD depth profiles to visual stimulation. This results from the fact that the activity of retinal ganglion cells is much less coherent in the visually evoked response than in the response to electrical stimuli (Grtisser and Grtisser-Cornelhs 1976; Schwippert et al. 1996; Witpaard and Keurs 1975). This is also consistent with the observation that the durations of sinks in the visually evoked response were much longer than those in the electrically evoked response [e.g., the mean ± SD of the duration of sink I and sink A were 23.2 ± 3.9 ms (n = 22) and 3.7 ± 1.6 ms (n = 7), respectively]. This study also shows that the deep current sinks below the retinoreceptive layer can be recognized not only in electrically
but also in visually elicited responses. Thus we can exclude the possibility that the deep sinks are artifacts due to unexpected activation of nonoptic fibers located close to the stimulating electrode.

**Correlations between current sinks observed in the frog optic tectum**

CSD profiles usually show relative variability depending on the preparation as a result of the relatively high intrinsic noise level (Mitzdorf and Singer 1978; Nakagawa et al. 1997). Moreover, the response to visual stimuli is often much less stable than that to electrical stimuli (Arieli et al. 1996; Gernert and Ewert 1995; Schwippert et al. 1996). Several methods for enhancement of the signal-to-noise ratio have been employed (Freeman and Nicholson 1975; Rodriguez and Haberly 1989). However, these methods focused on reduction of noise in potential profiles in each penetration. No attempt has been made to evaluate quantitatively CSDs obtained from more than one penetration. Our method for quantitative analysis (see METHODS) enables us to describe the spatiotemporal distribution of CSDs precisely and objectively. At first, using this procedure, correlations between sinks elicited by visual stimuli are examined. Seven sinks, I, II, IV, V, VI, VII, and VIII, could be subjected to a statistical analysis. A Laplace test revealed no statistical differences in the spatial distribution between sinks I and IV, and sinks II and VIII (P < 0.05). This suggests that sinks I and IV, and sinks II and VIII were generated by the same excitatory synaptic activity, respectively. Next, correlations were examined among five current sinks, A, B, C, D, and E, observed in the responses to electrical stimuli. The statistical analysis shows that only one set of sinks, C and E, exhibited virtually identical spatial distributions (P < 0.05). Again, it is very likely that sinks C and E were generated by the same excitatory synaptic response.

Electrical stimuli should activate all types of retinal ganglion cells nonselectively. This implies that the sink components observed in the visual response were involved in the CSD depth profile to electrical stimuli. However, between the responses to visual and electrical stimuli, our quantitative analysis could not reveal any set of sinks showing no statistical difference in distribution (P < 0.05). Possible explanations for this unexpected result are the following. The first is the greater temporal dispersion in visually evoked versus electrically evoked responses. We could attribute this to either or both of the following: intraretinal delay for the light-evoked responses, and the increase in conduction distance from the optic tract to the retina. Depending on light intensity, the latency and spike patterns of a retinal ganglion cell varied considerably between responses. Latencies also depend on adaptation and room temperature (Aho et al. 1993a,b; Varjú and Pickering 1969). A latency in the order of 50–100 ms was reported in the frog retinal ganglion cells in response to flashes of light (Varjú and Pickering 1969). A latency in the order of 50–100 ms was reported in the frog retinal ganglion cells in response to flashes of light (Varjú and Pickering 1969). Second, the electrical stimulation also activated other retinal ganglion cells than those excited in the visual response. Thus, although the discrepancies in timing could be accounted for by the physiology of the frog retina and the increase in conduction distance, it is very difficult to identify equivalents of each visually elicited sink in the electrically evoked CSDs in the previous study. To clarify the correspondence, we will compare the visually evoked CSD profile in the present study with that obtained with the electrical stimuli of the optic nerve head, to eliminate the effect of the increase in conduction distance (unpublished observations).

