

MEMORY PROCESSING IN CHICKENS AND GOLDFISH

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Abstract

Memory processing has been investigated in day-old chickens and in adult goldfish. In chicks, bilateral vs. unilateral storage of monocularly-acquired one-trial passive avoidance was examined using unilateral lesions of the avian telencephalon. Interocular transfer tested 24 hr after training was observed after lesions of the trained hemisphere delayed 2-18 hr after the training trial. Bilateral lesions abolished task performance. It is concluded that memory for one-trial passive avoidance is established bilaterally in the brain and that the telencephalon contributes either as a storage area or as a relay in retrieval. Results of unilateral lesions delayed 2 min after training also suggested bilateral storage, in contrast to published reports of apparent unilateral storage of the same task after unilateral injection of cycloheximide 2 min after training. The disparity between results may be the result of more general disruption of brain function produced by protein synthesis inhibitors; cycloheximide may interfere with transfer of sensory information or depress brain function so as to prevent the normal establishment of bilateral traces.

Adult goldfish were used to examine the roles of the optic tectum and forebrain in memory storage of color discrimination using conditioned respiratory suppression as the behavioral measure. Highly localized training of discrete tectal areas was followed by lesions of trained tectal regions or by bilateral forebrain lesions. Excellent intraretinal generalization to the remainder of the visual field of the trained eye was observed. Interocular transfer across the entire visual field of the untrained eye occurred after training in the posterior visual field. Complete tectal ablation after localized training did not produce deficits in interocular transfer. It is concluded that binocular regions of the

visual field in goldfish do not integrate color discrimination better than do monocular regions and that memory storage may occur bilaterally in the brain.

Findings of apparent bilateral memory storage in birds and fish are related to studies of memory in mammalian species. It is suggested that the parallel processing methods observed may reflect selection procedures which occurred early in the evolutionary development of behavior in vertebrates.

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General Introduction

The bulk of research on memory processing in animals has been focused on mammalian species; research in avian and teleost species has been carried out concurrently but on a smaller scale. In recent years, investigations of learning and memory in non-mammalian species have gained considerable importance, as the advantages of working with relatively simpler systems have been recognized. These advantages include the simpler organization of visual projections, decreased overall complexity in brain anatomy, availability of precocial avian species, regenerative capabilities in teleosts, and less expensive maintenance in the laboratory. There is a wealth of evidence that both birds and fish are able to learn tasks of comparable difficulty to those performed by at least some mammalian species (Bitterman, 1975). Among the more popular non-mammalian species have been the pigeon (Columba livia), domestic chicken (Gallus gallus), and common goldfish (Carassius auratus) as well as a variety of other teleost fish. A summary of findings in these species can indicate the contributions to the understanding of learning and memory processing of research on non-mammalian species.

The role of visual pathways and structures in discrimination learning has been investigated extensively in avian species, particularly in the pigeon, in which operant learning has been well documented. The anatomy of the visual system in pigeons has been studied in depth, and the development of an excellent stereotaxic atlas (Karten and Hodos, 1967) has greatly facilitated investigations involving specific lesions. Two primary visual pathways have been identified; tectofugal and thalamofugal pathways (Karten and Hodos, 1970; Karten, Hodos, Nauta and Revzin, 1973; Benowitz and Karten, 1976). Studies of the effects of lesions of these pathways have been carried out with somewhat perplexing

and unsatisfying results. Lesions of the tectofugal pathway (optic tectum, nucleus rotundus, ectostriatum) in pigeons result in marked deficits in postsurgical performance of intensity (Cohen, 1967; Hodos and Karten, 1970, 1974), pattern (Hodos and Karten, 1970, 1974; Jarvis, 1974) and color discrimination tasks (Hodos, 1969). Lesioned birds exhibit initial discrimination at chance levels with slow recovery rates, although performance does tend to return to preoperative levels after long retraining. The degree of deficit is greater in pigeons with large tectal lesions, and recovery does not occur. In contrast, lesions of the thalamofugal pathway (nucleus opticus principalis thalami, hyperstriatum accessorium, h. dorsale, h. intercalatus [the visual "Wulst"]) produce much milder deficits overall (Pritz, Mead and Northcutt, 1970; Hodos, Karten and Bonbright, 1973) although deficits approaching those reported with tectofugal lesions have been observed after very large Wulst lesions (Zeigler, 1963). Psychophysical experiments (see Hodos, 1976, for review) have indicated that lesions of the thalamofugal pathway result in small but significant increases in thresholds for intensity differences from which recovery is incomplete, while tectofugal lesions produce initially greater increases in threshold which are apparently transient. Further deficits in delayed matching response (Pasternak, 1977) and in reversal of discrimination (Stettner and Schultz, 1967) have also been reported after Wulst lesions. Reversal deficits were not due to reduced ability to discriminate, but were rather the result of increased perseverance of response to the positive stimulus, a finding which has been supported by related observations in forebrain-lesioned chicks (Benowitz and Lee-Teng, 1973; Oades, 1976). The general conclusions which can be drawn from these experiments are: 1. that the tectofugal pathway appears to play a more critical role initially in visual discrimination learning, but that recovery occurs, and 2. that lesions of the thalamofugal pathway produce

milder but permanent deficits in discrimination. The almost total recovery from lesions of the tectofugal pathway, excepting those of optic tectum, is probably the result of the animal's ability to adopt new strategies for problem solving. Presumably such lesioned birds can learn to suppress confounding influences produced by the lesion. No clear-cut roles have been established for the two relatively independent pathways; this ambiguity must be considered in the interpretation of other studies in birds, particularly of those which are directed towards questions of memory storage and processing.

Specific studies directed towards mechanisms of memory processing have been conducted in young domestic chickens. One reason why the chick has been used extensively has been the belief that a precocial species will have a brain less influenced by previous experience; hence, the learning experience might have more profound effects on the brain. The kinetics of memory consolidation for a one-trial passive avoidance task in day-old chicks (Lee-Teng and Sherman, 1966^{*}) has been investigated using electroconvulsive (ECS) and subconvulsive shock (Lee-Teng, 1966a; Lee-Teng and Sherman, 1966; Lee-Teng, 1970; Lee-Teng, Magnus, Kanner and Hochman, 1970; Benowitz and Magnus, 1973) and flurothyl, a convulsive agent (Cherkin, 1970b; Herz, Spooner and Cherkin, 1970). The task involves learning to suppress a natural pecking response to an attractive lure which has been coated with methyl anthranilate, an unpalatable liquid. ECS and related treatments have been demonstrated to produce retention deficits for one-trial learning if administered less than 30 sec after training; longer delays produce diminishing degrees of disruption. These results have contributed to the development of a multistage hypothesis of memory consolidation (Lee-Teng et al., 1970; Cherkin, 1971; Benowitz and Magnus, 1973; McGaugh and Gold, 1974); one stage lasting 10-30 min and not susceptible to ECS, and

a second stage, ECS-susceptible in very early post-training intervals and which develops into long term storage. These stages have been studied further using drug treatments, which will be discussed shortly.

Attempts to localize the sites of memory storage have focused on forebrain structures in chicks. Bilateral lesions of the forebrain have been reported to produce amnesia for the one-trial passive avoidance task (Benowitz, 1972, 1974; Greif, 1976). The suggestion has been that the forebrain acts either directly as a storage area or as a relay along the retrieval pathway. These two possibilities are difficult to separate with the methods currently available. As previously noted, forebrain-lesioned birds are able to learn and relearn discrimination tasks, suggesting that the deficit is not limited to the inability to perform operant tasks. One possible explanation which has not been adequately investigated is that lesions of forebrain structures result in deficits in response inhibition, perhaps akin to hippocampally-lesioned mammals (Izquierdo, 1975). Zeier (1971) has attributed response inhibition to archistriatal structures in the pigeon. Forebrain lesions in chicks, whether restricted to hippocampal or archistriatal regions (Oades, 1976) or encompassing both hyperstriatal and archistriatal areas (Benowitz and Lee-Teng, 1973) might produce perseverance syndromes, thus resulting in apparent amnesia for one-trial avoidance (Benowitz, 1972) and of delayed response tasks (Oades, 1976). The pecking response in chicks appears to be innate and of considerable strength in the first few days of life (Cherkin and Garman, 1977) and strong conditioning is required to produce the avoidance response (Cherkin, 1971). If lesions do produce deficits in response inhibition, the paradigm would not be a reasonable means for examining the role of forebrain structures in memory processing. However, unilateral forebrain lesions do not produce apparent amnesia (Benowitz, 1974; Greif, 1976), suggesting that an intact hemisphere

might prevent the appearance of a perseverence syndrome.

The second broad topic which has been extensively investigated in birds has been the mechanism of interocular transfer of learning after monocular training. Pigeons and chicks have been used because of the ease of lateralizing visual input in these species. The optic fibers cross completely at the optic chiasm (Hodos, 1976); to limit exposure to a single hemisphere one must merely occlude one eye. The capacity for interocular transfer in these species appears to be well developed. Visual discrimination tasks have in general been reported to generalize interocularly (Levine, 1945b; Catania, 1965; Mello, 1966a, 1966b, 1968), although failures have been reported (Levine, 1945a, 1952; Graves and Goodale, 1977). All failures of interocular transfer have involved the use of a modified Lashley jumping stand, requiring the birds to jump from a rotating stand to one of two platforms bearing the discriminanda; if the bird leaps to the incorrect stimulus, it falls through the door on the platform. The apparatus has been criticized as inappropriate for birds on the grounds that it requires stimulus viewing in portions of the visual field which are laterally displaced because of myopia in more central regions, causing conflicting proprioceptive cues from altered head and body position (Catania, 1964). Examinations of patterns of transfer of other types of learning have revealed an intriguing pattern. One-trial passive avoidance transfers interocularly (Cherkin, 1970a; Benowitz, 1974; Greif, 1976) as does visual imprinting (Moltz and Stettner, 1962; Horn, Rose and Bateson, 1973) and conditioned avoidance when shock is used as the aversive stimulus (Stevens and Klopfer, 1977). Visually-mediated food aversion learning also appears to transfer (Gaston, in preparation), but visual cliff habituation (Zeier, 1970), extinction of passive avoidance (Benowitz, 1974), and conditioned avoidance and extinction avoidance using aversive stimuli other than shock (Stevens

and Klopfer, 1977) do not. It appears that at least two classes of learning occur in birds, as measured by the capacity for interocular transfer. The nature of the dichotomy is at yet unclear; it has been suggested that failures of transfer may be the result of participation of brain areas which lack interhemispheric pathways (Benowitz, 1974; Stevens and Klopfer, 1977).

There is now considerable evidence that the pathway for interocular transfer in birds involves the dorsal supraoptic commissure (DSO). Section of the DSO prior to monocular training prevents the transfer of visually mediated learning in pigeons (Cuenod and Zeier, 1967; Meier, 1971; Cuenod, 1974) and in chicks (Horn et al., 1973). Unilateral lesions of the trained hemisphere combined with DSO section result in postsurgical deficits in the trained hemisphere only (Meier, Maier and Cuenod, 1972; Cuenod, 1974; Palmers and Zeier, 1976); the intact hemisphere is able to learn the task normally. Interocular transfer of monocularly acquired pattern discrimination in pigeons (Meier et al., 1972; Maier and Tanaka, 1973) and of passive avoidance in chicks (Benowitz, 1974; Greif, 1976) is not affected by unilateral forebrain lesions in the absence of DSO section. It has further been demonstrated that lesions of the trained hemisphere do not affect transfer when chicks are lesioned after monocular training, providing strong evidence that engrams for passive avoidance are established bilaterally (Greif, 1976).

Memory processing in birds has also been investigated using antibiotic protein synthesis inhibitors, notably cycloheximide (CXM) and anisomycin (ANI). It has been demonstrated in mammals that treatment with protein synthesis inhibitors shortly after training results in deficits in long-term retention (Flexner, Flexner and Roberts, 1966; Barondes and Cohen, 1967; Squire and Barondes, 1973; Flood and Jarvik, 1976). Bilateral injections of CXM or ANI in chicks have similarly

resulted in memory deficits, with retention unaffected for up to 3 hr after training, followed by a slow decline to chance levels (Mark and Watts, 1971; Watts and Mark, 1971; Bull, Ferrera and Orrego, 1976). The implication has been that protein synthesis is required for the establishment of long-term memory, but that short-term memory is independent of protein synthesis (see Barraco and Stettner, 1976, for review). However, there have been reports of recovery of retention after amnesic treatment (Quartermain, McEwen and Azmitia, 1972; Quartermain, 1976) and increasing evidence that protein synthesis inhibitors cause global changes in brain biochemistry (Barraco and Stettner, 1976), suggesting that the situation is far more complex than originally believed. Recent studies (Bell and Gibbs, 1977) have indicated that unilateral CXM treatment apparently produces unilateral storage of the normally bilateral (Greif, 1976) memory traces for one-trial passive avoidance in chicks. In parallel with the current belief concerning the effects of brain lesions (Isaacson, 1976), it is possible that drug treatments cause blocks in the retrieval of memory rather than prevent consolidation.

To summarize the generalizations derived from studies of memory and learning in avian species one might list the following: 1. that the capacity for visually mediated learning is well developed, 2. that performance and either retention or retrieval can be disrupted by lesions of identified visual structures in the brain, 3. that there is some evidence that forebrain structures may have limbic function akin to mammals, 4. that amnesic treatments can produce deficits in passive avoidance and discrimination learning similar to those observed in mammals, but the interpretation of these experiments is not certain, 5. that the pathway for interocular transfer involves the DSO, and 6. that memory for at least passive avoidance in chicks is established bilaterally in the brain.

Research in teleost fish has focused on similar questions; namely, the effect of lesions on learning and memory, and on the mechanisms of interocular transfer of discrimination learning. Lesions of the optic tectum (Springer, Easter and Agranoff, 1977) and of forebrain (Bernstein, 1961a, 1961b, 1962; Hainsworth, Overmier and Snowdon, 1967; Dewsbury and Bernstein, 1969; Savage, 1969) have been investigated in goldfish. Tectal ablation produces gross deficits in optomotor responses, food localization and respiration deceleration to shadow; animals appear to be functionally blind. However, optokinetic responses survive. Forebrain lesions appear to produce deficits in active avoidance (Hainsworth, Overmier and Snowdon, 1967; Dewsbury and Bernstein, 1969; Savage, 1969). Acquisition rates are somewhat slower and reaction time to conditioned stimuli is significantly lengthened (Savage, 1969). Fish are consistently reported to be behaviorally sluggish. Forebrain ablation after acquisition of an avoidance task results in postsurgical performance deficits, although performance does not drop to chance levels (Dewsbury and Bernstein, 1969; Savage, 1969). Cardiac conditioning is only slightly affected by forebrain ablation (Bernstein, 1962). Forebrain lesions have been reported to produce loss of color discrimination (Bernstein, 1961a, 1961b), although later studies have indicated that the deficit is transient and may be spurious (Bernstein, 1962).

