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SMR.853 - 7

ANTONIO BORSELLINO COLLEGE ON NEUROPHYSICS

(15 May - 9 June 1995)

"Sources of electrical transients in tectal neuropil of the frog, *Rana pipiens*"

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These are preliminary lecture notes, intended only for distribution to participants.

Brain Research, 560 (1991) 106–121
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ADONIS 000689939116978J

BRES 16978

Sources of electrical transients in tectal neuropil of the frog, *Rana pipiens*

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(Accepted 30 April 1991)

Key words: Frog; Optic tectum; Dendrite; Visual information processing; Extracellular recording

We have studied the outer neuropil layers in frog tectum where the unmyelinated optic nerve fibers terminate. At any point in the neuropil an extracellular microelectrode records several different visually evoked electrical transients, distinct by size and shape. When classified by shape alone, each transient falls into one of 3 distinct classes. Some of these transients are binocularly driven, as originally described by Finch and Collett. The aggregate of the receptive fields of all the elements recorded at a single point defines a multiunit receptive field (MURF). Each MURF is characteristically oval, and divided into 3 sections along its long axis. Each section represents the aggregate of the receptive fields associated with one class of transient; i.e. transients belonging to only one specific class can be evoked by stimulating that part of the visual field corresponding to the appropriate section of the MURF. All of the MURFs mapped by recording in a single tectum are radially arranged in visual space about a central point, or 'visual pole'. Several conclusions are made. First, the two larger types of transient are generated postsynaptically by electrically active dendritic elements, specifically the beaded dendritic appendages of tectal neurons. The smallest type of transient is of presynaptic origin. Second, these tectal elements have a local and global anatomical order across the tectum, which accounts for both the tripartite structure of the MURFs and their radial arrangement about a visual pole. Third, since the large transients are of postsynaptic origin, genuine recordings of single retinal ganglion cell (RGC) activity can be made only in the optic nerve or retina itself. Fourth, information is conveyed over the unmyelinated optic nerve fibers at pulse rates as high as 80/s and is transsynaptically effective at such rates. Finally, the electrically active tectal dendritic elements, with their highly organized spatial arrangement, are an important component of the frog's visual processing apparatus, instead of being merely relays or repeaters.

INTRODUCTION

Using extracellular microelectrodes to record in superficial tectal neuropil, Gaze[16] was first to find that the frog has a precise retinotopic projection from the eye to the contralateral tectal surface. Further study by Lettvin et al.[29] showed that the optic fibers from each of 4 funtional classes of retinal ganglion cell (RGC) terminate in 4 separate and parallel sublaminae of the tectal neuropil, and that the 4 maps are all in register. Maturana[31] had shown by electron microscopy that 97% of the frog's optic fibers are unmyelinated, and the physiology suggested that these terminate in the outer two-thirds of tectal layer 9[32]. Under the influence of Pedro Ramon's drawings of Golgi impregnations[6], Lettvin et al.[29] attributed the large transients, recorded in the neuropil, to terminal bushes of both the myelinated and the unmyelinated optic nerve fibers. Ramon[6] drew each arborization as a long, relatively thick central stalk giving off numerous branchlets. Invasion of this dense terminal bush by an action potential would presumably generate a relatively large extracellular current transient because of the large area of membrane involved. George and Marks[17] also concluded that the class III RGC responses, which are attributable specifically to myelinated fibers[32], are generated by optic fiber terminal arborizations.

It is now the general (though not universal) opinion that all of the electrical transients recorded extracellularly in the tectal neuropil are generated presynaptically. It follows that each pulse train so recorded is nothing more than a delayed account of the firing of a single RGC. This belief, combined with the retinotopic projection onto the tectal surface, and the ease with which relatively large transients are recorded in the neuropil has turned the tectum into an arena for studying RGC stimulus–response properties[15,23,24,43].

In this report we are primarily concerned with what the electrical transients tell us about the elements that generate them, at least for those neuropil layers wherein the unmyelinated optic nerve fibers terminate. Based on

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both intracellular and extracellular recordings, other authors have already concluded that, contrary to the original proposal by Lettvin et al.[29], these electrical transients may be generated postsynaptically by electrically active dendritic elements[13,30]. The anatomic foundation of Lettvin et al.'s[29] proposal, namely Ramon's[6] description of the optic fiber terminal arborizations, has also been contradicted by Potter[38], Székély and Lazar[42], and Hughes[25]. We now interpret the shapes of single unit transients, and the ensemble of units recorded at a single point (defining a multiunit receptive field) as indicating a dendritic origin of the activity. In addition, the results of our electrophysiological studies are consistent with and expand upon a recently recognized spatial order of tectal dendritic arborizations, first shown by Katz and Constantine-Paton using intracellular staining techniques[27]. Finally, we suggest that tectal dendritic elements, by virtue of their connectivity and electrical activity, are themselves important information processing devices.

MATERIALS AND METHODS

Surgery

Adult *Rana pipiens* were anesthetized by immersion in 0.3% tricaine-methane-sulfonate. The animal was wrapped in moist cotton gauze and placed on a cork platform. Xylocaine gel, 2%, was applied to the skin overlying the tectum, and a small flap of this skin was cut and folded back to expose the skull, a small piece of which was removed with a dental drill to expose the colliculi. Sometimes an additional piece of skull was removed rostrally and laterally to expose the optic nerve. Slight medial pressure, applied with cotton tipped fine forceps between the cerebrum and the skull in the opening for the nerve, created a clear passage for the microelectrode to the optic nerve, just as it enters the cranial cavity. The skin was replaced in its original position and the animal allowed to recover for 24–48 h.

Electrical recording

The animal was immobilized with light curarization, wrapped in moist cotton gauze (which was kept moist during the recording), and again Xylocaine was applied to the area of the incision. The skin was folded back and the meninges over the tectum were incised and reflected to expose the underlying tissue. For optic nerve recordings and stimulation the sheath of the nerve was slitted to permit passage of a microelectrode. The frog's head was centered in an aluminum hemisphere, 50 cm in diameter, having a matte grey coating on its inner surface and marked on its outer surface with lines of latitude and longitude. A small ball of moist cotton placed in the mouth was used to keep one or both eyes protruded. One eye was covered with an opaque occluder. Usually the animal was rotated so that either the snout faced the hemisphere's pole, or the projection of the optic nerve head (ONH) coincided with the hemisphere's prime meridian. In either case, the ONH projection onto the hemisphere was determined by a modification of the method described by Finch and Collett[13]. The horizontal position of this point was typically 45–50° from the point directly in front of the animal, which was consistent with the results of others[14,20]. In this way, receptive fields (RFs) mapped from different animals could be viewed with respect to a fixed physiological reference point – the optic nerve head.

Microelectrodes were a modification[19] of the metal-filled variety described by Dowben and Rose[11]. The indifferent electrode, a length of ordinary solder, was placed either between the animal and the swaddling cotton gauze, or, wrapped in the moist cotton ball, was placed in the mouth. Recordings were made within 150 μm of the tectal surface, from RGC class I and class II units, as identified by their response patterns and RF sizes. The recording band was 10 Hz to 3 kHz.

Electrical stimuli were applied to the optic nerve and n. isthmi through the same type of monopolar electrode as used for recording. The stimulus pulse was a diphasic square wave 160 μs in length, the first phase being negative. Adjusting the relative phase magnitudes significantly reduced the electrical artifact in the records. Permanent records of electrophysiological data were made either on diskette through a Nicolet digital oscilloscope, or on DAT tape through a Sony PCM 2500 digital tape recorder.

Mapping of receptive fields

The visual stimulus was a circular 2° black (magnetic) object moved about the hemisphere's inner surface by manipulating a magnet along the outer surface. Ambient lighting was provided by incandescent bulbs, and measured about 10 cd/m^2 at the frog's eye. Single unit and multiple unit receptive fields (MURFs) were mapped directly onto the hemispheric dome by conventional methods.

Rapid-Golgi impregnations

Rapid-Golgi impregnations of *R. berlandieri* (a species closely related to *R. pipiens*) generally followed the procedures of Valverde-Garcia[45], and Morest and Morest[34]. Best results were obtained with a single impregnation of two days followed by immersion in the silver nitrate solution for 5 days. The tissue was coated with a thin layer of gelatin before being placed in the silver nitrate so as to prevent blackening of the tissue perimeter. After dehydration and processing the impregnated tissue was embedded in LVN and sections were cut at 60–100 μm. Three-dimensional reconstruction of the Golgi impregnated material was performed with M.I.T.'s Neurotrace system.

RESULTS

Types of electrical transient

The elements recorded in the tectal neuropil are related to those recorded in the optic nerve stylistically, in that the size of the receptive field and the description of the stimulus that elicits response seem much the same. It is therefore useful to name the tectal units by the same classification scheme as is used for optic nerve fibers. When we talk of RGC classes as characterizing tectal units, there is no implication that the tectal transients are of optic nerve fiber origin.

It must also be emphasized at the start that our results pertain only to RGC class I and II responses, i.e. that information which is transmitted from the retina on unmyelinated fibers[32]. No distinction will be made between pulses evoked by excitement of class I or class II feature detectors, because with respect to the data presented here, there were no significant differences between those two response types.

