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Research report

Functional properties of regenerated optic axons terminating in the primary olfactory cortex

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Abstract

When the optic nerve of *Rana pipiens* is cut and deflected into the telencephalon, the regenerating fibers terminate selectively in the superficial neuropil of the primary olfactory cortex. These redirected fibers and their terminals on the dendrites of the cortical cells appear normal by LM and EM criteria. Electrical recording, done 2–16 months after surgery, shows visually evoked activity in the superficial neuropil (Layer I) of the olfactory cortex, and visually excited responses in the deep cortical cell layer (Layer II). In the normal frog, the electrical activity seen in the neuropil of the olfactory cortex consists of small transients about 2–3 × the noise level of the electrode contact. These occur spontaneously and are also excited by puffs of air to the nose. There is no such excitation by visual stimuli. Larger initially negative spikes cell above noise level are recorded in the cell layer next to the ependymal surface, and these are also spontaneous, or exicited by puffs of air to the nose, but not by visual stimuli. In the operated frog, the small transients in the neuropil appear and are excited by the puffs of air and by visual stimuli. Similarly the responses in the cell layer are excited by both sorts of stimuli. But new types of electrical signals appear in the neuropil; they are driven only by visual stimuli presented to the affected eye. These are very large transients of the kind found in the tectal neuropil and have the two characteristic shapes which were classified as B and C types in the tectum. Such large transients are never seen in the neuropil of the olfactory cortex in normal frogs. The receptive fields of the small visually driven transients in the neuropil are not easy to make out because the signal levels are so close to the noise level that different units cannot be reliably distinguished from each other. But the receptive fields of the much larger B and C type unit responses are as easy to classify and plot as they are in tectum, even though on the average they are only about 2/3 as large as in tectum. The single-unit receptive fields belong to one or another of the several types of retinal ganglion cell classes distinguished in optic-nerve recordings. Four of the major classes normally project to the tectum and a fifth projects to the lateral geniculate complex. But all five are present in the ectopic projection to the olfactory cortex. The various classes of ganglion cell operations are only crudely stratified in the olfactory cortex. The classes mediated by unmyelinated fibers (Classes I and II) are represented superficially in the neuropil; the myelinated contingent (Classes III and IV) are often found deeper. But a strict ordering by depth as seen in the tectum is not found in the cortex. In some of the operated frogs, several large, distinct B and C units, discriminable by size and shape, can be recorded at a single electrode position in the neuropil. Then both the single-unit receptive field (SURF) of each unit can be classified and plotted and also the multi-unit receptive field (MURF) of their distribution in the visual field. However, the MURFs are not densely filled with single units, as they are in the tectum, and they subtend much wider areas in the visual field. Generally, the SURFs are also not related systematically to the position of the recording electrode on the cortical surface. Thus, there is no finely ordered retinotopic map in the olfactory cortex. However, almost all the B and C units responded only to stimuli presented (monocularly) in the frontal part of binocular region of the visual field.

Keywords: Frog; Nerve transplantation; Aberrant synapse; Cross-modality rewiring; Alternative specificity; Neural plasticity

1. Introduction

When the optic nerve is surgically deflected into the base of the cerebral hemisphere in the adult leopard frog (*Rana pipiens*), the regenerating optic axons that grow anteriorly enter selectively the primary olfactory cortex and form a compact field of synaptic terminals in the

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superficial neuropil (molecular layer, Layer I) [24,25]. Like the normal optic projection to the tectum, the ectopic field includes axons of small diameter ending in thin, serially beaded terminals, and larger axons ending in more densely branched arbors [14,22,26]. When observed with the electron microscope, the terminals have a normal morphology and, like the optic fiber endings studied at normal locations, display the Gray type I configuration of excitatory synapses [14,17,34]. The projection is formed within the first 2–3 months after surgery and is apparently permanent [25]. The long-term retention of this redirected optic fiber projection and the normal appearance of its terminals suggested that the olfactory cortex is capable of maintaining trophic support for the projection and that physiological responses may be evocable in this foreign area by visual stimuli. Until the work reported here, however, nothing had been known about the physiological properties of the ectopic projection.

To characterize the ectopic retinal projection in the olfactory cortex physiologically, electrical responses to visual stimulation were examined by the same techniques used previously to study the properties of both the intact [12,19] and regenerated [18] optic axon projection to the contralateral optic tectum of *R. pipiens* and aspects of the normal retinothalamic projection [21]. This allowed us to determine the types of retinal fibers projecting to the cortex, whether they had the ability to evoke postsynaptic responses, whether their endings were spatially organized, and how the electrical waveforms associated with single unit activity in the ectopic field compared with those recorded in the tectum. The background for the comparisons that emerged from the present study are given briefly in the following.

Four of the five major classes of retinal ganglion cell identified by recording in the optic nerve project to the tectum, and one projects to the lateral geniculate complex. These classes are recognized by means of the selectivity of their responses to characteristic features of the visual stimulus. In the tectum, the activity sampled along single electrode penetrations in the superficial neuropil normal to the tectal surface shows that the four separate classes of retinal ganglion cell activity evoked from a common locus in the visual field are recorded in separate sublaminae of the neuropil. Penetrations deep to the superficial neuropil record the discharges of tectal cells and tectal efferent axons in response to visual stimulation. Another class of ganglion cell, characterized as a blue-sensitive ON-fiber, projects exclusively to the lateral geniculate complex of the thalamus [21].

