11. J. D. Swalen, *NMRPLT, NMR Spectrum Plotting*, Program 36 (Quantum Chemistry Program Exchange, Indiana University, Bloomington, 1972).
12. J. R. Van Wazer, *Phosphorus and Its Compounds* (Wiley-Interscience, New York, 1958), vol. 1, chap. 2.
13. For a comparable reaction involving the pyridine solvent, see T. M. Jacob and H. G. Khorana [*J. Am. Chem. Soc.* **87**, 368 (1965)]. Also for a related reaction involving ATP and Ba^{2+} ions in aqueous solution, see D. Lipkin, R. Markham, and W. H. Cook [*J. Am. Chem. Soc.* **81**, 6075 (1959)].
14. J. R. Van Wazer, *Colloques Internationaux du Centre National de la Recherche Scientifique, No. 106* (1962), p. 33; T. Glonek; J. R. Van Wazer, T. C. Myers, *Bioinorg. Chem.* **1**, 23 (1971).
15. This work was supported by the General Research Support grant awarded to the University of Illinois College of Medicine, a grant from the Research Board of the Graduate College, University of Illinois at the Medical Center, and PHS grant 11702. We thank S. Brudno and A. Kilburn of the Research Resources Laboratory–Computer Center for their assistance in the computer analysis aspect of this work.

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Visual Fields of Cats with Cortical and Tectal Lesions

Absrtact. *When cats were tested for visual field perimetry, the field of vision for each eye separately was from 45° contralateral to 90° ipsilateral. After either bilateral occipitotemporal lesions (with a split of the tectal commissure) or bilateral area 17, 18, and 19 lesions, the cats could see with each eye only from the midline to 90° ipsilateral. A cat that became nearly totally blind as a result of bilateral occipitotemporal decortication had a subsequent tectal split which enabled it to see with each eye from the midline to 90° ipsilateral.*

Sprague reported that cats with a large, unilateral, occipitotemporal cortex ablation develop a stable hemianopia (*1*), but that in such cats considerable visually guided behavior was restored for the previously blind hemifields after either an ablation of the superior colliculus contralateral to the cortical lesion or a transection of the commissure of the superior colliculus (*2*). As a tentative explanation for this remarkable phenomenon, Sprague suggested (i) that the midbrain subserves certain aspects of visually guided behavior; (ii) that each colliculus receives a facilitatory input from the ipsilateral cortex and a balancing inhibitory input from the contralateral colliculus via the collicular commissure; and (iii) that as a result of the imbalance caused by the cortical lesion, the function of the ipsilateral colliculus is inhibited. Under these conditions, the colliculus is nonfunctional for visually guided behavior, and this results in the hemianopia. Function is returned by destroying either the other colliculus or the collicular commissure.

There have been no reported confirmations of this phenomenon which Sprague reported in 1966 and few experimental attempts to understand it further [see (*3*)]. I now report a confirmation and extension of Sprague's finding.

I tested five cats with bilateral cortical lesions. In three (C3, C6, and C7), the lesion included most of the occipitotemporal cortex; in the other two (C10 and C11) it included only areas 17, 18, and most of 19 (Fig. 1). In addition, the collicular commissures were transected at the time of the cortical ablation in C3 and C7, and in a second operation 9 months later in C6. All cats except C6 were killed and perfused with 10 percent formol saline. Their brains were blocked stereotaxically, removed, photographed, and cut coronally in 40-μm sections that were stained alternately with cresyl violet for cell bodies and by the Mahon method for fibers. The lesions in cats C3, C7, C10, and C11 were reconstructed (Fig. 1). Cat C6 is being retained for further study, and I assume tentatively that its lesions are similar to those of C3 and C7 (Fig. 1), particularly since visualization was especially good during the two operations in C6.