At any point in the outer half of the tectal neuropil, an extracellular microelectrode records several concurrent pulse trains in response to a target moving in the corresponding region of the visual field. The pulses of the different trains are distinct by size and shape, and

the single unit receptive fields represented by the different pulse trains differ by position in the visual field. The spatial sum of the RFs represented by all the pulse trains recorded at a single point in the tectal neuropil defines a MURF.

When classified by shape, each pulse (also called a 'unit' or 'transient'), falls into one of 3 fairly distinct types, which we have termed types A, B and C, as shown in Fig. 1. All 3 types are usually recorded everywhere class I or II RGC responses are found. Type A is always small relative to types B and C. It can be preceded by a very short positive phase in the noise level. It rises sharply to a low negative peak and usually decays in ramp-like fashion. Types B and C are characteristically large. On slight displacement of the electrode vertically, the transients change size but not shape, i.e. type A is not a B or C observed from a distance. Type B is a triphasic transient with a large middle negative phase between two sizable positive phases. When a large number of type B units are compared, the first positive phase is the most variable in amplitude with respect to the other two phases. Type C is a diphasic transient with an initial large positive phase followed by a comparably large negative phase. Both type B and C transients are often followed by a slower rising and longer negative phase (Fig. 2), that, for any B or C transient is present inconstantly. The transient types are distinct enough in shape, but within each type there is a fair variability. On the whole there is remarkably little ambiguity in assigning each separate pulse train to one of the 3 unit types.

MURF structure

It is well known that class I or II RGC single unit responses recorded in the optic nerve or tectal neuropil have circular or slightly oval RFs 2–5 ° in diameter[23,29,32]. The MURFs, however, comprised of the many single units recorded at a single tectal locus, are distinctly oval[1,14,20] (Fig. 3). We often found that one end of the MURF is wider than the other, giving the MURF a tapered, wedge-like appearance. Near the frontal horizontal plane of the visual field MURFs typically measure about 5 ° × 17 ° of visual angle, while elsewhere in the visual field the long axis will extend up to 35 °. The relation between MURF size and its position in the visual field has been observed by others[1], and is consistent with a corresponding size change in the component single unit RFs, which in turn is inversely correlated with the density of photoreceptors in the retina[3].

Not previously reported by others is the fact that along its long axis each MURF is comprised of 3 distinct sections, giving the MURF an internal tripartite structure. Each section differs from the other two by the predominant pulse type, as shown in Fig. 1, evoked when the visual stimulus is applied in that part of the visual field corresponding to that section of the MURF (Fig. 3). If visual stimulation is restricted to one MURF section, then the evoked transients in the neuropil belong mainly to only one of the 3 fundamental unit types. The transients illustrated in each column of Fig. 1 were obtained by moving the target sequentially through the 3 sections of a single MURF, and then selecting one typical transient among the many evoked as the target moved through each section. At one end of the MURF are found the type A unit RFs, at the other end the type C unit RFs, while the type B unit RFs are in the middle (Fig. 3). The relative size of MURF sections is variable. Sometimes the sections are roughly equal in size. In other MURFs the section containing type B or type A unit RFs will be the longest. Almost never was the section containing type C units longer than both of the other two sections. In those MURFs which are wedge-shaped, the end containing the type A unit RFs is always the narrowest one. Some single unit RFs within the MURFs of Fig. 3 are shown. Both MURFs contained many other single units whose RFs could not be accurately mapped because of the difficulty had in isolating those units from the pulse trains of larger transients. Single unit RFs of different pulse types overlap at MURF section boundaries, but the transition from one type to another as the target moves through a MURF is nevertheless a dramatic one (Fig. 3).

One-hundred-and-twenty-seven MURFs of RGC class I and II responses were mapped in several animals. With one exception to be described later, the elliptical shape and tripartite structure of MURFs were always present. These properties were also found to be independent of MURF location in visual space, including MURFs mapped in the caudal hemifield and across the midline from the seeing eye. As discussed below, MURFs mapped by stimulating the eye ipsilateral to the recording electrode had the same shape and tripartite structure as those mapped by stimulating the contralateral eye, although they tended to be slightly larger. Within a MURF all the units seem qualitatively to have the same stimulus–response properties. For example, if the type B pulse trains in a MURF have class II RGC stimulus–response firing patterns, then the types A and C transients in the same MURF will also have class II RGC response properties.

MURF orientation

Since tectal MURFs have a major and minor axis, the question arises whether there is order to their orientation in space. Adamson et al.[1] observed that the long axes of MURFs mapped from neighboring electrode locations were similarly oriented. However, these authors

did not note any global order to MURF orientation. Fig. 4 illustrates the results of an experiment in which MURFs were mapped at over 25 different electrode locations in the left tectal neuropil of a single animal, and reveals a strong spatial regularity to MURF orientation. The MURFs are radially arranged about a point approx. 10° temporal (in visual space) to the ONH projection. This central point, defined by the radial orientation of MURFs about it, will be called the 'visual pole'. The MURF section containing the type A unit RFs, indicated by the open circle within each MURF, lies closest to the visual pole, and the type C unit section lies farthest away. The position of the visual pole was determined accurately in about 10 animals and always lay between the projection of the ONH and a point about 35° temporal to it in visual space. In these other preparations MURFs were also mapped in those portions of visual space outside of the hemifield shown in Fig. 4, particularly the regions above and behind the animal. In every case the MURFs were oriented towards a visual pole located near the projection of the ONH contralateral to the tectum in which the recordings were made, with the type A unit section within each MURF closest to the pole.

As can be seen in Fig. 4, two MURFs mapped by stimulation of the ipsilateral eye (labelled 'i') had the same organization as the MURFs mapped by stimulation of the contralateral eye. This consistent spatial orientation of MURFs mapped by recording in one tectum the units evoked by stimulation of either eye is seen more clearly in Fig. 5. Each pair of MURFs shown in this figure was mapped at a different electrode location within the binocular region of the left tectal neuropil. At each electrode position both the ipsi- and contralaterally evoked MURFs were mapped. Fig. 6 illustrates 3 representative transients, one of each unit type, evoked by stimulation within the corresponding section of an ipsilateral MURF. It is clear from Figs. 4–6 that MURF tripartite structure and radial orientation about a visual pole are a function of the colliculus in which the recordings are made, but not of which eye is stimulated. Since information from one eye reaches the ipsilateral tectum by way of the direct retinal projection to the contralateral tectum and an intertectal relay, the n. isthmi[22], these results suggest that MURF structure and orientation result from corresponding regularities in the anatomical and electrophysiological properties of those *tectal* elements which generate the type A, B and C units.

Very near the visual pole MURF structure is varied. Sometimes the tripartite structure and elongated shape are preserved. Other times the MURFs are only slightly

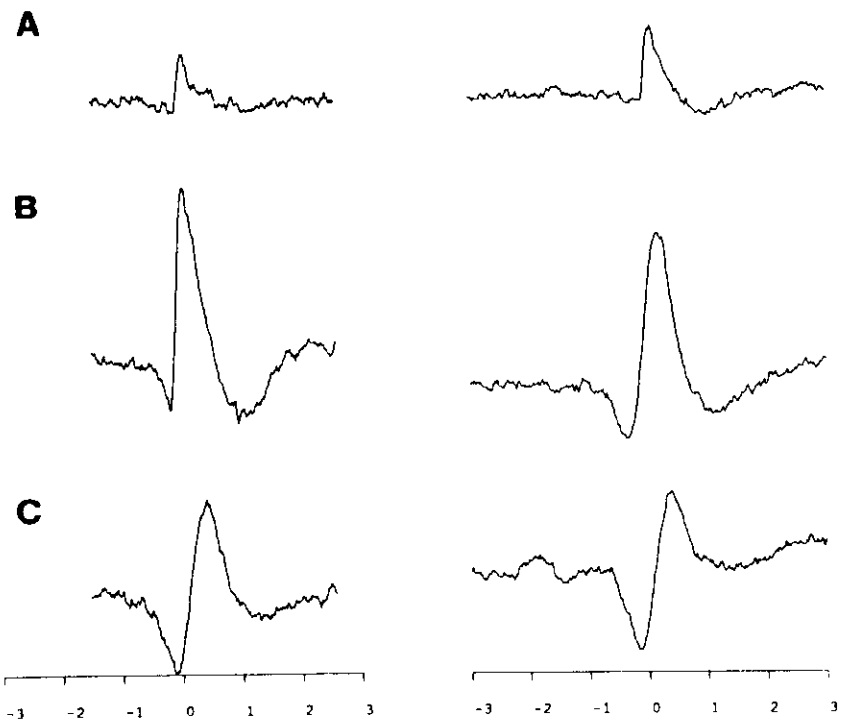

Fig. 1. The 3 fundamental unit types recorded in the outer layers of the tectal neuropil. A–C: each row illustrates two examples of the corresponding unit type (A,B or C). The 3 units in each column were recorded at a single tectal locus in response to stimulation in the corresponding section of a single MURF. See text for details. Ordinate scale is constant within each column, but differs slightly between the columns. Abscissa: time in ms. In this and other figures time is arbitrarily referenced to the oscilloscope's trigger point, and negative is up.