In the tectum, visual stimulation specific to the class I and II ganglion cells, which constitute the major tectally projecting population, elicits at a single position of the recording electrode trains of unit activity in which the individual transients have one of three distinct waveforms, designated type A, B and C [12]. The A transient is small, about twice the peak-to-peak noise level of recording, but the B and C transients are an order of magnitude larger. The B type is triphasic, with a small initial positive phase, a large negative phase and a small positive third phase. The C type is diphasic, with a large initial positive phase and large negative phase. Both types can be followed by an intermittently occurring slower negative phase. The type of waveform associated with the response of an individual single unit in the train depends systematically upon the spatial location of its receptive field within the aggregate receptive field of all the individual units comprising the response train. The aggregate of these individual receptive fields, termed a multiunit receptive field (MURF), has an elongated oval shape, the long axis of which always points toward the center of the visual field of the stimulated eye. The MURF is anisotropic in the following way: the single-unit receptive fields (SURFs) associated with type A responses are always displaced toward that pole of the MURF closer to the visual field center (its narrow pole), and the SURFs giving the type C responses are displaced toward the side opposite (its broad pole), each type of response occupying about a third of the MURF, the B transients in the middle.

Nerve-bridge and transplantation experiments have shown that optic nerve fibers can be induced to project into several different areas of the CNS normally foreign to them [2,5,6,11,15,36,37]. It has been argued that the new sites of projection may contain remnants of the molecular determinants of optic fiber target selection thought to operate during development [2]. However, only small numbers of optic fibers appear to have grown into the foreign targets in these studies, offering little opportunity to investigate their physiological properties and the types of ganglion cells capable of forming the connections. In other studies on mammals, ectopic retinothalamic projections of substantial volume have been induced in the lateral posterior nucleus, medial geniculate body and ventrobasal nucleus during development by ablation of the superior colliculus [30] or by means of surgical procedures that delete the normal sensory innervation to these nuclei and cause retrograde degeneration of the lateral geniculate body [4,9,10,20]. However, electrophysiological study has shown that input from the X and Y ganglion cells, which provide the main fiber complement of the normal retinogeniculate pathway is absent from some of these aberrant retinothalamic projections [23]. This is presumably due to the destruction of the lateral geniculate body. The present experiments differ from the above in that induction of the ectopic projection to the olfactory cortex does not require deafferentation of the new target, and transection of the optic nerve does not delete the retinothalamic fibers from the regenerated projection in the frog [32,33]. The current report presents the first physiological study of redirected retinal fibers after induction of a sizable ectopic projection into an otherwise normal area of the CNS, in which the capacity of the various types of ganglion cells to participate in the projection has been demonstrated.

An abstract of this work has been presented elsewhere [29].

2. Materials and methods

2.1. Subjects

Adult *Rana pipiens* (Northern, 3–3.5 inch body length) were prepared in advance for use in this study as experimental subjects. The frogs were anaesthetized with MS-222. The ventral aspect of the cranium was opened to expose the optic chiasma and basal telencephalon. The left optic nerve was detached from the optic chiasma, turned anteriorly and implanted into the posterior lateral surface of the fascicular prominence of the ipsilateral cerebral hemisphere (striatum), as described earlier [24]. In most cases, the continuity of the brain across the di-telencephalic junction was interrupted on the operated side by carefully controlled aspiration of brain tissue. This was done, as described earlier [25], to focus more of the aberrant projection into the telencephalon by reducing escape of the transplanted axons back into the diencephalon and midbrain. Transection of the di-telencephalic junction interrupts the lateral forebrain bundle and deletes the major afferent input to the striatum, but this should not directly affect the olfactory cortex since the striatum does not project to it [35]. The oral mucosa was sutured closed, and the frogs were returned to their holding pens. Recovery was uneventful, and the frogs resumed feeding (by the use of the unoperated eye) within a week or two. Normal, unoperated frogs were also available at the time of recording to establish baseline observations. All frogs were maintained in tanks with a constant supply of cool water, and were fed 1–3 times a week with a live insect diet.

2.2. Anatomical control

The projection from the left eye was traced in frogs sampled randomly from the operated group. In some cases the eye was injected intravitreally with 3–4 μl of a 1:1 mixture of [^{3}H]leucine (spec. act. 47 Ci/mmol) and [^{3}H]proline (36 Ci/mmol), total activity 40–60 μCi. These frogs were studied by the autoradiographic tracing technique. In one case, the projection of the left eye was traced with an injection of 9 μl of 15% biocytin-HCl in frog Ringer's. In other cases, the left optic nerve was severed in the orbit, and crystals of horseradish peroxidase (HRP; 2 mg; Boehringer-Mannheim) were placed against the central stump. The frogs were sacrificed after 24–48 h under MS-222 anaesthesia by transcardiac perfusion with 0.12 M phosphate-buffered saline, followed by 4% formaldehyde or 4% glutaraldehyde. The brains were sectioned frozen at 50 μm in serial order in a transverse or horizontal plane. For autoradiography, the sections were mounted on subbed slides, dipped in Kodak NTB-2 emulsion, exposed 2–3 weeks, developed in Kodak D-19 and counterstained with thionin for cells. Biocytin transport, was studied by the method of King et al. [16]. The filling of optic fibers with HRP was studied by the Ni–Co intensified DAB (diaminobenzidine) method of Adams [1]. Golgi stained [28] and other normal material was available for reference.

2.3. Electrical recording

The experimental frogs were examined two to sixteen months after surgery, using the methods described in earlier papers [12]. The dorsal surface of the cerebral hemispheres was exposed under MS-222 anaesthesia in the evening prior to the recording session. The skin was lightly sutured and the frog was kept overnight in refrigeration. The next morning, the frog was lightly curarized (0.2 mg D-tubocurarine injected into the dorsal lymph sac), the skin incision was coated with xylocaine gel, and the brain was reexposed. A careful drawing was made of the vascular pattern on the exposed surface of the brain to guide and map the placement of the electrodes. The frog was placed on a recording platform at the center of an aluminum hemisphere (50 cm diam.) of dull gray finish, the exterior surface of which was marked off in spherical coordinates. Visual stimuli consisting of circular black magnetic discs 1–4° of visual angle were held to the interior surface of the hemisphere and moved by means of a magnet applied to the exterior surface. These were used to detect and plot the receptive field location of active class I, II and III retinal ganglion cells (see below). ON, ON-OFF and OFF responses were elicited by switching the ambient illumination on and off.

