Spatiotemporal profile of synaptic activation produced by the electrical and visual stimulation of retinal inputs to the optic tectum: a current source density analysis in the pigeon (Columbia livia)

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Abstract

The optic tectum of the pigeon is a highly organized, multilayered structure that receives a massive polystratified afference of at least five different populations of retinal ganglion cells and gives rise to various anatomically segregated efferent systems. The synaptic organization of retino-tectal circuitry is, at present, mostly unknown. To investigate the spatiotemporal profile of synaptic activation produced by differential (electrical and visual) stimulation of the retinal inputs, we performed a high-spatial-resolution current source density analysis in the optic tectum of the anaesthetized pigeon. Electrical stimuli consisted of brief pulses of different durations applied to the optic nerve head, while visual stimuli consisted of light flashes of different intensities. Electrical stimulation generated sinks confined to retinorecipient layers. The temporal structure, spatial location and thresholds of these sinks indicated that they are all due to primary tectal synapses of retinal fibers with different conduction velocities. Sinks evoked by the fastest retinal axons were more superficially located than sinks produced by slower retinal fibers. Visual stimulation, on the other hand, resulted in a more complex pattern of current sinks, with various sinks located in the retinorecipient layers and also well below. Visual stimulation induced action potentials at superficial as well as deep tectal levels. We conclude that electrical stimulation activates most of the populations of ganglion cells as well as their primary tectal synapses, but is unable to elicit a significant activation of secondary tectal synapses. Visual stimulation, on the contrary, activates just some of the incoming retinal populations, but in a way that produces noticeable secondary activation of intratectal circuits. Laminar segregation of retinally evoked tectal activity, as reported here, has also been found in other vertebrates. Similarities and differences with previous studies are discussed.

Introduction

The first central stage of the retino-tecto-fugal pathway in all vertebrates is the optic tectum (called in mammals 'superior colliculus'). The avian optic tectum is a large, topographically organized, mesencephalic structure consisting of 15 clearly defined plexiform and cellular layers (Fig. 1B) (Ramón y Cajal, 1911; Cowan et al., 1961). In pigeons it receives at least 90% of all the ganglion cell axons (total 2.4 million) that originate in the contralateral retina (Binggeli & Paule, 1969; Mpodozis et al., 1995). These axons terminate in unequal numbers in layers 2, 3, 4, 5 and 7. Layer 5 receives the major portion of the retinal input, as demonstrated by the total volume and density of retinal arborizations it contains (Fig. 1A) (Hunt & Webster, 1975; Karten et al., 1997). The retinal input to the tectum is not homogeneous; it is made of at least five populations of fibers with different conduction velocities, ranging from 20 ms to 1–2 m/s (Mpodozis et al., 1995; Dye & Karten, 1996). These populations may have different visual properties, as a variety of responses have been described to exist among the optic nerve fibers of birds (movement-sensitive, direction-sensitive, pure 'off', pure 'on' and 'on/off') (Miles, 1972; Hughes & Pearlman, 1974; Holdén, 1977). Furthermore, retinal endings present characteristically distinct morphologies at each tectal layer (Ramón y Cajal, 1911; Hunt & Webster, 1975; Karten et al., 1997). This evidence suggests that the retinal input is spatially segregated and that each retinorecipient layer may contain terminals of a single specific kind of ganglion cell (Ramón y Cajal, 1911; Reperant & Angaut, 1977; Karten et al., 1997).

In birds, the optic tectum gives rise to various anatomically independent visually driven efferent pathways, which terminate in different structures such as the dorso-medial isthmus, the lateral pons, the dorsal and ventral pretectal complex, the nucleus rotundus and other thalamic nuclei (Benowitz & Karten, 1976; Reiner et al., 1979; Reiner & Karten, 1982; Hunt & Brecha, 1984; Uchiyama et al., 1996). Each of these pathways arises from specific cell groups located mainly in layers 9–14. In between the superficial retinorecipient layers and these output layers, a complex network of radial and horizontal local interactions exists (Ramón y Cajal, 1911; Stone & Freeman, 1971; Hunt & Webster, 1975; Hardy et al., 1985).