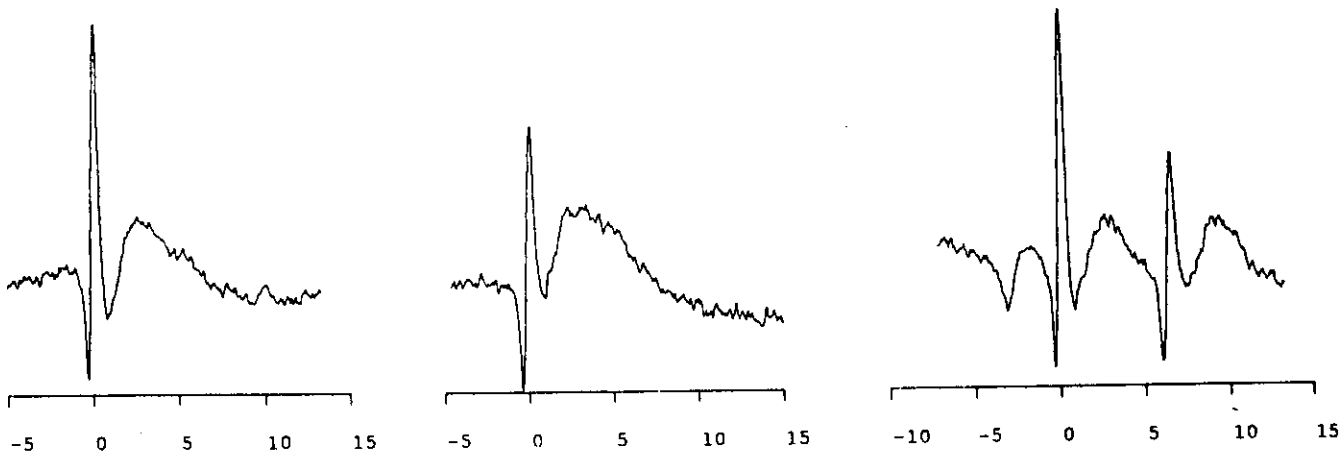

Fig. 2. Left: a type B unit followed by a slow negative potential. Middle: a type C unit followed by a similar slow wave. Right: the same type B unit as shown in the left panel, and a type C unit recorded a few ms. later, both followed by slow potentials. All 3 records were taken from a single electrode location.

oval, and occasionally they are irregularly shaped. When MURFs do not have the tripartite structure (two bipartite MURFs are shown in Fig. 4), the composition of their component single units is unpredictable. Two or 3 types of unit may be present, but there may not be a distinct partition of the MURF into segments. On a few occasions MURFs near the visual pole consisted primarily of type B units only. An exhaustive study of MURFs adjacent to the visual pole was not performed. It seemed that their shape and structure are compressed and unclear, but little more can be said about them at this time.

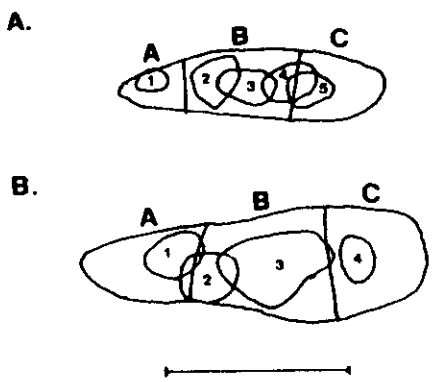

Fig. 3. A: a MURF from the region directly in front of the animal. Straight lines inside the MURF indicate the empirically determined boundaries of its 3 sections, as indicated by the corresponding letters above the MURF. Five of the MURF's many component single unit RFs are also shown. Unit 1 was a type A, units 2, 3 and 4 were type B, and unit 5 was a type C. B: a second MURF from the frontal field of the same animal but mapped about 20° of visual angle above the horizontal plane. Unit 1 was a type A, units 2 and 3 were type B, and unit 4 was a type C. All units were mapped by stimulating the R eye and recording in the L tectal neuropil. Scale bar shows 10° of visual angle.

Binocular units in the tectal neuropil

Finch and Collett[13] concluded that they had recorded electrical transients, at a depth 100 μm from the tectal surface, which were generated *postsynaptically* by electrically active dendritic structures. This conclusion was based upon the observation that the same transient could be evoked by independent stimulation of either eye. Since a single binocular unit could be evoked in the neuropil by activity in different tectal afferent fibers (which is guaranteed by the indirect pathway from retina to ipsilateral tectum), it was concluded that such units must be generated by a single postsynaptic structure. Since these authors did not show examples of the binocular units, or mention their shape, we repeated the experiment.

Distinct single units evoked by stimulation of one eye were stored in the memory of a digital oscilloscope. This allowed comparison of units subsequently evoked 'live' by stimulation of the other eye with those evoked earlier by stimulation of the first eye. The results of such an experiment in which a binocular type C unit was evoked are shown in Fig. 7. Several other repeatable binocular units have been similarly recorded in other animals. Classified by shape the binocular units were of type B, C or multiphasic. No type A binocular units were found. These experiments confirm the results of Finch and Collett[13], and we conclude, as they did, that the binocular units are generated by a postsynaptic structure.

Binocular neuropil units do not respond identically to identical stimulation of each eye. Responses evoked in the neuropil from stimulation of the ipsilateral eye habituate very rapidly and are virtually abolished after only a few passes of the target through the unit's RF. This

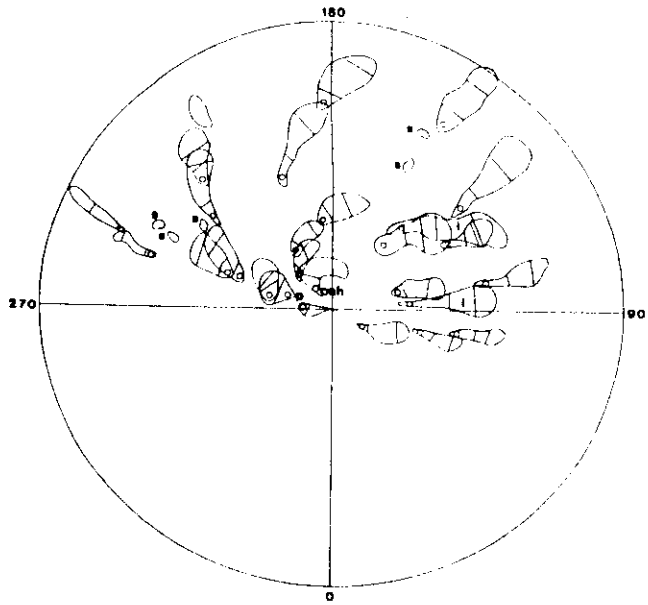

Fig. 4. Many MURFs mapped by recording at corresponding locations in the left tectal neuropil of a single animal. The frog was 'behind' the plane of the paper and was rotated so that its R ONH projected to the hemisphere's prime meridian. This point is labelled 'onh'. In this particular case, which is typical, the ONH projection is only a few degrees away from the hemisphere's pole. With the frog placed in this way the target hemisphere included a large portion of the uniocular visual field, as well as most of the binocular visual field. Along the perimeter of the hemisphere a target at 0° longitude would project to superior retina, and similarly one at 90° longitude would project to temporal retina. As in Fig. 3, lines inside each MURF indicate the boundaries between the MURF sections, and the open circle indicates the MURF section containing the type A unit RFs. i, these MURFs were mapped by stimulation of the ispilateral (L) eye. s, single unit RFs which occurred at some electrode locations in addition to a neighboring MURF. p, the 'visual pole', defined by the radial orientation of MURFs around it.

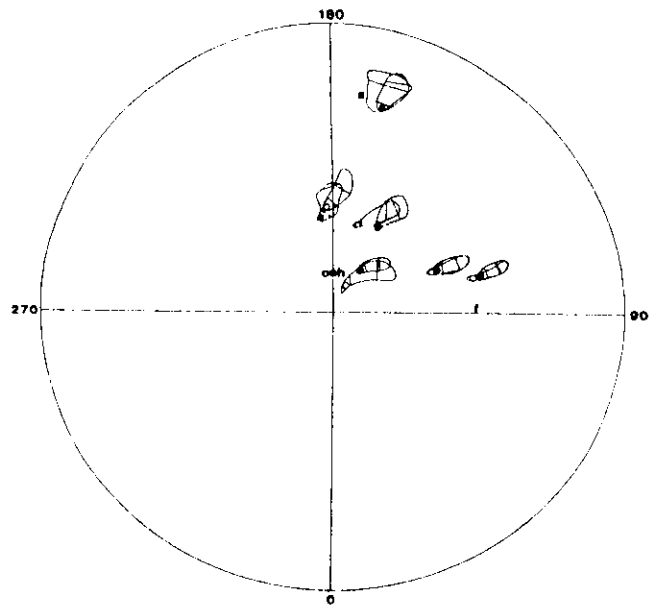

Fig. 5. Six pairs of MURFs mapped in a single animal at 6 different electrode locations in the binocular region of L tectal neuropil. As in Fig. 4, the circle in each MURF indicates the section containing predominantly type A unit RFs. In each MURF pair, single units in the MURF (filled circle) were evoked by stimulation of the contralateral eye, while units in the other MURF (open circle) were evoked by stimulation of the ipsilateral eye. The R ONH projected onto the hemisphere's prime meridian, and this point is labelled 'onh'. The point directly in front of the animal is marked with an 'f'. a: this ispilaterally evoked MURF had no section containing type A units.

rapid habituation, which is absent from contralaterally evoked responses, makes the isolation of binocular units unlikely unless one is specifically looking for them.