Recordings were made with a modification of the Woods-metal filled glass microelectrode described by Dowben and Rose [7], flashed with gold at the tip (5–10 μm) and coated with platinum black to achieve a low impedance in tissue. When desired, the final location of the electrode tip was marked by passing current (5 μA, 5–8 s) through the recording electrode. This produced a lesion of 10–20 μm diameter which was subsequently recovered in paraffin sections (10–20 μm thickness) stained with thionin. In addition, microglia were found to be aligned along the paths of the electrodes even when current was not passed. Although the final location of the tip was not readily indicated by this microglial reaction alone, the reaction did reveal where the electrodes had been positioned during recording.

3. Results

3.1. Anatomical background

The frog olfactory cortex consists of a compact, periventricular layer of cells (Layer II) whose dendrites, after branching several times, diverge obliquely toward the

external surface of the brain, where they terminate below the pia mater (Fig. 1A and B). Toward the outer third of the cortex, the radiating dendrites penetrate the lateral (or the medial) olfactory tract. The cortical cell dendrites, the olfactory tract, other unmyelinated axons, occasional small stellate neurons and the processes of the radial ependymoglia form a molecular layer (Layer I) that occupies the superficial one-half to two-thirds of the cortex. The olfactory tract and its terminals normally occupy the outer half of Layer I, while the inner half of this neuropil contains the efferent cortical and association axons and their terminals. The cortical neurons of Layer II are separated from the lateral ventricle by only a single layer of the ependymal cells.

When present, the ectopic retinal axons form a layer just beneath the pia mater (Fig. 1C), and their terminals

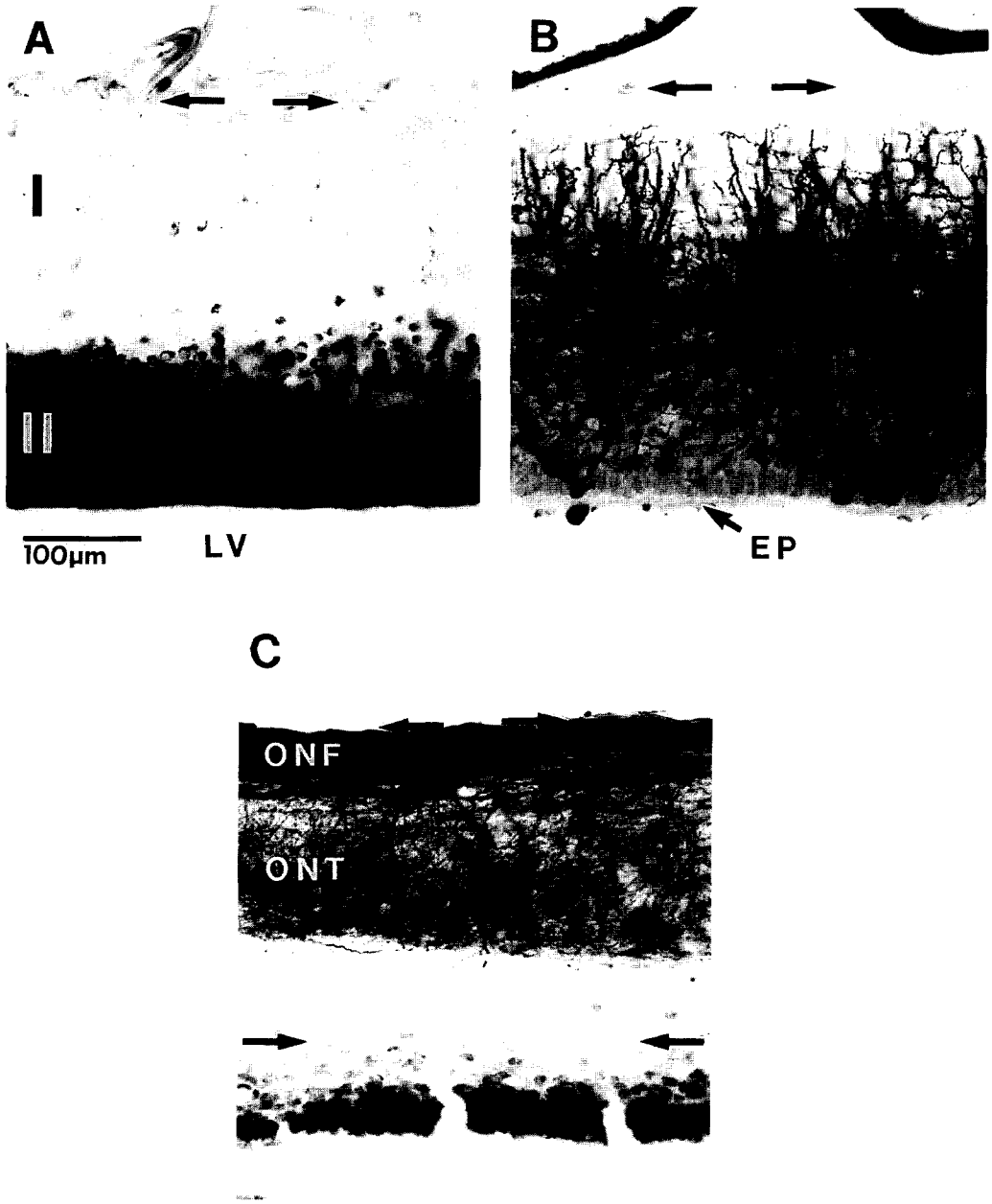

Fig. 1. Photomicrographs of frog olfactory cortex in horizontal sections of the brain. The level of the pia mater is indicated by the paired arrows at the top of the photographs. A: section stained for cells with thionin. The cell bodies of the cortical neurons form a compact layer (Layer II) adjacent to the lateral ventricle (LV), which is lined by a single row of radial ependymoglia (EP; see B). Few cells are found in the superficial neuropil (Layer I). B: section showing a comparable field impregnated by the rapid Golgi method. The branching dendrites of the cortical neurons extend obliquely through the superficial neuropil toward the pial surface. C: section showing a part of the ectopic projection in an operated frog 5 months after surgery. The optic nerve fibers (ONF) are grouped in a layer just beneath the pia matter, and their terminal field (ONT) extends downward into the superficial neuropil. The border between Layers I and II is indicated by the arrows facing toward each other. Bar in (A) applies to all the photographs in this figure.

extend through the outer half of the molecular layer. Characteristically, the inner border of the layer of optic terminals is sharply defined, and only an occasional optic axon penetrates beyond it into the deeper half of the neuropil. An electrode penetrating the olfactory cortex normal to the external surface would encounter the ectopic fibers and their terminals near the surface and the cell bodies of the cortical neurons at a deeper level, just before penetrating the ependymal layer.

