nuclei accompanying α-decay have such low kinetic energies (500 ev/nucleon) that they are essentially unstripped; these particles lose their energy in elastic, hard-sphere collisions between atoms, rather than in electronic processes. The radiation damage produced by α-recoils is probably best described by the "displacement-spike" concept (*8*). Measurements of track formation by recoils of different energy, slowed down perhaps by a thin air gap, and of different mass—produced, for example, by accelerator-scattering experiments—should elucidate the details of the solid state processes involved.

Much work needs to be done in order to establish the utility of α-recoil tracks. The α-recoil track-dating method, for example, requires the determination of the Th/U ratio. Although this determination can be made in principle with the track methods, with the use of independent irradiations in fast and thermal neutron fluxes, the precise techniques remain to be developed. The α-recoil tracks are also very small and not as unique in appearance as the larger fiission tracks; thus it remains to be shown that the full increase in sensitivity for age measurement, that is possible in principle, can be realized in practice. It further remains to be shown that α-recoils can be found in other substances; particularly, for archeological investigations, in glass.

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9. We thank J. Kardos for the preparation and examination of the electron-microscopy specimen. The use of Th^{228} was suggested by A. Wahl, and the experiment was carried out in cooperation with D. Sarantides. The cooperation of W. Johns was also essential. Supported by McDonnell Aircraft contract P.O. Z60046T.

12 January 1967

Retinal Ganglion Cells: Specification of Central Connections in Larval Xenopus laevis

Abstract. *In a series of* Xenopus *larvae (stages 28 to 35), the left eye cup was dorsoventrally and anteroposteriorly inverted. After metamorphosis, the retinotectal projection was mapped by recording action potentials evoked in the tectum by a small spot of light projected on the retina. Normal retinotectal projection was found following rotation of the eye cup at stage 28 to 29. Rotation of the eye cup at stage 30 resulted in anteroposterior inversion of the retinotectal projection; rotation at stages 31, 32, and 35 resulted in inversion of the projection in anteroposterior and dorsoventral axis of the retina. Therefore the retinal ganglion cells were unspecified at stage 28 to 29; spatial specification of ganglion cells occurred in the anteroposterior axis of the eye cup at stage 30 and in the dorsoventral axis between stages 30 and 31.*

The time of retinal specification has been studied in amphibians in which the eye cup is rotated 180° at different stages of development before formation of the optic nerve, and by observation of the visuomotor behavior of these animals after maturation. Rotation of the eye cup of the salamander *Amblystoma* before larval stage 34 (*1*) or of the newt *Triturus* before larval stage 22 (*2*) does not alter the development of normal visuomotor behavior, but rotation after these developmental stages results in reversal of visuomotor reflexes. The inference from these experiments is that, during "functional polarization" (*1*) or "functional specification" (*2*) of the retina, the retinal ganglion cells acquire an unknown kind of local property enabling them to connect with the appropriate places in the tectum. As a result, the retinal ganglion cells connect with the optic tectum in the retinotopic order found in adult amphibians (*3*). I have determined the patterns of connections between retina and tectum which form after the eye cup of the clawed toad *Xenopus laevis* is rotated at different stages of development.

In 52 larvae of *Xenopus laevis* at different stages from 28 to 35 (*4*) the left eye cup was excised, rotated 180°, and reimplanted so that the dorsoventral and anteroposterior axes were inverted. The operation was performed before the development of any nervous connections between the eye and the brain.

Only 12 animals survived metamorphosis. The left eye had been rotated at stage 28 to 29 in six, in three at stage 30, and in one each at stages 31, 32, and 35. These animals were used for mapping the projection from the rotated left eye and from the normal right eye to the optic tectum.

