SOME OBSERVATIONS ON THE BRAIN AND BEHAVIOUR OF THE GOLDFISH, CARASSIUS AURATUS

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ABSTRACT OF THE THESIS

A review is presented of the literature relating to the ability of fish to learn problems involving discrimination within a variety of sensory modalities, using a number of types of response. The literature concerning the functions of both optic tectum and forebrain is considered.

Various experiments are described which demonstrate the ability of goldfish (Carassius auratus) to habituate to new situations, to form simple conditioned responses, and to perform increasingly complicated avoidance tasks.

Results are described which suggest that quite complex representations can be formed and retained in the absence of the optic tectum, and that the deficits in retention described by earlier workers were due to a combination of motor and visual failures.

Bilateral removal of the forebrain causes a large and persistent deficit in various types of avoidance learning, and in retention, though some retention does occur. Results from animals with partial lesions suggest that some further localisation of function within the forebrain may be possible, and that memory may be lateralised.

These results are related to those in the literature, and the suggestion is made that the forebrain serves in two capacities, firstly, as an activator, to alert the animal in the presence of significant stimuli, secondly, as a system to address the reinforcement signals to the appropriate stimulus/response association, and to strengthen this link. Suggestions are put forward for further work to clarify the above hypothesis.

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DEFINITIONS.

AVOIDANCE: movement of an animal from the start box to the goal box, within the duration of the stimulus/shock interval, so that no shock is received.

ESCAPE: movement of an animal from the start box to the goal box when stimulated by a shock or shocks given at the end of the stimulus/shock interval.

SESSION: one day's training, equal in almost every case to 10 trials.

RECOVERY PERIOD: that period without training allowed animals after any procedure involving anaesthesia, being in all cases five whole days after the operation.

TRANSFER: the changing of the stimulus used for the training of an animal.

INTEROCULAR TRANSFER: the ability to respond correctly to stimuli when using the opposite eye to that used in training.

CS: conditioned stimulus

UCS: unconditioned stimulus

CR: conditioned response

UCR: unconditioned response

CAS: conditioned avoidance stimulus

UCAS: unconditioned avoidance stimulus

Limits of the forebrain: writers have differed as to the limits of the telencephalon, but these will be taken in the present work as being defined dorsally by the halenular nuclei, and ventrally by the posterior edge of the pre-optic nuclei.

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INTRODUCTION.

1. Apology.

A worker in this department has little need to spend much time giving the lie to the statement that "learning is confined to the Phylum Vertebrata". Nevertheless, it is only within this group, and only within the neurologically advanced members of the Class Mammalia, that the more complex modifiable behaviour patterns studied by the psychologist are observed. The emphasis, even in learning of simple tasks, has been on the mammals, under the apparent though unspoken assumption that any animals lacking a cerebral cortex would be incapable of learning beyond the simplest of reflexes. Indeed, Herrick, one of the most illustrious of the classical workers, considered that on the basis of his anatomical studies, he could state that modifiable behaviour would be virtually impossible in fish, on account of the lack of neo-cortical structures. (Herrick, C.J., 1957, "Neurological Foundations of Animal Behaviour", U. of Chicago Press.) The same criterion had led other classical anatomists to the equally false conclusion that learning could never occur in birds, on account of the absence of neocortex, and the basal nature of the "corpus striatum". One or two writers have even suggested that the mechanism of learning may even be completely different in the lower vertebrates. This last statement, whilst lacking the extreme mammalio-centricity of the previous ones, and admitting that the lower forms <u>can</u> learn, must be attacked on the basis of Occam's Razor, in that there has as yet been no convincing evidence to suggest this. A fuller discussion of this point will be given below, when some outline of fish learning has been gained from the review.

It would, of course, be naive to talk of fish as showing the "primitive pre-cortical" condition of the vertebrates, since actinopterygian and amphibian lines separated from one another in the Devonian period of the Palaeozoic era, and their respective lines have followed divergent paths since then. Each line has built up its particular specialisation of structure, albeit about a common framework. The amount of variation of crude brain form even within one Suborder, the Cypriniformes, the carps and goldfish, is very considerable (see, for example, Evans 1952) and this is only the most obvious and gross difference in size and proportion. It is, however, justifiable to consider the fish brain as being "simpler" than that of mammals, it has far fewer neurons, fewer connections, both on the basis of tracts observable, and of numbers of nuclei, even if these by themselves present a frightening level of complexity.

This is perhaps the first reason for working with lower vertebrates, or with invertebrates - relative simplicity, even though the emphasis on "relative" must never be ignored. The work of Wells and Young (Wells (1962), Young (1964)) on <u>Octopus</u>, of Horridge (1965) on cockroaches, of Vowles (1963) on ants, and of Evans (1963) on annelids shows how much can be gained from consideration of "lower" forms. The corresponding data on fish will be discussed in the review below. The use of simple situations with relatively simple animals has much to commend it, both from the point of view of control of experimental conditions and of interpretation of results. It may well be, of course, that even here, the problem is being attacked at several powers of complexity too high to allow resolution, and that the eventual answer may lie in the

/detailed

detailed study of populations of two or three neurons cultured *in vitro*. Whilst this may tell of the changes in single cells, it will not tell how these changes are fitted together to form a working nervous system. It is here, by consideration of the functions of various parts of the central nervous system with respect to one another, that lesion, learning and electrical techniques of more gross proportions have their use.

Those who would detract from fish as psychologically and neurologically worthwhile animals for investigation have, rather paradoxically, been at once correct and mistaken in their ideas. On the one hand, they speak correctly of the lack of higher forebrain developments homologous with the cerebral cortex, and identify the forebrain components as perhaps the homologues of the basal forebrain areas of higher vertebrates. Some have proceeded, as has been seen, to deny the existence of learning in fish; these can be dismissed on the basis of evidence to be presented below. Others admit that learning can occur, and make the quite reasonable assumption that areas other than the forebrain mediate it. They have then proceeded to test forebrain function using experimental techniques like those which have been found to yield deficits when used with cortical lesions in higher forms. In most cases the findings have been negative.

If such insistance is to be placed on *somewhat* dubious theories of homology, it would be as well to draw these accurately, so, bearing in mind the probable divergences of fish from the line of higher vertebrates, it would be reasonable to look for differences in mammals with lesions in the basal forebrain areas, the limbic system and its associated nuclei. Having some idea of types of deficit, it might be possible

/to extrapolate

to extrapolate these findings to fish, and to gain a valuable pointer to the sorts of questions to ask the system. There have been startling changes in all vertebrate systems since fish and amphibian lines diverged - one has only to consider those associated with the adoption of quadrupedalism - but though such an expectation of homologous function may be naive, at least there is some slight rationale for expecting it.

