Neuronal Locus Specificity: Altered Pattern of Spatial Deployment in Fused Fragments of Embryonic Xenopus Eyes

Abstract. *Before optic nerve outgrowth in* Xenopus laevis *embryos, a change of state occurs in the differentiating retinal cell population which renders the cells refractory to information about subsequent changes in their positions, and commits individual ganglion cells to develop specific position-dependent properties (locus specificities) which subserve the formation of orderly retinotopic connections in the optic tectum. When different parts of eye primordia from stages before optic nerve outgrowth are fused, each piece in such a reconstructed eye does not generate ganglion cells with the partial-set of locus specificities normally arising from that region of the intact eye. It is inferred that separate parts of the early embryonic retina do not contain stable programs for spatial deployment of locus specificity, and that development of definitive locus specificities in retinal ganglion cells requires additional cellular interactions among the retinal cells later in development.*

During embryonic development, individual retinal ganglion cells are believed to acquire unique position-dependent properties, termed locus specificities, which enable their axons to form a retinotopically organized pattern of synaptic connections in the midbrain optic tectum; this pattern is termed the retinotectal map (*1*). Precisely how and when each ganglion cell acquires and processes information about its retinal position is unknown, but a basic plan for spatial deployment of locus specificities appears to be set down during a "critical period" in the early embryo (*2*). In the clawed frog, *Xenopus laevis*, this critical period spans about 5 hours at embryonic stages 28 to 31, precedes the sprouting of optic nerve fibers by several hours, and occurs while the retina contains only a small fraction of its adult cell complement (*3*).

Our recent experiments (*4*) showed that a stable and irreversible change in the retina occurs between stages 28 and 31, which results in (i) the stabilization of a pair of orthogonal retinal axes, and (ii) the determination or specification of the positional information (*5*) which all the ganglion cells will ultimately use to derive appropriate locus specificities. Those experiments on intact eye primordia, however, could not show whether the stability of the specified state at stage 31/32 requires the structural integrity of the retina, or whether individual cells or cell groups would exhibit a