Multiphasic units

In addition to the types A, B and C units described earlier, 'multiphasic' units are often recorded extracellularly in the tectal neuropil, and have been mentioned by others[40]. These repeatable units are called multiphasic because they have more than 3 component phases. The additional phase(s) may be simply a hump or plateau superimposed on the falling side of the middle phase of a type B unit, as in Fig. 8, or it may be more pronounced and distinct, as in Fig. 9. Multiphasic units have been evoked in one tectum by visual stimulation of either eye, or by electrical stimulation of the contralateral optic nerve. The multiphasic transient shown in Fig. 8 was also a binocular unit. One of the two superimposed transients was evoked by visual stimulation of the contralateral eye, while the other was evoked by electrical stimulation to the contralateral n. isthmi. The transients shown in Fig. 9 were evoked by visual stimulation to the ipsilateral eye.

Patterned units

Occasionally we have observed two or more distinct tectal transients which occurred repeatedly with repeated stimulation, in a fixed temporal pattern. We have called these ensembles 'patterned' units. A patterned ensemble of 3 units evoked in the right tectal neuropil by visual stimulation of the left eye is illustrated in Fig. 10A,B. Fig. 10A shows one example of the set, while Fig. 10B shows 4 examples superimposed. Fig. 10C,D shows an ensemble of two units evoked similarly in the tectal neuropil by stimulation of the ipsilateral eye.

Patterned units are not as abundant as single units, but they are not so rare as to make them difficult to find if one is specifically looking for them. They are also stable, like single units, and once isolated can be evoked repeatedly by passing the target through their RF. In short, patterned transients seem to behave very much like single class I or II RGC units.

Optic nerve stimulation

In the frog's optic nerve it is possible to record single

unit responses from both myelinated and unmyelinated axons[29]. Therefore, before stimulation in the optic nerve began, RFs were found for units recorded by the nerve electrode. In this way the tectal electrode could be placed in a region of tectum likely to contain evoked responses when stimuli were applied to the nerve. It was first verified, as originally reported by Bishop[4], that there are 3 major peaks in the distribution of frog optic nerve fiber conduction velocities. In adult *R. pipiens*, single shocks delivered to the nerve just inside its foramen will evoke transients in the contralateral tectal neuropil with delays of about 2, 6–8, and 20–25 ms. The slowest set of fibers are presumed unmyelinated because of both their low conduction velocity (about 20 cm/s) and the fact that the evoked transients with the longest poststimulus delay are recorded from the same dorsal layers of the neuropil wherein terminate class I and II RGC axons, which were previously shown to be unmyelinated[32].

Optic nerve fiber firing rate

George and Marks[17] showed that class III RGC units could fire repetitively in the tectal neuropil, at frequencies up to 160/s, in response to electrical stimulation in the contralateral retina. The axons of class III RGCs are myelinated[32]. These authors argued that 160/s exceeded the synaptic firing rate, and based on this and other data concluded that class III units recorded in the superficial tectum were generated presynaptically by optic fiber terminal arborizations. Since we were interested in whether the transients evoked in the neuropil from excitation of *unmyelinated* optic fibers are generated pre- or postsynaptically, we decided to repeat their experiment, but to measure the maximum firing rate of the unmyelinated fibers as well. Paired and continuous stimulus shocks were applied to the left optic nerve through a microelectrode, and transients were recorded in the right tectal neuropil. First, it was confirmed that the class III responses fired at rates up to 160/s. In fact, stimulation of the fastest conducting myelinated fibers (which belong to the class IV RGCs[32]) at frequencies up to 200/s successfully evoked a repeatable transient in the tectum for periods exceeding 1 s. Second, stimulation (paired and continuous for > 1 s) of the relatively slow conducting unmyelinated fibers at frequencies up to 80/s successfully evoked repeatable type B and C units in the outer layers of the neuropil (Fig. 11). As discussed later, we do not conclude from these data that the neuropil transients must be generated presynaptically.

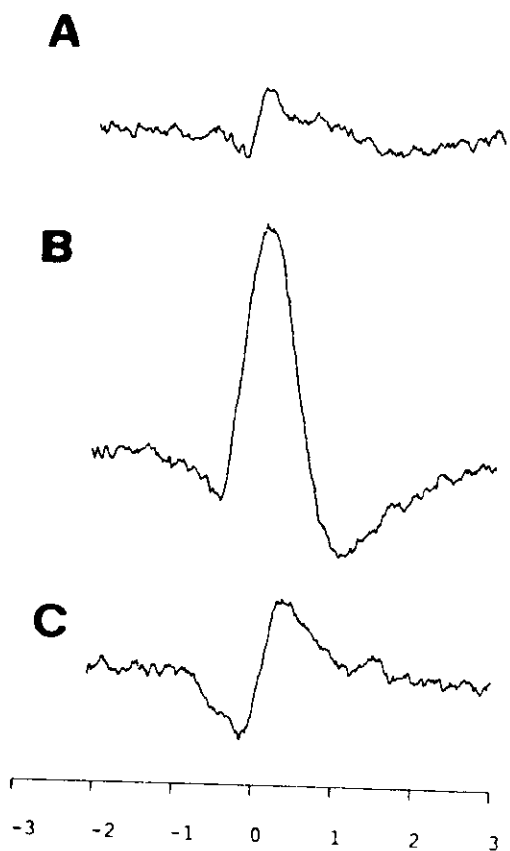

Fig. 6. A–C: similar to Fig. 1 but in this case the units were evoked by stimulation of the 3 sections of an ipsilateral MURF.

Rapid-Golgi-impregnations

Rapid-Golgi impregnations of frog tectum generally confirmed results reported by others[38,42]. We were unable to identify optic fiber terminal arborizations like those drawn by Ramon[6], even in sections where afferent axons (presumably myelinated) entering the rostrolateral tectum were clearly visible. Occasionally dense nests of fibers in the neuropil were stained. It is possible, as orginally suggested by Székély and Lazar[42], that Ramon mistook these fiber nests for axonal terminals, since they are parallel with the tectal surface and perpendicular to the rising apical dendrites of tectal neurons, whose somata lie in the cellular layers. At low magnification the fiber nests look as if they could be single arborizations, but at higher magnification it is virtually impossible to make sense of the component branches. A single fiber cannot be followed for any appreciable distance, nor can its relation to other fibers in the nest be determined. The relatively large thickness of these fibers suggests they are dendritic, yet there is no obvious connection between them and the rising dendrites. The disposition of these fiber nests remains unclear.

A few succesful impregnations were made of dendritic arborizations belonging to the pear-shaped tectal cells which Székély and Lazar[42] showed are the major recipient of optic terminals. Typically the apical dendrite will give off up to 3 or 4 secondary branches, which then branch repeatedly and form dense bushes studded with

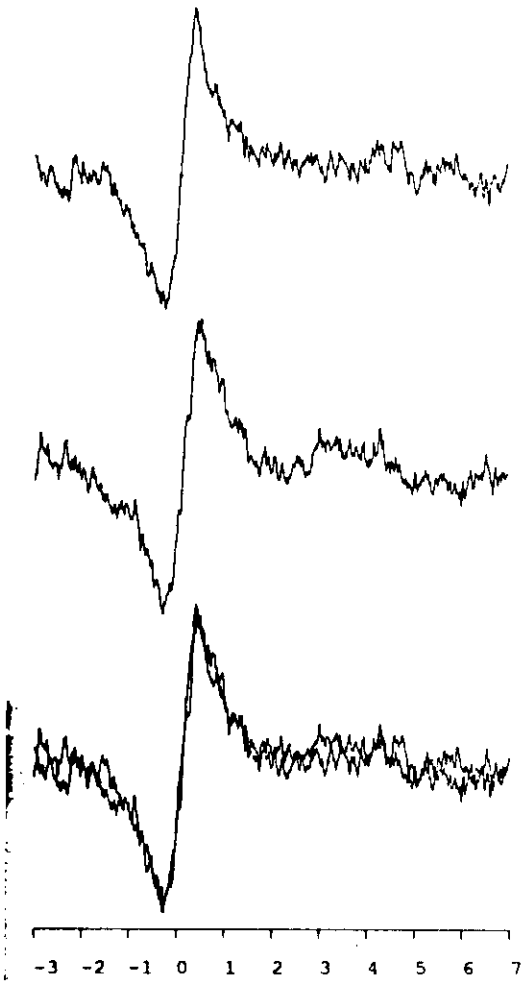

Fig. 7. A type C binocular unit. The top unit was evoked by stimulation of the contralateral (L) eye, while the middle unit was evoked by stimulation of the ipsilateral eye. The bottom record shows the superposition of the upper two records.