The mapping procedure was as follows. The animal was anesthetized by immersion in an aqueous solution (1:1000) of tricaine methanesulphonate (MS 222-Sandoz) and paralyzed with an injection of 0.01 mg of tubocurarine chloride. The cranium was opened over the tectum, and the meninges covering the tectum were removed. The animal was fixed in position with its left eye centered on the axis of a projection perimeter at a distance of 33 cm from the perimeter arc. By means of the perimeter, a spot of light (subtense 1°, duration 1 second, spot luminance 60 cd/m^2, background luminance about 20 cd/m^2) could be moved to any position in the visual field of the eye being stimulated. Action potentials evoked by the light were recorded from the tectum by means of a platinum-iridium microelectrode (*5*) with a tip diameter of about 1 μ. A micromanipulator moved the electrode to a succession of positions 200 μ apart on the tectum. The electrode penetrated the tectum vertically to a depth of between 0 and 50 μ. The depth was uncertain because of dimpling of the tectum by the electrode. At each electrode position, the position of the stimulating light was adjusted until responses of maximum amplitude were evoked in the tectum. The responses had latencies varying from 30 to 60 msec and consisted of bursts of action potentials occasionally from one, but usually from several units. The responses could be evoked from within a region subtending from 5° to 15° in the visual field. After the projection from the left (rotated) eye was mapped to the right tectum, the animal was positioned with its right (normal) eye centered on the perimeter, and the

retinotectal projection from the right eye to the left tectum was mapped. An attempt was made to record from positions on the left tectum symmetrical with those on the right tectum so that the projections from the two eyes could more easily be compared.

In Figs. 1–3, each number shown on the tectum indicates the position of the electrode at which action potentials were recorded when the stimulating light was at the position indicated by the same number in the visual field. The black and white arrows indicate the projection of the anteroposterior and dorsoventral axes of the retina onto the tectum. The convention has been adopted of showing the arrows in their normal positions on the tectum, since this had not been rotated, but showing by means of the arrows in the visual field diagram whether or not inversion of the axes of the retina had occurred as a result of rotation of the eye cup.

Normal retinotectal projection was found in four of six animals in which the eye cup had been rotated at stage 28 to 29 (Fig. 1). Therefore, the retinal ganglion cells formed tectal connections appropriate to their rotated position, and specification of the ganglion cells only occurred after stage 29. The left optic nerve had not developed in the other two animals in which the eye cup was rotated at stage 28 to stage 29.

Figure 2 shows the retinotectal projection from an animal with a normal right eye and a left eye which had been rotated at stage 30. The projection from the left eye is inverted in the anteroposterior axis but is normal in the dorsoventral axis. Similar results were obtained in three animals in which the eye had been rotated at stage 30. Since the left eye cup had been inverted in both axes at stage 30, the specification of the anteroposterior axis occurred before eye-cup rotation, while the dorsoventral axis was specified in accordance with the new position of the eye cup after rotation at stage 30. This interpretation is supported by the results of rotating the eye cup in three animals at stages 31, 32, and 35, in which total inversion of the retinotectal projection was found (Fig. 3). Therefore, specification both of the anteroposterior and the dorsoventral axis of

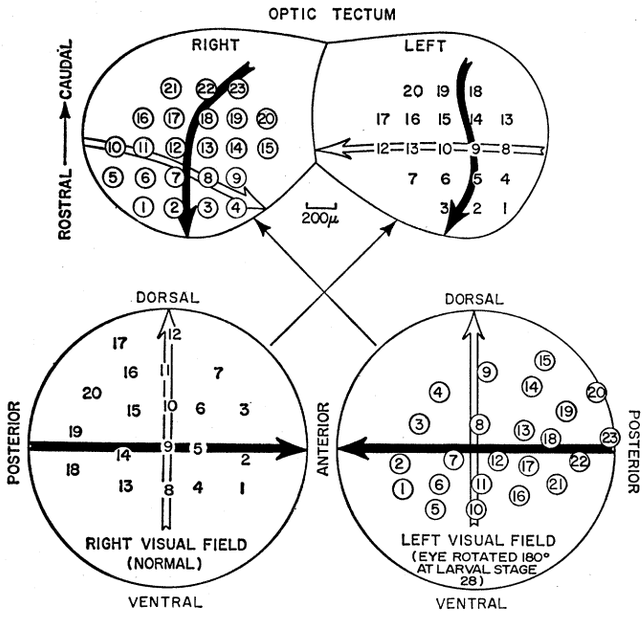

Fig. 1 (above). Map of the retinotectal projection in adult *Xenopus* to the left tectum from the normal right eye and to the right tectum from the left eye, which had been rotated 180° at larval stage 28 to 29. The projection from the rotated eye is normal.