Despite both the description of the tectal field potentials (TFP) evoked by electrical stimulation of the retinal input and the characterization of tectal receptive fields begun 30 years ago (Holden, 1968a; Jassik-Gerschenfeld & Guichard, 1972; Mori, 1973; Jassik-Gerschenfeld et al., 1975; Frost & DiFranco, 1976; Frost...
FIG. 1. Tectal organization and TFP profiles. (A) Labelling of the uppermost seven layers of the tectum after the injection of cholera toxin in the contralateral eye. The label is confined to the terminal arborizations of retinal axons and the bands mark the retinorecipient layers; layer 5 receives a particularly dense retinal input. (B) Correlation between the tectal layers, revealed by Nissl stain (left panel) and the electrically evoked TFPs obtained at representative depths (right panel). Compare the TFP profile at superficial layers (2–3) with the TFP recorded at layers 12–13, the inversion point is located approximately at layer 6. Scale bar in A, 200μm.
& Nakayama, 1983; Dye & Karten, 1996), the spatiotemporal characteristics of the tectal processing of retinal inputs remains an open field of exploration. Intracellular recordings of tectal cells have hinted at a complex circuitry in which optic nerve stimulation elicits monosynaptic excitatory responses as well as polysynaptic excitatory and inhibitory responses (Hardy et al., 1984; Leresche et al., 1986).

In this context, it is important to investigate whether the laminar segregation of tectal afferences and efferences is reflected as spatially and temporally segregated current generators when the retinotectal synapses are differentially activated. To this end we performed a high-spatial-resolution current source density (CSD) analysis of the local field potentials in order to: (i) describe the spatiotemporal structure of the current generators activated by electrical and visual stimulation of retinal fibers; (ii) compare synaptic events evoked by both types of stimulation; (iii) investigate if different classes of conduction velocity groups of retinal axons evoke laminarily segregated current generators.

Methods

Animal preparation

Adult feral pigeons (Columba livia) of either sex, with a body weight of 300–350 g, purchased from a local provider and kept in an institutional animal facility, were used. For surgical procedures, each pigeon was deeply anaesthetized with equithesin injected intramuscularly (0.3 mL/100 g body weight). A canula inserted in the pectoral muscle allowed supplementary doses. The pigeon was then placed in a head-holding device at the orientation defined by the stereotaxic atlas (Karten & Hodos, 1967). After opening the skin posterior to the orbit, the temporal muscle was retracted and a craniotomy was performed, exposing the centro-dorsal tectal region for electrical recordings. During the experiment, the ECG was continuously monitored and body temperature was kept at 40–41°C by means of a DC powered electric blanket. Anaesthesia was maintained by injecting 0.3 mL of equithesin every 2h. All procedures were approved by the Ethical Committee of the Facultad de Ciencias of the Universidad de Chile.

Stimulation and Recording

Tectal field potentials were recorded using tungsten microelectrodes (1–4 MΩ @1kHz, Frederick Haer, ME, USA) along radially orientated tracks that penetrated the optic tectum. The TFPs were elicited by direct electrical stimulation of the optic nerve or by light flashes. For electrical stimulation, the cornea was cut and the lens carefully removed. Bipolar electrodes, consisting of two parallel insulated tungsten wires separated by 1 mm, were placed near the optic nerve head. Electrical stimuli consisted of rectangular current pulses of 0.1–1 mA in amplitude, lasting 30, 100 or 300 μs. The pulses were produced by a constant current stimulator (CCU-8, Frederick Haer), controlled by a pulse generator and slaved to the main data acquisition program written in LabView 3.1 (National Instruments, TX, USA). Our standard procedure was to place the recording electrode just below the tectal surface and select the lowest current of a 100-μs stimulation pulse that produced stable TFPs. We then used 30, 100 and 300 μs pulses to record TFPs at every depth. In experiments where light flashes served as stimuli, the eyelid was maintained open by a suture, and two hemispherical diffusing screens were inserted in the light path to ensure homogeneous illumination. The light flashes were delivered by a conventional photographic flash located at 2 m from the eye. The intensity of the flash was graduated using calibrated neutral density filters in steps of 0.12 log units. Flash intensities covering a total of 4 log units were used.

Tectal field potentials were recorded each 30 μm (50 μm in the case of visual stimulation) starting from the surface until a depth of 1400 μm, which corresponds to tectal layers 14–15. At each depth, five stimuli were delivered at 0.033 Hz. The resulting TFPs were collected and averaged. The TFPs were collected at 10 Ksamples/s during 40 ms (200 ms for visual stimulation), starting 10 ms before the onset of the stimuli. At the end and middle of each penetration, small electrolytic lesions (5 s × 5 μA) were made for further histological analysis. To assess the stability of the preparation, TFPs were collected during the electrode withdrawal and compared with the initial ones. Only data from stable preparations were used for the subsequent CSD analysis.