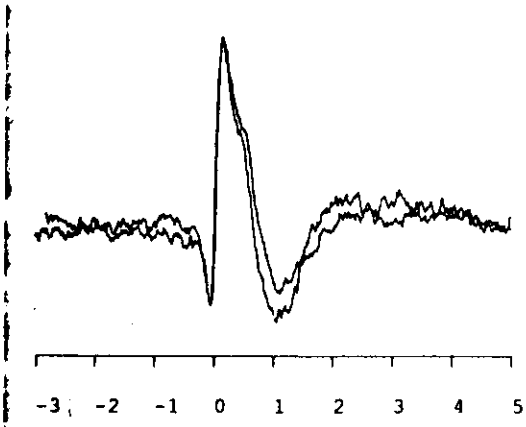

Fig. 8. Two superimposed records of a 'multiphasic' binocular unit. One spike was evoked by visual stimulation of the contralateral (L) eye, while the other was evoked by electrical stimulation of the contralateral n. isthmi (stimulus artifact not shown).

dendritic beads or swellings of 2–4 μm diameter, looking very much like loose clusters of small grapes. The dendritic beads are connected to each other by short and thin branch segments and terminal twigs. The diameter of those clusters best impregnated was typically about 30 μm.

Each dendritic bead cluster viewed under bright-field illumination in a single focal plane usually (but not always) appeared symmetrically distributed about the apical dendrite. However, when the focal plane was rapidly moved back and forth through the section there was a consistent anisotropy to the clusters in a neighborhood; they all came off eccentric to the dendrites and in the same direction. The same effect was produced when two half discs of polarizer, orthogonal in their axes of polarization, were placed side by side in the aperture plane of the microscope, and the eye pieces were each fitted with an analyzer, matched to the corresponding half disc, making the compound microscope stereoscopic. The polarizing discs cause the illuminant for the slide to pass through it at a different angle to each eye.

In order to demonstrate clearly the anisotropy of dendritic appendages a few of the better Golgi impregnations were subjected to 3-dimensional reconstruction. This effort was hindered by the difficulty in distinguishing between overlying components in the bead clusters. However, Fig. 12 is a good example of how the anisotropy of a single dendritic bead cluster is hidden when the cluster is viewed in a transverse section of tectum, but is beautifully revealed when the reconstructed image is rotated about the axis of the apical dendrite, or viewed from above the tectal surface. An elegant and more extensive survey of tectal cell dendritic arborizations was recently reported by Katz and Constantine-Paton[27]. The majority of tectal cells investigated by these authors had dendritic arborizations with a profound rostral orienta-

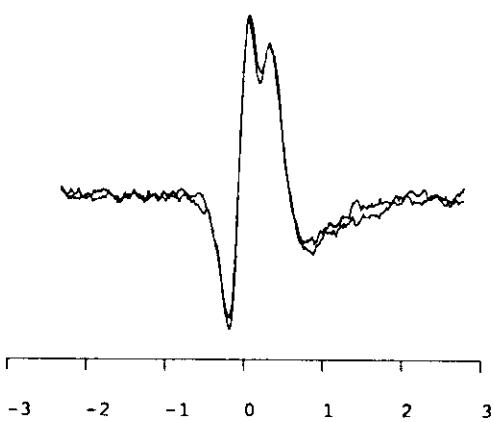

Fig. 9. Two superimposed examples of a repeatable multiphasic unit evoked by visual stimulation of the ipsilateral (R) eye.

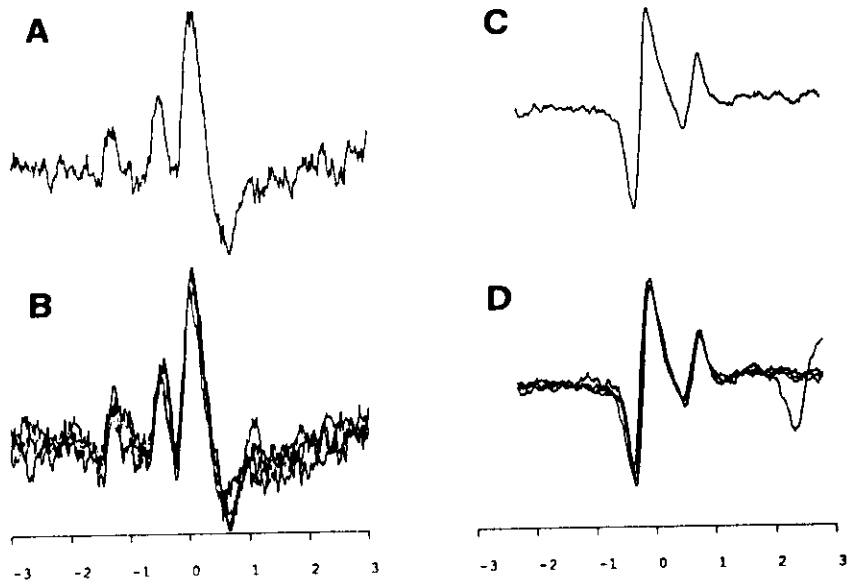

Fig. 10. Patterned units. A: a set of 3 transients, which occurred repeatedly as an ensemble, and were evoked by visual stimulation of the contralateral eye. B: 4 superimposed records of the patterned units shown in A. C,D: similar to A,B but illustrating patterned units evoked by stimulation of the ipsilateral eye (of a different animal).

tion with respect to the cell body. This rostral bias was noted everywhere cells were filled, but none of the cells investigated were near the tectal margins (Katz, personal communication). The reconstruction shown in Fig. 12 demonstrates that the dendritic bead clusters, in particular, belong to that group of arborizations with a clear orientation with respect to the apical dendrite.

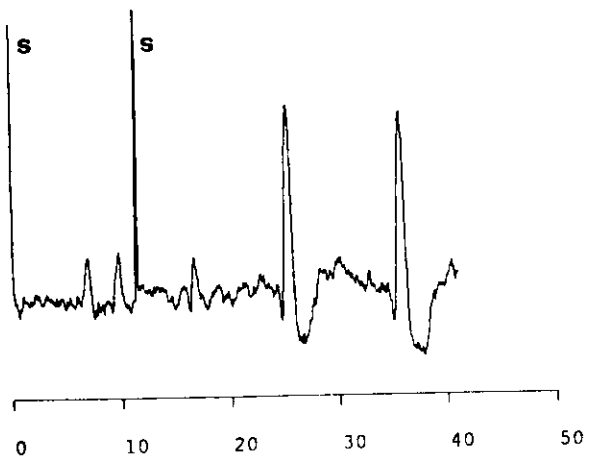

Fig. 11. A type B unit evoked twice in the neuropil by a pair of stimulus shocks to the contralateral optic nerve. The stimuli were separated by about 11 ms. s, stimulus artifact. Note the long delay between stimulus and evoked unit. Abscissa: time in ms.

DISCUSSION

Unmyelinated fibers, 0.2–0.6 μm in diameter, comprise 97% of the population in the optic nerve of frog[31]. Were they to lie in simple dense packing as in the olfactory nerve, no external electrical records could be made of any fiber because the external currents of the impulse are too small to set up voltage changes above the noise level of a microelectrode. But in optic nerve, small bundles of them are locally encased in non-conducting slings formed by the last turn of the spirally wound myelin around a myelinated fiber[31,41]. A properly constructed microelectrode, whose glass wall at the tip is flush with the end of the metallic conductor, can trap a few unmyelinated fibers between the tip and the glial sling after penetrating a bundle. The glass surround at the tip together with the glial wall constrain the lines of current around the trapped fibers and so bring the voltage signals well above the noise level of the electrode. Under these conditions the unmyelinated fibers of the optic nerve were classified 3 decades ago in terms of their receptive fields[29]. There are two classes: type I, or 'edge detectors' and type II, or 'bug detectors'. Once these fibers enter the tectum they lose their glial bundling and course parallel to the surface through the thick outer neuropil to synapse with the dendrites of tectal cells whose bodies lie deep to the neuropil.

A microelectrode thrust into the neuropil records relatively large transients, very like nerve spikes. Several such units are recorded at each tip locus, and can be

distinguished from each other not only by size and shape but also by associated receptive fields. As first described by Gaze[16] the retina is mapped along the tectal surface. Lettvin et al.[29] showed that there are 4 such maps, all in register parallel to the surface but distinguished in depth, and each is characterized by receptive field type. This mapping is known in detail only by electrical records — the anatomical foundations are still lacking.

Two of the maps lie between the surface and about 150 μm deep. The outer is that of the edge detectors, and the thicker inner one is of the bug detectors. The unit activity and receptive field size in each map is very like that recorded from the unmyelinated fibers in the optic nerve. It is as if the hard-won description of unmyelinated optic nerve fibers could have been more easily obtained from their terminations in the tectum. For, since no violation of point-to-point retinotectal mapping is observed, we can't be recording from fibers of passage, nor should we expect to record them above noise level.