**Physiology and tectal termination of the frog retinal ganglion cells**

Retinal ganglion cells in frogs have been divided into four main groups (R1, R2, R3, and R4), whose response properties are well characterized. Furthermore, Witpaard and Keurs (1975) reported an additional population of retinal ganglion cells that were named slow ON-OFF units. Here, physiological and morphological characteristics of these ganglion cells are summarized. Measurements of conduction velocity in single fibers showed that R1 and R2 have nonmyelinated axons that conduct at speeds of ≤0.5 m/s, whereas R3 and R4 have myelinated axons conducting at speeds from 1 to 8 m/s, the fastest being those of R4 class (Maturana et al. 1960). Although the conduction velocity of slow ON-OFF axons was not examined quantitatively, it was shown to be comparable with those of R1 and R2 (Witpaard and Keurs 1975). Thus the slow ON-OFF group is also thought to have nonmyelinated axons. Both R1 and R2 show no response to diffuse light onset and termination. R3 gives a phasic discharge to diffuse light ON and OFF stimuli. R4 gives a sustained discharge to diffuse light OFF stimuli. Like R3 cells, slow ON-OFF cells show a phasic response to both diffuse light ON and OFF. The distribution of optic nerve termination in the tectum has been examined by earlier workers. Maturana et al. (1960) first described the depth distribution of the axon terminations of different classes of retinal ganglion cells as a successive stratification of layers of major four classes of units. Gaze and Keating (1969) located R1/R2, R3, and R4 units at depths of 74 ± 59 μm, 290 ± 55 μm, and 385 ± 47 μm, respectively. On the other hand, Witpaard and Keurs (1975) separated R3 class into units of two classes, which are recorded at clearly different depths. Thus they located R1/R2, slow ON-OFF, R3 and R4 units at depths of 106 ± 17 μm, 199 ± 16 μm, 312 ± 24 μm, and 414 ± 29 μm, respectively.

**Neuronal mechanisms underlying the ON channel of the frog optic tectum**

R3 and slow ON-OFF cells show a phasic response to diffuse light ON stimuli, but others do not (Grüsser and Grüsser-Cornelis 1976; Lettvin et al. 1959; Maturana et al. 1960; Witpaard and Keurs 1975). Therefore current sinks observed in the ON response should be associated with EPSPs elicited by only R3 retinal ganglion cells and/or slow ON-OFF cells, whether they are monosynaptic or polysynaptic. The postulated circuits underlying the ON channel are demonstrated schematically in Fig. 10A.

The depth and the short latency of sink I strongly suggest that sink I reflects the monosynaptic activity derived from R3 retinal ganglion cells (I in Fig. 10A). The spatial distribution of its corresponding source extending under the retinorecipient layer strongly suggests that the tectal neurons generating sink I have their cell bodies in layer 6 or deeper.

It is possible that the postsynaptic event representing sink
II involves the EPSPs evoked by projecting neurons from another nucleus (Gruberg and Lettvin 1980; Kozicz and Lázár 1994; Lázár and Székely 1980; Scalia 1976; Schwippert et al. 1995) (2 in Fig. 10A). However, a more likely explanation is that sink II is inward current that generates the EPSP evoked monosynaptically by slow ON-OFF fibers (3 in Fig. 10A). Witpaard and Keurs (1975) showed that the initial burst of R3 units started at a latency of ~80 ms after the stimulus onset and was followed by slow ON-OFF units with a delay of 40 ms. This delay is comparable with that observed between sinks I and II. Furthermore, the depth of ~200 μm, where slow ON-OFF units generate the largest extracellular action potentials, shows good agreement with the spatial distribution of sink II (220 ± 50 μm). However, this explanation fails to account for the much longer duration of sink II than sink I (39.3 ± 13.4 ms and 23.2 ± 3.9 ms, respectively). The slow ON-OFF units showed a high degree of synchronization in the arrival of action potentials, as did R3 retinal ganglion cells (Witpaard and Keurs 1975). One possible explanation for the long-lasting duration is a proposed reverberating circuit based on an ultrastructural study by Székely and Lázár (1976) (4 in Fig. 10A). Their electron micrographs revealed, in laminae C and E, a whirllike synaptic group that comprises several dendrites of tectal neurons, occupying a central position, and the surrounding optic terminals. The dendrites are very often presynaptic to neighboring dendrites that may also contain synaptic vesicles. This assembly of synaptic structures may contribute to reverberating neuronal activity and generate the long-lasting current sink II. Unlike sink I, the spatial distribution of its corresponding source is restricted to the retinorecipient layer. This shows that the tectal neurons generating sink II have their cell bodies in layer 8. Thus sinks I and II must be generated by different types of tectal neurons.