CSD Analysis

Once the TFP data were collected, an off-line numerical procedure, performed with IGOR PRO (WaveMetrics, OR, USA) on a Macintosh computer, was used to construct the CSD map. The following procedures were applied: (i) following Golarai & Satulda (1996) the set of average TFPs was expressed as a two-dimensional matrix V in terms of time (t) and depth (d); (ii) in order to diminish the unavoidable artifact that affects the most superficial TFPs, some new TFPs, conceptually located outside the tectum, were defined by extrapolation (Vaknin et al., 1988); (iii) each time transect (V as a function of d, with t constant) was smoothed by convolving it with a 13-point Gaussian low-pass filter that had a 3-dB cut-off point at 3 cycles/mm to give a function G(d); (iv) the second spatial derivative, G"(d), of every transect was calculated by using the five-point formula (see Nicholson & Freeman, 1975; Mitzdorf, 1985), sinks corresponding to depths where G"(d)>0 and sources where G"(d)<0; (v) finally, we calculated, for each time t, the spatial integral of sink and sources to check our procedures. Step (iii) reduced the high spatial frequencies found in G(d) and diminished the unwanted noise that numerical differentiation introduces (Fig. 2). The CSD profiles were computed in relative terms, as the conductivity σ of the avian tectum has not been determined; CSD profiles are thus expressed in V/mm². We also assumed that the optic tectum was a homogeneous structure with respect to the conductivity tensor in the two tangential dimensions. The CSD distribution was visualized by constructing colour-coded maps that graphically displayed the sink and source profiles in space and time (see below Figs 4 and 5).

At the end of the experiment, the pigeon was injected with an overdose of anaesthetic and perfused with 600 mL of avian saline, followed by 600 mL of 10% formalin. The brain was removed and processed according to Nissl or Giemsa protocols (Iñiguez et al., 1985). The electrode tracks were reconstructed and the absolute tectal locations of the TFPs were estimated using the landmarks of the electrolytic lesions. To visualize the retinal terminals in the optic tectum, in some pigeons 50 μL of choleatoxin was injected in one eye. After 5 days of survival, the animals were perfused and the brains processed according to the standard ABC immuno-histochemical protocol (Karten et al., 1997).

Results

Description of the temporal course of electrically and visually evoked TFPs

To differentially stimulate the various groups of retinal axons, single electrical pulses of different durations were used. At short stimulus duration (30 μs) the superficial TFP exhibited a single negative peak that developed with a 4.3-ms onset latency (± 1.8, n = 18), had a peak latency of 5.1 ms (± 1.2, n = 18), and lasted 8.7 ms (± 2.1, n = 18)
(Fig. 3A, left panel). At increasing depths, \( \approx 300 \mu \text{m} \), the TFP profile changed. Its initial phase became positive and its maximum was reached at 4–5 ms. After correlating the readings of the micro-electrode advance with the histological analysis of electrolytic lesions, the inversion zone was located at the bottom of the retinorecipient layers (Fig. 1B). In the inversion zone, TFPs showed complex, low-amplitude (20–50 \( \mu \text{V} \)) features superimposed on the main positive component, and only visible for 60–100 \( \mu \text{m} \). Below the retinorecipient area the inversion was complete and the small features disappeared. Tectal field potentials remained positive until the bottom of layer 14 and their amplitude decreased gradually with depth.

At intermediate stimulus duration (100 \( \mu \text{s} \)) (Fig. 3A, centre panel), the amplitude of the initial negative phase increased between three- and five-fold. Furthermore, depending on the experiment, one or more secondary negative peaks appeared or were hinted at by an inflexion point in the trailing edge of the first negative peak, with peak latencies ranging between 7 and 15 ms. As was the case with short stimuli, at intermediate stimulus duration the first negative peak reversed its polarity at 300 \( \mu \text{m} \). The secondary negative peaks also changed their polarity in the same region and small features appeared around the inversion zone. Below the retinorecipient layers, only a single positive peak was visible.

