10. Paper electrophoresis with a 0.3*M* sodium phosphate buffer solution (*p*H 4.0) was used to characterize the radioactive compounds. Nonradioactive choline and acetylcholine were used as reference standards. The system was subjected to 26.3 v/cm for 2.5 hours. Acetylcholine moved 41.4 cm from the origin. The Rm of choline was 1.13.
11. Paper chromatography with *n*-butanol-water (9 : 1 by volume) was used to characterize the radioactive compounds. Descending chromatography was conducted for 18 hours. Nonradioactive choline and acetylchoine were used as reference standards. The $R_F$ of choline was 0.07 and the $R_F$ of acetylcholine was 0.10.
12. Supported by grants from the NIH (NB-04553 and 5T1-GM-153).
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## "Cytoplasmic" Sterility in Drosophila paulistorum Which Is Ultimately Dependent on Nuclear Genes

Abstract. *A case of hybrid sterility in* Drosophila paulistorum *is due to an incompatibility of the Y chromosome of certain strains with the cytoplasm of other strains. The constitution of the cytoplasm responsible for the sterility is not, however, independent of the chromosomal genes. After seven backcrosses of the hybrid females to males of the same strain, fertile male progenies are finally obtained.*

At least three different kinds of hybrid sterility occur within the superspecies *Drosophila paulistorum*. This superspecies consists of six races or incipient species; hybrids between the races are fertile as females but sterile as males (*1*). The hybrid females can be backcrossed to males of the parental races, and the backcross progenies consist again of fertile daughters and sterile sons. The sterility of the backcross males depends upon the genetic constitution of their mothers; all the sons of a female carrying any mixture of the chromosomes of the parental races are sterile, even if some of these sons themselves carry only the chromosomes of a single race (*2*). This is, then, an instance of a genic sterility operating through a maternal effect, the genes responsible being distributed in all three pairs of the chromosomes which the species possesses. Evidently, the sterility of the $F_1$ males is due to a different mechanism, since $F_1$ hybrids are descendants of pure rather than hybrid mothers.

A third kind of sterility has been reported (*3*), so far in only a single cross, between strains from Mesitas and those from Santa Marta, Colombia. Both the Mesitas and Santa Marta strains belong to the Transitional race of *D. paulistorum*. The cross Mesitas female × Santa Marta male gives fertile hybrids of both sexes, but the male progeny of the reciprocal cross is sterile. The hybrid females can be backcrossed to males of either parental strain; the male progenies of these backcrosses are sterile if they carry the Y chromosome of Mesitas in the Santa Marta cytoplasm, or the Y chromosome of Santa Marta in Mesitas cytoplasm. Genetic analysis consequently suggests that the sterility is caused by an interaction of the Y chromosome of the Santa Marta strain with the cytoplasm of Mesitas. This inference was based on a study of four backcross generations to both parental strains. Males with cytoplasm of Mesitas origin and a Santa Marta Y chromosome were sterile even when about 97 percent of their genome (other than the Y chromosome) were of Santa Marta origin. Since the earlier report (*3*) was published, additional backcrosses have been made to test the possibility that the cytoplasmic difference may eventually be overcome by the nuclear genes (*4*).

Fifteen new strains of *D. paulistorum* were obtained (*5*) from Mesitas, Columbia. These strains behaved like the old Mesitas strain (*6*), and were pooled into three stocks. All the experiments crossing Mesitas with Santa Marta were, therefore, made in triplicate; the results were uniform and can be described jointly. Mesitas females were crossed to Santa Marta males and the hybrid females were backcrossed repeatedly to Santa Marta males. The male progenies of the first four backcross generations were, as before, sterile. By the fifth or sixth backcross generation some motile spermatozoa were seen in the sperm-storing organs of the females with which the hybrid males were tested, but still none of the eggs deposited by these females hatched. The male progeny of the seventh backcross generation is, however, entirely fertile.