Sink III seems to be generated by the same neuronal activity as sink II (2–4 in Fig. 10A). Discontinuity of these two sinks could be explained by either of the following. The first possibility is that sink III represents a second burst of slow ON-OFF units after ~50 ms (Witpaard and Keurs 1975). The second possibility is based on a theoretical neural model

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**FIG. 9.** A: histograms of occurrence of electrically evoked current sinks in successive time every 2 ms. Five peaks are labeled according to the corresponding sinks. The 1st small peak labeled at 2 ms represents sink A. The 2nd and 4th labeled at 2 and 10 ms represent sinks B and D, respectively. The 3rd labeled at 8 ms represents sink C. The 5th labeled at 22 ms represents sink E. B: histograms of occurrence of electrically evoked current sources in successive time every 2 ms. Peaks are labeled according to the corresponding sources. A', B', C', and D' labeled at 2, 2, 8, and 10 ms represent the sources above and below sinks A, B, C, and D, respectively. E' labeled at 22 ms represents the source below sink E. The data were obtained from 6 CSD depth profiles. Bars on the right side in both A and B indicate occurrence of 12 suprathreshold bins.

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**TABLE 3. Spatial distribution of current sources in response to electrical stimulation**

<table>
<thead>
<tr>
<th></th>
<th>Upper</th>
<th></th>
<th>Lower</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean, μm</td>
<td>N</td>
<td>Time, ms</td>
<td>Mean, μm</td>
</tr>
<tr>
<td>A'</td>
<td>260 ± 50</td>
<td>42</td>
<td>2</td>
<td>560 ± 50</td>
</tr>
<tr>
<td>B'</td>
<td>20 ± 20</td>
<td>6</td>
<td>2</td>
<td>260 ± 50</td>
</tr>
<tr>
<td>C'</td>
<td>370 ± 40</td>
<td>32</td>
<td>8</td>
<td>600 ± 50</td>
</tr>
<tr>
<td>D'</td>
<td>40 ± 20</td>
<td>32</td>
<td>10</td>
<td>390 ± 30</td>
</tr>
<tr>
<td>E'</td>
<td>†</td>
<td></td>
<td></td>
<td>620 ± 50</td>
</tr>
</tbody>
</table>

Values in Mean are means ± SD. * Time bin at which the depth distribution was examined. † Not observed.
FIG. 10. Schematic diagrams of the postulated neuronal circuits underlying the various current sinks observed in response to diffuse light on and off stimulation are demonstrated in A and B, respectively. Morphology of a neuron generating each current sink is depicted based on the spatial distribution of the corresponding current source. In both diagrams, open circles represent excitatory synaptic connections. Stippled circles and ellipses represent the respective current sinks identified in this study. Numbers at the axonal projections indicate the postulated neuronal connections described in text. Thick lines show pathways well supported by data, whereas thin lines show more speculative pathways. Broken line indicates indirect connection. In B, for the sake of clarity, sink VIII has been neglected. PT, pretectum; L6, L8, cell bodies in layer 6 and 8, respectively.

study in which the delayed activity pattern was shown to be generated by an interaction between the reverberating circuits described above and negative feedback circuits involving tectal small neurons (Lara et al. 1982).