With maximal stimulus duration (300 \( \mu \text{s} \)), the peaks hinted at with intermediate stimulus durations became clearly visible (Fig. 3A, right panel) as three to five negative peaks could be elicited at the tectal surface. The mean onset latency of the first peak was 4.3 ms (\( \pm 1.5 \), \( n = 18 \)), while the secondary peaks had onset latencies of 8 ms (\( \pm 2.1 \), \( n = 18 \)), 11 ms (\( \pm 3.5 \), \( n = 18 \)), 15 ms (\( \pm 4.0 \), \( n = 18 \)) and 17 ms (\( \pm 4.0 \), \( n = 11 \)), respectively. The first peak had the largest amplitude, 1000–1500 \( \mu \text{V} \), similar to the amplitude reached with intermediate stimulation, whilst the amplitude of the longer-latency peaks was much smaller, in the range 100–200 \( \mu \text{V} \). This complex profile changed polarity with depth. Albeit not every peak reversed at exactly the same depth, at 600 \( \mu \text{m} \) the TFP polarity was completely reversed. Below 1000 \( \mu \text{m} \) a large positive peak was visible and, sometimes, a smaller positive peak could be detected at 20 ms latency.

The invariant features of electrically evoked TFPs could be summarized as: (i) an initial negative phase with a 4–5 ms latency that reversed polarity at 300 \( \mu \text{m} \) and remained positive until the end of tectal layers; (ii) three to five peaks with longer latencies elicited with longer stimulus durations, which reversed at the same region as the first peak; (iii) small features superimposed on the main phase around the reversal zone; and (iv) below 1000 \( \mu \text{m} \) a single large positive peak was the dominant characteristic of TFPs.

Compared with the responses produced by electrical stimulation, light flashes produced TFP profiles with a much longer latency, stretched over a longer period of time (Fig. 3B). At the tectal surface, two main negative components, an early one at 25.6 ms (\( \pm 4.0 \), \( n = 6 \)) and a late one at 53.6 ms (\( \pm 5.0 \), \( n = 6 \)) were always visible, even at threshold intensities. These two components lasted on average for 9.3 and 12.9 ms, respectively. At all stimulus intensities, but especially at high intensity, many secondary components were superimposed on the two main phases, thus producing TFP profiles of complex appearance. Visually evoked TFP series taken from different animals presented high variability in the number, amplitude and latency of these secondary features, making their characterization and analysis difficult. The main negative components of the visually evoked TFPs reverse their polarity at the bottom of retinorecipient layers. Below the reversal zone, usually new negative components appeared so, at increasing depth, the evoked TFP presented a mixture of positive and negative waves. The number, position, amplitude and latency of the negative components located below the retinorecipient layers were also variable and dependent on the stimulus intensity.

**Description of the CSD profiles evoked by electrical and visual stimulation**

To reveal the spatiotemporal features of the current flows that generate the TFPs, a current source density analysis was performed on a series of TFPs. The colour maps exemplified in Fig. 4 display the current sink and source patterns for electrical stimuli of 30, 100 and 300 \( \mu \text{s} \) pulse durations. These three sequences show that the main current sinks are restricted to the superficial layers located above 500 \( \mu \text{m} \) in the retinorecipient zone. Stimulus duration also affected the number of evoked sinks. Short stimulus pulses elicited only one or two sinks, while longer pulses elicited up to five. At short stimulus duration, a single sink (‘S’ in Fig. 4A) was detected at 230 \( \mu \text{m} \) in the retinorecipient
area, extended for 100 μm, had an onset latency of 3.0 ms and lasted for 6 ms. The histological analysis showed that this depth corresponded to tectal layer 5 with the lower source located between layer 7 and layer 9. No additional sinks were detected below the retinorecipient layers. In two cases a very small sink could be detected at the very surface with an onset latency of 3.0 ms (Fig. 4A, sink p).

At intermediate stimulus duration (100 μs), the CSD pattern usually exhibited more sinks with longer latencies. In some preparations two sinks were visible, while in others up to four sinks appeared. The first sink, S, resolved itself in two components (S1 & S2 in Fig. 4B) with peak latencies of 4.2 and 6 ms. Two additional sinks, with peak latencies of 7 and 12 ms, could be distinguished (S3 & S4). These subsequent sinks tended to appear 50–100 μm below the first sink (S1) (Fig. 4B).