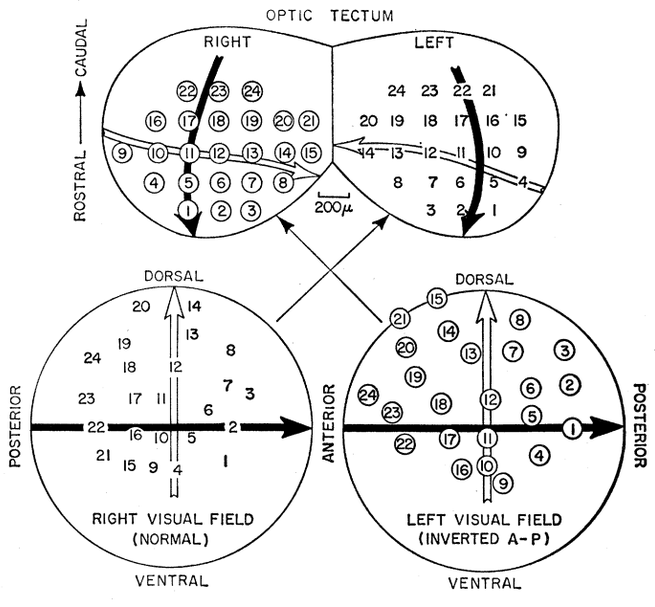

Fig. 2 (top right). Map of the retinotectal projection in adult *Xenopus* from the normal right eye and from the left eye, which had been rotated 180° at larval stage 30. The projection from the left eye is inverted in the anteroposterior axis but is normal in the dorsoventral axis of the eye.

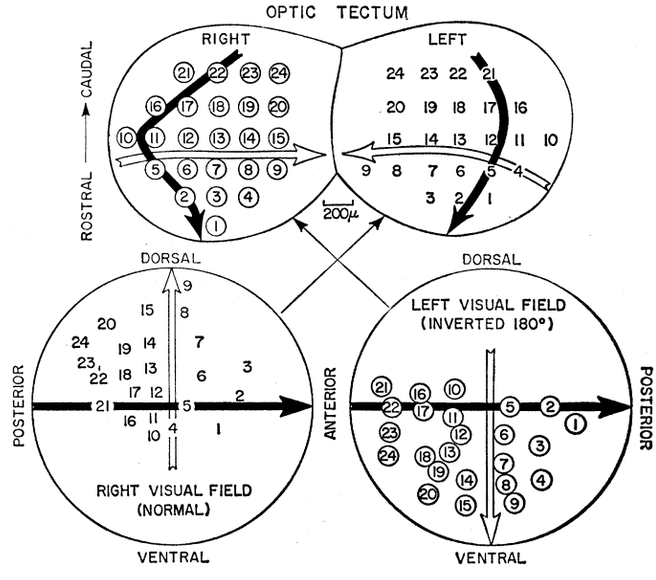

Fig. 3 (bottom right). Map of the retinotectal projection in adult *Xenopus* from the normal right eye and from the left eye, which had been rotated at larval stage 32. The projection from the left eye is totally inverted.

the retina had occurred before rotation of the eye cup at stages 31, 32, and 35.

My results show that before larval stage 30, the ganglion cells do not yet have the information about the positions with which they must connect in the tectum, and the retina can be rotated without altering the normal pattern of retinotectal connections. Specification of retinal ganglion cells occurs first in the anteroposterior axis of the retina at stage 30. After this, the ganglion cells have the information which enables them to form their connections in the correct order in the rostrocaudal axis of the tectum. During the next 5 to 10 hours of development the ganglion cells become specified in the dorsoventral axis of the retina and are able to form the correct connections in the mediolateral axis of the tectum. Inversion of the retina after this stage of development results in total inversion of the retinotectal projection. Specification in both axes of the retina occurs before outgrowth of optic nerve fibers from the ganglion cells.