For the most part, the olfactory cortex lies on the lateral surface of the cerebral hemisphere, medial to the orbit, where it is divided cytoarchitecturally into a pars ventralis and pars dorsalis. Since the ipsilateral eye cannot be removed, the recording electrode must be lowered from above to penetrate these areas tangentially. However, the anterior end of the olfactory cortex curves onto the dorsal aspect of the hemisphere, where it forms a belt separating the olfactory bulb from the dorsal cortex (Fig. 2). This anterior region is readily exposed by craniostomy, and electrodes thrust downward from above penetrate more-or-less normal to its external surface. Continuing medially, an extension from the anterior end of the pars ventralis enters the interhemispheric sulcus, where it becomes continuous with the postolfactory eminence. This is a narrow, dorsoventrally oriented strip of cortex, also bordering the olfactory bulb. Recordings from this small, medial part of the olfactory cortex are also obtained with tangential penetrations.

The experimentally induced projection was present in the pars ventralis (including its anterior region) and the postolfactory eminence in all experimental animals checked anatomically for the purposes of this study, and had all of the expected characteristics described above. However, in a few animals some fascicles of optic fibers entered the olfactory bulb in addition to olfactory cortex, and appeared to form terminals in the granule cell layer.

3.2. Visual responses

Unoperated controls

The electrical transients recorded in the superficial neuropil of the olfactory cortex were quite small in the normal

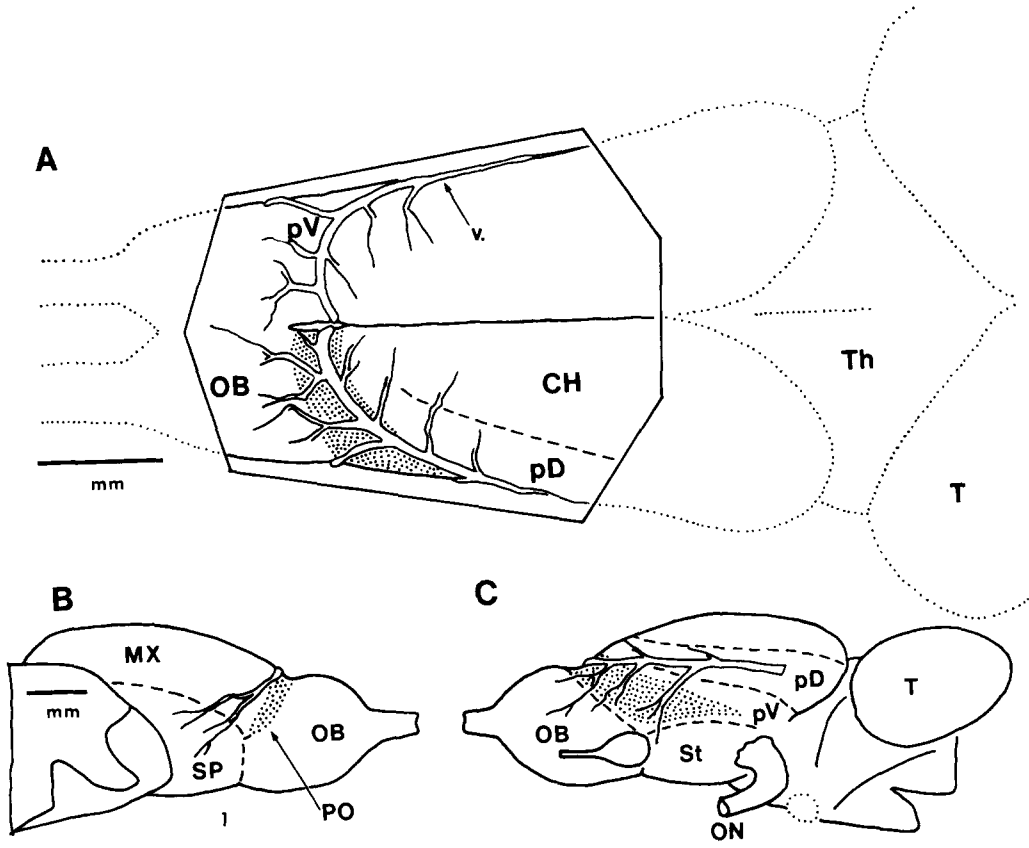

Fig. 2. A: dorsal surface of the telencephalon and the recording site, as viewed through the cranial window. The anterior end of the primary olfactory cortex, pars ventralis (pV), extends medially across the field to separate the olfactory bulb (OB) from the rest of the cerebral hemisphere, including the pars dorsalis (pD). The terminal field of the ectopic retinal projection, indicated by the stippled area in all the drawings, is crossed by the circumflex branch of the anterior cerebral vein and its anterolateral tributaries. B: medial view of the left cerebral hemisphere at a reduced scale, showing the extension of the ectopic terminal field into the postolfactory eminence (PO), which separates the olfactory bulb from the medial cortex (MX). C: lateral view of the left cerebral hemisphere, showing the site of implantation of the optic nerve (ON). The ectopic projection extends ventrally, laterally and posteriorly away from the cranial window.