I tested all cats pre- and postoperatively, and used previously described methods to study visual placing, visual following of moving objects, and the extent of visual field perimetry (*4*). For the perimetry, each cat was taught to fixate on one object while a second stimulus was rapidly introduced vertically from above into the visual field, and the presence or absence of the cat's orienting response to the second stimulus was noted. Every 15° sector of the visual field was thus tested numerous times, and a percentage of correct orienting responses was calculated. For a control, I computed for each cat the baseline percentage of apparent orienting responses in the absence of a second stimulus. Only regions of the visual field in which the second stimulus evoked a significantly ($P < .001$ on a χ^2 test) higher percentage of orienting than this baseline percentage are considered to be regions visually attended to by the cat. The cats were tested binocularly as well as monocularly by means of a contact occluder over one cornea.

The visual field perimetry data for

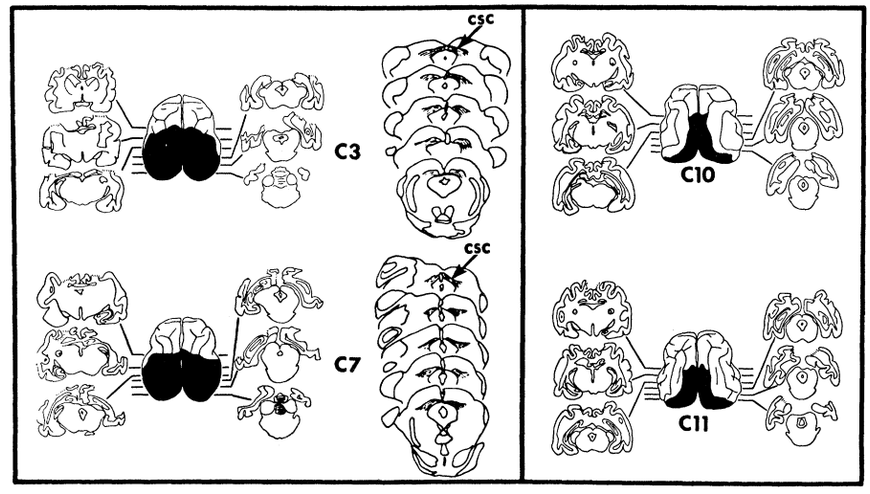

Fig. 1. Reconstruction of lesions. In all four cats, the lateral geniculate nucleus showed retrograde degeneration throughout its extent. For C3 and C7, the cortical lesions involved most of the occipitotemporal cortex including all known visual projection zones of the geniculate and pulvinar and lateral posterior nucleus complex (*11*); the commissure of the superior colliculus (*CSC*) was completely sectioned in each cat except for a few fibers surviving in the posterior quarter of the commissure. For cats C10 and C11, the lesion involved dorsally all of the lateral gyrus and medially all cortex superior to the lower bank of the splenial sulcus; all known visual recipient zones of the geniculate were removed, but the visual projections of the pulvinar and lateral posterior nucleus complex were largely spared (*11*).