As in so many other cases, the comparative method may be very useful. This is the second reason for interest in this sort of project, that it may yield some idea of brain function in the lower vertebrates, and allow some crude homologies to be drawn. This point will be discussed in more detail in a later section.

The third reason for working on fish is not as dramatic as those cited above; it is merely that very little is known about brain function in fish. Most of the more important papers to be quoted below have been published over the last ten years, for much of the earlier work was both ill-controlled and imbued with a bias towards description rather than quantitation. The goldfish is, as far as any fish is, the "rat" of teleost psychology. More behavioural work has been done with it than with any other teleost, both in variety and amount, and there has been quite a large amount of work studying its other attributes, physiological, genetic, and morphological, though in comparison with mammalian forms the total is scanty. <u>Carassius auratus</u> is very hardy - both the review and the results will bear this out, and it is easy to keep.

/Before

Before considering the method of attack, it is convenient to examine previous work and to formulate questions.

2. Early work on fish learning.

Contemporary fishermen and keepers of aquaria constantly sound the limits of credulity with tales of fish reasoning of Macchiavellian intelligence, and there is no reason to suppose that their predecessors in earlier centuries were any more objective. The first application of any scientific method to the study of fish behaviour is generally considered to be that of O. Amtsberg, working in Stralsund, Germany in 1873, whose classic experiment with a pike is widely misquoted as that of K. Möbius, in whose paper it appears.

A pike, one of the most vicious of freshwater fish, was living in a glass tank. A transverse glass screen was placed across the aquarium, so that the pike was confined to one end. After a period during which it had been observed that the glass screen did not disturb the pike, a number of small fish (presumably minnows) was placed in the other compartment. The pike immediately hurled itself at the fish and collided violently with the glass. It repeated this several times, and, at the end of a few minutes, "lay on its side as if dead" at the bottom of the tank. It soon recovered, and was seen to renew its futile attacks. The frequency of attacks decreased, over the next few days, and after three months there was no tendency to attack the small fish. After a total of six months in these separated conditions, the fish were allowed to mix, by the removal of the glass wall. The pike tended initially to remain within its previous limits, and to react to a "phantom wall" at the position of the partition. (It is unfortunate that Amtsberg gave

/no details

no details of the behaviour of the prey fish at any stage of the experiment; it would have been interesting to know whether or not they showed the same types of behaviour as the pike).

After some time, the fish mixed, and the pike made no attempt to attack the other fish. It would even take food (meat) thrown into the tank, and feed alongside the smaller animals without being motivated to attack them.

Since to the author's knowledge only one worker has repeated this work, that work is conveniently considered here. Triplett (1901) used two perch, one male, one female, which were fed on minnows for some time before the beginning of the experiment. (Perch, like pike, are very voracious predators). A transparent partition was placed across the tank, isolating both fish on one side. The tank was kept away from gross light changes, and the observer was concealed behind a black screen. On alternate days, for three times each week, four minnows were put in the empty compartment, and removed at the end of an half hour period. The perch made dives at the minnows, but the frequency of these decreased over the test periods, and, after a month, a single minnow was put in with the perch. The larger fish made one or two moves in the minnow's direction, but generally ignored it. If they did persist in their movements towards the minnow, they suddenly backed away as if encountering an obstacle.

In a second experiment, Triplett followed Amtsberg's experiment more closely in that the minnows were kept permanently in the other compartment. After one month, during which time the perchs' attacks had fallen to zero, the glass was withdrawn, and the fish mingled without

/the minnows

the minnows coming to any harm. He did observe, however, that if the minnows made sudden "escape" movements, the perch would pursue and eat them; as long as they swam slowly, they were not molested.

3a. More recent work.

Since Amtsberg's and Triplett's experiments, there has been a large amount of work on the behaviour of fish, and rather than deal with this as it has occurred chronologically, it will be more useful to deal with each particular facet as it has developed.

3b. Habituation.

Very few authors have considered the factor known as habituation in the behaviour of fish, that is in the sense of the decrease in activity due to increase in familiarity with a new situation. As Thorpe (1963) has observed, it is common to observe this phenomenon, but rare to make any quantitative study of it. As will be seen below, most workers have given their fish periods of habituation in the experimental situation, taking this to have occurred when the animals have ceased to show the initial continual agitated movement and exploratory behaviour. These periods for habituation have ranged from 10 minutes (Bernstein 1962) to hourly periods over twelve days (French 1942). Szymanski (1914) tied sticklebacks to the lever of a kymograph by a thin thread, and recorded their activity, but the attachment of the string to the dorsal musculature must have had a considerable effect on the animals. Spencer (1939) used a very similar configuration, which he called an "ichthyometer", and measured the activity of various fish, including goldfish. His curves show little sign of any waning of activity, and the same criticism can be levelled at him that was used for Szymanski.

It is worth noting here that Bull, whose series of papers will be referred to in some detail in subsequent sections, also used this system. He was trying to duplicate the work of Froloff (1925-1928) who measured fish's activity in conditioned reflex training by this means. Bull (1928) found that fish restrained in this fashion never became still enough for the extra activity of a reflex to be noticeable above the "noise". When he placed his fish in a tank, and did not restrain them, he found that they would become quiet within 15 minutes in darkness. Froloff's fish were also in darkness, and those of Syzmanski and Spencer were allowed normal daily lighting. Bull's and Froloff's work will be considered in more detail below.

French (1942) in a series of experiments to investigate the effect of temperature changes on retention in fish, placed goldfish in restricted bottles, with a number of paddles, so that pushing of a paddle caused a relay to close, and was recorded. Once again, however, it seems that the tactile effect of the recorder was too disturbing, for there was no decay of activity to zero. Most workers using training schedules have noted that fish became more easy to train as the trials progress (see, for example, Bull 1928 et seq., Perkins & Wheeler 1930, Janzen 1933 and Welty 1934) but the only paper actually devoted to the problem is that of Welker & Welker (1958) who worked with Eucinostomus gula. Individual fish were put into new tanks, whose bases were divided up into various equal areas by lines on the bottom. The activity of each fish was observed in terms of the number of lines crossed in equal times. This was at first low, but gradually increased over a period of

/minutes,

minutes, and this increase was taken to indicate a growing habituation to the tank. If new objects were dropped into the tank, the rate of crossing dropped sharply, then rose after some minutes. It is unfortunate, on the basis of results obtained, in the present work, that the observations were terminated so soon; there would have been a chance for comparison. Most workers would agree that habituation was cessation of movement, and would take hours, rather than minutes.