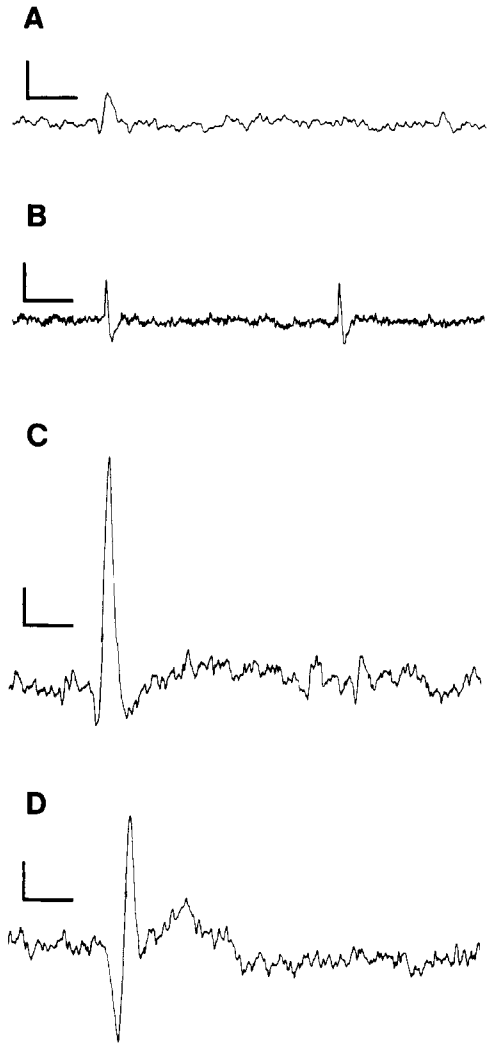

Fig. 3. A: small, spontaneously generated transient recorded in the superficial neuropil of the olfactory cortex of a normal frog (2 ms; 50 μV). B: Biphasic spike spontaneously generated in the cortical cell layer in the same preparation (10 ms; 50 μV). C: visually evoked type B wave from a train recorded in the superficial neuropil of the olfactory cortex in an operated frog. The response was evoked by a small moving target, but could not be elicited by switching the background illumination on and off. The response ceased if the target remained motionless within the receptive field (2 ms; 20 μV). D: visually evoked type C wave from a train recorded in the superficial neuropil of the same preparation. This unit responded to the same kind of stimulus as the unit in 3C (2 ms; 20 μV).

frogs, usually less than 40 μV, rising out of a background noise level of about 20 μV peak-to-peak, in the band of 10 Hz–3 kHz (Fig. 3A). They resembled the type A transient described in the tectum [12]. The transients occurred spontaneously, and they could be evoked by puffs of air directed at the nares. Although no systematic attempt was made to control the presumptive olfactory stimuli, the small transients were easily evoked by introducing into the air stream various odorous materials on hand in the laboratory. Spontaneous and odor-related responses were also recorded at the level of the cortical cell layer in these animals (Fig. 3B). No signs of visually related activity were seen in the normal olfactory cortex for any visual stimulus.

The ectopic projection

In all of the frogs, visual stimuli elicited trains of low amplitude transients in the superficial neuropil of the olfactory cortex, similar in height and shape to those evoked by air puffs. But there were also very much larger visually evoked single units recorded in the superficial

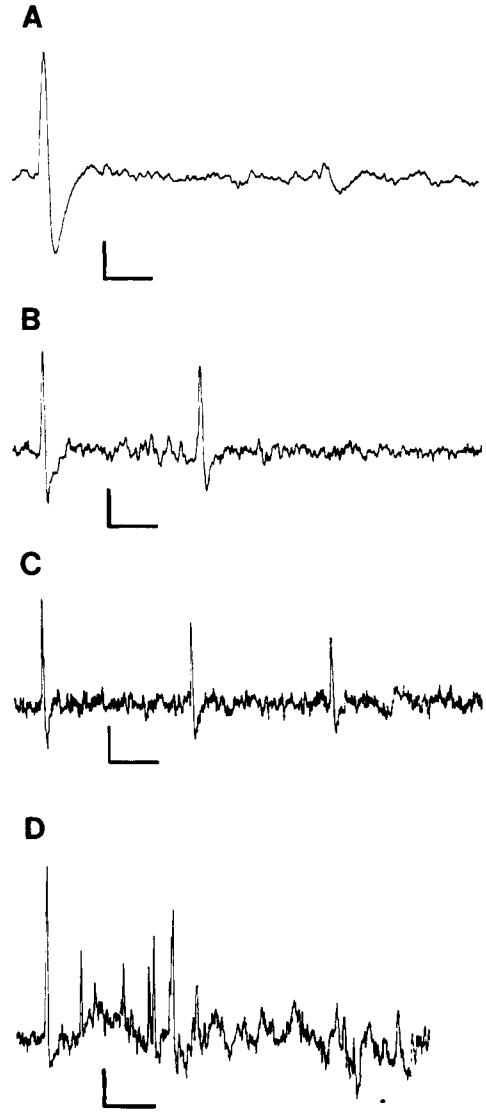

Fig. 4. Visually evoked responses in the olfactory cortex after innervation by the optic nerve, recorded at the level of the cortical cell layer. A: single transient sampled from a pulse train. Transients recorded at the level of the cortical cell layer have an initial negative phase, followed by a slower positive phase. This unit was activated by turning the background light on. (5 ms; 50 μV). B: another ON response unit shown in a slower sweep. (10 ms; 50 μV). C: this OFF unit was activated by a large moving stimulus, and responded maximally when the background light was turned off. (20 ms; 50 μV). D: ON response recorded just before making the lesion illustrated in Fig. 5 (20 ms; 50 μV).

neuropil; their amplitudes reached 150 μv. These transients never appear in the normal olfactory cortex. They seem identical to the B and C waves of single unit responses in the tectal neuropil (Fig. 3C and 3D). However, their maximum amplitudes were only about 2/3 as large as those usually found in the tectum. In addition, cell responses, driven by visual stimuli, were recorded just above the ependyma.