A similar situation was observed in the crosses with the Santa Marta cytoplasm. Six backcrosses to Mesitas males yielded sterile male hybrids, but the seventh backcross generation gave at least some fertile males. The progeny of the seventh backcross is expected to have more than 99 percent of the genes of the recurrent parent. Thus, by repeated backcrosses the origin of the cytoplasm is finally overcome by the chromosomal genes. If the properties of the cytoplasm were transmitted independently of the nucelar genes, the sterility of the backcross males would have to be retained irrespective of the number of the backcrosses made, as it was after some 60 generations in the experiments of Laven (*7*) on a cytoplasmic sterility in crosses between certain mosquitoes. This is, however, not the case in *Drosophila paulistorum*. Finally, the male progeny of the seventh backcross generation of (Santa Marta female × Mesitas male) × Mesitas male was crossed to pure Santa Marta females. All the hybrid males thus obtained were sterile, just as the initial Santa Marta female × Mesitas male $F_1$ males.

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### References and Notes

1. Th. Dobzhansky and B. Spassky, *Proc. Natl. Acad. Sci. U.S.* **45**, 419 (1959).
2. L. Ehrman, *Evolution* **14**, 2 (1960).
3. ———, *Proc. Natl. Acad. Sci. U.S.* **49**, 155 (1963).
4. Several colleagues, especially Ernst Caspari of Rochester University and Theodosius Dobzhansky of the Rockefeller Institute suggested that these backcrosses should be made.
5. Through the courtesy of A. S. Hunter, Universidad de los Andes, Bogata, Colombia.
6. Th. Dobzhansky, L. Ehrman, O. Pavlovsky, B. Spassky, *Proc. Natl. Acad. Sci. U.S.* **51**, 3 (1964).
7. H. Laven, in *Cold Spring Harbor Symp. Quant. Biol.* **24**, 166 (1959).
8. This work was carried out under contract No. AT-(30-1)-3096, U.S. Atomic Energy Commission.

20 April 1964

## Lateral Geniculate Nucleus and Cerebral Cortex: Evidence for a Crossed Pathway

Abstract. *Lesions were placed in the lateral geniculate nucleus of cats, and degeneration was traced in the brains after survival times ranging from 5 to 21 days. Degenerated fibers could be seen in the corpus callosum and in the lateral and suprasylvian gyri of the opposite hemisphere. Results suggest the presence of a crossed geniculocortical pathway.*

A number of earlier authors (*1*) suggested the possibility of a tract connecting each lateral geniculate nucleus with the contralateral cortex by way of the corpus callosum. Such a pathway was proposed to account for the paradoxical sparing of the macular portion of the visual fields in man after massive

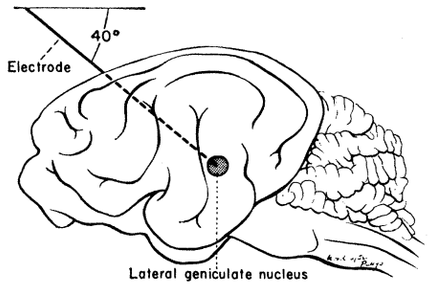


Fig. 1. Operative route for geniculate and control lesions.

unilateral occipital lesions. However, the presence of such a fiber system was questioned on the basis of two major anatomical observations. First, retrograde degeneration was not found in the opposite lateral geniculate nucleus after lesions had been made in the visual cortex (*1*). And secondly, the pathway's existence has been disputed on the basis of negative findings in secondary degeneration studies. Polyak (*1*) was unable to trace Marchi granules to the opposite visual cortex after unilateral lesions were made in the visual radiations or lateral geniculate nucleus of the monkey. However, these studies do not conclusively disprove that such a tract exists. Severing one branch of a corticopetal fiber does not invariably lead to retrograde degeneration (*2*). Moreover, the Marchi stain is rather selective for large caliber fibers.

Using a modified Nauta stain for degenerating axons (*3*), we reexamined the possibility of fibers arising in the lateral geniculate nucleus and traversing the corpus callosum to the opposite cortex. Anodal lesions (2.5 to 5 ma; 20 to 60 sec) were made in the lateral geniculate nucleus of five cats, and degenerating fibers were traced in the brains of cats after survival times ranging from 5 to 21 days. A control animal was also studied in which the lesion was placed ventrolateral to the lateral geniculate nucleus with little damage being done to the geniculate cells. In all cases, as shown in Fig. 1, the needle track was oriented on a 40-degree angle from horizontal to minimize commissural damage in the posterior portion of the hemisphere. We are now reporting only the degeneration in the corpus callosum and the lateral and suprasylvian gyri of the opposite hemisphere.