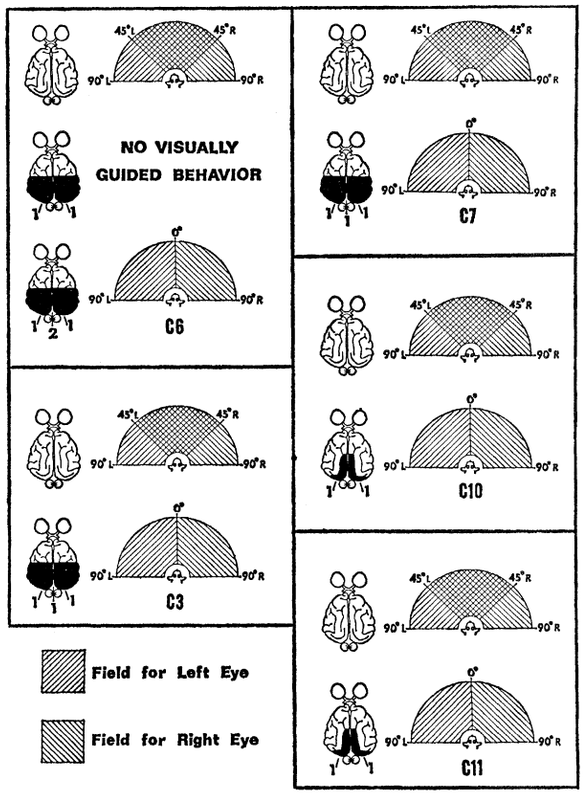

Fig. 2. Horizontal extent of visual field perimetry for each cat pre- and postoperatively. The numbers pointing to the lesions represent the sequence of operations. In all cases, the binocular field of view equals the sum of the monocular fields. Preoperatively, all cats with each eye saw from 45° contralateral to 90° ipsilateral. After its occipitotemporal lesion, C6 was virtually blind, but soon after a second lesion 9 months later which split the collicular commissure, the cat clearly saw with each eye from the midline to 90° ipsilateral. Cats C3 and C7, after the single stage occipitotemporal ablations and collicular commissure splits, behaved exactly as did C6 after its commissure split. Cats C10 and C11, with their smaller area 17, 18, and 19 ablations had monocular fields identical in extent to the final ones of C6, C3, and C7.

all of the cats and the data from C6 (Fig. 2) substantially confirm Sprague's result. Preoperatively, with respect to both binocular and monocular viewing, the cats had good visual placing, good following of moving objects in all directions, and typically normal visual fields (approximately these were: binocularly from 90° left to 90° right; left eye from 90° left to 45° right; and right eye from 45° left to 90° right) (*4*). Indeed, all of the cats during preoperative testing had indistinguishable visual placing, visual following, and visual fields (Fig. 2). After the bilateral occipitotemporal lesion, C6 appeared totally blind on all visually guided tests throughout the 9-month period (*5*). At this point, I transected the collicular commissure in a second operation, and within 4 weeks the cat showed a remarkable recovery of visually guided behavior. However, I did note certain deficits: (i) overall behavior was abnormally sluggish; (ii) visual placing seemed limited to the "visually elicited component" as described by Hein and Held (*6*); (iii) visual following could be elicited in both directions for binocular viewing, but with monocular viewing the cat followed moving objects only if they were directed from the midline to the periphery ipsiversive to the open eye; and (iv) although the cat had a full field of view binocularly, during monocular testing the fields were limited to the hemifield ipsilateral to the open eye (that is, the contralateral 45° was lost).

Both C3 and C7 had the cortical ablation and commissure transection during the same operation. Within 3 to 5 weeks after the operation, each animal showed visually guided behavior, and in the ensuing weeks this behavior, including the perimetry, stabilized to become virtually identical to that described above for C6 (see Fig. 2).

In all respects, cats C6, C3, and C7 behaved identically to cats reared with binocular deprivation (BD cats). I suggested earlier (*4, 7*) that in such BD cats the entire geniculocortical system failed to develop normally for visually guided behavior, so this correspondence in deficits between decorticate cats (C6, C3, and C7) and BD cats supports this hypothesis [see also (*8*)].

On these tests C6, C3, and C7 behaved as if only their nasal hemiretinas maintained functional central connections. The midbrain, or more specifically the superior colliculus, seems crucial to this behavior (*1*), and it is interesting to note that most of the retinotectal pathway in the cat is crossed. Sterling (*9*) has estimated the crossed component to be as much as 99 percent. Therefore, it may be that the few retinotectal fibers projecting ipsilaterally from the temporal retina are insufficient in number to sustain this visually guided behavior. Another possibility is that these ipsilaterally projecting fibers are related to behavior not tested here, such as brightness discrimination (*10*).

Given the above, it follows that the ability of the normal cat to use temporal retina for visually guided behavior is probably related to non-midbrain pathways, such as the geniculocortical pathway which has considerable temporal retinal representation via geniculate lamina A_1. I have arbitrarily and approximately divided the cat's visual cortex into (i) the geniculocortical region which mostly involves the lateral gyrus, and on the medial surface, all cortex above the splenial sulcus; and (ii) the region receiving input from superior colliculus and pretectum via the pulvinar and lateral posterior nucleus complex, and this lies mostly in the suprasylvian and parts of the ectosylvian gyri (*11*). Although it has been reported that unilateral lesions of areas 17, 18, and 19 do not produce long-lasting, obvious deficits in the cat's visually guided behavior (*1*), it followed from the above that such a lesion placed bilaterally might result in deficits similar to those of cats C6, C3, and C7 after their cortical lesions and collicular commissure splits. That is, such cats might be expected to lose central connections by which the temporal retina controls visually guided behavior.