Behavioral studies have also been associated with studies of regeneration in the visual system of teleost fish. Color and pattern discrimination has been found to be normal after regeneration of a cut optic nerve or brachium (Arora and Sperry, 1963; Cronly-Dillon, Sutherland and Wolfe, 1966; Francis, Bengston and Gazzaniga, 1976). Functional aspects of the phenomenon of "compression" in the goldfish retinotectal system, in which the entire retina comes to project onto the remaining half-tectum after removal of the caudal half of the optic

tectum (see Meyer and Sperry, 1976 for review), have also been studied using highly localized stimuli (Scott, 1975, 1977). The performance of a classically conditioned color discrimination task during compression was examined systematically. The recovery from half-field blindness proceeds in an orderly fashion across the region of scotoma, with recovery seen first in the anterior portion of the scotoma closest to the margin of the lesion. Once compression is complete, color discrimination reaches preoperative levels across the entire visual field (Scott, 1977). In a less elegant series of experiments, Yolen and Hodos (1976) reported that pattern and intensity discrimination also return to preoperative levels. Further studies of visual acuity suggest that compression occurs at the expense of spatial resolution (Hodos and Yolen, 1976).

Interocular transfer has also been extensively investigated in teleost fish, with less consistent results than those in birds. Interocular transfer in fish appears to be influenced by the type of task used and by stimulus complexity. Interocular transfer of color discrimination appears to occur (Sperry and Clark, 1949; McCleary, 1960; Ingle, 1965; Ingle and Campbell, 1977) but the degree and effectiveness of transfer is affected by the type of learning task. Incomplete transfer was found when complex motor behavior, such as swimming to food or over a barrier, was required (Sperry and Clark, 1949; McCleary, 1960; Lee-Teng, 1966b), while simple shuttle box tasks (McCleary, 1960; Ingle, 1968; Ingle and Campbell, 1977) and cardiac conditioning (McCleary, 1960; Yeo and Savage, 1975) show nearly complete transfer. McCleary (1960) reported that the use of eye occluders interfered with interocular transfer, a finding which has been confirmed (Lee-Teng, 1966b). He suggested that tasks which require active responses by the fish also need visual input to both eyes, even when the discriminanda are presented monocularly, in order to assure that the appropriate

visuomotor associations are established.

Pattern discrimination appears to be affected by stimulus complexity as well as by the type of task. Easy pattern discriminations transfer well (McCleary, 1960; Shapiro, 1965; Lee-Teng, 1966b; Campbell, 1971; Ingle and Campbell, 1977), but Ingle (1968) reported that while simple patterns transfer, stimuli which are initially difficult to discriminate transfer poorly to the untrained eye. In addition, Ingle (1965) reported that when fish learn to discriminate stimuli which differ both in color and pattern, they discriminate only on the basis of color cues with the untrained eye.

The pathways for interocular transfer have not been clearly determined in fish. While the tectal commissure has been reported to be necessary for the transfer of color and pattern discrimination using active avoidance tasks (Mark, 1966; Mark, Peer and Steiner, 1973), no deficits were observed after section of the tectal commissure using simple avoidance tasks (Ingle and Campbell, 1977) and cardiac conditioning (Yeo and Savage, 1975). Consistent deficits in interocular transfer have been observed after section of the postoptic commissure, which is analogous to the dorsal supraoptic commissure in birds (Yeo and Savage, 1976; Ingle and Campbell, 1977), using cardiac conditioning and simple avoidance tasks. The current hypothesis concerning mechanisms of interocular transfer in fish is that the postoptic commissure most likely mediates transfer of visual information, while the tectal commissure may be required for the transfer of visuomotor cues in tasks which require active responses (Ingle, 1967; Yeo and Savage, 1975, 1976; Ingle and Campbell, 1977).

Findings from research on memory and learning in teleost fish may be summarized as follows: 1. that teleost fish are able to learn visual discrimination tasks provided that stimulus complexity is limited, 2. that ablation of the

optic tectum produces functionally blind animals, 3. that lesions of telencephalic structures result in sluggish behavior and attentional difficulties and produce deficits in avoidance behavior, 4. that regeneration and compression in the teleost retinotectal system involve the establishment of functional connections, and that visual discrimination returns to preoperative levels, 5. that the occurrence of interocular transfer is dependent on both the type of task employed and of stimulus complexity, and 6. that the pathway for interocular transfer most likely involves the postoptic commissure.

The studies which will be described in the chapters to follow have attempted to answer several specific questions concerning learning and memory in non-mammalian species. Chapter I, which has been published (Greif, 1976) examined the question of bilateral vs. unilateral storage of memory after monocular training in day-old chickens. Previous studies had suggested that bilateral storage was likely (Benowitz, 1974), but the problem had not been examined directly. Chapter II, an extension of work reported in Chapter I, was prompted by the recent report of Bell and Gibbs (1977) that unilateral storage of memory for one-trial passive avoidance was found after unilateral treatment with cycloheximide. An attempt is made to reconcile the basic differences which might exist between lesion and drug manipulations.

Chapters III and IV are focused on the nature of the transfer of visual information in goldfish, and of the relationship of such transfer to the storage of memory. Chapter III examines the generalization of visual learning across the visual field of one eye after lesions of tectal areas receiving highly localized training. Generalization across the visual field has been reported in unoperated animals (Scott, 1977); we wished to examine the role of the directly trained portion of tectum on such generalization. In addition, the role of the forebrain

in the mediation of color discrimination and task retention is examined, prompted by our own studies of telencephalic lesions in birds and of reports of learning deficits after forebrain lesions in fish (Bernstein, 1961a, 1961b, 1962; Savage, 1969). Chapter IV investigates the effects of lesions of trained tectal areas on the interocular transfer of the color discrimination task. We wished to determine whether discrimination in portions of the visual field which lie within the region of binocularity would be better than that of regions which lie outside. Such patterns of discrimination have not been examined previously. We also have examined whether engrams for the classically conditioned color discrimination task are most likely established bilaterally or unilaterally in the brain, to determine whether fish process memory similarly to other vertebrates.

CHAPTER I

Bilateral Memory for Monocular One-Trial Passive Avoidance
in Chicks^{1,2}

Abstract

Numerous studies of monocularly-acquired interhemispheric transfer in birds suggest indirectly that memories are laid down bilaterally, but this has not been established by direct methods. The present experiment was designed to test directly the laterality of memory storage. Day-old chicks were taught monocularly a one-trial passive avoidance task, after which large hyperstriatal lesions were made either contralateral or ipsilateral to the exposed eye at time intervals ranging from 2-18 hr. Twenty-four hours after the training trial transfer and retention were tested by occluding the experienced eye of chicks with lesions contralateral to that eye. In chicks with ipsilateral lesions the same eye was trained and tested. Similar lesions made bilaterally in a control group abolished task retention. Both unilaterally-lesioned groups performed at near-normal levels. It is concluded from the results that memory for one-trial passive avoidance is established bilaterally in the chick brain.

Introduction

Whether memories are normally stored bilaterally or unilaterally in

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²This work has been published as Greif, K. F., *Behav. Biol.* 16:453-462, 1976. Copyright held by Academic Press, Inc.

the vertebrate brain has been the subject of considerable controversy in recent years. Extensive work in monkeys and cats on the role of the corpus callosum has produced a number of conflicting reports, some favoring bilateral memory storage (Myers, 1961; Downer, 1962; Ebner and Myers, 1962; Butler, 1968; Sullivan and Hamilton, 1973; Hamilton, 1977), others favoring unilateral (Gazzaniga, 1963; Webster, 1972; Doty and Negrão, 1973; Doty and Overman, 1977).

While a number of experiments in monkeys and cats have been directed towards elucidating storage mechanisms, studies of visual learning in birds have tended to focus on other aspects of memory, including interhemispheric transfer and the role of the forebrain. The capacity for interocular transfer in birds appears to be well established (Catania, 1965; Cuenod and Zeier, 1967; Cherkin, 1970a; Meier, 1971; Benowitz, 1974), although some tasks do not transfer following unilateral training (Zeier, 1970; Benowitz, 1974). Lesions of the "Wulst" of the hyperstriatal complex of the telencephalon in pigeons and quail result in difficulties in visual discrimination learning and reversal (Zeigler, 1963; Stettner and Schultz, 1967; Pritz et al., 1970; Cuenod, 1974). Further studies in chicks have shown that deficits in learning both visual discrimination and one-trial passive avoidance tasks result from bilateral lesions of the hyperstriatal complex (Benowitz, 1972; Benowitz and Lee-Teng, 1973; Benowitz, 1974). That this region is required for visual learning has been further supported by studies of "split-brain" pigeons. Cuenod (1974), for example, has shown that unilateral Wulst lesions combined with section of the dorsal supraoptic commissure (DSO), shown by Meier (1971) to mediate interhemispheric transfer, result in deficits in visual discrimination learning. In contrast, Benowitz (1974) found unilateral lesions of the hyperstriatum with the DSO intact did not affect acquisition or retention of a one-trial passive avoidance task regardless of which eye was trained. However,

extinction of the avoidance appeared to be mediated only through the eye contralateral to the intact hemisphere, suggesting that avoidance conditioning and extinction are processed differently in the brain.

Experiments on birds to date have not dealt directly with memory storage in the intact brain. Generally, lesions have been made prior to training or else emphasis was placed on finding particular learning rather than memory deficits. In the present study, dealing directly with memory laterality in the intact animal, surgery was carried out only after sufficient time for normal consolidation of the training had occurred. The specific aim was to determine whether memory for monocular one-trial passive avoidance is normally laid down bilaterally or unilaterally in the forebrain of day-old chicks.

Materials and Methods

A total of 625 white leghorn cockerels obtained when one day old from Pacesetter Hatcheries, Cucamonga, California, were used in this study. Upon arrival in the morning, the chicks' heads were shaved with electric clippers and the lids of one eye were closed with a drop of collodion. Chicks were housed individually in one quart cardboard cartons in a room maintained at 31-33°C and 45% RH. No food or water was provided as chicks have adequate nutrients in the yolk sac for the first few days of life. Lights were on from 8:00 a.m. to 8:00 p.m.

In the afternoon of Day I, chicks were taught a one-trial passive avoidance task (Lee-Teng and Sherman, 1966). Each chick was given a single presentation of a shiny metal lure which had been dipped in methyl anthranilate (MeA), an aversive liquid. Chicks would typically orient to the lure, peck once or twice and subsequently shake their heads and turn away. Any chick which failed to

peck in 10 sec or failed to shake its head was discarded (about 10%).

At different time intervals ranging from 2-18 hr after training, surgery was performed (see Benowitz, 1974). The chicks were anesthetized with halothane and placed between the ear bars of a small animal stereotaxic holder. The skin atop the skull was opened and retracted and a small triangular bone flap was cut in the skull above the forebrain; the dura was cut and folded back with the bone flap. The dorsal portion of the forebrain (hyperstriatal complex and adjacent structures) was gently aspirated away and the removed tissue replaced with small pieces of Gelfoam. The bone flap was then folded back, covered with a small piece of Gelfilm and the skin sutured with 4-0 gut. Surgery typically took 5-10 min per chick; mortality was less than 5%, almost exclusively due to excess anesthesia.

Twenty-four hours after the training trial the chicks were tested with a lure resembling the training lure but not coated with MeA. Chicks failing to peck within 10 sec or showing avoidance after one peck (head shake, backing away) were scored as showing retention. A small number of chicks were not scored because of apparent illness. Chicks were mixed by shuffling the cartons; testing was essentially blind.

Following testing, approximately 15% of the chicks, selected at random from each group, were sacrificed for histology with an overdose of chloroform and perfused through the heart with chick Ringers and Bodian (ethanol, acetic acid, formalin) fixative. The brains were removed, embedded in paraffin and the forebrain sectioned at 15 μ . One-third of the sections were retained, stained with cresyl violet and projected onto standardized brain outlines (Benowitz, 1974) to determine the extent of ablation. Individual behavioral performance was then compared with lesion size to determine if any correlations were present.

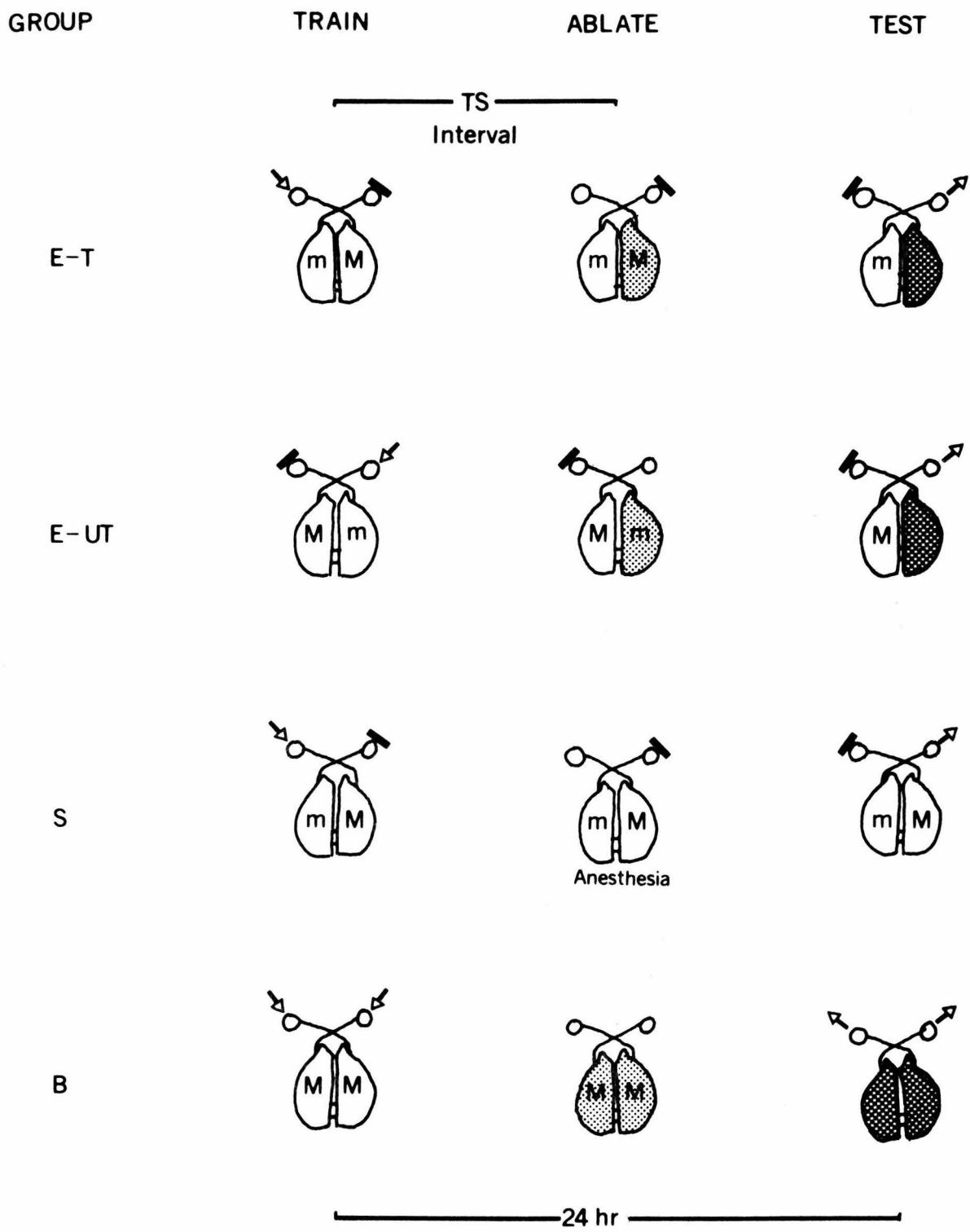


Fig. I-1. Experimental design: Inward arrows = training; outward arrows = testing; black bar = eye closure; M = memory in trained hemisphere; m = possible memory in untrained hemisphere; shading = lesions. See text for details.