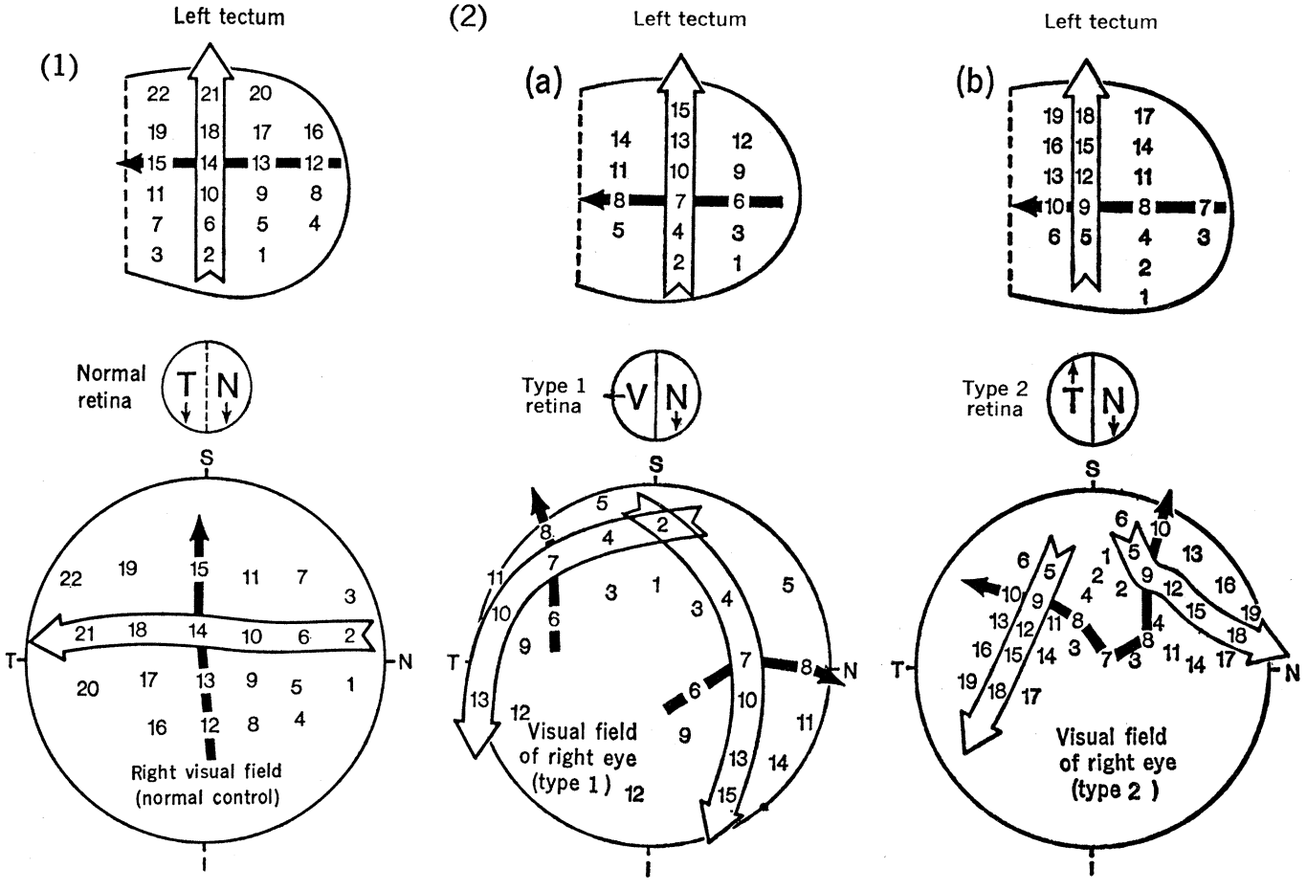

Fig. 1. Projection of the visual field of a normal intact right eye to the left optic tectum in adult *Xenopus*. Each number in the visual field shows the position of the stimulus that optimally evoked potentials recorded by an electrode at the position shown by the same number on the tectum. The smallest distance between tectal electrode positions is 100 μm. The visual field extends 100° from center to periphery. The composition of the embryonic eye is shown: *N*, nasal; *T*, temporal; arrows point ventrally. Fig. 2. (a) Tectal projection of the visual field of a type 1 eye in which the temporal half has been removed and replaced by a ventral half (*V*). (b) Tectal projection of the visual field of a type 2 eye in which the temporal half of the right eye has been removed and replaced by a temporal half of a left eye. The conventions are as in Fig. 1.

stable program independent of the whole retinal field.

Here we present evidence that, when pieces of eye primordia at stage 32 are fused to reconstitute a morphologically "whole" eye, the partial-set of locus specificities which arises from the respective pieces is very different from that which arises from the same region of the normal eye.

Xenopus laevis embryos were obtained, classified according to stage, anesthetized for surgery, and reared through metamorphosis as previously described (*5*). At stage 32, the right eye primordium was bisected along the vertical midline. The temporal half of the eye primordium was removed and replaced by either (i) the ventral half of another eye primordium, removed from a stage 32 sibling embryo (type 1), or (ii) the temporal half of a left eye primordium of a stage 32 sibling embryo (type 2). The paired half-eye rudiments fused to form morphologically "whole" eyes in the next 48 hours. In addition, the adult eye in each case showed the characteristic patchwork pattern of pigmentation expected from such a composite eye, and type 1 eyes possessed two choroidal fissure scars, 90° apart.

After metamorphosis, we mapped the projection of the experimental (right) eye's visual field onto the left tectum. In the mapping procedure, which has been described in detail (*5*), a platinum-iridium microelectrode is moved across the tectum in 100-μm steps and, at each electrode position, the optimal stimulus position in the visual field is determined. The projection of the frog's left eye (unoperated) to the right tectum was always normal and is not shown. Behavioral tests demonstrated the existence of functional synapses between the experimental eye and the brain.

Figure 1 shows a normal retinotectal projection that developed from a control eye, which had been excised and replaced intact at embryonic stage 32. The projection is orderly and continuous, and the ganglion cells at each retinal locus have expressed a unique locus specificity. Now let us consider the projections expected from the type 1 and type 2 experimental eyes; we assume that each of the original embryonic eye-fragments went on to generate the half-set of locus specificities that it would have generated in its original context in the intact eye.