3C. Visual problems.

A. Classical conditioned reflex formation.

The phrase "classical conditioned reflex" is used here in the sense of an undirected activation of the subject, caused originally by a naturally motivating stimulus, that comes to be elicited by a neutral stimulus alone, on account of that stimulus having preceded the motivating one in previous experiences. Hence any conditioning experiments which require the animal to make a directed response, no matter how simple, will be dealt with in later sections.

The earliest experiments on learning of conditioned reflexes were, not surprisingly, by a Russian worker, Froloff (1925, 1928). He used a variety of fish, including Gadus morrhua, Corvina nigra and Carassius carassius. Subjects were suspended in water at the end of a wire, which transmitted their movements to a Marey capsule, thence to a recording drum. As has been remarked above, and by Bull (1928), it is difficult to see how Froloff managed to keep his animals still, so that he could record their reactions to the CS. Experiments were well controlled in that the experimenter was concealed behind a wall, and that the tank and its contents were well insulated against variations in light,

/vibration,

vibration, and movements of the experimenter. The wire to the capsule also constituted one pole of the shocking system; the other electrode was placed some distance from the fish. The electrodes were connected to the secondary circuit of an induction coil. Visual stimuli were at first the switching on of the overhead light, but in later work, to be described below, coloured lights were used. CS/UCS intervals of about 5 seconds were used, and one specimen of Gadus aeglifinus acquired a good CR to the light after about twelve trials, spread over two days, with intertrial intervals of about two hours. One Gadus morrhua acquired the same conditioning in six trials in one day. Froloff found that the response, once well established, took about 30 trials to extinguish.

Bull, in a famous series of experiments, examined conditioned responses of fish to a wide variety of CS's; in most cases, however, the responses were directed ones, and do not, therefore, come within the scope of this section. He did, however, try to repeat Froloff's method, and failed, as has been noted. He therefore developed his own method, still using light as the CS, and shock as the UCS. The fish could swim freely, and was shocked by agar electrodes set at either end of the tank, which carried the current from an induction coil. Activity was recorded by a cover-slip which floated in the surface film of the water, and was attached to a lever with a recording drum at its far end. These changes represented great improvements over Froloff's method, in that the fish was as far as possible unimpeded. Using a 2 second CS/UCS interval, he trained a Blennius gattorugine to show a CR within 20 trials, with a green light as CS. Another fish of the same species was trained

/to show

to show a CR to a red light within 40 trials. After 20 more trials with this fish, he left the CS unreinforced, and extinction of the response occurred within 36 more trials.

Sears (1934) used the onset of an overhead light as CS, with a jet of water at the animal's side as the UCS, the duration of the latter was 0.5 second. All his control fish reached 8/10 correct responses within 100 trials, some taking as little as 15 trials to show the response, the mean being about 50 trials. Time taken for the CR to extinguish was from one to 62 trials, and there was no correlation of resistance to extinction with speed of learning. Vanderplanck (1938), investigating the effects of hormones on CR's, used a method similar to that of Bull, and produced CR's in rudd (Leuciscus leuciscus) after about 25 trials, though he gave no indication of any training to criterion. His training technique was rather odd in that the CS/UCS interval seems to have been near zero, which paradigm does not generally produce any learning save for a certain "sensitisation" (see a later discussion of the work of Bitterman (1965)).

Frolov (1941) used a technique similar to that of Froloff, except that he ran a separate shocking wire to the fish, and used a more sensitive Marey lever. He obtained CR's from fish in response to various coloured lights. (Most of these more recent Russian papers have not been available, and are quoted from Pavlovskii (1964)). Frolov is reported as having found that the response to shock waned, and finally extinguished, but this is a very odd result, and is not in accordance with most of the literature, or with results quoted below. Popov (1953) used the UCR shown by fish to a jet of air on the surface of water, and, with air jet

/as UCS

as UCS, and light as CS, obtained good CR formation in carp. In the same year, Chernova (1953) and Sokolev published papers on fish CR's. The former used various gases dissolved in water as UCS's, and light as the CS. Gases used included carbon dioxide, hydrogen sulphide, and oxygen. (Details of oxygen sensitivity of fish are given in a later section). Sokolev, using an unspecified method, changed the pressure of the gas in the swim bladder, and used this as the UCS, with light as the CS.

In 1959, Noble, Gruender and Meyer carried out the first quantitative investigations into the time relations of CR formation in fish. They used Molliensia sp., and tested the formation of CR's using a light/shock schedule, with two days' training at 30 trials per day. Every ten trials, a dummy trial was run without shock, and the CR was measured. The greatest rate of learning, and the highest percentage of responses attained, were in the group with a CS/UCS interval of 2 seconds; rate and performance fell off on either side of this peak.

Otis, Cerf & Thomas (1957) used a method of autonomic conditioning which shows considerable promise as regards quantitation of results. They measured heart and respiratory rates electrically, and gave the fish light/shock associations with a CS/UCS interval of 5 seconds. Shock intensity was adjusted to be sufficient to just flick the tail. Conditioning took place with rapidity, most animals taking less than ten trials to show responses to light. After twenty trials, there was consistent inhibition of respiratory activity and deceleration of the heart rate when the light was presented.

/Adler

Adler & Hogan (1963), working with <u>Betta splendens</u>, used the reflex of gill membrane erection as the UCR and shock as the UCS. The CS was the sight of another fish, or of the subject itself in a mirror. The stimulus was concealed behind a door, which was raised to initiate the trial. Stimuli were presented for 15 seconds, and a shock of one second's duration was given at the beginning of this period. The extreme number of trials, as compared with previous work, to attain the reflex (about 86) was no doubt due to this failure to give a CS/UCS interval, as has been remarked above. In a subsequent experiment, fish were punished if the gill-erection response occurred within 120 seconds of the presentation of the stimulus. Two groups were used in this experiment, one in which fish were shocked immediately the gill response occurred, an other whose members were shocked at the same time as those in the first group, so that they received the same number of shocks, but with no relation to gill cover erection. The supression of this response occurred in the first group, not in the second.