With longer pulses (300 μs) a total of five sinks was normally obtained. A new sink (S5) (Fig. 4C) with an onset latency of 17.5 ms and a peak latency of 19 ms appeared between 200 and 300 μm. The four sinks (S1, S2, S3 and S4) detected with 100 μs pulses continued to be present at the same locations and latencies but with higher amplitudes. In the case displayed in Fig. 4C, sink S4 appeared spread out indicating the possible presence of other sinks with even longer latencies and high thresholds. As was the case with 30- and 100-μs stimulation pulses, no sinks were detected below the retinorecipient layers.

A cluster analysis of onset latencies of the sinks obtained from five animals distinguished five separate sinks that appeared at 2.9 (n = 5), 5.2 (n = 3), 6.3 (n = 4), 9 (n = 3) and 14.3 (n = 3) ms and had corresponding durations of 1.4, 1.5, 2.3, 3 and 3.3 ms. Onset latency and duration covaried, in such a way that sinks with longer latencies lasted longer (r = 0.92). In three of the five animals some initial sinks were found with a latency that gradually increased with depth at an average rate of 6.7 ± 2.5 ms/mm (mean ± SD, n = 5).

The histological data indicated that every sink in every animal was located above layer 7. The sinks had an average vertical span of 130 ± 76 μm (mean ± SD, n = 18) and a range between 40 and 300 μm. In all cases the sink with the lowest onset latency was also the most superficially located. We normalized each set of sink depths by dividing by the depth of the first sink and tested the null hypothesis that secondary sinks, those with latencies greater than 4 ms, were located at the same depth as the first one. The null hypothesis was rejected (Student’s t-test, at P < 0.01) thus indicating that the first sink is more superficially located than the sinks with longer latencies. The average relative depth of all secondary sinks is 1.44 ± 0.25 (mean ± SD, n = 13) thus indicating that, as a group, secondary sinks are deeper than the first.
All sinks had a corresponding source located below them, thus forming a sink/source pair. In only two animals a source/sink/source triplet was detected and in both cases the superior source was much less intense than the inferior source. The mean extent of the sources was $188 \pm 96 \mu m (n = 18)$, and correlated with the mean extent of sinks ($r = 0.80$). The neutral zone separating each sink from its
corresponding source was 60 ± 45 μm (mean ± SD, n = 18). The temporal course of each source matched the sink’s profile.

Near the tectal surface, the visually evoked CSD profiles appeared qualitatively similar to electrically evoked CSDs. Below the retinorecipient layers, visual stimulation triggered large sinks that were never observed with electrical stimulation. These visually evoked sinks exhibited more variability than the CSD pattern obtained by electrical stimulation (Fig. 5). Despite this variability, the following invariant features were always present: (i) appearance of early sinks in the retinorecipient area; (ii) at least some of these sinks were followed, after extinction, by strong sources located at the same depth (see the succession of sinks/sources located at 300 μm in Fig. 5); (iii) appearance of late polysynaptic sinks below the retinorecipient area, especially at 800 μm (equivalent to layer 9).

**Multiunit activity evoked by visual and electrical stimulation**

In experiments with visual stimulation, multiunit visually driven activity was recorded as soon as the electrode entered the surface of the tectum. Many observations indicated that these responses came from retinal fibers and/or their terminal arborizations on the tectum. Firstly, the activity was composed of many potentials of different sizes responding synchronously, as expected from the recordings of the densely packed branches of ganglion cell terminals. Secondly, the responses to the ‘on/off’ of spots of lights or to the movement of small bars presented on the receptive field were very brisk, and with no accommodation to repetitive stimulation. Thirdly, the neural activity had no spontaneous discharge, in agreement with direct recordings of avian ganglion cells or their axons (Miles, 1972; Hughes & Pearlman, 1974). Fourthly, the receptive field of these...
multiunitary responses was small, always <3°, and changed its location in space only minimally as the electrode advanced in depth. This small change in receptive field position was attributed to drifts in the eye or to a tangential component of the penetration. All these elements indicated that retinal axons or their terminals were the main contributors to the multiunitary activity recorded superficially. In simultaneous recordings, obtained by applying low- and high-pass filters to the same signal, the multiunitary activity occurred in phase with the negative TFP components. Furthermore, in cases where isolation of single spikes was possible, it was observed that the same unit fired a burst of spikes repetitively during each negative phase. This synchronous firing correlated with the periodic temporal profile of the compound action potential to a flash recorded in the optic tract (see Discussion and Fig.6A and B). Thus, the main negative components of the TFPs triggered by the flash are at least partially explained by the repetitive firing of some ganglion cells.