In type 1, the two fragments should each have generated a nasoventral quarter-set of locus specificities and, in addition, one unique quarter-set apiece: a temporoventral quarter-set from the original ventral fragment, and a nasodorsal quarter-set from the original nasal fragment. Thus, part of the tectum should be shared by fibers from both halves of the adult type 1 retina, and other parts of the tectum should be occupied exclusively by fibers from one half or the other (*6*). As shown in Fig. 2a, the predicted result was not observed. Instead, each half of the adult type 1 retina projected to the entire tectum, with each tectal locus receiving input from two visual field positions, one on each side of the vertical meridian.

Similarly, the two fragments which had been combined to form a type 2 eye should have generated completely different half-sets of locus specificities: a nasal half-set in the one case and a temporal half-set in the other. Thus, the two halves of the resulting adult retina should have projected to different, nonoverlapping regions of the tectum. As shown in Fig. 2b, the predicted result was not observed. Instead, each half of the adult type 2 retina projects to the entire tectum, with each tectal locus receiving input from two positions in the visual field, one on each side of the vertical meridian.

These results have three immediate implications. The first relates to the nature of the specified state at stage 31/32, which in the intact eye is stable and irreversible (*3, 5*). These results show that a separate region of the stage 32 eye does not possess a stable program for generating the partial-set of locus specificities normally arising from that region. This means that the change of state which occurs from stages 28 to 31 (*5*) must be a change in the retinal cell population as a whole and must, at the very least, require further intraretinal changes before stable local programs are established within the cell population.

The second implication relates to the problem of how positional information is handed down to the descendants of the mitotically active cells at the retinal margins during the protracted period of retinal growth following stage 32. That an orderly, but unexpected, pattern of locus specificities developed in the type 1 and type 2 eyes argues against a simple cell-lineage mechanism, in which ganglion cells with predetermined qualitative differences are produced each time a precursor cell divides. That interactions occurred between the two fused half-eyes suggests that cellular interactions may be important in transmitting positional information to ganglion cells born during later phases of retinal growth.

The third implication relates to the expression of locus specificity in the morphogenesis of retinotectal connections. Investigators of this problem have frequently examined the mechanism by which the retinotectal projection adapts to deletion or amplification of specific regions of the retina or tectum, often in an attempt to infer the "rules" by which neurons with "known specificities" choose between various potential synaptic sites. The possibility that the specificities themselves may adapt to the changing context of the system has been suggested (*7*), but there has been no previous definitive demonstration of alterations in the spatial deployment of locus specificities. Perhaps because of the absence of such a definitive precedent, some authors have preferred to attribute adaptive changes in the retinotectal map to some flexibility in the rules governing the formation of the retinotectal system (*8*). Without minimizing the latter possibility, the results reported here leave no doubt that an altered set of locus specificities can result from surgical disruption and rearrangement of the retina. Therefore, experiments must control for such an effect before alternative interpretations can be advanced to account for the altered retinotectal maps. We should keep in mind Harrison's warning in his paper on the problem of determining cellular phenotype (*9*): "There is no way of finding out with certainty whether the particular quality which the cell seems to have is finally fixed, for there may always be new conditions, not yet tested, under which other potencies may be revealed." We should, therefore, not take evidence of stability of the differentiated state in an integrated mass of retinal cells for evidence of stability of cytodifferentiation of the individual retinal cell. Nor should we attribute this stability of differentiation to the properties of individual retinal cells when it might be due to properties of the tissue as a whole. Thus, in the extreme case, neuronal specificity may, throughout the life of the nerve cell, remain a function of its multicellular context. At the very least, the emergence of neuronal specificity as a con-

text-free property of the individual retinal ganglion cell must occur at a later stage of development some time after the original program for spatial organization has been established at the tissue level.