In each animal with a lesion in the lateral geniculate nucleus, degenerated fibers could be seen in the corpus callosum and in the white matter underneath the lateral or suprasylvian (or both) gyri of the opposite hemisphere. Degeneration appeared more marked in animals that survived 21 days; the longer the cats survived, the more frequently could contralateral preterminal degeneration be seen in the deepest cortical layers of the lateral gyrus anteriorly and in the suprasylvian gyrus posteriorly. The distribution of the observed preterminal fibers appeared to coincide roughly with the boundaries of visual area II as described by Talbot (*4*). In the control animal, few, if any, degenerating fibers were found in the contralateral white matter; nor could preterminal fiber degeneration be observed in the contralateral visual cortex, although other degenerating pathways in this brain were well stained. Figure 2 shows sections through three of the brains studied and high-power photomicrographs of the apex of the lateral gyrus opposite and slightly posterior to the lesion. Geniculate lesions and contralateral degeneration in brains from cats that survived 5 and 21 days are shown in Fig. 2, *A* and *B*, and *C* and *D*, respectively; the control lesion with absence of degeneration in the lateral gyrus is shown in Fig. 2, *E* and *F*.

Evidence in the literature indicates indirectly that a crossed geniculo-cortical pathway may exist and be of considerable functional importance. As mentioned previously, sparing of the macular portion of the visual fields in man has been demonstrated even after surgical amputation of the occipital cortex (*5*). Additional support has re-