Four time intervals were used between training and surgery (TS interval): 2-4 hr, 6-8, 12-14, 16-18. The intervals were arranged in 2-hr blocks to allow sufficient time for surgery on approximately 20 chicks (see Table I-1 for total numbers in each group). The chicks in each TS interval were divided into three groups. two experimental groups and a sham-operated group. The first experimental group (E-T) was trained monocularly and had the trained (contralateral) hemisphere lesioned. The second experimental group (E-UT) was trained monocularly and had the untrained hemisphere lesioned. Sham-operated chicks (S) were similarly monocularly trained, anesthetized for a corresponding length of time and had the skin above the skull opened and reclosed with collodion.

One-half hour before testing, groups E-T and S had the experienced eye closed with a drop of collodion and the naive eye opened by softening the collodion cap with acetone. This was done to allow for the testing of memory in the previously untrained hemisphere. Group E-UT, which had the same eye trained and tested, was handled similarly to equalize testing conditions; acetone was applied around the eye to be tested but no collodion was used. The procedure is summarized in Fig. I-1.

Two additional groups were used as controls. Bilateral lesions (B) were made following binocular training to confirm that the forebrain was required for retention of the task. A sham-operated group (S'), anesthetized for the corresponding longer period, was combined with this group. In addition, a group of 50 chicks was monocularly sham-trained with a dry lure to see if eye opening and closure caused any disruption of the normal pecking response. Half of the chicks were tested with the same eye and half with the other eye.

TABLE I-1

Raw Data for All Groups at Different TS Intervals

TS	Group	Total	#	%
		N	No Peck	Retention ^a
2-4	E-T	48	31	65
	E-UT	44	26	59
	S	31	22	71
	B	26	4	15
	S'	20	18	90
6-8	E-T	50	37	74
	E-UT	46	27	59
	S	29	22	76
	B	27	5	19
	S'	18	15	83
12-14	E-T	48	33	69
	E-UT	44	22	50
	S	31	26	81
	B	26	7	27
	S'	18	18	100
16-18	E-T	48	29	60
	E-UT	42	22	52
	S	33	18	55
	B	26	4	15
	S'	20	18	90
Pooled	E-T	194	130	67
	E-UT	176	97	55
	S	124	88	71
	B	105	20	19
	S'	76	69	91

^a# No Peck/Total N x 100

Results

Evidence for bilateral memory storage was found at all TS intervals (Fig. I-2). The TS intervals had been used in the expectation that different periods of intact brain activity would produce different strengths of memory traces as a result of decay of the memory laid down in the untrained hemisphere following surgery, but no consistent trends were observed. Data were therefore pooled and Chi square tests using Yates' correction for continuity were performed on the grouped data (see Table I-1 for raw data). In all cases, unilaterally lesioned chicks showed significantly greater retention than those with bilateral lesions (E-T vs. B: $\chi^2(1) = 60.78$, $p < .001$; E-UT vs. B: $\chi^2(1) = 33.73$, $p < .001$). The retention levels of group E-T were equal to those of monocularly-trained sham-operated chicks (S) ($\chi^2(1) = 0.38$, $p > 0.5$). However, there was a significant difference between E-UT and S ($\chi^2(1) = 7.08$, $p < .01$).

Bilateral lesions essentially abolished task retention (B vs. S': $\chi^2(1) = 87.94$, $p < .001$), confirming the results of Benowitz (1972). Binocularly-trained chicks (S') showed significantly higher retention levels than the corresponding monocularly-trained group (S) ($\chi^2(1) = 9.83$, $p < .01$), although eye opening and closure by itself had no effect on pecking performance.

Histological Analysis. Summary diagrams, shown in Fig. I-3, were constructed by superimposing all reconstructions of individual ablations. The median lesion contours were determined for all cases by discarding the cases in which the lesioned area exceeded $\pm 5/6$ of the area of the average lesion. The two contours drawn are thus a representation of the mean area of the lesion plus or minus one standard deviation. Bilateral ablations encompassed approximately twice the area of unilateral lesions. The area ablated included the hyperstriatal complex

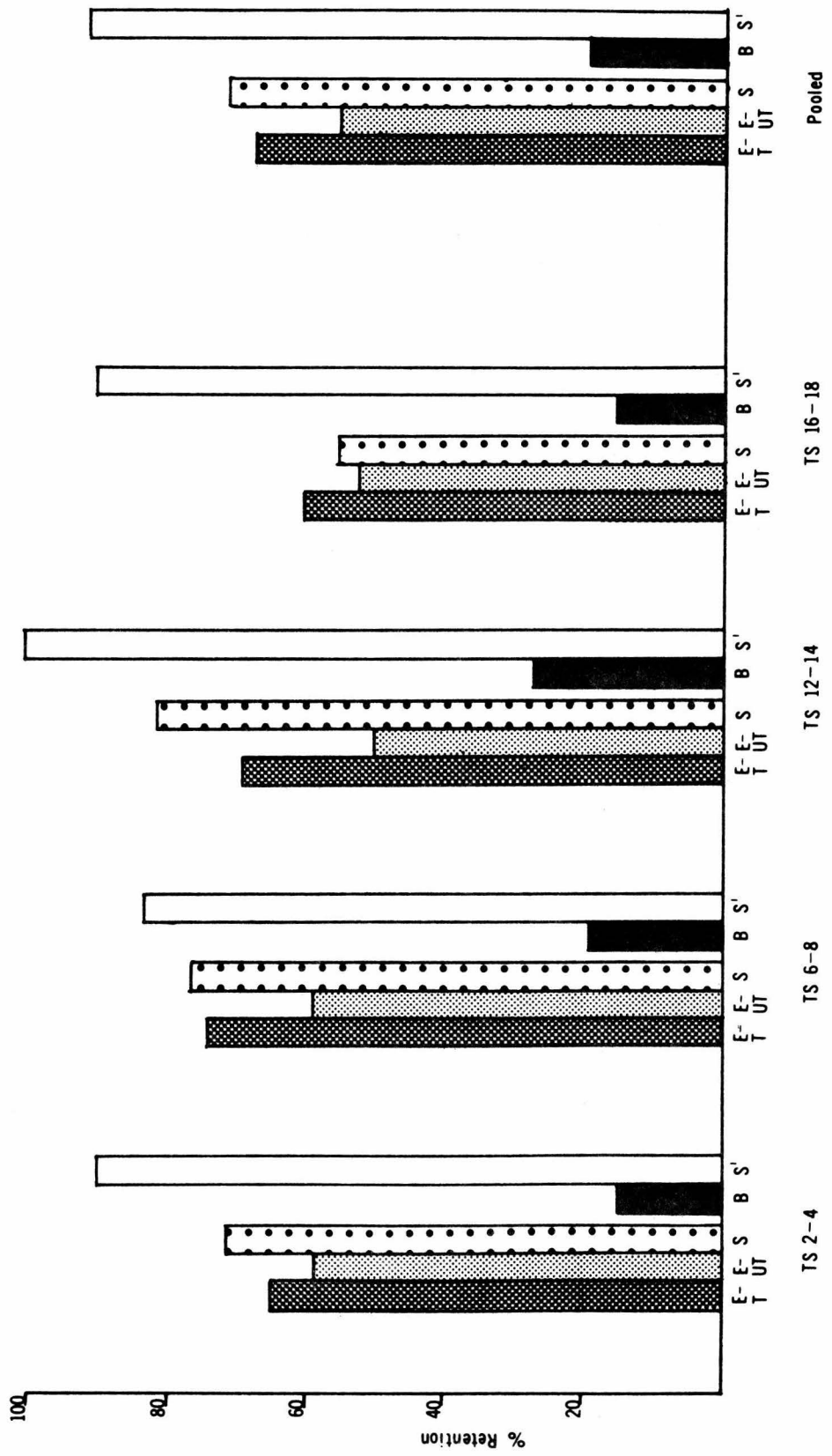
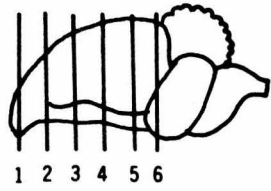
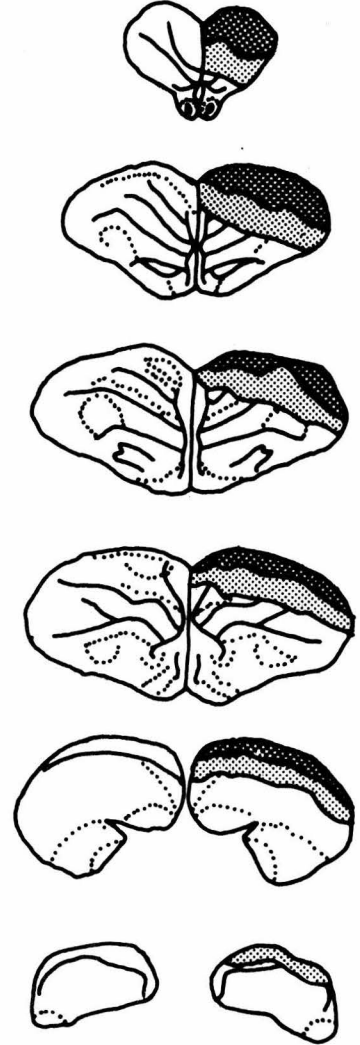
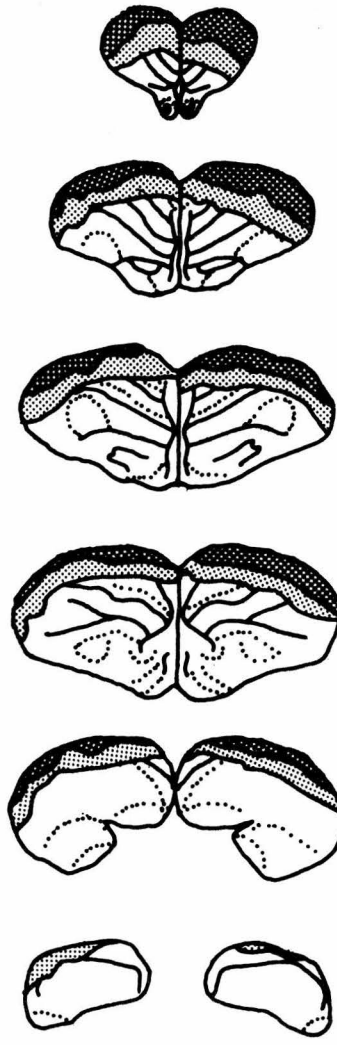
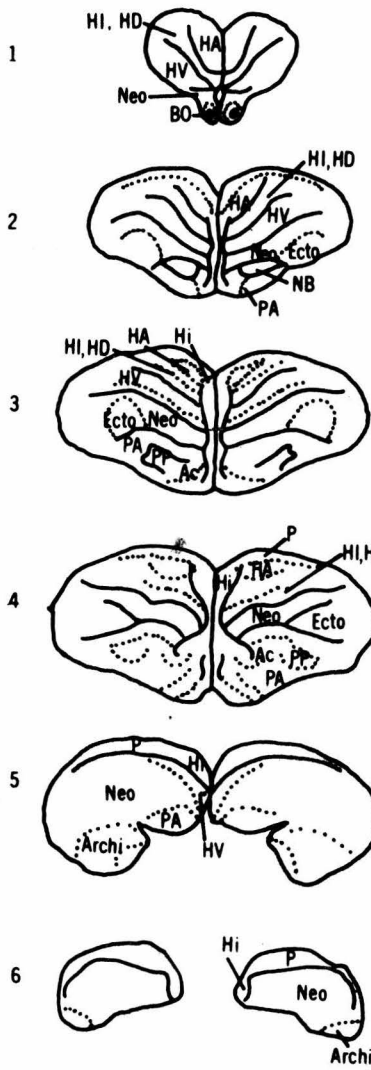


Fig. I-2. Retention levels at different TS intervals: Groups indicated below bars. Pooled data = sum of individual groups. % retention = # No Peck/Total N x 100.



BILATERAL
LESIONS

UNILATERAL
LESIONS



(a)

(b)

(c)

Fig. I-3. Ablation reconstructions a: Cross-sections through the four-day-old chick brain. Ac = nucleus accumbens; Archi = archistriatum; BO = bulbus olfactorus; Ecto = ectostriatum; Hi = hippocampus; HA = hyperstriatum accesorium; HD = hyperstriatum dorsale; HI = hyperstriatum intercalatus; HV = hyperstriatum ventrale; NB = nucleus basalis; Neo = neostriatum; P = para-hippocampal area; PA = paleostriatum augmentatum; PP = paleostriatum primitivum. b and c: Mean extent of surgery (dark shading) \pm S.D. for bilaterally- and unilaterally-lesioned chicks.

(h. accessorium, h. dorsale, h. intercalatus, h. ventrale), hippocampus, parahippocampal area, and portions of the frontal neostriatum, with a varying degree of damage to the more caudal neostriatum. No apparent correlation between the extent of surgery and retention was found within groups.

Discussion

The results give clear evidence that monocularly-acquired memory for passive avoidance is laid down bilaterally in the intact chick brain. Interocular transfer of the task occurred in all groups, as judged by retention levels following forebrain lesions in either hemisphere. Tapping of a unilateral memory is ruled out by the high retention levels exhibited in chicks which had the trained hemisphere lesioned. The result is not an artifact produced by decreased pecking after surgery; chicks which had the untrained hemisphere lesioned showed, if anything, an increased propensity for pecking compared with those with the trained hemisphere lesioned. The lower retention levels of chicks with lesions in the untrained hemisphere are puzzling, and no explanation is apparent. However, despite the small difference in retention levels between the two unilaterally-lesioned groups, both showed near-normal memory for the task. A role of the forebrain in memory storage for this task is indicated by the lack of retention in bilaterally-lesioned chicks.

The forebrain in one-trial passive avoidance appears to be required either for storage or retrieval of the task. It is not possible on the basis of the present evidence to distinguish between its possible role as a direct storage area and that of a relay in the retrieval system. The data also do not rule out the unlikely possibility that memories are stored in subcortical regions which are depressed by bilateral but not unilateral forebrain lesions. The present findings

argue against the notion that deficits following bilateral lesions of the hyperstriatum are a result of deficits in learning ability per se. In the present experiment, the chicks were given the opportunity to learn normally and had ample time in which to consolidate memories (Lee-Teng, 1970; Benowitz and Magnus, 1973). The present findings are consistent with reports of bilateral memories found in mammals after unilateral training followed by section of the corpus callosum (Ebner and Myers, 1962; Butler, 1968; Hamilton, 1977) and with the report (Horn et al., 1973) that monocularly-induced imprinting in chicks results in equivalent biochemical changes in both halves of the forebrain.

Further investigations are necessary to determine whether other types of learning in the chick are also stored bilaterally. Benowitz (1974) has suggested that different kinds of learning may involve anatomically distinct systems which may or may not have commissural pathways for bilateral memory. This notion appears to find support in studies of transfer of different types of learning in fish (McCleary, 1960; Lee-Teng, 1966b). It is also suggested (Benowitz, 1974) that different motivational systems (hippocampus vs. amygdala) may mediate these differences. The technique employed in the present experiment can be used to further elucidate storage mechanisms, particularly in cases where failure of interhemispheric transfer has been reported.