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Brain Aluminum Distribution in Alzheimer's Disease and Experimental Neurofibrillary Degeneration

Abstract. *Neurofibrillary degeneration is an important pathological finding in senile and presenile dementia of the Alzheimer type. Experimentally, aluminum induces neurofibrillary degeneration in neurons of higher mammals. Aluminum concentrations approaching those used experimentally have been found in some regions of the brains of patients with Alzheimer's disease.*

Aluminum is one of the most abundant elements in the earth's crust, and biological systems probably evolved in the presence of appreciable concentrations. Nevertheless, a number of observations indicate that high concentrations of aluminum may be toxic to the nervous system. The brain of an aluminum ball-mill worker, with progressive encephalopathy accompanied by dementia and convulsions, was found to contain 20 times the normal concentration of aluminum (*1*). In experimental animals, aluminum hydroxide applied to cerebral tissues induced epileptogenic foci (*2*), and subarachnoid injection of trace amounts of salts of aluminum resulted in a progressive encephalopathy characterized by a unique cellular change, that of neurofibrillary degeneration (NFD) (*3*). A similar but not identical cellular change is one of the hallmarks of Alzheimer's disease, a disease occurring after the age of 40 and producing progressive dementia (*4*, *5*).

Work in our laboratory has shown that, in the early stages of an aluminum chloride–induced encephalopathy in cats, an apparently selective impairment in short-term memory and associative learning preceded the appearance of focal neurological signs (*6*). The decline in higher nervous functions resembled in part those noted in the human conditions of presenile and senile dementia of the Alzheimer type, although the time course was shorter.

To appraise further the role of aluminum in this animal model of a human dementia, a study of the aluminum concentration in cat brain with acquisition defects was undertaken in order to establish the tissue concentration at which morphological and behavioral changes occurred. In addition, the aluminum content of the brain has been determined in humans with Alzheimer's disease. We now report the results of these studies.

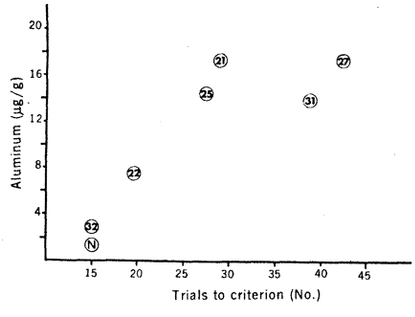

Fig. 1. Relation between acquisition of a conditioned avoidance task and brain aluminum concentrations [μg/g (dry weight)].

Eighteen cats (2.5 to 3.5 kg) were given 150 to 225 μg of aluminum (as aluminum chloride) which was injected into the ventral hippocampus, internal capsule, or cisterna cerebellomedularis (*6*). In six aluminum-treated animals behavioral testing was carried out in a one-way avoidance response acquisition task (*6*). Acquisition performance was measured on three consecutive days, beginning on day 9 after injection of aluminum chloride. These animals were killed between day 11 and day 18, and the brains were bisected in the sagittal plane. One-half of the brain was prepared for histology and stained for NFD by the method of Bielschowsky. The other half was prepared for aluminum assay. Special precautions were taken to prevent aluminum contamination of tissue during necropsy.

Human tissue was collected from deceased patients in which the clinical history, physical examination, and pneumoencephalogram were compatible with the diagnosis of Alzheimer's disease. Pathological examination of a portion of brain revealed brain atrophy, extensive neurofibrillary degeneration, senile plaques, and hippocampal pyramidal cell granulovacular degeneration. Removal of tissue was supervised to minimize possible aluminum contamination.

Aluminum was assayed by the atomic absorption method (*7*). By this technique as little as 0.1 μg/ml can be detected. The only measurements excluded from this report were from samples in which (i) all of residue could not be redissolved (three cat samples and one human sample) and (ii) the signal-to-noise ratio was less than 2 in the spectrophotometer (one human sample).

In our initial experiments we found that aluminum was not homogeneously distributed in the tissue. In order to establish that the variation was of biological origin and not due to lack of precision of the analytical technique, repeated assays were made for aluminum in homogenized brain and liver. The aluminum content was found to be reproducible in different samples, within 10 percent, even at the level of 1 μg/g (dry weight).

The aluminum concentration in several regions of the feline brain is listed in Table 1. The samples denoted frontal and occipital poles and were largely cor-