In addition to the multiunitary activity recorded in the superficial layers, single units, especially between 700 and 1000μm, were frequently recorded. The characteristics of these responses were: presence of spontaneous discharge, large receptive fields (between 3° and 40°), selectivity for motion stimulus and strong habituation to repetitive stimulation; all suggest that they originate from tectal cells. In particular, the frequency of these recordings coincided with the crossing of the electrode through the tectal cellular layers (especially layers 6–8, 10 and 13). Light flashes were able to trigger these tectal spikes, at least in a fraction of the units recorded.

In the case of electrical stimulation, multiunitary activity in phase with the negative component of TFPs was recorded at the level of retinorecipient layers. This indicates that the recordings were taken from the tectal area activated by the stimulus. Below these layers no spikes, time-locked to stimulus onset, were found, even at the level of layers 10 or 13, where well-resolved recordings of isolated spikes are common because of the large size of the neurons. These findings correlate with differences in the CSD profiles obtained for the two types of stimulation, as explained in the Discussion.

Discussion

Analysis of TFPs and CSD

Tectal field potentials evoked by short electrical pulses (30μs) exhibit a single negative peak at the most superficial tectal layers. This peak reverses its polarity around 300–400μm, and becomes a positive wave toward the deeper layers. Increasing the duration of the pulses increases the number of negative components, until four or five peaks are obtained with 300μs pulses. This correlation probably resulted from the recruitment of additional populations of retinal fibers with progressively slower conduction velocities. The classic results of Bishop (Bishop et al., 1953) showed that in the optic nerve of cats, brief pulses of 30μs stimulate thick myelinated fibers with fast conduction velocities, whereas longer pulses recruit unmyelinated fibers with slower conduction velocities. In pigeons, recordings made in the optic nerve of the compound action potential while antidromically stimulating the optic tectum have directly shown that increasing the duration of the stimulating pulse produces the recruitment of slower conduction velocity groups, up to five for the tectum (Mpodzi et al., 1995). The wave of activity of each population arriving sequentially in the tectum would then produce a sequence of postsynaptic potentials recorded as negative components in the TFPs. Another mechanism that may contribute to the generation of delayed negative components is the synchronized activation of postsynaptic intratectal circuits. However, as we argue next, our CSD analysis and spike recordings do not favour this interpretation.

The spatio-temporal structure of electrically evoked synaptic currents shows that a single stimulation pulse delivered to the optic nerve can trigger many sinks located in the retinorecipient layers and none below layer 7. The recruitment of longer latency sinks as stimulus duration increased (Fig.4) reflects both the range of conducting velocities found among retina-tectal axons (20–2 m/s) and the increasingly higher threshold of slow-conducting retinal axons. Also, the temporal coherence of longer latency sinks is lower than that of earlier sinks. This time-spread is correlated with the wider distribution of conduction velocity among slow-conducting retinal fibers (Clare et al., 1969). Taken together all this evidence (dependency on stimulus duration, relation between temporal coherence and onset latency, location inside the retinorecipient layers) strongly suggests that, with electrical stimulation of retinal fibers, every sink detected in the retinorecipient layers is monosynaptically driven by a specific group of retinal axons. Furthermore, the absence of sinks between layer 7 and layer 13 correlates well with the absence of neuronal responses locked to the electrical pulse in that region. Nevertheless, because one-dimensional CSD analysis only
reflects synchronized activations of currents in the vertical dimension, we can not discard the possibility that electrical stimulation may mobilize non-synchronous intraretinal polysynaptic circuits, or polysynaptic circuits involved in lateral interactions.

The properties of the very early sink p (early latency, short duration and anatomical localization) indicate that it corresponds to the transversal current generated by the synchronous and massive activation of fast retinal axons, as they bend to enter the tectum. Similar presynaptic sinks have been described in the visual cortex of the rat (Kenan-Vakin & Teyle, 1994).