CHAPTER II

Further Evidence for Bilateral Memory Processing in Chicks^{1,2}

Cycloheximide (CXM), a potent protein synthesis inhibitor, has been implicated extensively to be an effective disrupter of memory consolidation processes. Because of the profound effects of CXM and other antibiotics on brain protein synthesis, it has frequently been suggested that protein synthesis is critical for the establishment of long-term memory traces in the brain (Agranoff, 1967; Barondes and Cohen, 1968; Roberts and Flexner, 1969; Squire and Barondes, 1973; Barraco and Stettner, 1976; Quartermain, 1976). In general, CXM and closely-related acetoxy-CXM have been administered bilaterally via intracranial (Barondes and Cohen, 1967; Daniels, 1971; Mark and Watts, 1971; Quartermain, 1976) or subcutaneous (Barondes and Cohen, 1968; Squire and Barondes, 1973) injections. Recently, Bell and Gibbs (1977) used unilateral intracranial injection of CXM in chicks and concluded from their results that memories for monocular one-trial passive avoidance learning are apparently unilateral. This conclusion is in direct conflict with the results of the author's study of unilateral hyperstriatal lesions from which it was inferred that memory storage for the same task in chicks is normally bilateral (Greif, 1976).

Bell and Gibbs (1977) contended that by delaying drug treatment until 2 min after training, normal consolidation was allowed to occur and that the observed restriction of memory traces to the trained hemisphere under these

¹I thank K. Gaston for valuable discussion, J. Macenka for histology and Dr. R. W. Sperry for helpful criticism of the manuscript. Supported by NIH Grant GM 00086 and USPHS Grant MH 03372.

²A revised version of this chapter has been submitted as a short communication to Brain Research.

conditions reflected the normal tendency for engrams to be established unilaterally after monocular training. On the other hand, in our own experiments (Greif, 1976) surgery was delayed a minimum of 2 hr after training and the engrams were found to be bilateral. We wished to further investigate the discrepancies between the unilateral lesion and unilateral cycloheximide results by examining the effects of unilateral lesions made 2 min after training. If retention deficits were found, then one might conclude that a 2-min interval was insufficient to allow for complete transfer of visual information necessary for the establishment of bilateral engrams. Alternatively, if no deficits were observed, it would be necessary to consider what basic differences might exist between lesion and drug treatment.

A total of 76 one-day-old chicks, obtained from a local hatchery, were used, divided into unilaterally lesioned and sham-operated groups. Chicks were individually housed in 1 quart cylindrical cardboard cartons in a room maintained at about 33°C and 40% R.H. Room lights were on from 8:00 a.m. to 8:00 p.m. Chicks were given a small amount of water upon arrival to reduce an observed hyperactivity in some chicks. No further water or food was given, as chicks have sufficient nutrients in the yolk sac for the first few days of life. One eye was closed prior to training by sealing the lids with a drop of collodion.

Chicks were trained a one-trial passive avoidance task (Lee-Teng and Sherman, 1966) on the afternoon of the day of arrival. Chicks were given a single exposure to a metal lure coated with methyl anthranilate, an aversive liquid to chicks. Those which pecked at the lure gave a characteristic disgust response of head-shaking and distress calls. Chicks which failed to peck within 10 sec or to exhibit the disgust response were discarded (less than 10%).

Chicks were anesthetized with Halothane (Fluothane) beginning 1 min 50 sec after the head-shake in the training trial. Actual surgery began as soon as the anesthesia took effect, at almost exactly 2 min after training. The skin was opened above the forebrain and a bone flap was rapidly cut in the skull. Large hyperstriatal lesions were made by aspiration; surgery to this point usually took less than 1 min. The removed tissue was replaced by small pieces of Gelfoam to control bleeding, the bone flap closed and the skin sutured shut. Sham-operated chicks were anesthetized, had the skin above the forebrain opened and closed with sutures. Recovery from surgery was rapid; mortality was less than 5%, exclusively due to excess anesthesia.

Testing occurred 24 hr after training. All chicks were tested for interocular transfer. The untrained eye was opened by softening the collodion cap with acetone, and the trained eye closed with a drop of collodion, 30 min before testing. No anesthesia was used for this procedure. Chicks were pseudorandomly mixed by scrambling the housing cartons before testing. Each chick was presented with a lure resembling the training lure but not coated with methyl anthranilate. Chicks which failed to peck in 10 sec were scored as having retention for the task. Occasionally a chick would peck at the lure and give an immediate strong disgust response (whether the lure was actually contacted or not). These chicks were also scored as showing retention. Following testing, one-fourth of the lesioned chicks were killed for histology.

Results are shown in Fig. II-1. Lesioned and sham-operated chicks showed equally good interocular transfer following surgery delayed 2 min after training indicating that sufficient time was allowed for transfer of information. The slightly lower retention levels as compared with those previously reported (Greif, 1976) may be due to a depressive effect of Halothane anesthesia, which

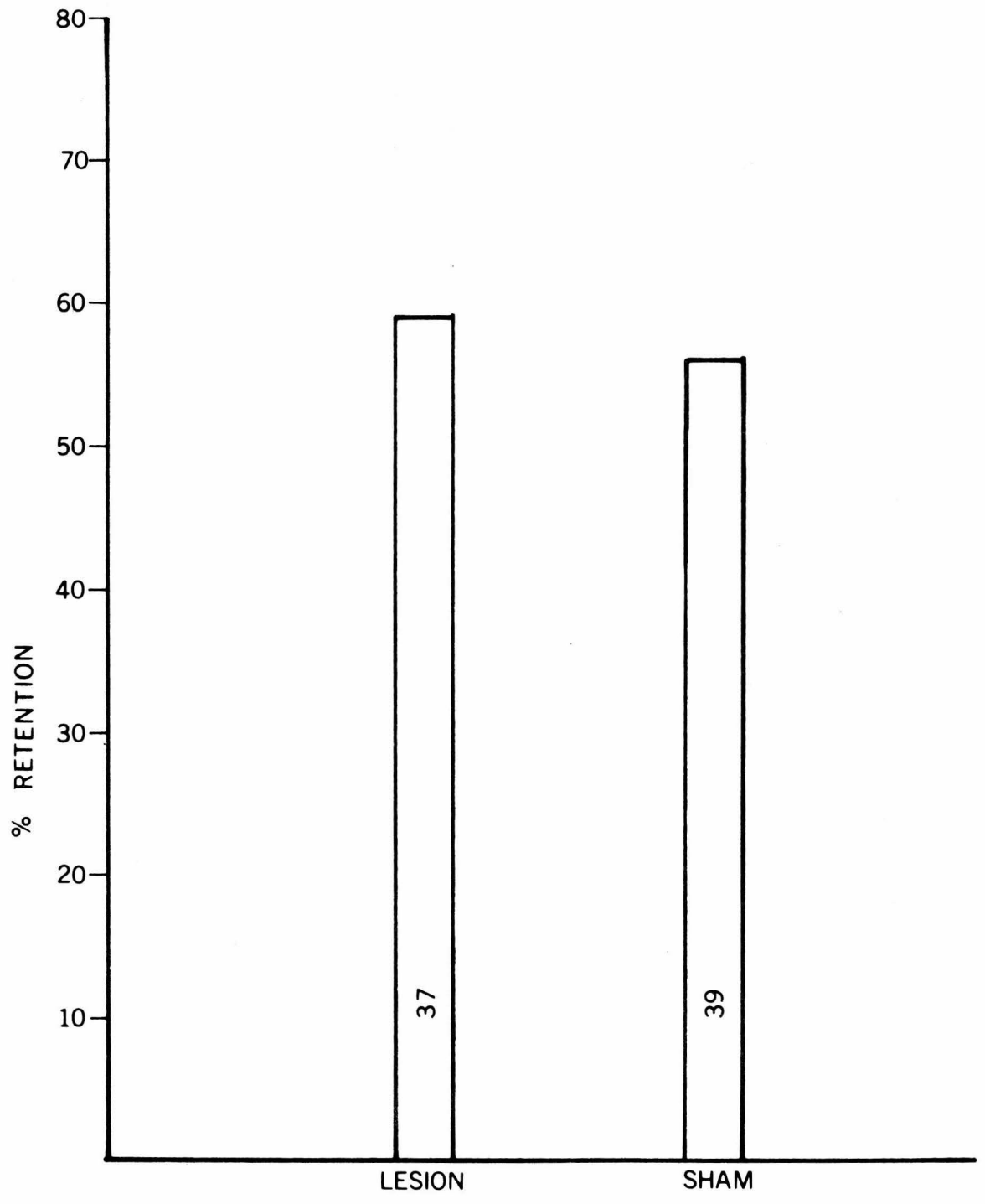


Fig. II-1. Retention levels after unilateral forebrain lesions and sham operations. Number within bars is the number of chicks per group. % retention = (# no peck/total N) x 100.

has been reported to interfere with memory consolidation when administered shortly after training (Cherkin and Lee-Teng, 1965). The results are in agreement with our earlier study (Greif, 1976) in which bilateral memory for one-trial passive avoidance was found and which confirmed that bilateral hyperstriatal lesions abolished task retention (Benowitz, 1974). Ablation reconstructions confirmed that lesions were comparable to those of our earlier study.

The finding of long-term engrams in the untrained hemisphere after lesions of the trained hemisphere 2 min after training is in marked contrast to the absence of such memory traces after CXM treatment with a similar delay. One is left to consider what basic differences might exist between lesion and drug treatments. It is generally conceded that large brain lesions produce effects beyond the direct removal of brain tissue (Isaacson, 1976). Such secondary effects include the overall depression of brain function and secondary damage as the result of interruption of normal brain circulation. Hence, it is rare to observe postsurgical performance levels equivalent to presurgical levels until considerable time is permitted, even when the lesions apparently do not cause profound deficits initially. Similarly, cycloheximide is known to cause wide-ranging changes in brain biochemistry, involving the disruption of all brain protein synthesis, including that of transmitters, inhibitors, and hormones, as well as of new proteins (Flexner, Serota and Goodman, 1973; Barraco and Stettner, 1976; Quartermain, 1976). Protein synthesis inhibition by cycloheximide is transient, having a time course of hours instead of days, as is often the case with lesions. However, one might argue that at the time of peak protein synthesis inhibition, the degree of brain disruption is more profound, involving gross reduction in nearly all biosynthetic processes.

The results of the present experiment, when compared to those of Bell and Gibbs (1977), suggest that CXM treatment somehow prevents the establishment of normally bilateral engrams. Alternatively, it has been suggested that CXM may cause deficits in retrieval, and that memory consolidation may be independent of protein synthesis (Quartermain, 1976). It is possible to argue that the drug interferes sufficiently with overall brain function as to disrupt the integration of visual information which has already been transferred across the commissures to the untrained hemisphere. As Bell and Gibbs (1977) did not measure relative levels of protein synthesis in the two hemispheres, it is possible that sufficient diffusion of the drug to the untrained hemisphere occurred to prevent the establishment of the normal secondary memory trace. A secondary memory trace might be more labile and hence susceptible to levels of protein synthesis inhibition lower than those generally held to be required for amnesic effects (Agranoff, 1967; Roberts and Flexner, 1969; Barraco and Stettner, 1976). In the control experiments in which the trained hemisphere was the "untreated" one, a primary memory trace would be established, subject to the usual rules pertaining to levels of protein synthesis inhibition required. Based on the Bell and Gibbs result (1977), it is not possible to rule out that a secondary trace would be established in the treated hemisphere once the CXM had worn off.

The results of this experimental comparison point out the problems plaguing current research in memory processing. It is exceedingly difficult to determine the primary effect of amnesic agents, particularly those of drugs. It is this difficulty which has resulted in the current controversy as to the amnesic mechanisms of antibiotics (Quartermain, 1976). It now appears that the results of drug experiments are also incompatible with the results of lesion studies, suggesting that even more caution will be required in the future.

CHAPTER III

Intraretinal Transfer of a Color Discrimination Task after
Tectal and Forebrain Lesions in Goldfish^{1,2}

Since early studies by Lashley (1929), investigators have sought to determine whether brain regions which are specific for memory processing exist. Many studies focusing on the effects of lesions of identified brain structures on postsurgical acquisition or on the retention of tasks have had difficulty separating sensorimotor deficits from those directly reflecting memory losses. We have used a technique which partially overcomes the problem by selectively creating sensory deficits which should not affect performance.

In the goldfish, the optic tectum is the primary processing center for vision, which maps the visual field of the contralateral eye in an orderly topographic manner (see Meyer and Sperry, 1976, for review). Thus, if visual stimuli are presented to only a portion of the visual field, a discrete portion of tectum receives direct visual input from the retina. The generalization of visual information from one part of the visual field to the remainder is clearly beneficial for adaptive behavior. Without such intraretinal transfer, an animal would require multiple exposures to similar stimuli in order to establish a generalized association. In spite of its apparent adaptive value, intraretinal transfer does not always seem to occur. Transfer has been reported in goldfish (Cronly-Dillon, Sutherland

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²This work was done in collaboration with Dr. Margaret Y. Scott. It will be submitted for publication under the joint authorship of K. F. Greif and M. Y. Scott.

and Wolfe, 1966; Scott, 1977) and in octopus (Muntz, 1963). Limited transfer has been reported in monkeys (Ganz and Wilson, 1967) and the complete failure of transfer from the lower to upper hemiretina in pigeons (Levine, 1952) has been observed.

Our interest in intraretinal transfer is focused on its implications concerning the localization of memory. We were interested in determining whether the retinotectal system contributed to memory storage of visual discrimination tasks. The apparent generalization of learning across the visual field in goldfish may be due to the tapping of memory stored only in the directly trained portion of tectum, or in a localized extratectal association area. Alternatively, memory for a learning experience may be established diffusely across the entire tectum. A primary problem with most studies to date is that investigators have tried to locate storage or processing areas after unrestricted training procedures. Thus, it has been difficult to reliably determine the specific roles of structures under study.

We have reasoned that it may be easier to determine whether the capacity for localized storage exists by limiting direct training to relatively identifiable portions of the optic tectum and examining retention and generalization of the tasks after lesions of trained brain regions. A behavioral training technique has been developed which permits the restriction of stimuli to highly specified loci in the visual field of goldfish (Scott, 1975; Scott, 1977). Using this technique, we have examined the role of the optic tectum in memory storage of a color discrimination task, using conditioned respiratory suppression as the behavioral measure. If storage occurs only in the trained region of tectum, or in a closely associated extratectal area, lesions should abolish task performance in the remainder of the visual field. Preliminary results have been reported (Greif and

Scott, 1977; Scott, Greif and Sperry, 1977).

A possible extratectal site for memory processing in goldfish is the forebrain, a telencephalic structure. The forebrain has been implicated to be involved in avoidance learning, with lesions producing deficits in acquisition and postsurgical performance (Hainsworth, 1967; Dewsbury and Bernstein, 1969; Savage, 1969). It has also been suggested that the forebrain is critical for color vision (Bernstein, 1961a, 1961b, 1962). Fish trained to perform a color discrimination reportedly discriminate on the basis of brightness after lesion of the forebrain. However, color discrimination appears to recover after relatively short intervals after surgery (Bernstein, 1962). We have also examined the effects of forebrain lesions on memory for color discrimination and of forebrain interactions with tectal structures in the mediation of intraretinal transfer.