I tested this hypothesis on cats C10 and C11. Within 4 days of bilateral removal of most of the geniculocortical region (Fig. 1), both cats showed clear visual responses. Their behavior during binocular testing was in every way normal, and only monocular testing demonstrated any deficits. With one eye, each cat could follow objects only if they moved from the midline to the periphery ipsiversive to the open eye, and each cat attended to objects only in the ipsilateral hemifield (Fig. 2). While these cats shared with C3, C6, and C7 an apparent inability to use temporal retina effectively, they had notably brisker responses, normal visual placing, and required no collicular commissure split for this behavior. The intact suprasylvian or ectosylvian gyri (or both) evidently maintained the brisker behavior and

prevented suppression of the colliculi.

Four major conclusions are drawn from this work. First, these data confirm Sprague's dramatic report (*2*) that occipitotemporal decorticate cats are capable of extensive visual behavior provided that intercollicular influences via the collicular commissure are prevented. Second, such decorticate cats behaved as if only their nasal retinas maintained functional central connections, and it is noted that most —up to 99 percent (*9*)—of the cat's retinotectal pathway is crossed. Third, cats with lesions largely limited to geniculocortical areas also behaved as if they have no functional temporal retina, and presumably this obtained because the remaining cortex had visual afferentation only from the midbrain via the pulvinar and lateral posterior nucleus complex. Fourth, the cats with large occipitotemporal lesions plus a split of the collicular commissure behaved on all tests precisely as did binocularly deprived cats (*4*), and this strengthens an earlier suggestion (*7*) that such deprived cats develop visually guided behavior only through retinotectal and not geniculocortical pathways.

S. MURRAY SHERMAN

Department of Physiology, University of Virginia School of Medicine, Charlottesville 22901

References and Notes

1. J. M. Sprague, in *The Thalamus*, D. P. Purpura and M. D. Yahr, Eds. (Columbia Univ. Press, New York, 19(——— and T. H. Meikle, Jr., *Exp. Neurol.* **11**, 115 (1965).
2. J. M. Sprague, *Science* **153**, 1544 (1966).
3. M. A. Goodale, *Exp. Brain Res.* **17**, 75 (1973); R. D. Kirvel, R. A. Greenfield, D. R. Meyer, *J. Comp. Physiol. Psychol.*, in press.
4. S. M. Sherman, *Brain Res.* **49**, 25 (1973); *ibid.*, in press.
5. I have observed such long-term blindness in three other cats with large, bilateral, occipitotemporal lesions. These cats, like C6, are being retained for further study. In the context of Sprague's suggestion that each colliculus receives balancing facilitatory corticotectal and inhibitory collicular commissure inputs, it is interesting that bilaterally decorticate cats remain blind. This implies that, although each colliculus loses a facilitatory input and is "depressed," it still manages to inhibit the other through its commissure.
6. A. Hein and R. Held, *Science* **158**, 390 (1967).
7. S. M. Sherman, *Invest. Ophthalmol.* **11**, 394 (1972).
8. ———, K.-P. Hoffmann, J. Stone, *J. Neurophysiol.* **35**, 532 (1972).
9. P. Sterling, *Brain Res.* **54**, 347 (1973).
10. J. P. Baden, J. C. Urbaitis, T. H. Meikle, Jr., *Exp. Neurol.* **13**, 233 (1965); B. S. Wood, *Brain Res.* **53**, 428 (1973).
11. L. J. Garey and T. P. S. Powell, *Proc. R. Soc. Lond. Ser. B* **169**, 107 (1967); A. M. Graybiel, *Brain Res.* **44**, 99 (1972); A. C. Rosenquist, S. B. Edwards, L. A. Palmer, *ibid.*, in press.
12. Supported by NSF grant GB-38264. I thank Mrs. Lodi Smith for her excellent technical assistance.