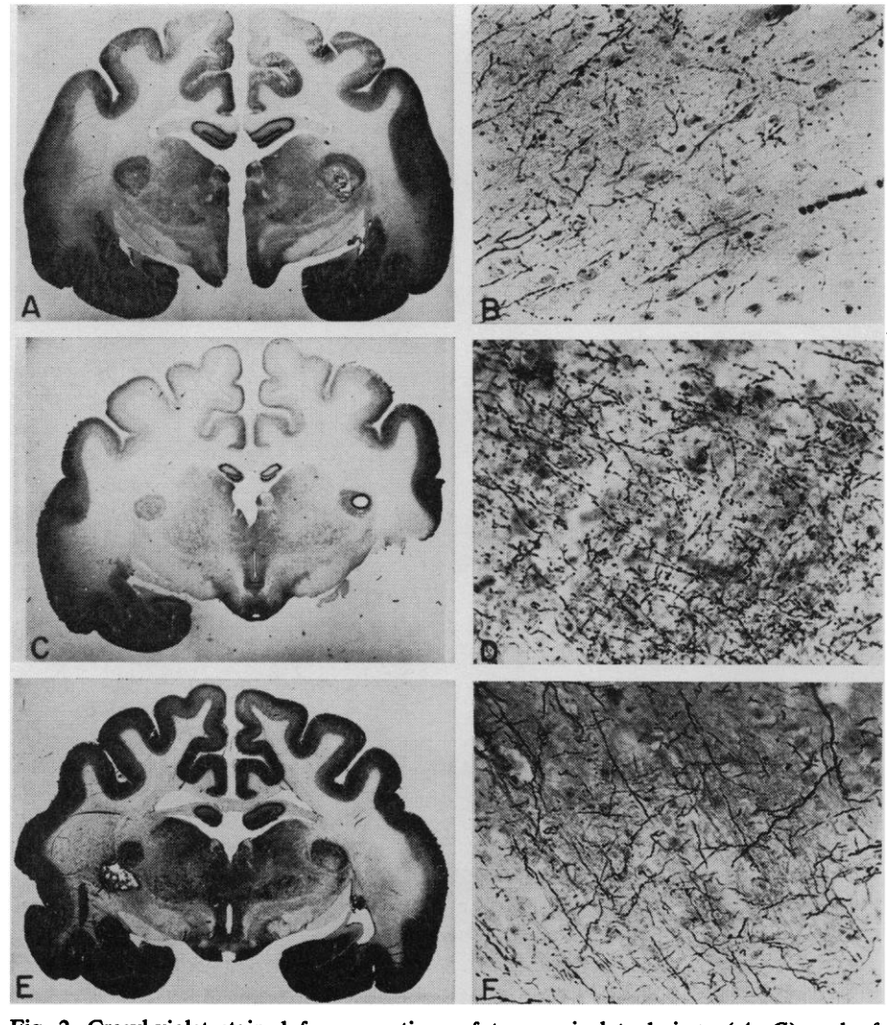


Fig. 2. Cresyl-violet stained frozen sections of two geniculate lesions (*A*, *C*) and of the brain from the control cat (*E*) along with corresponding areas of degeneration (*B*, *D*, *F*) stained by a modified Nauta technique. *B*, *D*, and *F* were taken from apex of lateral gyrus, slightly posterior to plane of whole brain section (× 210). Cats from which *A–B* were obtained survived 5 days, *C–D* and *E–F*, 21 days. Damage to corpus callosum in *A* and temporal lobe and habenula in *C* are postmortem artifacts.

sulted from behavioral studies. Glickstein *et al.* (*6*) observed an apparent recovery from hemianopia in monkeys after unilateral section of the optic tract. Subsequent section of the corpus callosum gave the appearance of reinstating the hemianopia. A similar observation had been reported in the early literature by Imamura (*7*) and Yoshimura (*8*) who noted a "hemiamblyopia" in dogs after unilateral lesions were made in the occipital cortex. The condition seemed to disappear with time. However, if the corpus callosum of these animals was subsequently sectioned, the visual defects were reinstated. In terms of the proposed crossed pathway, interpretation of these studies of cortical lesions and sectioning of the corpus callosum is relatively straightforward. However, problems of interpretation arise in the case of optic tract sectioning since, according to the classical understanding of retinal-geniculate anatomy, optic tract section alone should lead to a complete hemianopia.

Such a pathway could be important in interpreting interocular transfer of visual learning in cat and monkey. Myers reported that a visual discrimination, learned monocularly, is transferred to the untrained eye in cats with the chiasma sectioned. Such discriminations are not transferred if the corpus callosum is sectioned as well (*9*). A similar effect in the monkey has been described by Downer (*10*) and Sperry (*11*). These authors have assumed that the interhemispheric mechanism by which a contralateral trace is established in the chiasma-sectioned animal involves a cortico-cortical commissural route. The evidence presented here suggests that interhemispheric transfer may be brought about in part by a simple bifurcation of the visual projection pathway from the lateral geniculate nucleus.

There are two major possible sources of artifact in our experiment. The observed degeneration may have resulted from inadvertent damage to commissural neurons or their axons. However, the lesions were located far from the route of typical commissural fibers of the lateral gyrus. Moreover, our control case exhibited little or no degeneration in the lateral and suprasylvian gyri of the opposite hemisphere, a situation which further suggests that commissural damage did not occur. The second possibility for artifact, that of transneuronal degeneration, is unlikely since contralateral degeneration was observed after survival periods as short as 5 days. Thus, we believe the anatomical evidence indicates the existence of a pathway crossing from the lateral geniculate nucleus of one hemisphere to the lateral and suprasylvian gyri of the other by way of the corpus callosum.

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**References and Notes**

1. S. L. Polyak, *The Vertebrate Visual System* (Univ. of Chicago Press, Chicago, 1957).
2. J. Rose and C. N. Woolsey, in *Biological and Biochemical Bases of Behavior*, H. F. Harlow and C. N. Woolsey, Eds. (Univ. of Wisconsin Press, Madison, 1958), p. 127.
3. June L. De Vito and O. A. Smith, Jr., *J. Comp. Neurol.* **111**, 261 (1959).
4. S. A. Talbot, *Federation Proc.* **1**, 84 (1942).
5. W. C. Halstead, A. E. Walker, P. C. Bucy, *Arch. Ophthalmol.* **24**, 948 (1940).
6. M. Glickstein, H. Arora, R. W. Sperry, *J. Comp. Physiol. Psychol.* **56**, 11 (1963).
7. S. Imamura, *Arch. Ges. Physiol.* **100**, 495 (1903).
8. K. Yoshimura, *Ibid.* **129**, 425 (1909).
9. R. E. Myers, *Brain* **79**, 358 (1956).
10. J. L. de C. Downer, *Federation Proc.* **17**, 37 (1958).
11. R. W. Sperry, *Anat. Record* **131**, 297 (1958).
12. This study was supported in part by USPHS grant MH 06722-02.