Methods - Experiment I

Experiment I was designed to investigate the intraretinal transfer of a classically conditioned color discrimination task after lesions of trained tectal regions. Twelve goldfish (Carassius auratus), approximately 6-9 cm in length from snout to base of tail, were used, housed in glass aquaria under a 12 hr light-dark cycle throughout the course of the experiment. Fish were trained a red-green color discrimination using conditioned respiratory suppression as the behavioral measure (Scott, 1977). Fish had the extraocular muscles of one eye cut, were immobilized in an elastic sling and placed in the training apparatus, a small aquarium with a water-filled hemisphere mounted against one wall. The optic disc was visualized via transillumination and centered at 0° of a perimeter mounted around the hemisphere, which had been sandblasted on the interior to reduce reflected images. If necessary, readjustment of the eye during the

course of training occurred. The training lights, equated for brightness, were projected onto the hemisphere by a fiber-optic source mounted on the perimeter. Stimuli subtended 1.5° visual angle. Respiration was monitored through external electrodes placed in front of the mouth and behind the gills contralateral to the exposed eye. Resistance changes were amplified and recorded on a paper polygraph (Beckman).

Six fish were trained at -60° along the horizontal meridian (posterior visual field) and six at $+60^\circ$ along the vertical meridian (superior visual field). Before training, each fish was anesthetized with 0.05% tricaine methanesulfonate (Finquel) solution and had the extraocular muscles of one eye teased apart with jeweler's forceps. A 30 min period of adaptation was allowed, after which the fish was habituated to the two stimuli by approximately ten presentations of each color without shock. Most fish responded once or twice and several did not respond at all. The fish was then given five consecutive simultaneous pairings of shock across the tail with the red light (CS+), with 15 min rest periods between shocks. Exposure to the green light (CS-) was intermittent. Following the block of five shocked trials, the fish was given a second 30 min rest period, after which shocked trials were interspersed with unshocked trials. Subsequent training sessions consisted of mixed shocked and unshocked trials. No more than ten shocks were given in a single session, which normally took about 4 hr. Fish required on the average three training sessions spaced four days apart to establish a clearly differentiated response (in general, greater than 90% response to CS+ and less than 20% response to CS-, over two blocks of at least ten presentations of each color without a shocked trial). Three fish were discarded because of failure to learn the discrimination; two others died during the training period.

A minimum of three days after the final training session, fish were

anesthetized with Finquel and the tectum exposed through a reflected bone flap. The caudal half-tectum, in fish trained along the horizontal meridian, or the medial half-tectum, in those trained along the vertical meridian, was removed by gentle aspiration after having been separated from the remainder of the tectum by an incision reaching down to the ventricle. The size of the lesion was varied to determine whether any correlation existed between lesion size and retention. The bone flap was wedged into place and the fish revived by infusion of water through the gills. Fish recovered rapidly with no signs of motor deficit.

On the day following surgery, fish were reanesthetized and any regenerated extraocular muscles were cut. A 30 min adaptation period was allowed before testing for generalization of the discrimination across the remainder of the visual field. Testing began at $+60^\circ$ along the equator or at -60° along the vertical meridian in the corresponding groups, with 3-5 presentations of each color at each test position. Test sites were selected alternately in the anterior and posterior* (superior/inferior) fields at 20° increments except near the border of the scotoma, where 10° steps were used to map out the extent of the lesion. After the fish had been tested once in all positions, another 30 min rest period was allowed before the fish was retested at the same loci.

Results

The results of Experiment I are shown in Fig. III-1. Good generalization of the task was observed across the remaining portion of the visual field of the trained eye in both groups. Differential response scores were obtained by subtracting the percent response to the green light (CS-) from that of the red light (CS+) and averaging these values over all fish within a group. Absolute ranges for individual fish are shown. Individual data for loci at the margin of the scotoma

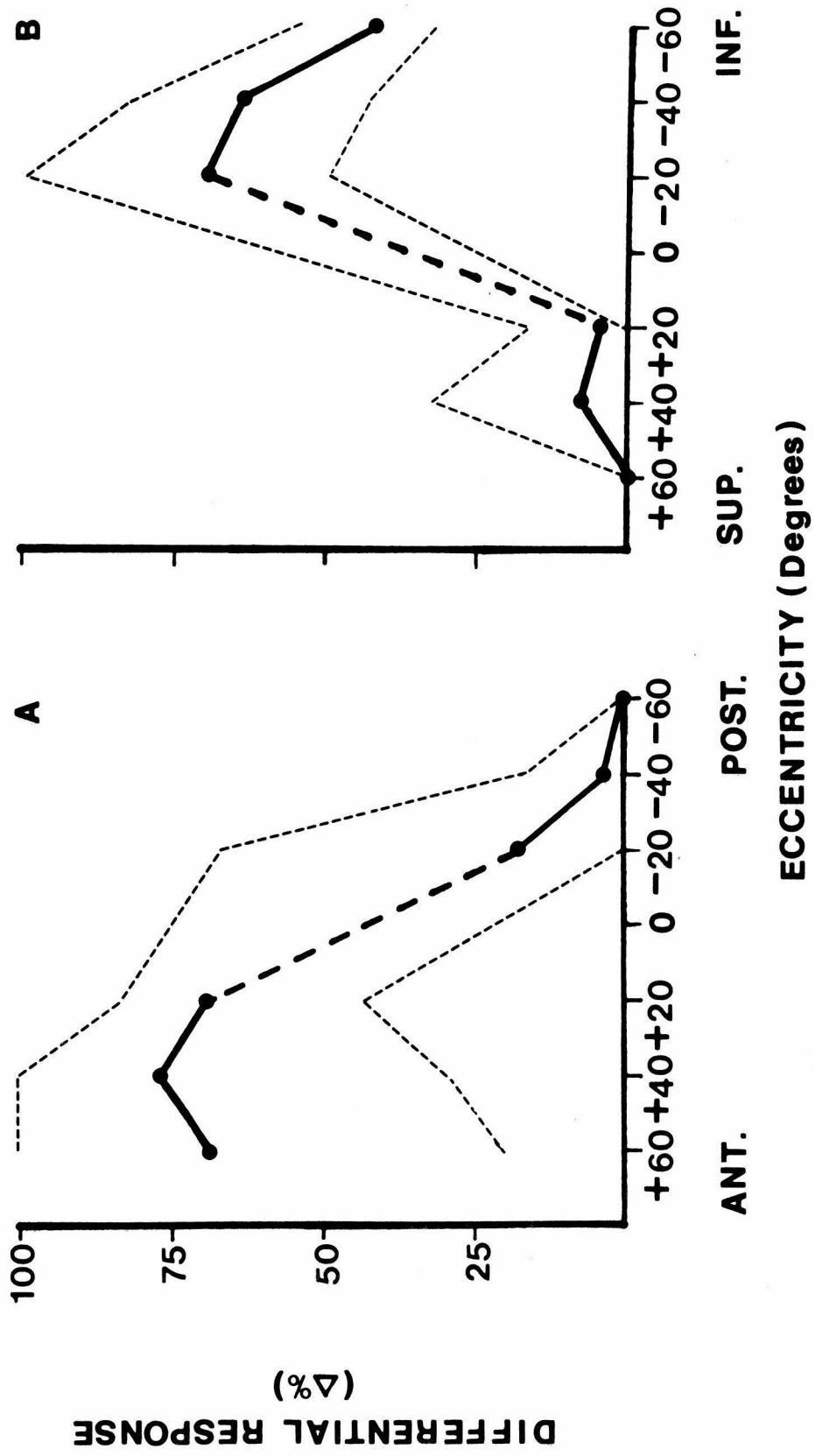


Fig. III-1. Intraretinal generalization after lesions of trained tectal regions. Eccentricity measured in degrees from the optic disc. Ant. = anterior visual field, Post. = posterior visual field, Sup. = superior visual field, Inf. = inferior visual field. Differential response derived by subtracting % response CS- from % response CS+, averaged across fish. Dotted lines indicate absolute ranges for all fish. A. Response in fish trained at -60° along the horizontal meridian, N=6. B. Response in fish trained at $+60^\circ$ along the vertical meridian, N=6.

were discarded to eliminate bias in averaged scores.

Fish trained along the horizontal meridian showed consistently high differentiation throughout the remainder of the visual field. Slightly lower differentiation overall was observed in fish trained along the vertical meridian, with a marked decline in the extreme inferior visual field. Both groups showed significant differentiation outside the region of scotoma, in which virtually no responses were recorded. One fish in the horizontal group failed to respond during testing; this fish died one day after testing. One fish in the vertical meridian group made a large number of responses to CS- as well as to CS+; retesting five days later gave identical results.

No trends were observed relating performance on the final training day and that on test day. There were no significant correlations between the speed of learning and final performance, or between lesion size and retention.

Discussion

Using a classically conditioned red-green color discrimination, goldfish showed excellent generalization across the visual field of task which had been learned in only a small portion of the field. The transfer occurred despite the lesion of the portion of tectum receiving direct visual input. The generalization appeared to be better along the horizontal meridian than along the vertical meridian. One fish in the latter group lost the differential response entirely; this probably marks an extreme case in the variability in response level. The overall differences in the response level may indicate a natural preference for viewing objects along the horizontal axis of the visual field. Secondary damage may have occurred during lesion of the medial tectum as a result of greater bleeding with this type of surgery. There has been a report (Marc and Sperling, 1976)

of decreased cone density in the extreme superior fringes of goldfish retina; the decrement in response in the extreme inferior visual field may be the result of decreased color vision in this portion of the field. One fish in the horizontal meridian group apparently failed to generalize at all. However, as this fish died on the day following testing, it is likely that illness produced the response depression.

The results indicate that memory storage for this task must lie outside of the region of tectum which receives direct visual input during training. To determine if storage might occur in the forebrain, Experiment II was initiated.

Methods - Experiment II

Experiment II was designed to determine whether the forebrain played a role in storage or processing of the color discrimination task. Five fish were trained and tested similarly to those in Experiment I. Surgery was performed through a bone flap cut in the skull above the forebrain. Large bilateral lesions were made by separating pieces of tissue with a sharpened tungsten wire and removing them by gentle suction. Care was taken to avoid damage to underlying fiber tracks and thalamic regions. Fish were revived as previously described and exhibited transient motor deficits, which disappeared within 24 hr.

Fish were tested as in Experiment I. Two fish were subsequently tested for performance of the task as a brightness discrimination. Neutral density filters (Wratten No. 96, 0.30 N.D.) were inserted to reduce the brightness of either the positive or neutral stimulus by 50% with each filter. Filters were inserted until fish ceased to respond or showed reversal of response.

Following testing, fish were killed with an overdose of anesthesia and the brains removed for histological section. Brains were fixed in Bodian fixative

(alcohol, acetic acid, formalin), imbedded in paraffin and sectioned in the sagittal plane.

Results

Results are shown in Fig. III-2. Fish showed consistent transfer across the entire visual field of the trained eye. No deficits were observed as a result of forebrain lesions, although respiration tended to be somewhat more erratic than in tectal lesioned fish. The variability of individual responses at single loci was quite high.

The two fish tested for brightness discrimination gave no evidence for a shift away from color discrimination. Responses to CS+ weakened with reduction of brightness and generally ceased after brightness was reduced to 12.5% but no increase in response to CS- was observed. Addition of neutral density filters to the CS- did not affect responses.

Ablation reconstructions are shown in Fig. III-3. Approximately 60% of forebrain tissue was ablated. Minor damage to underlying structures was limited to distortions probably produced by excessive bleeding.

Discussion

The results of Experiment II indicate that the forebrain does not play a major role in memory processing for this task. Retention is excellent after large telencephalic lesions and performance is at near normal levels. The variability observed in forebrain lesioned fish is likely the result of unavoidable secondary damage to thalamic structures as a result of the considerable bleeding which occurs during the surgery. Such excessive bleeding could produce damage both by interruption of blood circulation and by pressure produced by external

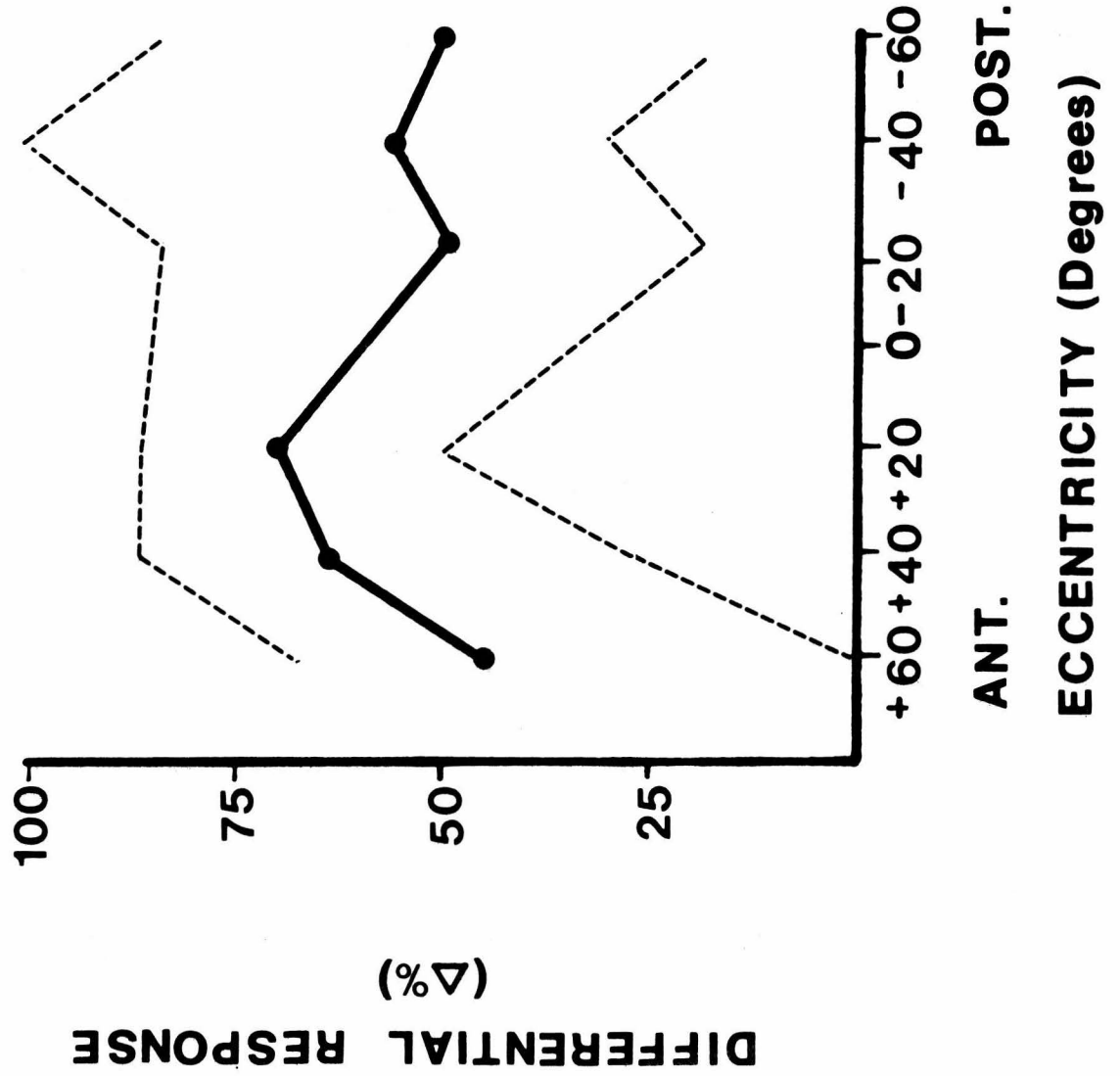


Fig. III-2. Intraretinal generalization after bilateral forebrain lesions. Conventions same as in Fig. III-1. Fish trained at -60° along the horizontal meridian, N=5.