Klinman & Bitterman (1963), prompted by earlier findings (discussed below) that in goldfish the percentage avoidance rose over the 0.5 - 2 second range, and remained fairly constant thereafter, examined the role of the CS/UCS interval in classical conditioning in an attempt to relate their data to that of Noble, Gruender and Meyer cited above. Their first experiment, with <u>Molliensia</u>, duplicated that of those workers, except that a constant dummy test was used, so that differences in length of time to respond in the test would not affect the results. (It is doubtful whether this was a very valid critiscism, since if the

/results

results had been biased in this way, it would have been expected that there would have been a direct relation of CS/UCS interval to magnitude of response; this did not occur). In this first experiment, trials were massed in groups of 31, with test trials at I, II, 21 and 31, and it was found that a 0.5 second interval produced much greater responses than the longer periods. In the second experiment, the inter-trial interval was increased from one to four minutes, and it was found that there was very little difference between the groups. A third experiment was run with goldfish as subjects, using an inter-trial interval of one minute as in the first series, and it was found that there were no differences between the various time intervals. Klinman and Bitterman concluded that the CS/UCS interval and the inter-trial interval interacted in mollies, but not in goldfish.

In a later paper Bitterman (1965) showed that goldfish trained with complete contiguity of the CS and the UCS showed no response to the CS when tested with it alone. (This finding bears out the criticism made of the papers of Vanderplanck, and Adler and Hogan). The degree of activity produced, and the speed of its production, differed little over CS/UCS intervals of 0.5 - 2 seconds. Further experiments in which the post -UCS duration of the CS was varied showed that this interval was far less important than the CS/UCS interval, as would be expected. Bitterman went on to examine the importance of trace conditioning as a method of training. Fish were given CS's of 0.5 second's duration, and CS/UCS intervals of 5, 10, and 20 seconds. There was some acquisition of CR, but the level of response was not nearly as high as in previous groups. He drew the conclusion that in avoidance conditioning, there was a prior acquisition of a CR, which came to be associated with an avoidance response. This will be referred to again in the section dealing with avoidance learning.

Roots and Prosser (1962) and Prosser and Farhi (1965) used a method of measurement similar to that used by Otis et al (1957). The CS was a dim light, and the UCS shock,applied via the electrodes which also served to restrain the fish. The CR was an inhibition of the respiratory rate, and was measured by a balsa paddle resting lightly against the operculum, whose movement disturbed a transducer, and was recorded. Using a CS/UCS interval of 5 seconds, it was found that a consistent inhibition of the respiratory rate occurred within five trials.

B. Avoidance learning.

In this section only those types of avoidance requiring a constant "go" response to stimuli will be dealt with; "go/no go" situations, with discrimination between stimuli, will be dealt with in later sections.

The use of avoidance techniques for training fish is surprisingly recent. As Bitterman (1965) has pointed out, an avoidance task is merely a classical conditioned reflex with a directed motor act appended, and, again, it appears to have been the Russian school which first developed the use of this type of training in fish.

Prazdnitkova (1953) trained fish to avoid a light stimulus, succeeded by a burst of bubbling, by swimming from a start box, through a swing door, into a goal box. Animals apparently learned to do this with some ease, and would soon pass from one box to the other when the light came on. Sokolov (1955) used a more precise UCAS, shock. The box used was the type of shuttle box used by Prazdnitkova, but there were large

/electrodes

electrodes imbedded in the walls. Using a 3 - 5 second CAS/UCAS interval, and a shock which stayed on until the escape was accomplished, it was found that light alone could evoke the response after some training. Passage of the fish through the door operated a microswitch which allowed trials to be recorded automatically. More recently, Sokolov has used a more unusual form of punishment. Using light as the CAS, and the same type of shuttle box as described above, he used rotating plexiglass combs, which beat the water, the resulting disturbance constituting the UCAS.

McCleary (1960) used a simple avoidance technique when testing for interocular transfer in goldfish. Animals were fitted with bilateral translucent blinders, and placed in a shuttle box, whose halves were interchangeable, and joined by a small swing door. A small spot of light was projected on to the blinder of one eye, serving as the CAS, whilst the UCAS, shock, followed after an interval of 10 seconds. Fish learned to avoid the light in a few trials, and attained a criterion of 5/5 in an average of about 60 trials.

Bitterman and his associates have made extensive studies of the conditions affecting the learning of simple avoidances. Behrend and Bitterman (1962) found that trials to criterion fell off rapidly as the CAS/UCAS interval was increased, being about 60 to 9/10 correct for a 5 second interval, and 40 for a 40 second interval. They also found that the shorter intervals in training produced a more persistent response in extinction. Fish which were trained with various CS/UCS intervals, without opportunity for escape, and were then transferred to avoidance with an interval of 20 seconds, avoided well, those with the longer

/intervals

intervals performing better on transfer than those trained with short intervals. Thus the association was acquired independently of the opportunity for response. Wodinsky, Behrend and Bitterman (1962) showed that both Carassius and Pomacentrus showed the same negative acceleration of trials to criterion as the CAS/UCAS interval was raised.

Behrend and Bitterman (1963) also studied the acquisition of Sidman avoidance by fish. Fish were shocked every 20 seconds, but could postpone shock for 20 seconds by escaping into the other box. The rate of movement from box to box increased, whilst that of a control group, shocked at the same times as the experimental fish, did not. When a warning signal was introduced, in the last five seconds of the 20 second interval, the number of responses in the previous 15 seconds fell off, whilst the number of movements in the last five seconds increased. The same workers in 1964 investigated the effect of type of reinforcement on the formation of avoidance responses, Warner versus Hunter reinforcement. In the former, a continuous pulsed shock (0.25 sec. every 1.5 sec.) was given until the fish avoided, in the latter, a single 0.5 second pulse was used. There was no effect of type of reinforcement on the level of performance. In their last experiment, they found that Molliensia was significantly inferior to goldfish on forming conditioned avoidance responses, and in view of the differences found for classical conditioning, they concluded that there was a species difference in learning ability.

Aronson and Kaplan (1963, 1965) and Aronson and Haberman (in preparation) have used techniques similar to those of Bitterman. Using Tilapia as subjects, they trained these fish to swim from one

/compartment

compartment to another in response to a light stimulus when the light was reinforced by shock. CAS/UCAS intervals were 2.5 and 5 seconds. Unfortunately these are short reports, and no details of the rate of learning, or of the asymptote are given. Agranoff and his co-workers have used the avoidance technique of Bitterman in their interesting work on the effects of puromycin on memory, to be discussed below. Their fish were given 20 trials daily, and trained again some days later. They did not train to criterion, but when their fish were trained later, there seemed to be good retention of what level of avoidance had been attained. (Agranoff and Klinger (1964), Agranoff, Davis and Brink (1964 ~~a~~ & ~~b~~ 1966), and Davis, Bright and Agranoff (1965)).