Neuronal mechanisms underlying the off channel of the frog optic tectum

In response to diffuse light off stimuli, R3, R4, and slow on-off retinal ganglion cells elicit discharge (Grüsser and Grüsser-Cornehls 1976; Lettvin et al. 1959; Maturana et al. 1960; Witpaard and Keurs 1975). This shows that the current sinks observed in the off response were generated by a combination of EPSPs elicited by these retinal ganglion cells, whether they are monosynaptic or polysynaptic. The postulated circuits underlying off channel are demonstrated schematically in Fig. 10B.

The depth and the short latency suggest that sink IV reflects the excitatory monosynaptic activity derived from R3 and R4 retinal ganglion cells (1 in Fig. 10B). However, a statistical difference in the spatial distributions was not present between sinks I and IV (P < 0.05) even though the terminals of R4 are located below those of R3. The durations of sinks I and IV are also comparable (23.2 ± 3.9 ms and 18.8 ± 2.8 ms, respectively). This shows that R4 retinal ganglion cells give a small contribution, if any, to sink IV. The terminal arborizations of R4 cells exhibit a less profuse branching pattern than those observed more superficially (Hughes 1990; Potter 1972). It is also possible that there are fewer R4 cells than others (Witpaard and Keurs 1975). Combined with a tonic discharge pattern, these properties of R4 retinal ganglion cells could explain the small contribution to sink IV. The spatial distribution of its corresponding source extending under the retinorecipient layer indicates that the tectal neurons generating sink IV have their cell bodies in layer 6 or deeper.

Because, so far, no response to diffuse light off stimuli has been shown for retinal ganglion cells from the sink V depth region, this sink should involve polysynaptic activity. The onset latency and its rather short-duration strongly suggest that sink V mainly reflects excitatory disynaptic activity derived from the neuronal population that generates sink IV (2 in Fig. 10B). What retinal ganglion cell type is responsible for this sink? If target cells of R3 and R4 retinal ganglion cells are different, sink V must be derived from only R4 input. Otherwise the corresponding sink should be observed in response to diffuse light on. However, R4 cells give only a small contribution to sink IV and are unlikely to produce subsequent polysynaptic activity. The more likely explanation is that inputs from both R3 and R4 converge on the common target cell and elicit its suprathreshold response (1, 2 in Fig. 10B). If so, the convergence of both types of retinal ganglion cells must be essential for generation of sink V. Many pear-shaped neurons in the deep tectal layer send their ascending axons to the tectal surface (Antal et al. 1986; Székely and Lázár 1976). These axons could mediate sink V.

Except for slow on-off units, no response of retinal ganglion cells has been shown to diffuse light off stimuli from the depth of sink VI. The onset latency of slow on-off units is much longer than that of sink VI (see Neuronal mechanisms underlying the on channel of the frog optic tectum). Thus, again, sink VI should be composed of polysynaptic activity. Considering the onset latency and duration, sink VI is most likely to represent excitatory trisynaptic activity via the neuronal population generating sink V (3 in Fig. 10B). Because no morphological correlate has been found to transmit action potentials from the upper to lower layer 9, sink VI also must be mediated by the ascending axons of the tectal neurons in the deeper layer. As for the relevant retinal ganglion cells, the same consideration applies as in the case of sink V. The combined source observed under the retinorecipient layer shows that either or both of sinks V and VI are generated by tectal neurons whose cell bodies are located in layer 6 or deeper.

Sink VII is observed below the retinorecipient layer. Therefore this sink should represent exclusively polysyn-
aptic activity. Considering its onset latency, sink VII seems to represent further excitatory polysynaptic activity mediated by the neuronal population generating sink VI (4 in Fig. 10B). Either or both of descending axons from layer 8 tectal neurons and ascending axons from those in layer 6 or deeper could be responsible for sink VII (Antal et al. 1986; Székely and Lázár 1976). Again as for the relevant retinal ganglion cells, the same consideration applies as in the case of sink V. The spatial distribution of its corresponding source extending up to almost the periventricular layer shows that the tectal neurons generating sink VII have their cell bodies in layer 6 or deeper.