LEFT

RIGHT

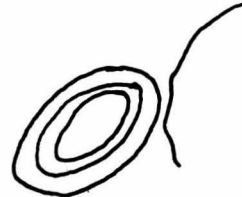
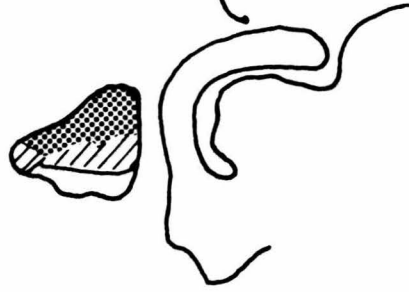
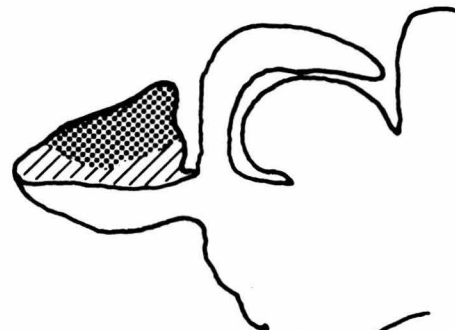
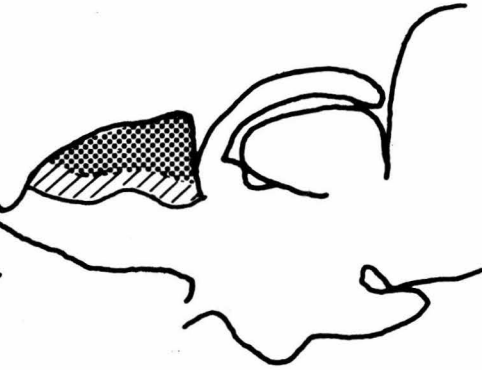
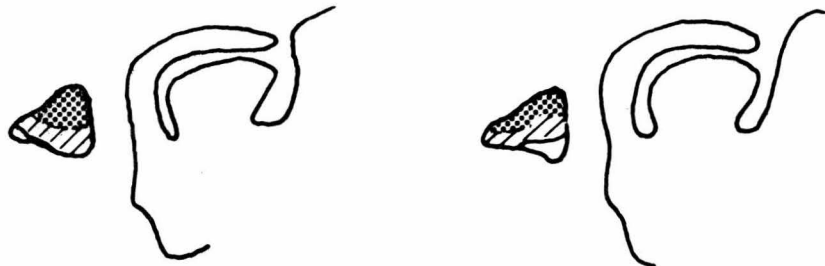
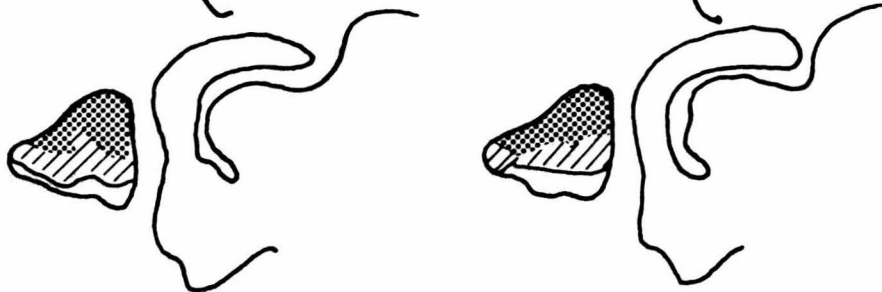
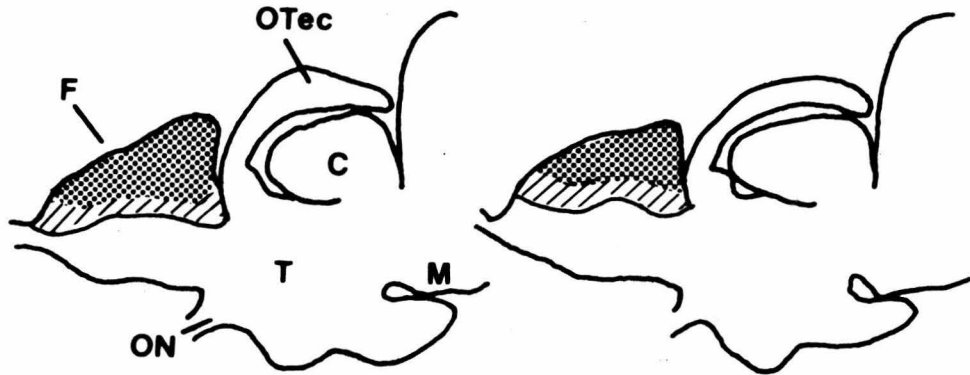


Fig. III-3. Ablation reconstructions (sagittal sections) of five forebrain-lesioned fish. Dark shading = tissue removed in all fish. Diagonal lines = area removed in at least 3/5 of all fish. Abbreviations: C = cerebellum, F = forebrain, ON = optic nerve, OTec = optic tectum, M = midbrain, T = thalamus.

accumulation of clotted blood. That at least minor damage occurs is evidenced by the transient motor difficulties observed immediately after surgery. Motor deficits have not been observed after partial tectal ablation.

No evidence of a reduction in color discrimination after forebrain lesions (Bernstein, 1961a, 1961b, 1962) was observed. Bernstein (1962) noted that the effect was transient with recovery complete in 4 hr after surgery, suggesting that initial reports may have been the result of depression of response or inadequate controls.

It appears that the forebrain does not play the major role in retention that the analogous structure performs in birds. Considerable evidence in birds (Benowitz, 1974; Greif, 1976; Cuenod, 1974) points to the importance of the forebrain as either a retrieval relay or memory storage center. The forebrain in birds makes up a considerably larger portion of total brain tissue than does the forebrain in goldfish. It may be that this size difference is an indication of relative unimportance of the structure in goldfish.

That consistent discrimination across the remainder of the visual field in goldfish was found is in accord with previous findings in fish (Cronly-Dillon et al., 1966) and octopus (Muntz, 1963). Most investigators have been unable to limit stimulus presentation to a small portion of the visual field; seeking to prevent stimulus overlap by presenting stimuli above vs. below, or in front vs. behind the animal. In addition, as animals had unlimited eye movements, a great deal of overlap was possible between trained and untrained half-fields. Cronly-Dillon et al. (1966) approached the problem of stimulus localization by cutting one of the two optic brachia projecting from retina to tectum in goldfish before exposure to the training patterns, thereby creating a scotoma in half of the visual field. After training, the cut brachium was allowed to regenerate and its mate

cut before transfer of the discrimination task was tested in the previously blind hemi-field. Excellent intraretinal transfer was observed. However, as the visual projections of the two brachia overlap, and the rate of optic brachium regeneration is variable, it is likely that inadvertent training of at least part of the "blind" half-field occurred. In the single highly controlled experiment, using juvenile monkeys, Ganz and Wilson (1967) maintained the localization of stimuli by projecting them onto the retina via a contact lens with an attached mirror. Intraretinal transfer limited to 2-5° from the training site was found, suggesting that less rigid stimulus localization might produce spurious results. Localization of stimuli close to the fovea apparently limited generalization in the periphery. The difference in results after stimulus localization in goldfish is likely due to basic differences in the organization of the visual system; it is not surprising that stimuli are "viewed" differently in the peripheral regions of retina and in the fovea, because of greatly reduced acuity in the periphery.

Reports of failure of intraretinal transfer in pigeons (Levine, 1952) have been criticized because of the questionable value of the task employed. In these experiments, pigeons were trained using a modified Lashley jumping stand, which requires the birds to leap off a rotating stand to one of two platforms bearing the discriminanda, located either in the superior or inferior visual field. Catania (1964) has criticized the paradigm as inappropriate for birds on the grounds that it requires the birds to view the stimuli in more lateral portions of the visual field in order to compensate for myopia in more central regions. Conflicting proprioceptive cues would be developed because of head cocking, possibly interfering with stimulus recognition.

The findings of the present study indicate that memory storage is not limited to the portion of the tectum which receives direct visual input during

training, nor does the forebrain play a major role. Muntz (1963) found in octopus that knife cuts across the optic lobe before training could prevent transfer of learning to the untrained portions of the visual field; he proposed that localized training results in the establishment of an engram across the entire lobe. The present study also suggests that diffuse memory is produced across the entire tectum after highly localized training, but does not rule out storage in an extratectal structure. It may be possible to argue that the optic tectum acts only as a relay area and that a discrete memory is laid down in an unidentified area. However, if this is the case, one must argue that in spite of the absence of the primary relay, the remaining tectum manages to retain access to this discrete area. A possibility which cannot be eliminated is that extratectal visual projections are critical for memory processing. While visual projection areas have been identified in the thalamus and midbrain (Sharma, 1972; Finger and Karten, 1977), the roles of such areas have not been determined.

The finding of apparent generalized memory for color discrimination is in accord with the failure to find discrete storage in earlier studies. The present study, in which no localization of memory in tectum or forebrain was found after highly localized training supports the general concept that memory is not stored in discrete sites but rather is the result of more global changes in the brain (see McGaugh and Gold, 1974; Barraco and Stettner, 1976, for review).

The results of this study also have implications for further study on the mechanism of memory processing in goldfish. Since the posterior visual field lies outside the region of binocular overlap in goldfish, and there is limited direct communication between caudal portions of the tectum, it becomes essential to study the interocular transfer of the color discrimination task after lesions of trained caudal tectal regions. Such studies have been carried out (Chapter IV).

Excellent interocular transfer to the entire visual field of the untrained eye was found after lesion of trained tectal regions. In addition, good transfer was observed after removal of the entire trained tectum. These results suggest that binocular interaction is not required for interocular transfer, and that memory storage for the task must either occur bilaterally in the tectum or in extratectal regions.

CHAPTER IV

Interocular Transfer of Color Discrimination after Tectal
Lesions in Goldfish^{1,2}

Interocular transfer, the generalization of monocular learning from the trained to untrained eye, has the potential for providing insight into the mechanisms of learning and memory. By analysis of patterns of transfer, it may be possible to determine the functional roles of structures involved in the transfer of information and to determine whether memory storage involves one or both halves of the brain. Unfortunately, there is frequently disagreement concerning interocular transfer due to variations in procedure and interpretation of results. Such is the case in teleost fishes.

Interocular transfer in teleosts has been studied since Sperry and Clark (1949) reported partial transfer of active discrimination of lures which differed in color, size, and position, for food reward. While transfer of learning occurred, it was by no means complete or instantaneous. Color discrimination has in general been reported to show transfer in goldfish (McCleary, 1960; Ingle, 1965; Ingle and Campbell, 1977). Interocular transfer of simple patterns using both active avoidance and cardiac conditioning has been confirmed (McCleary, 1960; Shapiro, 1965; Ingle and Campbell, 1977). However, Ingle (1965) found that fish trained with stimuli which differed both in color and pattern cues discriminated only

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²This work was done in collaboration with Dr. Margaret Y. Scott. It will be submitted for publication under the joint authorship of K. F. Greif and M. Y. Scott.

on the basis of color when tested with the untrained eye, using an active discrimination paradigm. In a later study (Ingle, 1968), easy pattern discriminations were found to show transfer, but stimuli which were initially difficult to discriminate transferred poorly to the second eye.

The nature of transferred information and the pattern of performance have not been examined directly or in depth in previous studies. It is not necessarily reasonable to assume that transfer occurs equally well to all portions of the visual field of the untrained eye. If, for example, fish normally attach greater importance to stimuli which appear in the region of the visual field for which it has binocular vision (anterior visual field, about 50° from the midline), then interocular transfer from the posterior visual field of the trained eye might be expected to be less effective than from portions which do interact binocularly. Previous studies have not restricted initial training to limited regions of the visual field. If innate differences between binocular and monocular portions of the visual field exist, careful testing of performance across the visual field of the untrained eye may reveal systematic differences in the effectiveness of interocular transfer. Using an apparatus which permits highly localized presentation of visual stimuli (Scott, 1977), generalization of task performance, across the visual field of the trained eye after lesion of trained tectal regions, has been reported (Chapter III) using conditioned respiratory suppression as the behavioral measure. Using the same procedure, we have investigated the interocular transfer of the color discrimination task after lesions of the trained posterior tectal regions. Preliminary reports have been filed (Greif and Scott, 1977; Scott, Greif and Sperry, 1977).

Methods - Experiment I

Experiment I was designed to investigate whether a color discrimination task learned in the posterior visual field of one eye would be generalized across all portions of the visual field of the untrained eye after lesion of the trained portion of the optic tectum. Six fish, approximately 6-9 cm in length from snout to base of tail were used. Fish were housed in glass aquaria under a 12 hr light-dark cycle throughout the course of the experiment except when placed in the training tank.

Fish had the extraocular muscles of one eye teased apart with jeweler's forceps after anesthetization with 0.05% tricaine methanesulfonate (Finquel). An elastic sling was placed around the body of each fish, the sling mounted on a block of clay and placed in the training tank (see Scott, 1977; Chapter III, for details). The optic disc was visualized by transillumination and centered at 0° of a perimeter mounted around a water-filled hemisphere, which had been sand-blasted on the interior to reduce reflected images. Respiration was monitored by external electrodes mounted in front of the mouth and behind the gill of the fish. Resistance changes were amplified and recorded using a paper polygraph (Beckman). The stimuli were 1.5° red or green lights, equated for brightness, and projected onto the hemisphere by a fiber-optic source mounted on the perimeter.

Before training, a 30 min period of adaptation to the apparatus was allowed, after which the fish was habituated to the stimuli by several presentations of each color without shock; fish responded at most once or twice to either color. Previous studies have indicated no innate aversion to either color (Scott, 1977). Stimuli were presented at -60° along the horizontal meridian (posterior visual

field). After habituation, fish then received five consecutive simultaneous pairings of shock to the tail with the red light (CS+) with 15 min rest periods between shocks. Exposure to the green light (CS-) was intermittent. Following the block of five shocked trials, a second 30 min rest period was allowed, after which shocked trials were interspersed with unshocked trials. No more than ten shocks were given in a single training session. Subsequent training sessions consisted of intermittent shocked trials only, until the differential response level reached a criterion of greater than 90% response to CS+ and less than 20% response to CS-, over two blocks of at least ten exposures to each color without a shocked trial. Attainment of the learning criterion took on the average three training sessions, spaced four days apart. In some cases, difficulty in suppressing responses to the neutral stimulus was observed; these fish were given up to four 15 min periods of exposure to the neutral stimulus alone in an effort to extinguish the unwanted responses. An unusually large number of fish, twelve in all, were discarded because of failure to reach criterion.