One-hundred and twenty-two large amplitude B and C type visual unit responses that could be assigned definite SURFs were obtained in about half the operated frogs tested ($n = 9$). Of these frogs, eight had sustained transection of the ipsilateral di-telencephalic junction at the time of initial surgery, but one had not. Only one of the frogs giving positive visual responses was studied at an earlier postoperative time (3 months); the others were studied after 5 months or more, including the frog with an intact di-telencephalic junction. The rest of the frogs ($n = 7$) gave fewer and lower amplitude responses, or had no large visually evoked responses at all. Four of these frogs, although studied after more than 5 months survival, were specimens in which the forebrain lesion had not been made. The remaining three were studied after less than 5 months survival.

The visually evoked activity was obtained from the accessible parts of the pars ventralis and postolfactory eminence of the ipsilateral olfactory cortex, and occasionally from neighboring areas of the olfactory bulb. No evoked optic activity was detected in any adjacent cortical areas or in the contralateral olfactory cortex. All visual

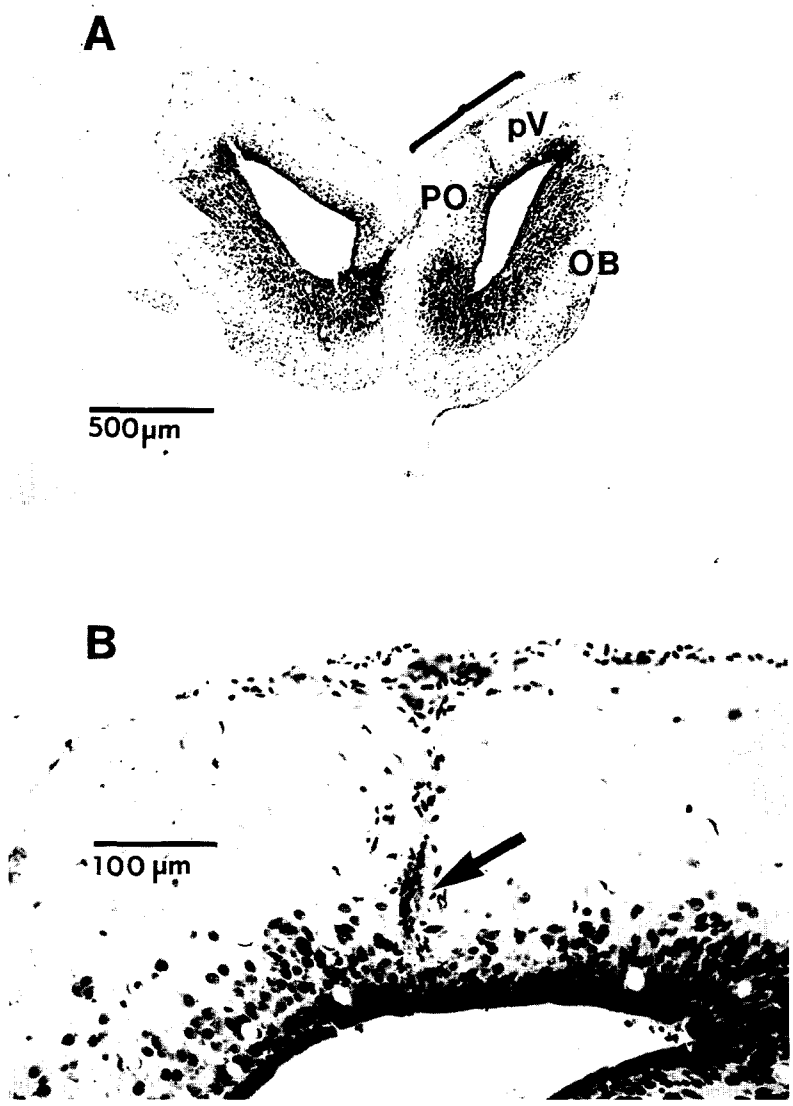

Fig. 5. A: low-power photomicrograph of a thionin-stained transverse section to show the lesion made at the end of the electrode track after recording the unit illustrated in Fig. 4D. The bracketed area is the part of the olfactory cortex shown at higher magnification in B, and rotated to return it to its orientation during the experiment. B: the lower end of the electrode track is located at the level of the cortical cell layer, as shown by the position of the lesion (arrow). Labeling as in Fig. 2.

responses were evoked through the left eye. When the left eye was occluded and stimuli presented to the right eye, no activity was evoked in the olfactory cortex.

Four kinds of visual stimuli were used to classify the units recorded in the cortical neuropil. (1) A small, dark target was moved across a white background. (2) A large target was moved across the same background. (3) The general illumination was turned off. (4) The general illumination was turned on. Classes I and II respond to the first two kinds of stimulus, but not to the last two. The responses are sustained for a while if the target stops in the receptive field, but cease abruptly if the light is turned off. Most of the units recorded in the ectopic projection to the cortex were of classes I or II (Fig. 3C and D), but no systematic attempt was made to discriminate between the two classes of unmyelinated fiber inputs. Class III responds phasically to all four kinds of stimulus, and such units were also found in the responsive cortex, but appeared much less often than the combined classes I and II. Class IV responds only to the second and third kind of stimulus. The response to dimming the light is relatively sustained, but ceases abruptly with brightening. Only a few class IV units were found in the ectopic field. Sustained responses to the fourth kind of stimulus (ON-response), were absent from the cortical neuropil, but could be found in the cell layer of the experimental animals.