The sources, corresponding to the electrically evoked sinks, were mostly located below these sinks and extended well into the non-retinorecipient area. This spatial arrangement, valid for all sink/source pairs, indicates that the dendrites of the postsynaptic cells are radially orientated and that their length is similar to the extent of the sources. Many tectal cell classes, especially projection cells, have their dendrites and axons orientated radially; for example, layer 9 neurons that project to the Istmic nuclei, layer 10 cells that project to the ventral nucleus of the lateral geniculate and the principal optic thalamic nuclei, and lamina 13 neurons that give rise to the tecto-rotundal projection (Hunt & Künzle, 1976; Woodson et al., 1995; Karten et al., 1997). Some of the neurons of this last group are particularly relevant because they have long dendrites that ramify in layer 5 (Luksch et al., 1998).

The spatio-temporal structure of sinks evoked by light flashes shows a clear difference with respect to electrically triggered events in that superficial and deep sinks are always evoked by light flashes. As expected from the initial negative TFP component, a prominent sink appears in the retinorecipient layers. Its latency is at least 25 ms and depends on flash intensity. At the intensities used in these experiments, other sinks, with longer latencies, also appeared in the retinorecipient layers. These superficial sinks are in phase with respect to the main negative TFP components recorded at the tectal surface. Some of these superficial sinks probably represent post-synaptic potentials produced by the repetitive firing of retinal fibers.

Our multiunit recordings (Fig.6B) and data from experiments describing the discharge characteristics of retinal axons to whole-field illumination (Duff & Cohen, 1975) indicate that retinal ganglion cells respond with a train of spikes to a single light flash. This is also corroborated by direct recordings made in the optic tract, as the compound action potential evoked by a flash showed a very regular waveform with four or five evenly spaced peaks (8 ms) of similar amplitude (Fig.6A). Thus, some of the superficial sinks are probably due to a complex interaction among the bursty structure of spike trains of retinal ganglion cells, their different conduction velocities and the nonhomogeneous visual properties of these cells. This complexity of the retinal input makes it difficult to investigate whether some of the superficial sinks, especially the later ones, could correspond to polysynaptic activation (or postsynaptic firing). Although the overall pattern of superficial sinks was similar in all the preparations stimulated with light flashes, the precise spatio-temporal pattern of sinks and sources is more variable than the pattern obtained with electrical stimulation. Below the retinorecipient layers, light flashes triggered clear and intense sinks. The most intense sinks appear at 700 μm. In association with these clear sinks, neuronal responses, time-locked to the flash, appear at the same level, thus providing evidence that secondary circuits are triggered by light flashes.

Spatial segregation of retinal inputs

Besides the temporal segregation of electrically evoked sinks, our results also demonstrate spatial segregation. The earliest sink, produced by fast-conducting axons, is more superficially located than sinks with longer latencies produced by slower-conducting axons. This difference indicates that, on average, fast retinal fibers arborize more superficially than slow ones. Recent anatomical findings (Karten et al., 1997) indicate that layer 5, the main retinorecipient layer, contains terminal arborizations of the w-5b population of ganglion cells that have the finest unmyelinated axons and would thus have the slowest conduction velocity. In agreement with these observations, we found that the long latency sinks elicited by electrical stimulation are commonly located at the level of layer 5. On average then, most of the activity elicited in the tectum by electrical stimulation of retinal afferences takes place at that tectal layer.
The presence of strong sinks near layer 5 also correlates with the anatomical observations showing that the more abundant kind of tecto-rotundal projecting neurons, Type I cells, have dendritic expansions in layer 5, and that they are probably monosynaptically connected with the retinal endings at that layer (Karten et al., 1997; Lukesch et al., 1998). Type I neurons project to the anterior and centralis subdivisions of the nucleus rotundus (Karten et al., 1997). Extracellular recordings have shown that each of these regions of the rotundus responds to different visual dimensions (Wang et al., 1993). Taken together, the aforementioned evidence, the results presented here and the well-known correlation between conduction velocity classes and functional types [exemplified by the X, Y, W subsystems (Bishop et al., 1964; Stone, 1983)] suggest that the retinal ganglion cells projecting into layer 5b, instead of forming a single definite functional class, are a heterogeneous collection of ganglion cells that might contribute to synthesize some of the various visual properties detected upstream in the ascending tectofugal pathway.