Statistical analysis showed that there is no difference between spatial distributions of sinks VIII and II (P < 0.05). The durations of sinks II and VIII are 39.3 ± 13.4 ms and 35.0 ± 10.4 ms, respectively. Moreover, the delay of sink II from the onset of sink I and that of sink VIII from the onset of sink IV ranged from 40 to 110 ms and from 60 to 100 ms, respectively. Thus the time courses of these two sinks are also almost comparable. These facts strongly suggest that neuronal activities underlying sinks II and VIII are common (2–4 in Fig. 10A).

The last sink, sink IX, was observed under the retinorecipient layer. This sink may represent polysynaptic activity generated by the neuronal population producing sink VII indirectly (5 in Fig. 10B). Alternatively, sink IX may represent postsynaptic activity mediated via the extratemporal brain region including the pretectum (Kozicz and Lázár 1994) (6 in Fig. 10B).

Functional meanings of current sinks observed in the on and off responses

In previous work, field potentials have been recorded from the surface of the toad’s optic tectum in response to the onset and offset of diffuse illumination (Gernert and Ewert 1995; Schwippert et al. 1996). In both on and off responses, the field potentials were composed of two main negative waves (N1 and N2), interrupted by positive wave P2 and terminated by positive wave P3. Thus, field potential analysis failed to reveal any essential difference between these responses. On the other hand, the present CSD analysis could demonstrate obviously different spatiotemporal patterns of neuronal activity underlying responses to diffuse light on and off stimuli. CSD analysis can disclose and localize the generators of evoked potentials in which physiologically relevant information is obscured by the dissipated nature of the potential fields (Mitzdorf 1985, 1986).

Every natural stimulus activates two or more types of retinal ganglion cells. The combination of activated neuronal types changes depending on the stimulus. Therefore adaptive behavior of the frog depends on the combined overall activation of different retinal neuronal types (Grüsser and Grüsser-Cornehls 1976). Here, in this context, we discuss the functional roles of tectal current sinks observed in visually evoked responses.

The restricted spatiotemporal distribution of sink I implies that this sink alone does not have an important role in frog visually guided behavior. On the other hand, the long duration and the depth of sinks II, III, and VIII make possible effective integration with tonic excitatory synaptic activity derived from R1 and R2, which is believed to play an important role in prey catching behavior (Ewert 1989). Another possible functional role of these sinks is the facilitatory effect of prey-catching activity, which was found by Ingle (1975) and which was simulated by means of a neural model (Lara et al. 1982). In frogs disinhibited by thalamic lesions, within the most superficial 200 μm of the tectal neuropil, a new class of units was found to continue to discharge for a few seconds after a brief stimulus motion (Ingle 1975). Thus sinks II, III, and VIII are likely to correspond with the subthreshold activity underlying the long-lasting spike discharge that appears to play an important role in prey catching facilitation.

Sink IV was shown to be accompanied by the following synaptic activity generating current sinks V, VI, VII, and IX. The extensive spatiotemporal distribution even below the retinorecipient layer implies that these successive excitatory synaptic activities have a significant role in eliciting visually guided behavior. What is their behavioral correlate? Here, it may be useful to consider properties of visual stimulation that activate R3 and R4 retinal ganglion cells simultaneously. A large fast-moving object leads to a stronger activation in R3 and R4 cells if it is darker than the background (Grüsser and Grotssser-Cornehls 1976). More specifically, a looming object is appropriate stimulation to activate these types of neurons. The sensitivity of R3 cells to the leading edges of a visual stimulus provide information about expanding edges in all directions. The sustained activity of R4 cells to the dimming of light ensures the continuity inside the expanding edges (Liaw and Arbib 1991). It is well known that these are typical stimulus patterns that elicit avoidance responses in frogs and toads (Ewert and Rehn 1968, 1969; Ewert and Traud 1979; Schiff 1965; Schneider 1954). Moreover, if the information of R3 and R4 cells contributes to the central command sequence controlling avoidance behavior, a good correlation exists between neuronal and behavioral findings (Grüsser and Grotssser-Cornehls 1976). These facts strongly suggest that the series of sinks originating from afferent inputs of R3 and R4 cells play an important role in eliciting avoidance responses.