Offered to explain the remarkable signal amplification was the Golgi picture by Ramon[6], which showed optic nerve fibers ending in long dense brushes, very like a test-tube cleaner. But later Golgi studies by others[38,42] and ourselves have shown these brushes to be dense bundles of varicose tubes that have been tentatively identified as of dendritic origin. A recent and comprehensive HRP study[25] of the retinal axons of *R. pipiens* revealed no axonal terminals resembling those drawn by Ramon. The synapses formed by the optic fiber terminals and tectal dendrites are eminently visible by electron microscopy[25,42].

Several reasons have impelled us to consider that the large transients were not of presynaptic origin, but postsynaptic. First was the work of Luk'yanov[30], who recorded intracellularly from the somata of tectal cells a relatively small transient, often superimposed on the cell spike, which he attributed to a regenerative dendritic potential. Second was the existence of units in the neuropil that were equally excited by input from either eye as reported by Finch and Collett[13], and verified by us (Fig. 7). Since these binocular units cannot be distinguished from monocular units on the basis of shape, stimulus–response properties, or electrode position in the tectum, there is no reason to suppose they are generated by a mechanism different from that of the monocular units. Last was a marked distinction in pulse time series description between the optic nerve fibers of

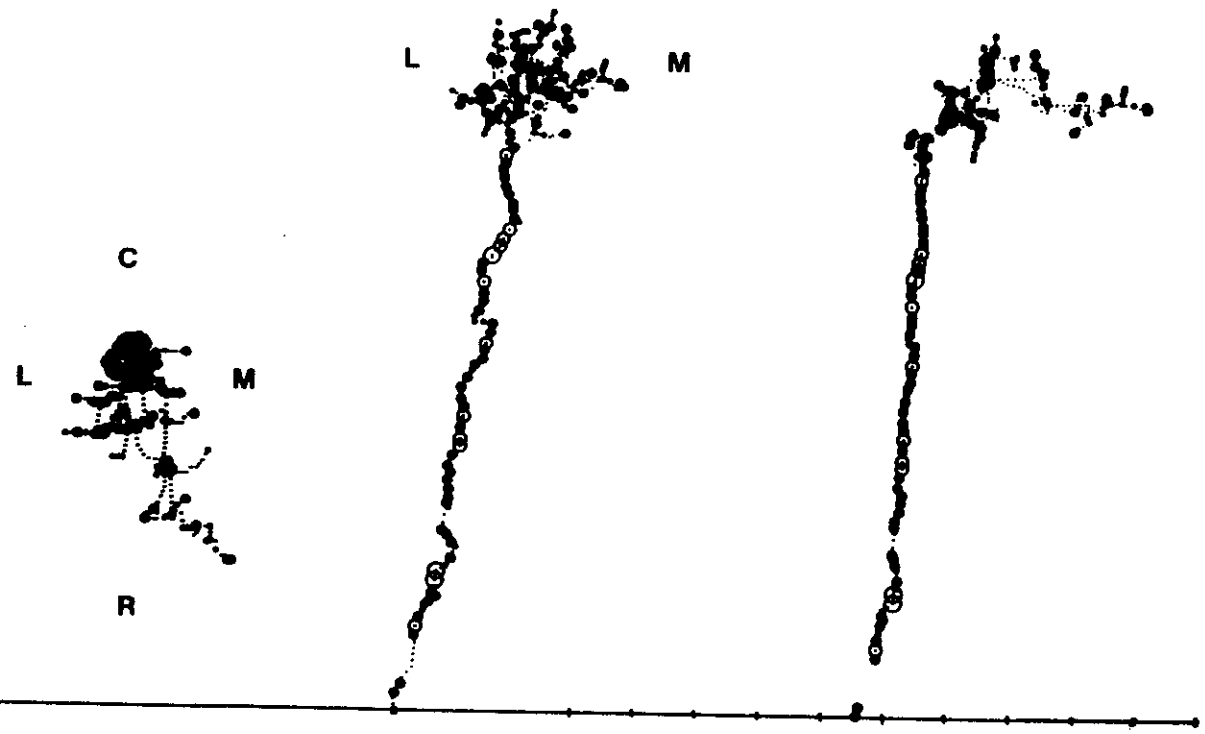

Fig. 12. 3-D reconstruction of a single dendritic bead cluster and its parent apical dendrite impregnated by the rapid-Golgi method. The apical dendrite is in the rostral half of the right tectal neuropil and its cluster lay just under the pial surface. Middle: the cluster as it looked in the original transverse section. Note that no anisotropy of the cluster is apparent. Rostral is towards the reader. Left: the cluster as seen looking down onto the tectal surface. From this perspective the cluster has a clearly asymmetric orientation with respect to the apical dendrite. Right: the middle image was rotated about the dorsoventral axis 70° so as to best demonstrate the cluster's directional orientation. R, rostral, C, caudal, L, lateral, M, medial. Tic marks denote 10 μm.

class IV RGCs, and the class IV units found in the deepest layer of tectal neuropil[39].

Accordingly we began the work reported here, the classifying of electrical transients recorded at a single position of the microelectrode anywhere along the tectum in the outer two physiologically defined layers, which are synaptically fed overwhelmingly by unmyelinated optic nerve fibers[25,32]. Before proceeding to the detailed exposition, we want to make a specific point. The 3 fundamental electrical transient shapes (Fig. 1) do not convert into each other by change of scale. Reducing a type B transient to the scale of a type A transient always shows that the A is not a B seen from a greater electrical distance. The rise to peak for the type A is always faster and the falling phase more ramp-like than for the type B. The type C response is different enough in shape from both the A and B as to make it easily distinguishable from them with any scale change.

The different spike shapes, the spatial organization of these response types in the MURF, and the clear radial arrangement of the MURFs with respect to some common pole in the visual field is firmly attested by Figs. 1–5. These data called for an underlying anatomical basis. But there lay the first problem: to account for the different electrical transient shapes with the nervous elements present in the neuropil.

The type B transient is triphasic and looks very like the external record of a passing impulse in an axon. Type C is diphasic and looks very like the arrival of an impulse at an axonal end. The 3 phases of the type B can be seen as the approach of an impulse, the local invasion, and the departure. The two phases of the type C can be seen as the approach and local invasion; there is no further membrane into which to depart.

The B and C transients are well above noise level, implying large currents. If we were recording from axons this would ordinarily bespeak a large space constant. But the localization of these transients is remarkably sharp. It is not as if the same type B transient can be seen in any direction away from the point of recording. The precise mapping of receptive fields by these transients tells us that a 0.1 mm displacement of the electrode in any direction away from the point of recording will not permit us to find a particular transient again. Such a small space constant, with so high a current that implies an approach, an invasion, and a departure might just be possible with a highly branched varicose terminal structure such as Ramon[6] showed. That is why it seemed reasonable 3 decades ago to imagine such a large convoluted active area to serve as the current amplifier[29]. But Ramon was wrong, and the teledendra of the unmyelinated optic nerve fibers are certainly much more tenuous than he portrayed[25].

The most obvious candidate for the generator of type B and C transients is the dendritic 'appendage' or bead cluster described by Székély and Lazar[42]. These issue from the apical dendrites of the large pear-shaped neurons in the cellular layers of the tectum. They are best described as an interconnected cluster of grapes on a twig. Each dendritic grape or ball is 2–4 μm in diameter, and the balls in a cluster are everywhere interconnected among themselves by thin branches. The clusters are about 30 μm in diameter. Many of the balls have both presynaptic as well as postsynaptic apparatus[42]. Their input is from retinal or isthmic[21] terminals, or from the balls of neighboring clusters. Their synaptic output is to other balls or to dendritic collaterals of cells of a different type. They have a reasonable amount of mitochondria each. The axons of these cells spring from the apical dendrites, not the cell bodies[42].

Interestingly, a similar arrangement exists in the olfactory bulb, where the dendritic processes of mitral cells are postsynaptic to olfactory nerve terminals, presynaptic to the dendrites of interneurons, and some distance from the parent cell somata[35]. Mori et al.[35] have shown these dendritic arborizations to be electrically active, and propose they serve a dual purpose of magnifying the signals reaching the distant cell somata, and boosting local synaptic output. The theoretical development of signal amplification and propagation by dendritic elements, e.g. a set of electrically active dendritic spine heads attached to passive spines, is crisp and convincing[33,37].

We suppose the dendritic grape clusters to be constructed similarly to the active head/passive spine arrangement[33,37]. The balls themselves are presumed electrically active, and the connecting threads between the balls of a cluster are presumed to be passive membrane. In a sense, taking the balls as nodes and the passive threads as internodes, the arrangement can be regarded as equivalent to a crumpled, highly branched myelinated fiber where the internodes are exiguous. If the attaching twig to the apical dendritic stalk is also passive membrane, but the zone of the apical dendrite to which it is attached is active membrane, the whole operation is that of a collecting amplifying system setting up unit signals in an active segment of dendrite. This latter point, that there is an active segment of apical dendrite, was suggested by Luk'yanov[30].