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9 November 1966

Calcium-Induced Activation of Phosphorylase in Rat Hearts

Abstract. *Isolated perfused rat hearts exposed to increased calcium concentrations demonstrated a marked activation of phosphorylase, the maximum occurring about 15 seconds after the peak of the positive inotropic response. These observations provide an explanation for the failure of earlier investigations to demonstrate a significant calcium-induced increase in the active form of phosphorylase in the intact heart.*

The evidence that calcium is capable of inducing a significant activation of phosphorylase in the *intact* heart has been inconclusive. In the investigation described here, a marked activation of this enzyme has been demonstrated by studying the time course of the effects of calcium on phosphorylase activity in the isolated perfused rat heart.

Phosphorylase is an important enzyme, since it regulates the breakdown of glycogen to glucose-1-phosphate, thereby providing the heart with a substrate which can be catabolized to utilizable energy (*1*). This enzyme exists in two forms: phosphorylase *b* is the inactive form of the enzyme (it is active only in the presence of adequate amounts of adenosine-5′-monophosphate) and phosphorylase *a* is the active form. Phosphorylase is considered to be activated when the percent of phosphorylase *a* activity increases; this is computed in the following manner:

$$\frac{\text{phosphorylase } a \text{ activity} \times 100}{\text{phosphorylase } a + b \text{ activities}}$$

The activation of phosphorylase is a relatively complex biochemical process (Fig. 1) involving a number of enzymatic reactions (*2*).

Several investigators (*2*) have shown that calcium activates striated muscle phosphorylase *b* kinase in vitro by a mechanism that appears to be independent of that involving adenosine triphosphate (ATP) and magnesium and accelerated by adenosine-3′,5′-monophosphate (3′,5′-AMP). If such a process occurred in the *intact* heart it should lead to an enhanced conversion of phosphorylase *b* to phosphorylase *a*. However, Belford and Feinleib (*3*) observed that increasing the calcium concentration produced only a relatively small increase in the percent of phosphorylase *a* activity in isolated guinea pig atria. Similarly, Mayer and Moran (*4*) failed to demonstrate a significant activation of this enzyme in the open-chest dog preparation with doses of calcium that produced maximal increases in the contractile force of the heart.

It must be emphasized that in *all* of the above-mentioned experiments heart samples were taken at the moment the positive inotropic response reached its maximum. Hence, the failure of these experiments to demonstrate a significant activation of phosphorylase by calcium could be due to the fact that this activation process occurs sometime after the peak increase in contractile force. Such a delay in the maximum activation of cardiac phosphorylase following epinephrine administration has been recently demonstrated (*5*). We have therefore studied the time course of the effect of calcium on phosphorylase activity in the isolated perfused rat heart.

The methods used in the above experiments are briefly described below. Hearts obtained from female Wistar rats were perfused by the Langendorff technique with Tyrode's solution (37°C) at a flow rate of 5 ml/min. Five grams of diastolic tension was applied to each heart. All hearts were perfused for 8 minutes with control Tyrode's solution containing 1.8 mmole of calcium prior to exposing them to Tyrode's solution containing higher calcium concentrations. Subsequently, at a predetermined time interval, a portion of left ventricular tissue was frozen with a modified Wollenberger clamp (*6*) prechilled in liquid nitrogen. All samples were stored under liquid nitrogen until assayed. Phosphorylase *a* and total phosphorylase activities were assayed according to the procedure described earlier (*5*). Since no statistically significant change in total phosphorylase was observed during the course of any of the experiments, all data are presented in terms of the percent of phosphorylase *a* activity.

The data summarized in Table 1 demonstrate that when samples were taken at the peak of the inotropic response no significant activation of

Table 1. Effect of calcium on the percent of phosphorylase *a* activity, with means and standard errors, in isolated rat hearts.*

Calcium concentration (m*M*)	Phosphorylase *a* activity (% ± S.E.)
1.8†	11.1 ± 0.8
3.6	10.7 ± 0.7
7.2	13.7 ± 1.3
14.4	10.3 ± 0.5

* Heart samples were taken at the peak of the inotropic response. See text for details. † Control samples were taken after 8 minutes of perfusion.