When recording from the anterodorsal region of the olfactory cortex, where penetrations could be made normal to the surface, the class I and II responses were obtained within 150–200 μm of the surface. When recording from the more lateral or medial regions of olfactory cortex, where penetration was necessarily made increasingly oblique or tangential to the contour of the surface, the units were located at increasing depths. At some electrode positions, several of the large B and C units were recorded together, each characterized by a separate receptive field in visual space. The individual receptive fields in these MURFs were small, on the order of 3–5° across, as is characteristic of classes I and II, but were often separated widely from each other, sometimes by as much as 30° of

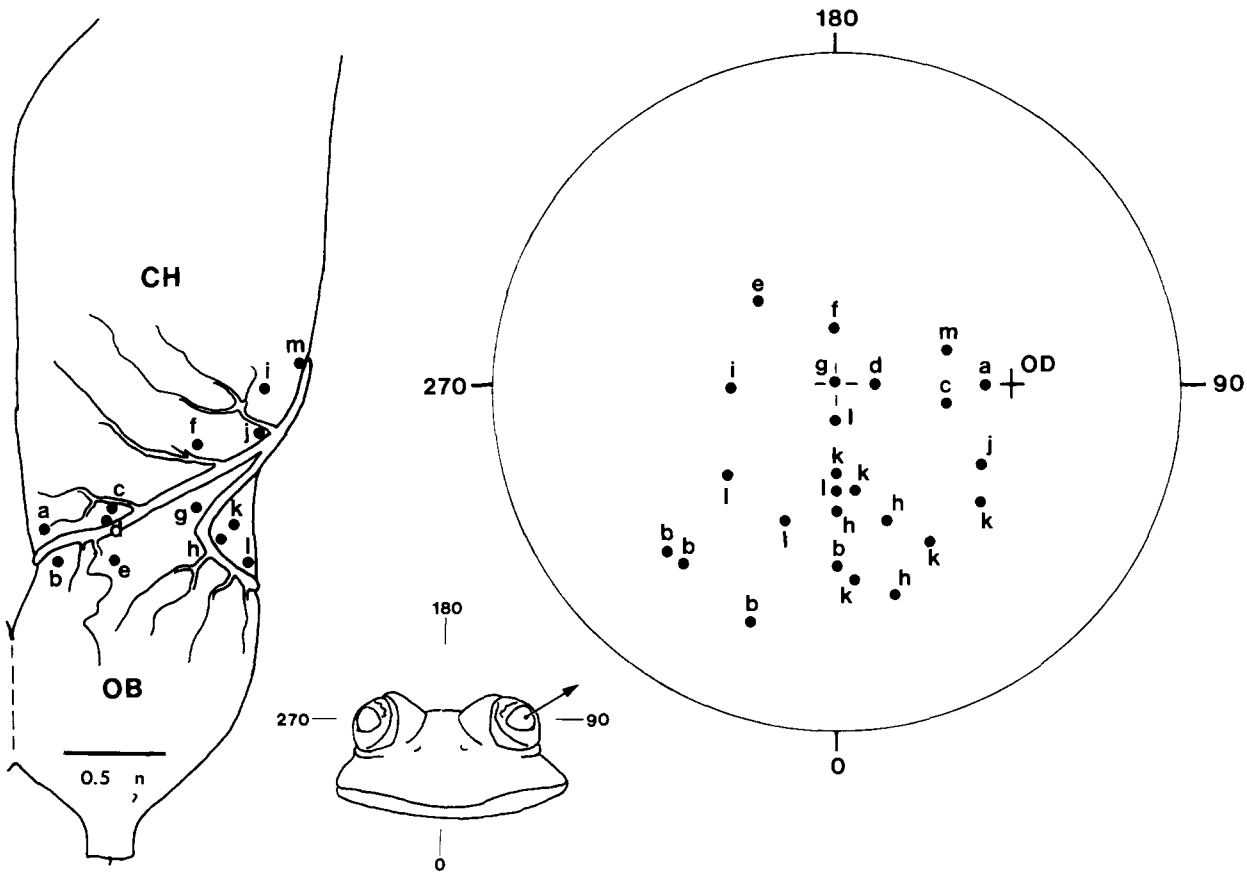

Fig. 6. Receptive field locations of single units recorded in the olfactory cortex of frog ST-124, mapped to the surface of the cortex at the site of penetration by the electrode. Left: Exposed dorsal surface of the left cerebral hemisphere (CH) and olfactory bulb (OB). Recording sites are marked (●) and lettered (a–m). Right: polar coordinate plot of the frontal region of the visual field of the left eye, centered at the intersection of the vertical midplane and the horizontal plane containing both pupils. Projection of the optic disc (see inset) is indicated by the cross (OD). Multiple appearances of the same letter identify the single unit receptive field loci defining a multiunit receptive field. No responses were found in the part of the visual field lateral to the projection of the optic disc in this frog.

visual angle. MURFs were found in the olfactory cortex much less frequently than in the tectum, and at no place were the individual receptive fields of a MURF present at the same high density found in tectum. At most penetrations, only 2 or 3 unit responses with distinct receptive fields were found, and sometimes only 1.

Class III and IV responses were found occasionally in the same MURFS as class I and II, but more often they were found at a deeper level along an electrode track when penetration was being made normal to the surface. The receptive fields of the class III units, on the order of 8–12° across, were much larger than that of class I or II. Only a few class IV units were noted over all of the animals studied, and no attempt was made to map the extent of their receptive fields.

When the electrode penetrated to the deep cell layer, there was an immediate increase in spontaneous activity, and the noise level rose noticeably at the ependymal margin, as expected at a barrier of high electrical resistance. With the electrode in the cell layer above the ependyma, bursts of single-unit and multiple-unit activity were evoked by a variety of visual stimuli (Fig. 4). This included switching the room lights on and off or moving the black target stimuli within or into the visual field. Some units responding to the black targets also showed sustained ON-responses. These units had definite receptive fields which were larger than the SURFs mapped in the superficial neuropil. Other units gave only the ON-response, and had no localized receptive field. Units responding to such combinations of stimuli or giving a pure ON-response were never observed in the neuropil. Electrolytic lesions made at these deep recording sites confirmed the inference that the final recordings obtained at the end of a penetration had been taken in the cortical cell layer (Fig. 5).