C. Detour learning.

The learning of detour and maze situations almost always involves the performance of delayed responses, since the interval between the initiation of the trial and the attainment of the goal is occupied by negotiating the situation, which means either losing sight of the goal or having to move away from it. For this reason, detour, delay, and maze situations will be dealt with together. Some discrimination situations, of course, involve quite complex detours, but these will be dealt with only insofar as the detour aspect of the task is involved.

Thorndike (1899) found that Fundulus sp. would seek out and rest in the most shaded part of their tanks. If an animal were placed in the lighted part of its tank, it would swim quite rapidly to the dark section. He placed an opaque partition across the tank, with a hole big enough to allow passage of the fish. Fish were placed in the light half, and had to negotiate the barrier before they could rest in the

/dark.

dark. At first, fish would swim to and fro in front of the screen, bumping it with their heads. After some minutes, they would find the hole, and go through. Once they had done this, reaction times fell rapidly, and attained a fairly constant low level after 20 trials. Other screens, with holes in different places, were introduced, and the reaction times rose considerably. Again, once the fish had learned the new situation, their times fell rapidly. In later experiments, Thorndike used food reward, and found that fish learned just as well. Goldsmith (1914) trained specimens of Gobius and Gasterosteus to go to one part of their tank for food using a light stimulus, and found that the response was not impaired after 18 days' abstinence. She placed a stickleback and its nest on opposite sides of a glass partition, which had a small central hole. In first trials, the fish took up to 3½ hours to reach the next, but this dropped rapidly to a matter of a minute or so. In later trials, she used a gauze partition, and the rates of learning and the final reaction times of fish decreased further. She concluded that visual stimuli played a considerable part in the formation of the motor patterns necessary for the performance of detours.

Churchill (1916) also studied the effect of the nature of the barrier on the learning of mazes. He used goldfish, which were fed at first from a shelf at the end of their tank. When fish fed regularly from the ledge, he interposed two partitions between the animals and their goal. In the first experiment, the barriers were of gauze. As the two previous authors had observed, fish seemed to learn their way through the maze by feeling with their snouts, rather than using their eyes. They would swim past the hole, and go through only when they nosed along the wall of the gauze and found the opening. Reaction times for four animals dropped from an initial 110 minutes to 5 minutes over 60 trials, though most of this decrease occurred in the first ten trials. As fish learned the problem, they used touch less, and swam straight towards the holes, but would sometimes bump around them before going through. Two more fish were trained using the same situation, except that the edges of the holes were outlined with black tape. These animals showed a startling drop in reaction times, taking half the time of the previous group to reach the same level of performance. Their final level of time was also lower, being about 3½ minutes. Churchill then replaced the wire barriers with ones of wood, and trained two more animals, with the rather surprising result that they took much longer to learn the maze than the previous group, but less than the first group. Since the partitions were of plain wood, it may have been that the edges were not as obvious to the fish as had been the black edges on the gauze. When the two barriers were changed so that the first was second, and vice versa, animals showed great increases in reaction times. Churchill concluded that maze learning was far less visual than supposed, and that there was a large kinaesthetic component to it.

Bull (1928) trained Labrus bergylta (wrasse) to swim to a particular part of the aquarium once a light came on, in response to food. Food was dropped in exactly the same place each day, some seconds after the onset of the light. For the first few days, the onset of the light caused the fish to show signs of agitation, but after five

/days,

days, and some 20 trials, the fish would swim to the correct point immediately the light came on, and look upwards towards the surface. Later he used a feeding bottle, which required fish to swim into the base of a bottle, a semicircular portion of which had been removed to facilitate access. Food was introduced into the bottle by way of a covered tube at the neck. A wrasse was trained that 15 seconds after a light came on, food was introduced into the feeding bottle. On the first trial, the fish saw the food through the wall of the bottle, and dived at it immediately, banging itself against the glass. It then felt around the bottle for 5½ hours, till it entered and fed. Over the next few days' trials, the time to enter the bottle fell rapidly, and the wrasse rarely took more than a few seconds to enter and feed. The bottle was next made opaque, and the fish trained as before, so that it would have to respond to the light rather than to the sight of the food entering the bottle. In initial trials, there were no responses but after about twenty trials, the fish started to make correct responses. This was both a detour and a delayed situation, in that food was given 15 seconds after the onset of the light irrespective of the position of the fish. *Crenilabrus melops* was also trained on this problem, and learned rapidly.

In a later series of experiments on detection of temperature and salinity changes, Bull (1936, 1937) used a very simple maze. Fish were kept in the deeper end of a sloping tank. Near the shallow end, a partition with a large door marked off a food chamber, into which animals could swim or wriggle only with difficulty. This area, needless to say, was normally shunned by the fish. After presentation of

/the heat

the heat or saline stimulus, a piece of mussel was placed in the food chamber, and, as soon as its smell had reached the fish, the animals would swim up the concentration gradient, and finally splash through the door into the food box to get their reward. The time taken to do this dropped rapidly, and the subjects soon made the trip when the CS appeared.

Russell (1931) performed a series of experiments with Gasterosteus aculeatus. He first tested fish with dead worms in test tubes, and found that they were approached, concluding that visual rather than olfactory stimuli were operating. He then put a worm in a wide necked bottle lying on its side with the end to the fish's left. Times taken to retrieve the worm were initially as high as one hour, but dropped steeply to about one minute. On account of the effect of the initial position of the fish on the reaction time, he tried a second experiment in which the bottle was put resting on its base, so that the difficulty of the problem did not depend on the relative position of the fish. As in the previous experiment, the number of "direct path" attacks was initially high, but fell off, and the time taken to get the food dropped rapidly. Russell concluded that "insight" was not involved in this learning, on account of the fact that the response did not remain constantly low, but was erratic. If the animal had grasped the total situation, it would have been expected that it would perform well once it had first managed the task properly.