11 May 1964

## Thermal Inactivation of the Primer in DNA-Dependent Synthesis of RNA in Animal Tissue

Abstract. *An enzyme preparation obtained from bovine lymphosarcoma tissue catalyzes the DNA-dependent synthesis of RNA. Native DNA is a more efficient primer in the reaction than heat-denatured DNA.*

The RNA polymerase of chicken embryos, like the bacterial enzyme, is soluble, and it is also dependent on the addition of DNA for maximal activity; the added DNA influences the rates at which each of the four ribonucleotides are incorporated (*1*).

In order to investigate further DNA-dependent RNA synthesis in animal tissues, we have studied the reaction with a soluble enzyme preparation obtained from bovine lymphosarcoma tissue (*2*). With this enzyme preparation, the synthesis of RNA requires DNA and all four ribonucleoside triphosphates. For example, in an experiment similar to that outlined in Table 1, 0.19 m$\mu$mole of uridylic acid was incorporated into an acid-insoluble form. When calf thymus DNA or the three unlabeled ribonucleoside triphosphates were omitted, less than 0.02 m$\mu$mole of uridylic acid was incorporated. In this experiment the reaction was inhibited 95 percent by the addition of 20 $\mu$g of ribonuclease, 84 percent by 20 $\mu$g of deoxyribonuclease, and 89 percent by 5 $\mu$g of actinomycin D.

Since DNA, unless it has been denatured, is a poor primer for DNA polymerase from calf thymus (*3*), native and heat-denatured DNA were compared as primers of the lymphosarcoma RNA polymerase. As shown in Table 1, native DNA is a better primer than heat-denatured DNA. The heat-denatured calf thymus DNA is still able to prime the RNA polymerase from *Escherichia coli*, as has been reported (*4*). It is not considered likely that this primer specificity is the result of a nuclease preferentially degrading heat-denatured DNA; for, when the two enzymes are incubated together,

Table 1. Comparison of native and heat-denatured DNA as primers for RNA synthesis. The complete reaction mixture (0.5 ml) contained: 80 $\mu M$ $\alpha$-$P^{32}$-UTP (*10*) (3.1 $\times$ $10^6$ count/min per $\mu$mole); 160 $\mu M$ each of CTP, GTP, and ATP; 8 m$M$ $MgCl_2$; 4 m$M$ $MnCl_2$; 2 m$M$ 2-mercaptoethanol; 50 m$M$ tris-maleate buffer at *p*H 8.0; DNA and enzyme. The amounts of DNA added, expressed as millimicromoles of deoxynucleotides, were: calf thymus DNA, 72; *H. influenzae*, 60 (*5*). The DNA preparations were denatured by heating at 100°C for 4 minutes followed by cooling in an ice bath. The amounts of enzyme protein added were, lymphosarcoma polymerase, 530 $\mu$g; *E. coli* polymerase, purified through the first ammonium sulfate step of procedure B (*6*), 49 $\mu$g. After incubation for 20 minutes at 38°C, the reaction was terminated by the addition of 0.2 ml of 7 percent perchloric acid, albumin (0.5 mg) was added, and the acidified mixture was centrifuged. The precipitate was washed twice with 3-ml portions of 1 percent perchloric acid, dissolved in 1.5 ml of 0.2$N$ $NH_4OH$, decanted into metal planchets, and dried; the radioactivity was measured in a windowless Geiger-Muller counter.

| Conditions | Uridylic acid incorporated | | |
|---|---|---|---|
| | Lympho-sarcoma polymerase | *E. coli* poly-merase | Both enzymes |
| Omit DNA | < 0.01 | 0.08 | |
| Calf thymus DNA | 0.56 | 0.68 | 1.39 |
| Calf thymus DNA, heat-denatured | 0.05 | 0.46 | 0.39 |
| *H. influenzae* DNA | 0.19 | 0.46 | |
| *H. influenzae* DNA, heat-denatured | 0.01 | 0.09 | |