A clearly organized map of the visual field was not observed in any of the subjects. As a rule, the individual SURFs in a MURF recorded at a single electrode position were widely separated over the visual field, and the receptive fields of the units found at neighboring sites in the olfactory cortex were not ordered in any systematic pattern (Fig. 6). However, the receptive fields of almost all of the large amplitude units of classes I–III and the receptive fields of the target-responsive cells were located frontally, in an area that extended from the projection of the optic disc to the far contralateral edge of the binocular field (Fig. 6). Most of the SURFs in the frontal area were centered directly forward of the nose. Although few recordings were obtained from the most posterior lateral region of the cortex the receptive fields of these units and the occasional unit seen in the olfactory bulb were also located in the same frontal field. Among all the animals explored only two units had receptive fields outside this frontal area. In one case, the receptive field was located directly above the animal in the midplane; the other receptive field was on the horizon far to the rear.

4. Discussion

Visually evoked large electrical transients were recorded in the superficial neuropil and deep cell layer of the primary olfactory cortex of *Rana pipiens* ipsilateral to the stimulated eye when studied 5 months or more after deflection of the optic nerve into the telencephalon. These visual responses were evoked only from the affected eye, and were never observed in normal frogs. Such findings are taken as evidence that the ectopic synaptic field induced in the olfactory cortex contains a population of retinal terminals fully capable of exciting the cortical neurons on which they terminate. Further, the responses were qualitatively similar to those observed in tectal recordings from the superficial neuropil of normal frogs and frogs with regenerated optic nerves [12,18,19]. All of the different types of retinal ganglion cells that project axons to the tectum (class I–IV) were represented in the ectopic projection. As in the tectum, the most numerous units belong to class I and II, and each of these units has one of three waveforms (types A, B and C), as recorded in the tectum. The ON-fiber type that projects to thalamic targets, but not to the tectum, is also present in the redirected projection. These observations show that the ectopic field includes a broad sample of the retinal fiber population, including fibers that ordinarily project into different branches of the visual pathway, and that this population is not discriminated on the basis of physiological class in its regeneration into the foreign territory. The co-projection of ordinarily segregated retinothalamic and retinotectal fibers to the olfactory cortex, suggests that this area may contain a widely varied complement of synapse-specific molecular markers.

Some quantitative differences exist between the retinotectal and ectopic projections. First, the amplitude of the cortical B and C waves is only half to three-quarters their size in the tectum. The density of these visually responsive large unit transients recorded at any position is less in the cortex than in the tectum. Correspondingly, the number of SURFs in a MURF is much lower, as is the probability of finding a MURF. The result of this is that the shape of MURFs in the cortex is not well-defined. The individual SURFs in a MURF are generally widely separated, and a map of the visual field was not apparent in any specimen. This result differs from a previous finding that optic fibers innervating a part of the cerebellum after bilateral tectal ablation are visuotopically organized [31]. It was notable, however, that almost all the large amplitude visual responses obtained from the class I–IV ganglion cells and the localizable responses from the cell layer were evoked from a limited window in the frontal part of the visual field. Although class III responses are sometimes found deep to the class I/II responses in the cortex, a distinct layering of responses by class, as seen in the tectum, was not noted in the cortex. Finally, the ON-responses, normally recorded in the neuropil of the lateral geniculate

complex, could only be found in the cortical cell layer of the ectopic field, suggesting that the small, A-type transients are also indications of effective synaptic action.

The absence of a detailed retinotopic map in the ectopic projection field is not surprising. First, the olfactory cortex does not appear to be spatially organized with respect to its normal afferent fibers from the olfactory bulb [27]. Moreover, the cortical cell dendrites diverge widely and obliquely through the neuropil and do not have the columnar arrangement of dendrites that characterizes tectal cells. This may interfere with the organization of a topographic map in the olfactory cortex. However, the clustering of the SURFs of the large B and C transients in the frontal visual field shows that the ectopic projection is not random.

The prominent question raised by our findings is how to account for the emergence of the large B and C transients in the ectopic visual projection to the olfactory cortex. No such electrical signals ever appear in the normal cortical neuropil, but develop slowly over about five months, well after optic fiber synapses appear anatomically. The projection is established in the first 13 weeks [24], during which only low voltage electrical activity can be recorded. That these small transients are synaptically effective is shown by the deep cell responses to visual as well as olfactory stimuli in frogs in which none of the large transients have yet appeared in the neuropil. In the tectum the small transients (called A-type response) can be shown to be associated only with the endings of fine fibers not with fibers of passage [12]. Furthermore, the unmyelinated fibers (97% of the optic nerve population) end in fine serially beaded terminal filaments [14] in the tectum, rather than the dense terminal arbors that were once supposed [3]. The same holds true in the ectopic projection.

Studies on the tectum suggest that the large B and C transients are post-synaptic signals arising from an active dendrite membrane. This was established by showing that the identical transient (by size and shape) could be evoked from the corresponding points in the visual field of either eye [8,12] by way of an intertectal relay involving the nucleus isthmi [13]. Certain dendrites in the tectum possess a densely focussed, locally branched and beaded appendage onto which the optic fibers synapse, forming a kind of glomerulus [34]. The glomerular appendage and the segment of dendrite from which it projects, if taken as having active membrane accounts well, not only for the three types of electrical transients, the A, B and C types, but also for the anisotropic spatial arrangement of these transients in a MURF. Just as with the ectopic projection of optic fibers to olfactory cortex, the regrowth of fibers into the tectum during optic nerve regeneration establishes synaptic connections [33] long before the appearance of B and C transients [18]. However, no such structure is seen in the normal dendritic anatomy of the olfactory cortex [28]. Yet, the same arguments apply as they do in any neuropil, that the unmyelinated terminal stretches of synapsing fibers are intrinsically incapable of producing the larger B and C electrical transients recorded external to them in the neuropil.

The evidence and arguments discussed above lead us to the hypothesis that the appearance of both B and C type transients in the ectopic projection signifies that optic fiber terminals can induce a change in the membrane properties of the dendrites on which they terminate. This is a question for further study.

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