Welty (1934) trained goldfish to swim through a hole in a partition from one box to another, to receive food. The stimulus to do this was the onset of an overhead light. After the first high reaction times, the response became rapid, and less erratic. He went on to consider the effect of various groupings of fish on the rate of learning. Ono (1937a) tested the maze preferences of various fish, mainly <u>Oryzias latipes</u>, a fighting fish. Single animals were allowed to escape from the start box into a large box, which had a row of eleven goal boxes set in its far wall. (This initial movement into the large box is rather reminiscent of Ingle's spontaneous escape method described below). Although the goal boxes were identical, fish tended to choose the extreme boxes; thus, for example, in one series, 59 out of 73 fish chose the extreme left or right boxes. In a subsequent experiment, where the RHS overhead light was switched off, of 38 fish, 23 went to the left side, 8 to the right side. He next put individual fish into goal boxes, and took their times to escape. The smaller the door aperture, the longer the escape took. Ono next used a reduced number of goal boxes, five in this case, and put groups of fish into the start box. Of two groups, of 26 and 39, 25 and 35 fish respectively were found in the extremes, indicating that there was some social effect on the animals' movements.

In a subsequent work, Ono (1937b) trained a single specimen of <u>Macropodus opercularis</u> to swim from the start box into one of five goal boxes. The correct box was indicated by a white triangle over the door, the other boxes were blocked by glass doors. The animal was fed when it had entered the goal box, and took 500 trials to form a good response to the triangle, an extremely long period in view of the general speed of learning in fish; this would not seem to be a very difficult problem. Three other similarly-trained fish attained 70-90% in 500 trials. Fish trained to go left or right attained 100% in 50-60 trials, so it seems

/likely

likely that there were some factors interfering with the other learning situation. For example, if the position of the triangle were changed (it was kept above the same door each time in the previous experiment) fish showed complete return to chance level, which would not have been expected if the figure had been a cue.

Spooner (1937) performed an extensive series of experiments using wrasse (Ctenolabrus rupestris) as subjects. The first task required animals to circumnavigate a series of vertical plates, in the form of an eight-pointed star. One set of four was arranged as a cross, with the centre edges meeting. The plates of the second set were set each one between a pair of the others, with a space on the inner side to permit animals to move round. The maze was put into the aquarium in such a way that it lay between the fish and food which was introduced into the tank. The fish had then to move round two partitions in each direction in order to get the food. In first cases, the animals would rush straight at the food, and bang their heads on the glass. They initially took about 15 minutes to reach the goal, but after 50 trials the reaction times dropped to 10 seconds. After the reaction had become stabilised, a prism was introduced into the tank, on the path of the fish, so that as the animal passed, it would momentarily lose sight of the food. This had no effect at all on the performance. As the situation was in this case, fish had to move through two quadrants of the maze to reach its food. One animal went left in all cases, that is, clockwise. The food bait was then placed in the next compartment anti-clockwise, so that there were three compartments if the fish persisted in moving clockwise, but only one if it moved anti-clockwise. The

/animal

animal did in fact continue to go left, taking the long way round, although it could see the food at all stages of the experiment. Spooner next painted the edges of the glass with black paint, in the expectation that the task would be made easier, but this had little effect on the running of the maze. He also tried etching the glass, so that it was visible to fish, and the average reaction times fell sharply.

A further type of maze was used, rather like that used by Russell. It consisted of an horizontal glass bottle, of constant diameter, which was open at one end, and held some way off the ground. Food could be introduced via a dorsal pipe at the closed end, and a pattern of black criss-crosses on the glass, whilst serving to outline the bottle, did not prevent the food from being seen. Fish generally took about seven minutes to enter and take the food in initial trials, but the time fell to a few seconds in ten to fifteen trials. When fish were performing well, the tube was turned round, so that the entrance was at the opposite end to previous trials. Fish approaching from the same direction persisted in trying to enter from the LH end, and only with difficulty learned to use the RH end. Spooner concluded that vision was less important than kinaesthesia, a conclusion which has been noted for Churchill and Ono.

One animal was placed behind a glass plate, and food was dropped on the opposite side. The fish learned to go round the glass to get the food, but would use one way only, i.e. it would go left, never right, although the routes were of equal length. If the glass wall were made longer, the time taken to reach the food increased out of all

/proportion

proportion to the amount of detour added, and he supposed that the difficulty inherent in this type of behaviour was that at some point the animal had to move away from the food, and that this was a difficult task for the animals. Examining his findings in the light of current ideas on learning, Spooner concluded that fish showed too much variability in their solution of problems to allow consideration of such concepts as the Thorndike "Law of effect". (On the basis of this, movements which resulted in success should be printed into the memory, and others eliminated). He showed that the type and direction of movements used in the early stages of learning were very different to those used eventually.

Beniuc (1938) examined detour behaviour in *Betta splendens*. The fish was confined on one side of a coloured glass screen, in which was a circular hole. When a light had been switched on, the fish was allowed to swim freely, and a worm was dropped into the water on the far side of the plate. The fish behaved as noted by other workers using this sort of situation; it dashed at the worm, and collided violently with the barrier. After some repeats of this, it would run along the side of the glass, at varying levels, until by chance it came upon the hole. The animal would then generally back away from the hole, and then swim slowly through; once through it would rapidly swim to and eat the worm. Times to do this fell from 2½ minutes to 10 seconds in ten trials. Beniuc trained a number of animals to swim through the hole, and then presented the worm on the far side, supposing that the learning of the path should facilitate performance; in fact these animals took longer to reach the food than the totally

/naive

naive animals. Animals of the first group, which had been trained to a hole at the bottom of the glass on the right hand side, were then transferred to other barrier problems, where the position of the hole was different. Initially, all the animals swam straight at the bottom RH corner, and banged their heads there for some time. They then retired to a corner, and after some minutes, again approached the screen, though without the certainty of the first trials. As soon as they found the hole, in the process of exploration, they went into the other box, so learning was retained to this extent. After fish had mastered this task, they were all transferred to yet another position of the hole. All animals showed the same pattern of behaviour, in that they went first to the position of the previous (second) problem, then to the position of the hole in the first problem. Failing on these approaches, they swam off, but finally came back to achieve success. It seemed likely that visual and tactile cues predominated initially, but that positional cues were used once the task had been learned.

Beniuc then performed a L/R experiment very similar to that used by Spencer. A fish was presented with food behind a blue screen, such that it could reach the food either by going left or right. The food was presented in such a way that it was always quicker to go right. If the food was then placed at the left end of the screen, the shortest path was to go left, but the fish persisted in going right. This fish was then trained to go left, but with a partition of clear glass; it learned this quite well, and when the blue glass was replaced, and the animal required to go left again, it did so without any trouble. In his next experiment, Beniuc used the same glass screen, but it was

/placed

placed against one wall of the aquarium, so that fish could pass only on one side. An opaque plate was put against the open end, at right angles to it, so that fish had to negotiate an L-shaped detour. In initial tests, fish would go to the hole when they saw food introduced on the far side, but would retreat because as soon as they rounded the bend, they were out of sight of the goal. With more training, the task was learned, and the opaque plate was then moved so that there was an opening between the clear and opaque plates which allowed fish to make a short cut. Animals were presented with this situation, but showed no tendency to use the new path, though this was visible to them as they passed it on the detour. One of the plates used in Beniuc's first experiment was one with a hole high up. Fish had to learn to swim up, then down for food. When fish had been trained to do this, the water level was gradually lowered, so that in the end, the hole was just above the water. Several fish jumped from the water into the air, through the hole into the goal chamber. Naive animals could never be trained to do this. Beniuc's experiments emphasise the importance of known paths, and the difficulty of changing these when new and more accessible ways become available.