22 March 1974

Assembly of Chick Brain Tubulin onto Isolated Basal Bodies of Chlamydomonas reinhardi

Abstract. *Basal bodies isolated from* Chlamydomonas reinhardi *will serve as initiation centers for the assembly of chick brain microtubule protein subunits (tubulin) into microtubules. The rate of microtubule assembly is tubulin-concentration dependent; this assembly occurs onto both distal and proximal ends of the basal body microtubules, with distal assembly greatly favored. In vitro assembly of brain tubulin also occurs onto the mid-lateral aspects of the basal bodies, presumably onto the fiber connecting the two basal bodies.*

Basal bodies are the assembly sites for the microtubules of all eucaryotic cilia and flagella. Because of the difficulties which have been encountered in their isolation, little is known about their biochemistry or how they function in the organization and assembly of microtubules (*1*). By contrast, their ultrastructure (see Fig. 1), development, and movements in the cell are well defined, and there are excellent reviews on these topics (*2*). One approach to the determination of the role of basal bodies in the assembly of microtubules has been the use of flagella-regenerating systems in flagellated cells such as the biflagellate alga *Chlamydomonas*. In these systems the flagella are detached by physical or chemical procedures and new flagella regenerate from the remaining basal bodies. There have been a variety of studies on the synthesis and assembly of flagellar proteins during this regeneration process, including some which demonstrated that the flagellar microtubules elongate by distal assembly of subunits (tip growth) (*3, 4*). In order to learn more about the role of basal bodies as sites for microtubule protein (tubulin) assembly it would be advantageous to be able to study the assembly of tubulin onto basal bodies in vitro. It became possible to carry out such studies with the development of methods for the in vitro assembly of brain tubulin by Weisenberg (*5*). By use of these methods for the in vitro assembly of tubulin along with new procedures described here for the isolation of basal bodies from *Chlamydomonas*, we now show that the isolated algal basal bodies can act as assembly sites for chick brain tubulin. As with the distal directionality of microtubule assembly seen during flagellar regeneration in vivo, the in vitro assembly of tubulin onto isolated basal bodies occurs principally by distal addition of subunits, although there is also some assembly onto the proximal ends of the basal bodies.

Cultures of strains 21 gr (+ mating type) and 6145C (− mating type) of *Chlamydomonas reinhardi* were grown to a cell density of approximately 2×10^6 cells per milliliter in Medium I of Sager and Granick (*6*) supplemented with 3 g of sodium acetate per liter and three times the normal amount of phosphate buffer. Growth was at 25°C with continuous aeration on a cycle of 13 hours of light and 11 hours of dark. Four liters of each strain were harvested, resuspended in 4 liters of M-N medium (Medium I of Sager and Granick without NH_4NO_3, at *p*H 7.6), and placed in continuous light for 18 hours, during which time the cells differentiated into gametes (*7*). The two strains of gametes were harvested and mixed together in 1 liter of M-N medium for 30 minutes to allow mating to occur (*8*). During the mating reaction a cell wall lysin is released into the medium which causes dissociation of the cell walls from approximately 90 percent of the cells (*9*). These wall-less cells were then deflagellated in M-N medium using the *p*H shock method (*4*). The suspension of cells, cell walls, and flagella was centrifuged at 1500*g* (2700 rev/min, IEC PR-6 centrifuge, rotor No. 253) for 3 minutes at 25°C, sedimenting the cell bodies and leaving the cell walls and flagella in the supernatant. The supernatant was removed, the cell bodies were resuspended in 500 ml of TE buffer (10 m*M* tris, 1 m*M* EDTA, *p*H 7.5 at 25°C) at 4°C and centrifuged again as above. All subsequent steps were carried out at 4°C. The wall-less, flagella-less cells were resuspended in 50 ml of TE to which was then added 50 ml of 1 percent Nonidet P-40 (Shell) in TE. The suspension was stirred vigorously for 10 minutes, and homogenized by hand in 20-ml aliquots in a 25-ml glass-Teflon homogenizer (Arthur H. Thomas Co.) with five up-and down strokes; 20-millimeter aliquots of this suspension were layered over 20 ml of 25 percent sucrose-TE in 50-ml, conical, polycarbonate centrifuge tubes.