Comparison with previous studies in the pigeon

The TFPs obtained in this study differ from those described in previous studies of the pigeon's optic tectum where only a single 'N' wave has been described (Holden, 1968a; Stone & Karten, 1971; Mori, 1973; Bagnoli et al., 1977; Holden, 1980; Dye & Karten, 1996). Here we report the existence of more complex waveforms with many negative components. We found a direct relation between the number of peaks detected and the duration of the stimulus. At short stimulus duration, our TFPs showed a single 'N-like' negativity whose features (latency, duration and vertical location of the inversion point) resemble that of the previous studies. Mori (Mori, 1973) also reported that increasing the strength of the stimulation produces multipeaked TFPs. However, in his studies he only used data from experiments where a single peak was elicited.

Another result of the current study, the absence of evoked action potentials by electrical stimulation, differs from intracellular recording of tectal cells performed in anasthetized pigeons (Hardy et al., 1984; Leresche et al., 1986) and from the work of Holden (Holden, 1968b). In the intracellular studies it was found that 25% of tectal cells responded to an electrical pulse delivered to the optic nerve with an EPSP followed by at least one action potential. In this study we explored intensively below the retinorecipient layers and specifically at and near the output layer 13, and no cellular responses were found. Besides differences in experimental procedures, two reasons could explain the difference between this study and previous studies. Firstly, because the depth of the intracellular responses is not specified in the original study, it could be that some of the responses were obtained from the retinorecipient layers. Secondly, a well known side-effect of cellular impedance by a microelectrode is to render the cell more excitable because the puncture slightly depolarizes the cells. Thus, EPSPs that would, under normal conditions, be subthreshold might generate an action potential. In any case we feel that this question requires further research.

Comparison with other vertebrates

In the frog, electrical stimulation of the optic nerve elicits five sinks located at different tectal levels (Fig. 7). The first sink to appear (sink A) is located at the bottom of the retinorecipient layers (layer 8) and is produced by fast, myelinated retinal fibers (Witpaard & Keurs, 1975). The second and fourth sinks to appear (B and D) are located more superficially (at retinorecipient layer 9) and appear to be a mixture produced by the activation of slower retinal axons and of polysynaptic intratectal circuits. The third and fifth sinks (C and E) are both located outside the retinorecipient layers, and can be exclusively attributed to nonretinal postsynaptic intratectal circuits (Nakagawa et al., 1997).

Similarly, five tectal (collicular) sinks have been described after the electrical stimulation of the optic nerve in the cat. Sink A, located at the lower part of the stratum griseum superficialis (SGS) is solely produced by the activation of fast Y retinal fibers (30 m/s), while sink D, produced exclusively by the activation of slow W fibers (5–4 ms), is located at the upper part of the SGS. Sinks C and E, located at the middle part of the SGS, are produced by the activation of fast W retinal fibers (10–7 ms) mixed with indirect activation of cortico-collicular inputs. Finally, sink B is solely produced by an indirect corticocollicular input. No synaptic currents attributed to the activation of intracollicular circuits were found (Freeman & Singer, 1983).

If we compare our results with these studies, we find important similarities and differences (Fig. 7). Our results indicate that, as in the frog and the cat, the sinks produced by activation of retinal synapses are spatially segregated according to the conduction velocity of the incoming fibers. However, unlike the situation found in frogs and cats, in the pigeon all the sinks resulting from the electrical stimulation of the optic nerve appear to be monosynaptically driven by the retinal inputs. Our results are similar to results with cats in that we found no evidence of massive activation of intratectal circuits. In the cat, some of the more delayed sinks are produced by the rebound of activity generated in the visual cortex. We think that in our case this is improbable, as it has been shown that the stimulation of the Walst (the avian equivalent of the visual cortex) produces evoked potentials not in the superficial but in the deep tectal layers (Bagnoli et al., 1977).

Summary and conclusions

Our data indicate that the sink and source pattern of the superficial layers, for both electrical and visual stimulation, are mostly monosynaptically generated, and mainly reflect the wave of activity that these stimuli trigger in the optic nerve. Electrical stimulation generates a wave of activity composed by the single firing of fibers belonging to most of the velocity groups that go to the tectum. Visual stimulation, on the other hand, triggers a sequence of bursts in fibers from only some of these velocity groups. Electrical stimulation reveals a laminar segregation of retinal input, with fast-conducting axons terminating more superficially than slow ones. We also show that light flashes are more efficient than electrical stimulation in mobilizing intratectal circuits, as they clearly trigger current generators and action potentials in deep tectal layers.

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Abbreviations

CSD, current source density; SGS, stratum griseum superficialis; TFP, tectal field potential.

References


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