Sanders (1940) trained goldfish to swim into a goal box for food in response to a light signal given in the start box. Reaction times dropped sharply from 800 to 50 seconds within 20 trials. He then placed animals in a third box, which gave access to the original start box, and used an olfactory stimulus to induce animals to swim into that box, and into the goal box. His "second-order" conditioning will be discussed below. French (1942) trained goldfish to navigate a maze involving four L/R decisions, passing through four doors. The start box and the maze were brightly illuminated, to motivate animals, whilst the goal box was in dim light. Fish took about 30 trials to reach a criterion of 5/5 errorless runs.

Schiller (1948, 1949) using the mud-minnow Gambusia affinis, investigated delayed responses using a modified T maze. Fish were shown food through one of two windows, left or right, and were then allowed to swim up a central passage, to make a L/R decision at its end, and turn into passages leading back into the boxes with windows. Since only these windows were transparent, fish had to remember to which side to turn. It is unfortunate that Schiller used a strict alternation system, using one side until fish achieved success on that side. This introduced a considerable element of doubt as to whether fish were learning to remember during a delay or merely learning an alternate left/right problem. In a later experiment, he used delays of 4-5 seconds before fish were allowed into the choice passage, and this decreased the number of successful trials. Schiller went on to examine other maze problems, using the same animals as experimental subjects. A long tank was used, with a glass partition fixed along its length, abutting against the wall at one end, open at the other, forming a long "U". Fish were placed at the far end of one arm of the U, and food was placed at the other. Animals quickly learned to pass round the maze, and when they could do this, the relative positions of fish and food were changed, so that a fish having previously to go left round the U now had to go right. There was apparently no difficulty in doing this. For the next detour, he used a T piece with walls of glass, placed so

/that

that the base of the T abutted against the side of the tank. Food was placed in the compartment under one arm of the T, the fish under the corresponding arm opposite. Fish could learn this detour only with great difficulty, and when the relative positions of fish and food were exchanged, were unable to perform at all. If one top bar of the T was removed, to produce an inverted L, fish found this easier, taking about 90 seconds to reach the food. He then made the top bar opaque, and reactions times dropped to about 20 seconds, but rose again to 90 seconds as soon as the glass top wall was replaced. Passages to, or from, the inside of the arm of the L did not have the same reaction times, since the latter involved moving away from the food, and also had a longer time when the food was out of sight, since the fish had in the first case to turn away from the goal. Using a transparent top wall increased this difference, since the fish moving away from the food could see the goal throughout this period. When fish had learned well the detour using an opaque top wall, Schiller introduced the apparatus into a much larger tank. Though, of course, the length of detour was in no way affected, the reaction times increased considerably.

Zunini (1954) summarised his work on learning in fish, and referred to the use of a Russell-type bottle situation with minnows. The bottle with food inside it was initially placed on its base. Fish took long periods to enter, but times fell rapidly over the first ten trials, and thereafter remained low. Thinking to increase the ease of solution of the problem, he turned the bottle onto its side, but found that animals persisted in trying to enter from above. The reaction times of these animals were very high, and the rate of decrease of these was much

/inferior

inferior to that seen in the same fish on their initial learning; there was no sudden drop, but a slow decrease. Hale (1956a) used a Welty-type maze in his experiments on social facilitation, which will be dealt with in the section dealing with the effects of forebrain lesions, and he reported the use of very similar situations by Mussey (1942) and Hillowitz (1945).

Munn (1958) re-examined Schiller's work, quoted above, and used a U maze in his experiments. In this case the barrier, in the shape of a U, with a glass end at the base, was placed in a large tank; food was placed either inside or outside the U. Thus fish had either to enter or leave the inside of the U. Fish found more difficulty in leaving than in entering the U. In a later experiment, Munn used the same technique as Schiller, but with the introduction of a Gellerman alternation of side, so that no cues should be given by the temporal distribution of trials. Fish trained with this schedule did not learn very well, but fish which were trained in Schiller's conditions, with strict alternation of side, performed better than the previous group. Munn concluded that Schiller's results were due to extraneous cues, and that delayed learning was impossible. Although Munn did not specifically refer to the cue of alternation, it is obvious that this cue was the main one operative. On the other hand, as was stated at the beginning of this section, any maze situation of necessity involves delays between stimulus presentation and completion of response, so that to try to divide maze from delayed response behaviour is rather artificial. The problem is worth re-examination.

Warren (1960 and 1961) used a T maze, with lateral return corridors

/from

from goal boxes to the start box. This will be dealt with more fully below. Ingle (1965a) has used an ingenious continuous Y maze, which allows fish to be trained for long periods without the use of an extensive layout. At the end of each arm of the Y there is a circular turning chamber so that fish can be moved round, then back down the entry corridor. Glass doors can be dropped into position just inside the entry to each arm of the Y, so that fish collide with them if they try to enter. A constant turning, say to the left, is easily learned, but an alternation problem is more difficult.

D. Visual discrimination.

(I) Brightness discrimination.

Although, of course, the conditioned reflex and avoidance learning situations outlined above have been dependent on the discrimination of changes in level of illumination, there has been only one level of illumination, discriminated against zero or very low illumination, so that the magnitude of change has been enormous. In addition to this, the response has been a constant one. In the following experiments there are two intensities as well as the inter-trial intensity, and there are required different responses to these.

Graber (1885) placed specimens of Gasterosteus spirachia in an aquarium whose overhead illumination was arranged so as to give very different levels of intensity at different parts of the tank. Fish were not reinforced for any particular movements, but their distribution through the tank was noted. There tended to be a close correlation between light intensity and number of fish in that sector; animals would generally seek out areas of dim illumination in preference

/to those

to those of high or medium illumination, and would also avoid areas of almost total darkness. Hess (1909) trained specimens of Atherina hepsetus to feed at the brighter of two plates, presented simultaneously. Initial differences were that one plate was about five times brighter than the other, but fish transferred to lesser differences, and the smallest difference which was constantly discriminable was 1:1.23.