If this is the case the shapes of types B and C transient are directly accounted. For an electrode tip lying within a dendritic bead cluster, those balls excited in remoter stretches of the cluster make of the balls at the electrode a source. As the depolarizing wave spreads, the whole cluster is seen as a sink. When the active dendritic segment of apical dendrite is invaded while the active state declines in the cluster, the sink state at the

electrode is followed by a source state. The number of balls in a cluster, and relative sizes of cluster and electrode insure that most of the time the first few balls excited will be remote to the electrode. If excitation does begin near the electrode, the initial current source will be small. It is not surprising then that among the type B units, the initial positive phase is the most variable in relative amplitude. This accounts for the type B transients. For an electrode tip lying adjacent to the active dendritic segment, the firing of the cluster makes the segment a large initial prolonged source, and the subsequent firing of the segment makes of it a sink. Since there is no active membrane beyond the segment, the sink dies without being followed by a source. This accounts for the type C units.

That any B or C transient is often but not constantly followed by a slower rising and longer negative transient (Fig. 2) can be accounted as follows. It has been shown that cell body firing does not invariably attend axonal firing among the tectal cells[21]. The length of inactive dendrite between the active dendritic segment and cell body is at most 0.3 mm, so that there is good coupling of the receptive structure — grape cluster and active dendritic segment — to the axon and cell body. As elsewhere in neurons, the axon fires first and the cell body may or may not fire in response to the axon discharge[2, 5,7]. The externally taken records of cell-spikes in deep tectum show a sharp negative transient of about the same duration as the externally recorded negative transients of the receptive dendritic structure, but it is followed by a large slower positive transient which represents the recovery phase of the cell. This swing of hyperpolarization of the cell makes a sink of the receptive dendritic structure, and accounts for the relatively slow externally recorded negative wave at the receptive structure (Fig. 2). This inward current probably aids in the recovery of the receptive structure from its own activation. On the other hand, the firing of the cell, in making of the receptive structure a source, would sharpen the falling phase of the negative wave recorded at the active dendrite, and, under appropriate conditions, even re-excite it — as we suspect occurs in the occasionally encountered complex waves of Fig. 10. The negative after-wave, as described, in hyperpolarizing the receptive structure, raises its electrical threshold. This view of the slow afterpotential is reinforced by the observation that for a single type B or C transient watched over a reasonably long period, it will sometimes be followed by a slow potential, sometimes not — but the slow potential is always of fairly constant height when it occurs.

In accounting for the type A transient, several points can be made. First is that it is often preceded by a small and quite short positive swing, small enough to be below the noise level but observable by its association to the larger negative transient. The negative transient most often rises quite sharply. Scaled to the type B transient it is usually markedly steeper. Its fall is sometimes ramp-like, sometimes almost exponential. Occasionally there follows a long, very low positive phase. The type A transient looks rather much like the external record of peripheral nerve frog axons reported by Del Castillo et al.[8] which have active Na^+ conductance but no active K^+ conductance. But similarities of this sort are not supportive evidence, merely suggestive.

As remarked earlier, impulses in single unmyelinated fibers of passage generate external currents whose signal strength in the volume conductor of neuropil lies at or below the thermal noise level of the electrode. But where a fiber terminates, the end acts as a reflecting barrier; the impulse reaches it, then dies in place. This magnifies the recorded negative phase of the externally recorded impulse and the signal goes above noise level — the impulse comes but doesn't pass. The negative phase of the type A spike is thus attributed to the terminal stretch of the afferent axon. The frequently observed short initial positive phase, well below the broad-band thermal noise in the recording electrode but easily seen by its association with the sharp rise of the larger negative phase, is fully explained by supposing it to represent the approaching impulse to the terminal stretch of an afferent fiber. Near their terminals the ultimate and penultimate twigs of the unmyelinated optic nerve fibers and of the thicker axons from contralateral n. isthmi are probably of the same final size so that no distincion would be seen in the records of their excitements. There is no simple way of explaining the type A transient if we suppose it to be generated by the postsynaptic structures.

A provisional account has been given for the generators of the 3 fundamental electrical transient types. Under this account the origin of the neuropil binocular units (Figs. 7,8) is readily apparant. There is a many–many connection between optic nerve fibers and the tectal dendrites postsynaptic to them. Székély and Lazar[42] point out that the same grape cluster receives input from two or 3, and perhaps a few more optic nerve fibers, and the terminals of each optic fiber are synaptically connected to a few neighboring dendritic bead clusters. It was recently shown in *Xenopus* that the crossed isthmotectal fibers and optic fibers synapse onto common dendritic structures in the neuropil[44], which we suppose to be the bead clusters seen in *R. pipiens*. Since, taken as a unit, a cluster can be postsynaptic to both optic and isthmic terminals, and any effective input to the cluster results in a unit signal, there ought to be binocularly driven type

B and C units in the neuropil, and the data confirm this (Figs. 7,8).

It is difficult to account for the binocular neuropil units without attributing them to an active dendritic process. They can not be generated presynaptically, for that would imply that two adjacent axonal terminal bushes, were they capable of producing the currents necessary to record the large type B and C units, had exactly the same extracellular current transient at the electrode. But it is well known that the probability of this is of measure zero, especially when it is considered that the binocular units are not lost by small dorsoventral displacement of the electrode. Cell somata are rare in the neuropil, and there is no evidence that the few cells present are postsynaptic to both optic and isthmic terminals. It could be argued that the binocular units are recorded from the terminal arborizations of tectal cells with ascending axons, a group to which the pear-shaped neurons with dendritic appendages belong. Székély and Lazar[42] draw these arborizations as relatively sparse. But even if they were not the question again arises how these axonal twigs could generate the large type B and C units. Lastly, there is both the data of Luk'yanov[30], which is strongly suggestive of an electrically active dendritic process, and the tripartite structure and radial orientation of the MURFs, which is also consistent with postsynaptic generation of the type B and C units, and to which we now turn.

Several points can be made in accounting the MURFs. First, the observation that visual stimulation in a MURF evokes units of all 3 types in the neuropil (Figs. 3–5) is consistent with the proposed origin of the transient types given above. The electrode lies simultaneously among the terminal structures of some optic nerve fibers, within the interweaving of some dendritic grape clusters, and next to the electrically active segment of apical dendrites of other pear-shaped neurons, and thereby records in a MURF transients of each type. However, that MURFs have a tripartite structure such that pulse trains of each unit type are excited in sequence by moving the visual target along the long axis of the MURF implies a specific anatomical order to the spike-generating elements.

One level of organization is provided by the retinotopic distribution of optic terminals across the tectal surface. This anatomical order, still best demonstrated by the electrical records, makes possible the mapping onto the tectum of both the visual pole and the radial lines along which the MURFs are oriented. Secondly, the electrophysiological data presented here imply that each dendritic grape cluster is not randomly distributed around its parent apical dendrite, but rather that it must come off that dendrite in a specific direction, as shown for the cluster in the reconstructed image of Fig. 12. In any one region of tectum the clusters should be oriented similarly with respect to the primary dendrites, a phenomenon we also observed in those few rapid-Golgi preparations where several adjacent dendrites were impregnated. This local order is carried into a global order by the systematic alignment of the grape clusters, with respect to their parent apical dendrites, along the projection of the radial lines centrifugal to the projection of the visual pole (Fig. 13). In a physiologically connected triad of optic fiber teminal arborization, dendritic grape cluster, and active segment of apical dendrite, the latter must lie closest to the projection of the visual pole. As shown in Fig. 13, this arrangement guarantees MURF shape, polarity, and tripartite structure. Parenthetically, the loss of MURF tripartite structure near the visual pole presumably reflects the expected local breakdown of the anatomical ordering principle for the spike generating elements near that singular point in the neuropil which corresponds to the projection of the visual pole. In this scheme MURF structure and orientation are a direct consequence of the anatomy and electrophysiology of tectal, not retinal, structures.

The anatomical order we have proposed would be difficult to notice by chance with conventional staining techniques. The terminal arbors of single unmyelinated axons are not easily visualized by any method. However, Constantine-Paton et al.[9] concluded from their developmental studies that the terminal arborizations of the myelinated fibers are oriented in a manner remarkably similar to that we are proposing for the unmyelinated fibers. Specifically, these authors show the terminals as essentially radially oriented around the tectal perimeter, and 'facing' in towards central tectum. The dendritic arborizations are seen in Golgi and other preparations, but because the alignment of the dendritic elements is radial in visual space, and therefore curved on the tectal surface, no single plane of sectioning will make apparent the systematic alignment of the elements. Katz and Constantine-Paton[27] overcame this obstacle by looking at whole mounts of tecta in which individual cells had been stained intracellularly with Lucifer yellow. With entire dendritic arborizations visible in the plane of the tectal surface, these authors were first to notice that these arborizations were non-circular and had a long axis. It was especially gratifying that not only did they report a rostral bias to the dendritic arborizations with respect to the cell body, but also this bias was most pronounced for those cells with grape cluster-like arborizations (Katz, personal communication). Since the visual pole maps to a point in caudolateral tectum, the anatomic implications of our electrophysiological data for the dendritic grape clusters (Fig. 13) would most likely be observed as a rostral bias to these clusters for most cells in central tectum. But

Katz and Constantine-Paton[27] filled only these cells, and their data do not include cells around the perimeter of the tectum, where other orientations would be most pronounced. For example, in the region of tectum immediately caudal to the projection of the visual pole we would expect the dendritic clusters to be oriented more or less caudally with respect to the parent apical dendrites and cell somata. The results of these anatomic studies[9,27] are complementary to our electrophysiological studies on MURF structure and orientation, and taken together, reveal a previously unrecognized order, both local and global across the tectum, for the terminals of the unmyelinated optic fibers and the dendritic elements postsynaptic to them.