Three days after the final training session, fish were anesthetized with Finquel solution and a bone flap was cut above the tectum. Lesions of the caudal half-tectum were made by gentle suction after cutting down to the ventricle with a sharpened tungsten wire. The size of the lesions was varied to determine if any correlation existed between performance and lesion size. The bone flap was folded down and wedged into place. Fish were revived by infusion of water through the gills; fish recovered rapidly with no signs of motor deficits.

The day following surgery, fish were reanesthetized and the extraocular muscles of the untrained eye were cut. The untrained eye was aligned as previously described and a 30 min rest period allowed before testing. Testing began in the posterior visual field, with 3-5 presentations of each color at each locus.

Stimuli were presented alternately at sites in the posterior and anterior fields along the horizontal meridian at 20° intervals. After testing at each locus, 30 min was allowed before repeated presentation at the same sites.

Approximately one week after initial testing, generalization in the remaining portion of the visual field of the trained eye was examined. Stimuli were presented as described along the horizontal meridian, beginning in the anterior visual field. At the margins of the scotoma produced by the tectal lesion, 10° increments were used to permit more accurate determination of the edge of the lesion.

Results

Results are summarized in Fig. IV-1. Good transfer of the visual discrimination task was observed across the entire visual field of the untrained eye (Fig. IV-1a). Variability was greater in the posterior visual field, with some fish performing well and others poorly in this region. The differential response score was derived by subtracting the percent response to CS- from the percent response to CS+ and averaging these values across fish. The absolute ranges of scores for individual fish are shown. No overall deficit of response was observed in the posterior field, contrary to the expected result.

Generalization of response within the trained eye is shown in Fig. IV-1b. Good intraretinal transfer was observed across the remainder of the visual field, as has been previously reported (Chapter III). Data for loci immediately adjacent to the edge of the scotoma were discarded to eliminate bias in averaged values.

No trends were observed relating lesion size and postsurgery performance, or final performance before surgery and transfer performance. No improvement in retention was observed with a longer recovery period before intraretinal

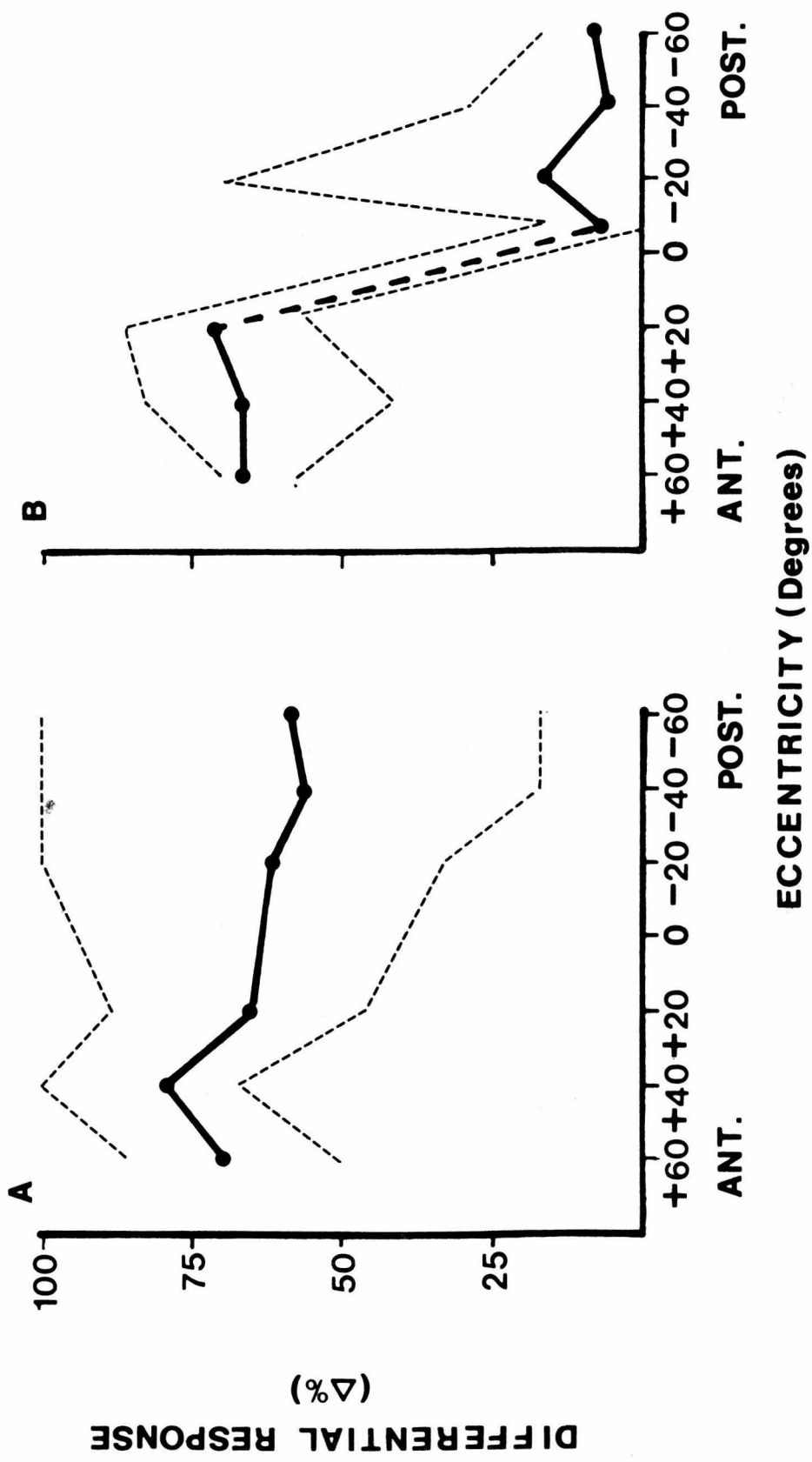


Fig. IV-1. Interocular and intraretinal transfer after lesions of trained tectal regions. Six fish were trained at -60° along the horizontal meridian. Eccentricity measured in degrees from the optic disc. Ant. = anterior visual field, Post. = posterior visual field. Differential response = % response to CS+ - % response to CS-, averaged across fish. Dotted lines = absolute ranges for all fish. A. Responses in the untrained eye. B. Responses in trained eye, approximately one week after interocular transfer testing.

generalization was tested, as compared with fish tested one day after surgery (Chapter III).

Discussion

The finding of consistent interocular transfer of color discrimination after lesions of trained tectal regions has several implications. The absence of a deficit in transfer from the posterior visual field of the trained eye to that of the untrained eye indicates that binocular interaction is not required for the transfer of color information. It also appears that color is recognized in a similar fashion throughout the visual field, in terms of content and importance. This conclusion is supported by previous findings of consistent transfer of color discrimination learning within one eye after highly localized training (Chapter III).

The absence of transfer deficits suggests that memory storage for this task occurs bilaterally in the goldfish brain. It is clear that the portion of the tectum receiving direct visual input is not required for retention of the task; after lesions of directly "trained" tectal regions, good retention is still observed. A possibility which cannot be eliminated is that engrams are stored in the remaining portion of the trained tectum, or in an association area, and that information is tapped by the naive hemisphere at the time of testing. Experiment II was initiated to examine this possibility, by studying the effects of complete tectal ablation after localized training on the interocular transfer of the color discrimination task.

Methods - Experiment II

Three fish were used in this study, designed to investigate whether

complete ablation of the trained optic tectum would result in deficits in the interocular transfer of a color discrimination task. An additional fish was discarded because of failure to reach criterion. Fish were housed, trained and tested as in Experiment I. Surgery was performed through a slightly larger bone flap. Removal of the optic tectum was accomplished by essentially peeling the tectal tissue off down to the ventricle by gentle suction. Care was taken to remove extreme rostral and lateral portions while avoiding damage to underlying structures. Fish were revived as before, and showed minor transient motor defects, which disappeared within 24 hr.

After testing of the untrained eye, the fish were reversed in the training tank and tested for residual vision in the trained eye. If no responses to stimuli were noted, attempts were made to elicit startle responses using larger objects. At the conclusion of testing, fish were given an overdose of anesthesia and brains removed for histology. Brains were fixed in Bodian (acetic acid, alcohol, formalin) fixative, imbedded in paraffin and sectioned in the sagittal plane.

Results

Results are shown in Fig. IV-2. Good interocular transfer was observed across the visual field of the untrained eye. No deficits were observed in the posterior visual field and the increased variability in the posterior field in fish with partial tectal ablation was not observed.

Fish showed little residual vision in the trained eye. One fish (CTL 2) made a few weak responses in the extreme anterior visual field ($+80^\circ$), but did not respond elsewhere. The two remaining fish made no responses to the stimuli, to a waving hand or to a looming body shadow.

Ablation reconstructions of histological sections are shown in Fig. IV-3.

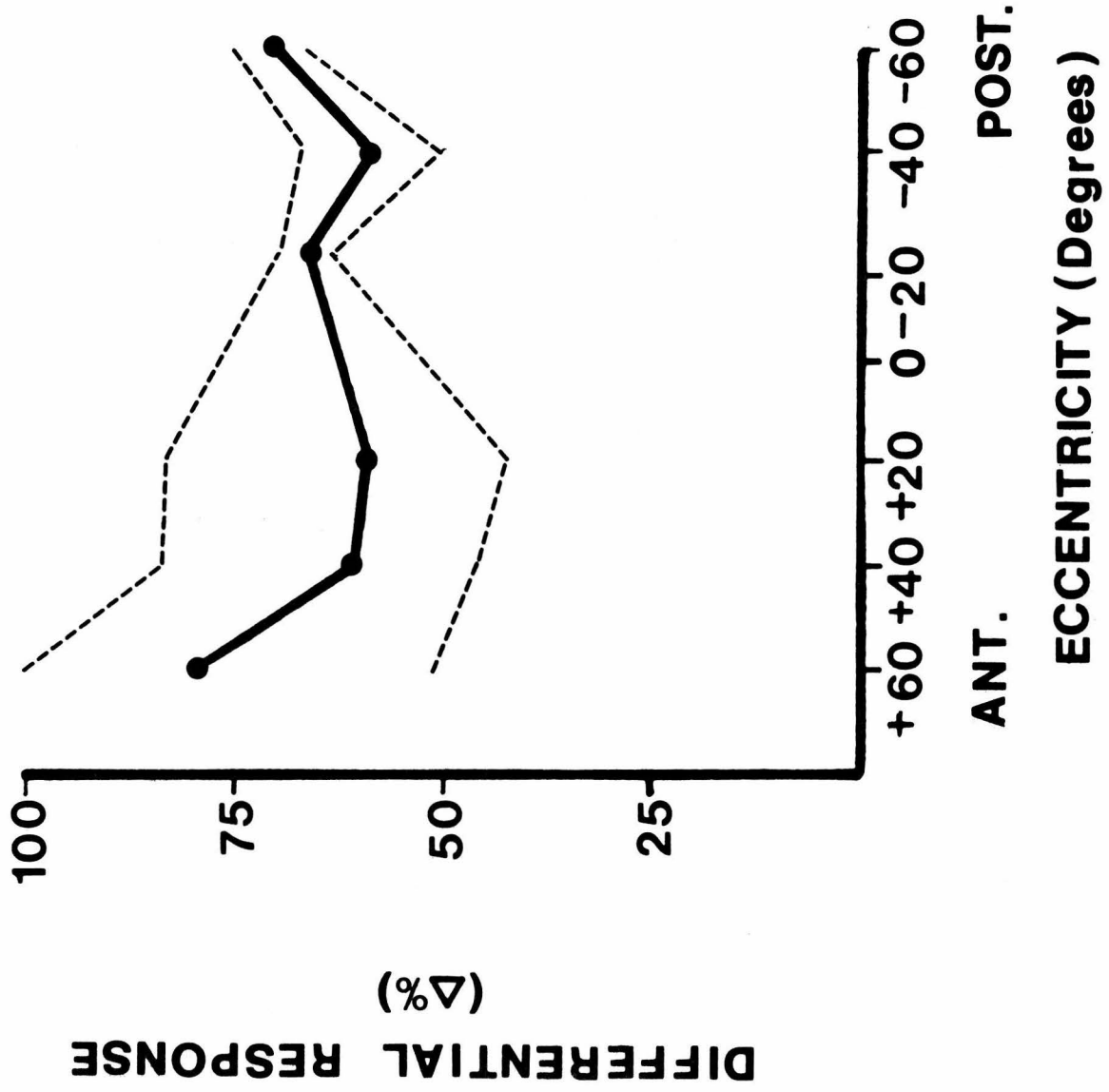


Fig. IV-2. Interocular transfer after complete tectal lesion, N=3. Conventions as in Fig. IV-1. Fish trained at -60° along the horizontal meridian.

CTL 2

CTL 3

CTL 4

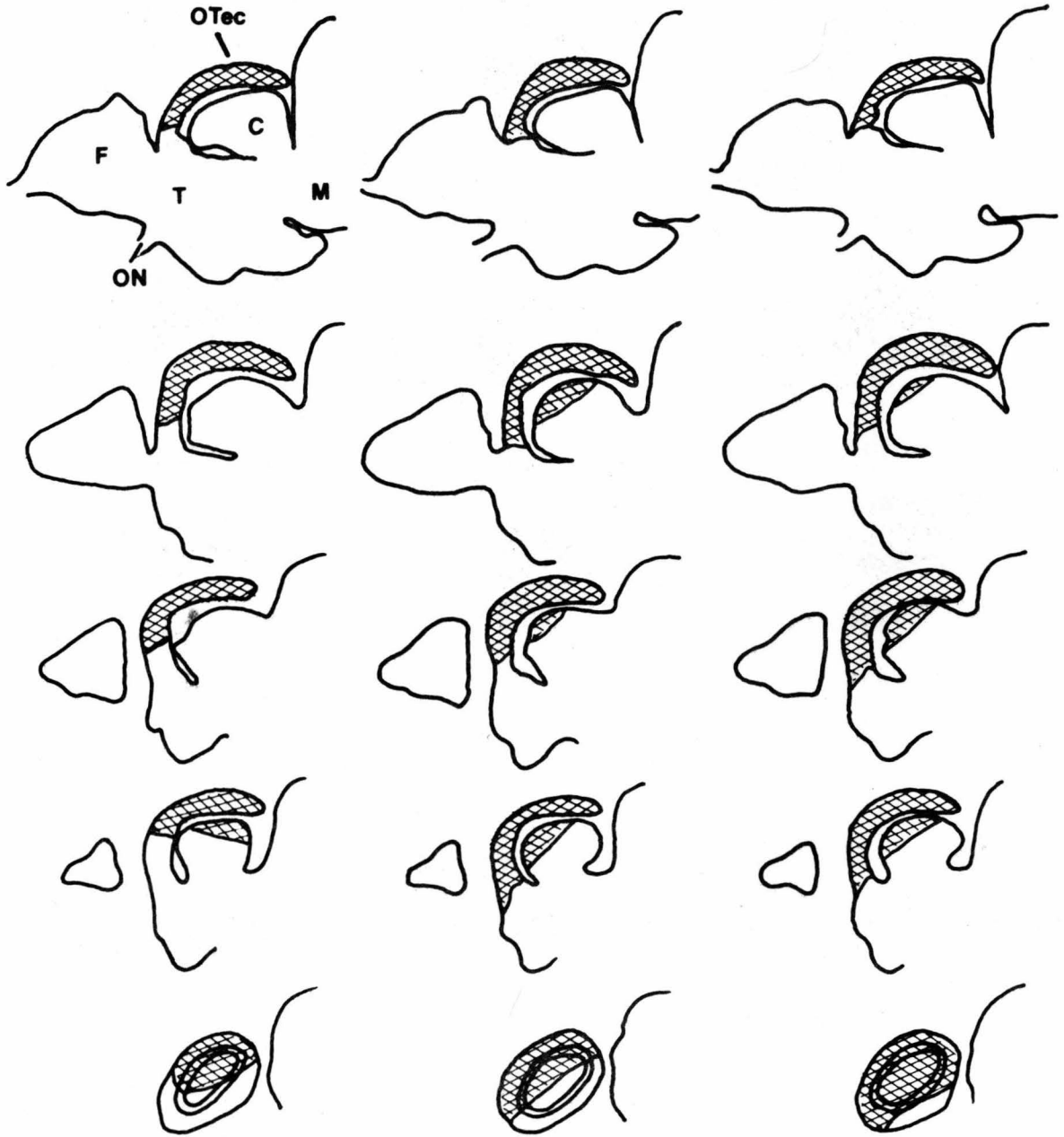


Fig. IV-3. Ablation reconstructions (sagittal sections) of complete tectal lesion (CTL) fish. Diagonal lines encompass tissue removed in each fish. Abbreviations: C = cerebellum, F = forebrain, ON = optic nerve, OTec = optic tectum, M = midbrain, T = thalamus.