Ewert (1967, 1984) has shown that electrical stimulation of the pretectal region elicits avoidance behaviors that closely resemble natural ducking, side-stepping, or running away from threatening stimuli. Moreover, large lesions placed in the toad’s pretectal region can totally abolish visually elicited avoidance behaviors (Ewert 1968). This indicates that the pretectum is the center for frog avoidance behavior. Brown and Ingle (1973) reported that ablation of the optic tectum totally eliminates the most common type of caudal thalamic unit, which has a large receptive field. This shows that a large part of the visual input to the pretectum comes from optic tectum. Thus it is very likely that the tectopretectal connections mediated by the output the four early successive current sinks IV–VII activate the pretectal command system for avoidance behavior. It is possible that, when animals perform avoidance responses, the pretectopretectal projecting neurons activated by R3 and R4 retinal afferents strongly inhibit the long-lasting current sinks II, III, and VIII and suppress the elicitation of feeding behavior. This hypothetical neuronal mechanism could explain the influence of pretectal lesions on frog visually guided behavior. After disconnecting the pretectum from the ipsilateral optic tectum, the output from early sinks generated by R3 and R4...
retinal ganglion cells cannot activate the pretectal command sequence for eliciting avoidance behavior. Simultaneously, the pretectotectal projecting neurons cannot suppress the late long-lasting sink anymore, and the tectal excitatory process related to prey catching is hyperexcited. Thus, following pretectal lesions, the avoidance behavior fails to occur, and the animal orient and snaps toward any moving visual object, even though they are normally recognized as predators (Ewert 1984; Ewert et al. 1996; Ingle 1973a).

Frogs must be aware of the substrate on which they live and of stationary objects with which they must not collide. Furthermore, the animals must discriminate between the real object motion and self-induced object motion. These are also situations in which R3 and R4 retinal ganglion cells are activated simultaneously (Pigarev et al. 1971). This implies an alternative functional role of the early successive sinks in these behavioral situations. However, frogs suffering tectal ablation are able to avoid stationary objects very well, although they cannot orient toward prey and visual threats properly (Ingle 1973b, 1976). Furthermore, the frog whose regenerated optic nerve projects to the remaining ipsilateral tectum performs a barrier task normally although mirror image responses are produced for prey catching and avoidance behavior (Ingle 1973b). These observations show that it is unlikely that the early successive current sinks have an important role in detection of stationary objects. As many pretectal ablation studies have suggested, the retinopretectal system may mediate these behavioral tasks (Ewert 1984; Ewert et al. 1996; Ingle 1970, 1976). The behavioral pathway hypotheses discussed in this section are summarized schematically in Fig. 11.

So far, explanations of neuronal mechanisms underlying visually guided behavior of the anuran cannot have been based on the activities of individual neurons in single-unit analyses. In this study, we first demonstrate that there is crucial differ-

![Schematic diagram of the hypothetical pathway underlying frog visually guided behavior. Sinks II, III, and VIII mediate prey catching behavior, whereas sinks IV, V, VI, and IX mediate avoidance behavior. However, sink IX, due to its late onset, is not likely to trigger the command system for avoidance behavior in the pretectum. Open circles show the excitatory synaptic inputs relevant to the respective sinks. Arrows and dashed lines indicate excitatory and inhibitory influences, respectively. The tectal and the pretectal command systems for various visually guided behavior are shown in italics. OT, optic tectum; PT, pretectum.](image-url)