The tentative explanation given above for the provenance of the types B and C transients suggests at least two ways to account for the multiphasic (Figs. 8,9) and patterned units (Fig. 10). First is the possibility of re-excitation between cell body and receiving unit, cluster and active dendritic segment, about the time of cell excitement. That re-excitement occurs by reflection has been seen in axons and neurons before[28]. A second explanation for these units is that since the dendritic ball clusters are synaptically connected locally to each other[42], there is the possibility of circus action in that connected net restricted to that level of the neuropil in which it occurs. In either case the likelihood of such reverberatory phenomena can be subtly controlled by other influences that affect the damping factor or threshold of such oscillation, as we have observed with some pharmaceuticals. If it is so controllable, the reverberation becomes a legitimate operation in the processing of visual data.

One important point from the experiments is the high reliability of the unmyelinated channels. There has been an impression that dense-packed unmyelinated fibers block at relatively low frequencies. This was based on a study where the whole optic nerve was massively stimulated[36]. Similar results are obtained in the olfactory nerve[12], and Gesteland[10,18] believes that some of the rapid adaptation in the smell pathway from the mucosa may be due to such action — the outpouring of K^+ from the enormous surface of the dense-packed fibers. But in the optic nerve, as Maturana[31] showed, the unmyelinated fibers weave between the glial slings along their course so that the proximation of any two fibers as neighbors only occurs over short stretches. This is borne out by the wide distribution of receptive field centers recorded within a single glial sling in the optic nerve. Also the nature of the receptive field of class I and II RGC fibers is such that no natural stimulus can set more than a small fraction of them firing over the same period. Thus it was not surprising that quite local stimulation in optic nerve turned out to give reliable conduction up to

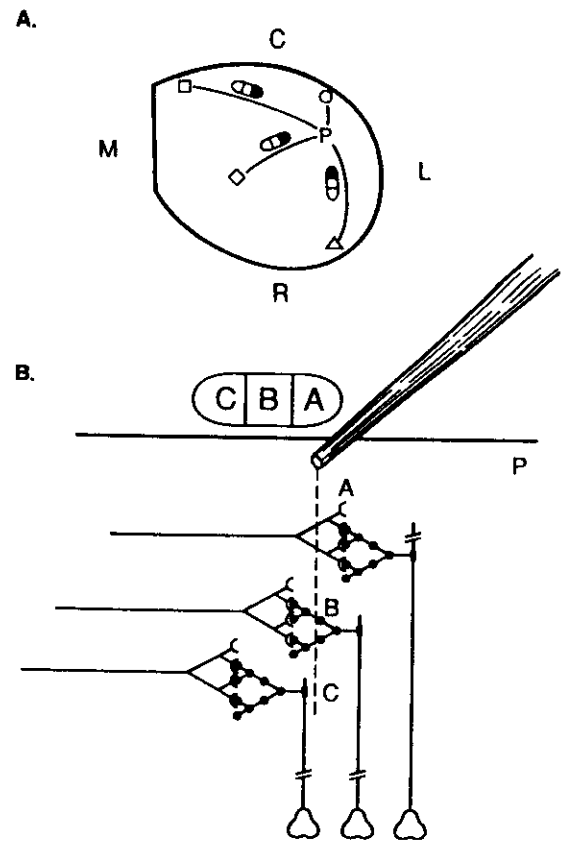

Fig. 13. A: a diagram of the left tectal surface showing the approximate location of the projections onto it of the visual pole (marked with a 'P'), and several of the radial lines along which MURFs are oriented in visual space. MURFs adjacent to these lines indicate the relative position of each MURF section in visual space with respect to the visual pole. The blackened segment of each MURF would contain the type A unit RFs. The single units comprising a MURF are recorded at a single tectal locus. Symbols indicate the projection of the 'poles' of visual space around the frog as follows: △, directly in front; ◇, above; and □, behind the animal; ○, the intersection of the horizontal and coronal planes on the frog's right side. B: a hypothetical section of optic tectum along the projection of a radial line. The 'P' and heavy horizontal line represent the visual pole and tectal surface, respectively. Optic nerve fibers shown on the left arborize and synapse onto the electrically active dendritic beads which are depicted as filled circles. The thickened segment of apical dendrite where the stalk to the bead cluster attaches signifies the electrically active zone. A,B,C within the hypothetical section indicate the type of unit recorded by the electrode at each type of nervous element. The axon–dendrite connections are separated vertically for clarity, but are actually intermingled at the recording point in the neuropil. If the neuronal elements in the neuropil have the anatomical order shown with respect to the visual pole, the ensemble of single units recorded at any point in the tectum will comprise an elongated tripartite MURF whose long axis intersects the pole, and whose section containing type A units lies closest to the pole, as shown in the MURF drawn above the tectal surface. The RFs of the different unit types in the MURF will be separated in visual space due to the anatomical separation in the neuropil of the optic terminals whose activity triggers the different transient types. Movement of a small target through the MURF along its long axis would excite in sequence each unit type. This diagram is highly simplified and illustrates neither the many–many connection between optic nerve fibers and tectal cell dendrites nor the large number of nervous elements from which an electrode normally records.

80/s as viewed transsynaptically in the tectum, with fibers whose conduction velocity was 20–30 cm/s.

The important point here, which was overlooked by George and Marks[17] in their study of the clas:s III responses, is that no single synapse must conduct reliably at these relatively high frequencies. The redundant connectivity between the terminals of a single optic fiber and the active dendritic clusters postsynaptic to them means that succesful transmission at a subset of the synapses may be adequate to evoke the unit response in the dendrite. By multiplying the number of connections between the optic fibers and the tectal cells upon which they terminate, the frog has produced an admirable system for conveying information reliably over unmyelinated channels. This reliability is coupled to a relative maximizing of representational resolution, since an optic nerve of equal size but containing only myelinated fibers would necessarily contain many fewer fibers indeed.

A few final comments, implicit in the discussion so far, are now made explicit. First, by identifying the B and C transients with specific dendritic structures, we show the source of the regular and sharply distinct functional lamination recorded in the tectum. It is not the distribution of optic nerve terminals. These can be of mixed type, from both myelinated and unmyelinated fibers, at all depths. Rather it is the distribution of dedicated receiving elements to which *some* of the axonal endings are synaptically applied. This subset of optic fiber terminals can not be distinguished from other optic terminals by forward staining of the optic nerve. Signals transmitted by optic fibers which synapse with dendritic structures other than the beaded dendritic appendages may be unrecordable with contemporary methods. For example, the tectal neurons whose projections form the crossed tectobulbar pathway receive direct retinal input onto ascending dendritic structures which entirely lack the densely branched beaded arborizations[26].

Second, it can no longer be taken for granted that single unit class I and II responses recorded in the tectal neuropil are in fact a delayed account of single RGC firing. While such a one to one correspondence is not ruled out by our data, it also can not be taken as certain, now that the large extracellular electrical transients recorded in the neuropil have been shown to be of dendritic origin. If, as we suppose, the type A units are generated presynaptically by the axonal terminal arbors, they should provide a true measure of RGC firing. But reliable observation of the firing pattern of a single type A unit is very difficult due to its small amplitude and the simultaneous excitement by the target of many other transients. Therefore, until the physiology of the connections between optic terminals and dendritic structures is better understood, it seems prudent to return to recordings from the optic nerve for reliable measures of RGC activity.

Lastly, the information processing capability of the active dendritic structures which we believe to be present in the neuropil must be recognized. That these structures amplify locally the afferent signals is a direct consequence of our data. That more complex processing takes place is suggested by responses such as those shown in Fig. 10, and the fact that the dendritic bead clusters are synaptically interconnected. In addition, there is the analysis by Pratt, who noted differences in the temporal firing patterns of class IV RGC axons recorded in the nerve and class IV responses recorded in the neuropil[39]. The specific nature of dendritic information processing for the sensorimotor interface and the frog's visually guided behavior was not investigated. However, we feel that the tectal cell dendrites play a much larger role in the processing of visual information in the frog than has previously been assumed.

Acknowledgements. Three-dimensional reconstructions of tectal dendritic trees were done by Wendy Yee and Gerald E. Schneider using M.I.T.'s. Neurotrace system. We are also indebted to Prof. Schneider and Pat Harlan for their help with the rapid-Golgi impregnations.

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