Approximately 85–90% of the optic tectum was removed, with remnants mainly in the extreme ventro-lateral portion. This tissue was visually isolated by lesion of the rest of the tectum, but presumably retained efferents to other structures. In fish CTL 2, which showed slight residual vision, the extreme anterior portion of the tectum was spared.

Discussion

The results of Experiment II indicate that removal of the entire trained tectum does not prevent interocular transfer of a learned color discrimination. This suggests that memory for the task is established bilaterally in the brain, but does not rule out an extratectal storage area for memory, which may or may not be bilateral. If storage does occur outside of the tectum, it is reasonable to suggest that such storage is not in a unilateral localized area, because of the resulting complexity in neuronal circuitry. A highly discrete area would require visual connections from the entire visual field or a means to integrate visual information, and access to a pathway across the midline to the contralateral hemisphere. While extratectal visual projection areas have been demonstrated (Sharma, 1972; Finger and Karten, 1977), the roles of such areas have not yet been determined and further projections to the contralateral hemisphere have not been delineated.

The pathways involved in the interocular transfer of information have yet to be unequivocally determined. The tectal commissure has been reported to be necessary for the transfer of both color and pattern discrimination using tasks which required an active response on the part of the fish (Mark, 1966; Mark, Peer and Steiner, 1973). Ingle and Campbell (1977) found no deficits in pattern discrimination using active avoidance with tectal lesions and Yeo and Savage

(1975) found excellent transfer of pattern discrimination using cardiac conditioning as the behavioral measure. These conflicting results may have been produced by methodological differences; failure of transfer was reported in tectal commissure-lesioned fish only when reversal learning was used as the behavioral measure (Mark, 1966; Mark et al., 1973). Ingle (1968) found that fish were able to learn conflicting discriminations with each eye individually even when commissures were left intact, suggesting that reversal learning rate is not a valid measure of transfer. In addition, such a finding suggests that while interocular transfer can occur, contradictory information does not necessarily inhibit learning of a new task in the untrained eye.

Deficits in interocular transfer have been observed consistently with lesions of the post-optic commissure, using both active avoidance (Ingle and Campbell, 1977) and cardiac conditioning (Savage, 1969; Yeo and Savage, 1976). It has been suggested (Ingle and Campbell, 1977) that the post-optic commissure may mediate discrimination, while some "emotional" information may be sent via the tectal commissure, since post-optic commissure sectioned animals appear to exhibit an emotional response to stimuli without being able to perform the task discrimination.

By analogy to studies using cardiac conditioning (Yeo and Savage, 1976), it seems reasonable to suggest that transfer is mediated by the post-optic commissure. However, the roles of intertectal commissures have not been established for this task, and a contribution by them cannot be entirely eliminated. If the post-optic commissure does mediate discriminative learning, as has been suggested (Ingle and Campbell, 1977; Yeo and Savage, 1976), then it may well be that extratectal visual projections are critical for information transfer, as the efferents from the tectum do not project through the post-optic commissure.

The finding of an apparent bilateral memory for color discrimination in goldfish is in accord with most studies in mammals (Hamilton, 1977; Myers, 1961) and birds (Greif, 1976) where bilateral storage has been found after monocular training and either section of commissural pathways or specific lesions. A parallel finding in teleost fish suggests that a universal strategy for memory processing may exist which does not require specific brain structures. Despite considerable evolutionary divergence and profound differences in brain anatomy, most animals appear to use redundancy of storage as a general rule. Rather than increase capacity by limiting traces to a single hemisphere, it appears that even after monocular training, engrams are laid down bilaterally. It may be that if an experience is worthy of retention, efforts are taken to assure that important information is not lost.

General Discussion

Is it possible that a general strategy for memory processing exists in vertebrates? For the purposes of this discussion, man will be omitted because of unequivocal evidence for hemispheric specialization and asymmetric processing of different aspects of learning (see Sperry, 1974, for review). In animals other than man, however, no strong evidence for asymmetric processing has been observed, so that one may assume that the two hemispheres are equipotential for the processing of memory.

The current prevailing view, although not without critics, is that memory storage in mammals occurs bilaterally in the brain, even when input is lateralized to one hemisphere during training. Many studies to date have focused on visual discrimination learning, which can be lateralized by section of the optic chiasm. In monkeys, discriminations learned by intact or split-chiasm monkeys show good retention after completion of section of the forebrain commissures; corpus callosum, splenium and anterior commissure (Sullivan and Hamilton, 1973; Hamilton, 1977). Contrary findings have been reported using electrical stimulation of the visual cortex (Doty and Overman, 1977), but in general it appears that visually-mediated discriminations are retained in both hemispheres. Using postsurgical performance as a reflection of the degree of retention, it appears that retention is better in the directly trained hemisphere, suggesting that at least some information is lost during transfer through the commissures (Hamilton, 1977). Other modalities have not been thoroughly investigated and the pattern of storage is uncertain. A recent report using lesions of auditory cortex (Dewson, 1977) has suggested that combined auditory and visual stimuli in conditional tasks may

be processed asymmetrically, and that hemispheric specialization may exist in primates for complex tasks involving more than one sensory modality.

Research in cats has not been as systematic as in monkeys, but most reports have been similar to those in primates. In the original series of experiments in split-brain cats (Sperry, Stamm and Miner, 1956; Myers, 1961) it was found that simple tasks were retained in both hemispheres when training was followed by commissure section, but that the pattern of transfer using more difficult tasks was not clear. While Webster (1972) claimed to have found evidence for hemispheric specialization by studying cats' performance of difficult tasks, he found "lateralization" in only three of the eight tasks used. The remaining tasks all showed comparable retention levels in both hemisphere after section of the corpus callosum.

In lower mammals, indirect evidence for bilateral storage has been provided by studies of cortical spreading depression (CSD). CSD can be produced by stimulation of the cortical surface mechanically or by chemical treatment. By restricting treatment to one hemisphere it is possible to obtain functionally split-brain animals. Rats trained binocularly and then having CSD initiated in one hemisphere retain the ability to perform the tasks with the other (Ray and Emley, 1964; Buresova and Bures, 1965). Animals which are trained while under unilateral CSD do not show retention in the treated hemisphere, at least initially, suggesting that cortical structures are indeed involved in learning (Bures and Buresova, 1960). The findings of CSD have been sharply criticized because of uncertain methodology (Petrinovich, 1976) but nevertheless do suggest that bilateral memory storage occurs in rats.

The experiments reported in the preceding chapters offer evidence that at least two non-mammalian species process memories similarly to mammals.

It appears that chicks and goldfish may establish bilateral memory traces after monocular training of visually-mediated tasks. No evidence for lateralization of storage was found in either species. The forebrain appears to play a critical role in the processing of one-trial passive avoidance in birds, as has been reported previously (Benowitz, 1972; Benowitz, 1974). In goldfish, no structures critical for memory processing were identified, but the results do suggest that memory may not be stored unilaterally in an unidentified area.

The integration of bilateral memory storage with the process of interocular transfer should be considered. The failures of interocular transfer under controlled conditions may not reflect conditions occurring in the natural state. Under normal conditions, it is rare for information to be totally lateralized to a single hemisphere; any difficulties observed experimentally in the transfer of information would not necessarily be a problem. Training situations in the laboratory put unnatural constraints on the integration of visual information; nevertheless, there is obvious value in reducing the number of parameters to be considered, while bearing in mind the deviation from the natural state.

A principal question might be whether the occurrence of interocular transfer is an indication of the establishment of bilateral memory traces, or whether one can observe such transfer when unilateral storage occurs, as a result of tapping of information through the commissures. A possibility which has not been thoroughly investigated is that retrieval mechanisms operate bilaterally, but engrams are established unilaterally. Cases of failure of interocular transfer may be a reflection of the absence of bilateral engrams or of the need for interactions between structures which lack a route for interhemispheric communication. It appears from studies in split-brain humans that the latter occurs as a result of hemispheric specialization (see Sperry, 1974, for review). Failures of interocular

transfer in animals may be due to the inaccessibility of certain factors (emotional, motivational) to the second hemisphere; sensory information may reach the untrained hemisphere but its importance may not be recognized. One might argue that this may be indicative of genuine "unilateral memory." The restriction of "emotional" factors has been suggested to be the reason for failure of transfer of certain types of tasks in birds (Benowitz, 1974) and in fish (Ingle, 1967; Dewsbury and Bernstein, 1969; Savage, 1969; Ingle and Campbell, 1977). Ingle (1967) has suggested that thalamic integration of nonvisual information may be required for interocular transfer, although the nature of the required information has not been determined.

Parallel processing methods have important implications concerning the development of brain structures and of anatomical and functional parallels in evolutionarily divergent species. If one adopts a viewpoint based on anatomical structural analogy, it is possible to consider the brain of birds and fish as a mammalian brain essentially stripped of neocortex, except for a small remnant above an expanded midbrain. It is obvious that such a "simplified" brain does not imply a lack of "intelligence"; studies of comparative learning employing a variety of tasks have indicated that both birds and fish can perform a broad range of tasks (provided that adaptations are made for species differences) and that frequently the more "intelligent" birds (crows, magpies, owls) outstrip the mammals in performance (Stettner and Matyniak, 1968; Bitterman, 1975). Non-mammalian species have clearly enlisted structures other than neocortex to do the "thinking" for them.

Anatomical comparisons have been frequently made between brain structures of mammalian and non-mammalian brains using connectivity as an estimation of anatomical homology. Similarities in the connectivity of the avian visual "Wulst" and thalamofugal pathway and of the mammalian geniculostriate

system have been noted, as well as of the correspondence of tectal and collicular connections (see Hodos, 1976, for discussion). However, it has become evident that anatomically homologous structures may not necessarily have the same function. Visual system lesions do not produce the same degree of deficits in fish, birds, and mammals. There is a general trend towards increased participation of cortical structures, but even this pattern is incomplete. Tectal lesions in fish result in functionally blind animals with sparse sparing of optokinetic responses (Scott, 1975, 1977; Springer et al., 1977; Chapters III and IV), while telencephalic damage produces no apparent effect on visual discrimination (Dewsbury and Bernstein, 1969; Savage, 1969; Chapter III). Tectal lesions in birds produce gross deficits in pattern discrimination, with little recovery of performance, although the animals do not appear blind (Hodos, 1976). Lesions of the thalamofugal pathway produce small increases in thresholds for discrimination tasks (Hodos, 1976), reminiscent of deficits observed in de-striate cats (Berkley, Sprague and Warmath, 1976; Sprague, Berkley, Tunkland and Berlucchi, 1976). Interocular transfer is not affected by telencephalic lesions (Benowitz, 1974; Meier et al., 1972; Greif, 1976), again similar to findings in cats (Berlucchi, Sprague, Lepore and Mascetti, 1977). In mammals, lesions of the superior colliculus produce deficits in a number of measures, including attention to visual stimuli, visual following, and varying degrees of pattern discrimination, with greater deficits observed in cats than in monkeys (see Sprague, Berlucchi and Rizzolatti, 1973, for review). Recovery from these lesions is usually observed. The geniculo-striate system appears to play a greater role in discrimination learning in monkeys, with more profound defects observed after removal of the striate cortex, although animals do retain some ability to discriminate (see Doty, 1973, for review). It is clear that the similar visual systems in vertebrates do not have equal importance in processing

of visual information and that caution should be used when questions of functional analogy are considered.

In addition to visual system comparisons, forebrain structures have been compared with limbic structures of the mammalian brain. While forebrain lesions in goldfish have some effect on active avoidance learning, the degree of deficit is small when compared with the gross deficits observed in birds. Such differences may reflect teleost dependence on other structures for analysis of visual information. Bilateral forebrain lesions in birds produce marked deficits in avoidance learning and retention (Benowitz, 1972; Greif, 1976; Oades, 1976) and of reversal of discrimination learning (Benowitz and Lee-Teng, 1973). There has been an effort to relate the deficits in forebrain-lesioned birds to hippocampal "syndromes" observed in mammals (Benowitz, 1974; Oades, 1976), after lesions which in general have been poorly localized. It should be noted, however, that the nature of such "hippocampal syndromes" is a source of considerable controversy among investigators (see Izquierdo, 1975, for discussion), suggesting that comparisons of this type may be inappropriate, at least at the present time.

Since evidence for bilateral processing of memory has been found in mammals and non-mammals alike, it appears that such patterns of storage or retrieval may be a reflection of a generalized system present in vertebrates lacking hemispheric specialization. Redundancy of storage has not been a popular idea (see Doty and Overman, 1977, for example) because it can reduce the storage capacity of the brain by as much as 50%. Such redundancy may be valuable to an animal for several reasons, however. By the duplication of memory traces in the brain, an animal may assure that at least portions of important information can be retained after trauma to brain structures. There is also evidence that information transferred to the untrained hemisphere in monkeys may not be

as complete as that retained in the trained hemisphere (Hamilton, 1977). Bilateral storage could limit the apparent loss of information which occurs during transfer through the commissures by requiring that such information be transferred only once after lateralized input. Obviously, in the natural state, lateralized input is not likely to occur; information transfer via the commissures might not be required at all. Complete transfer of information may be too slow and inefficient in situations which require rapid analysis and response. Particularly in animals with large amounts of monocular vision, one might argue that if information is stored in the inappropriate (ipsilateral) side of the brain, the animal might still be analyzing when it should be fighting or fleeing.

The possibility of generalized rules for memory processing in vertebrates may be reflection of selection which occurred at much earlier stages of evolution. Animals with the ability to process information quickly and efficiently, store important features and retrieve them easily would have obvious adaptive advantages over those which could not. What we observe today in widely divergent species may be the most efficient strategies established during preliminary stages of behavioral development in vertebrates.

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