Mention has already been made of Bull's method of using a feeding bottle with light as the CS. When the subject, a wrasse, had learned to enter the feeding bottle when the light came on, there was presented at intervals a second stimulus, the onset of two lights, giving twice the previous level of illumination, which stimulus was not reinforced with food. The wrasse's first reaction was to enter the feeding bottle for every stimulus, but after about 15 trials it reversed this, and for ten trials, it made no responses. Over the next fifty trials, performance was mised, but the fish finally achieved an almost errorless level of performance. As Bull admitted, there was the possibility with this arrangement that fish were responding to the configuration one versus two lights rather than to degrees of brightness, so he introduced a transparent screen over the tank, and used a system of raising and lowering lights to produce changes in illumination. Using the same animal as before, and the same differences of light intensity, he found that there was no discrimination in this modified situation. He increased the intensity difference of the huge value of 7,420 metre candles, and found that the animal could perform well. Values of difference much less than this were generally not discriminated. Bull concluded that fish could not discriminate brightness to any extent, but it would be

/suggested

suggested that there are two points that he did not take into account. The first, and most obvious, is that he was using a sequential situation, whereas Hess, using simultaneous discrimination, found very good acuity. The second is that perhaps the motivation of the situation was wrong. "Go/no go" situations tend to need reinforcement values associated with both the stimuli; for example, to take a case outside the vertebrates, learning occurs faster in Octopus if food and shock are used, than if food alone is used, in Boycott and Young's "attack/don't attack" schedule. In addition to this, most workers have found that long reaction times are not suitable for use with these paradigms, so that Bull's 15-20 second CS/UCS interval may have been too long for the discrimination to be possible. Inhibition of response to the neutral stimulus, especially as it was unreinforced, would increase in difficulty as the CS/UCS interval increased.

Scharrer (1928) working with minnows (Phoxinus sp.) arranged a number of fish in a large tank, each separated fromits fellows by black partitions. The whole area was diffusely illuminated. A travelling light was so arranged as to slide over the compartments, and was operated by a soundless switch, to illuminate the area beneath it with a very thin beam. Although the fish were blinded, as Scharrer was investigating the other area responsible for the perception of light, he found that if he paired onset of the light, directed on to the top of the head, with administration of food a few seconds later, he could elicit feeding responses to the light alone after some days' training. A stable response was established after 20-30 trials, and changes in illumination and in the intensity of the stimulus light did not abolish the reaction.

/Herter

Herter (1930) using his feeding prong method of simultaneous discrimination, trained minnows to discriminate between two shades of grey. One fish, trained with light grey as positive, took 80 trials to attain 100% accuracy, another, trained with dark grey as positive, took 130 trials to reach the same criterion. Perkins (1931) avoided the difficulty of yellowing inherent in electrically-dimmed lights by using holes of various aperture in front of one light, which projected on to a milk-glass screen. Fish were presented with three compartments, each of which was baited with food. If fish entered the correct chamber, they were allowed to feed. If they made a mistake, a grid was raised from the floor, hinged at the anterior edge of the box, which both prevented them from feeding, and moved them from the box into the discrimination chamber. Two training schedules were used; a "static" one, where fish had to choose one of three levels of brightness, whose absolute intensity remained constant, and a "dynamic" one, where fish had again to choose one of three intensities, but the absolute values of these were changed, although their relative values remained constant. Fish trained to go to the bright light, in preference to the medium or dim lights, were on average slower in learning, but all fish, in both static and dynamic groups, attained nearly 100% correctness within 200 trials. The poorer learning of the bright-trained fish was accounted for on the basis of intensity preference. Test fish tended to gravitate into dim rather than light boxes if no reinforcement were provided, this bore out the work of Graber quoted above.

Von Schiller (1933) trained minnows to feed at the brighter of two lights presented simultaneously. Fish had to swim into one of two

/goal

goal boxes, each backed by milk-glass, and illuminated from behind by the same lamp. If fish entered the correct box, they were rewarded with food. Unfortunately, von Schiller appears not to have controlled for olfactory cues from food, and did not use dummy bait in his trials. Hager (1938) trained various fish with stripe discriminations, but his work may be mentioned here since he investigated the effect of contrast reversal on the learned responses. *Phoxinus*, *Xiphophorus* and *Macropodus* were trained with food rewards and simultaneous presentation. A discrimination of 3 from 4 black vertical stripes on a white background transferred at 90% correctness to the same number of stripes, but with white stripes on a black background. Privol'nev (1956) testing preferences of fish, used a simple maze, with two goal compartments, lit at different intensities. He found that in summer *Leucaspius* sp. tended to remain in the brighter of the boxes, but would seek out the dimmer in winter. These findings are interesting not only from the point of view of discrimination, but because they suggest that the preferences found by Graber and Perkins may have been of a transitory nature, and that tests at other times of year might have yielded opposite results. Warren (1960, 1961) trained *Macropodus opercularis* in the T maze described above. Fish were rewarded with a shrimp for going left to the white box, and were not reinforced for going to the opposite black box. In three groups of 24 fish, a criterion of 18/20 correct trials was attained in 23, 30 and 20 trials respectively. Since Warren was trying to make up an easy learning situation, rather than to study brightness discrimination, he confounded side and brightness cues, but there is little doubt from the speed of learning that the brightness cue was of

/considerable

considerable individual importance.

(ii) Colour discrimination.

The literature associated with colour discrimination in fish is voluminous, and it will be seen that not only has there been disagreement as to the acuity of the discrimination between wavelengths, but that most of the controversy has been as to the actual existence of that discrimination. Rather than deal in very brief fashion with each of the large number of papers on the subject, it is perhaps better to consider the more important publications in more detail. The basic difficulty throughout the half century since the first publication has been the problem of dissociating discrimination of brightness from discrimination of colour, and it is only recently that methods have been used which have proved colour vision beyond all reasonable doubt. That fish can discriminate red from green, for example, has been accepted for many years, and recent electrophysiological investigations have shown that cones in the retina respond maximally at wavelengths which suggest a trichromatic sensitivity similar to that found in higher vertebrates with colour vision (see, for example, Marks (1963) and Cronly-Dillon and Muntz (1965)). Neither of these approaches answers the question, since neither describes the subjective impression of colour on the fish, which may be just as difference of intensity.

The first experiment using colour appears to have been that of Graber (1885) who illuminated an aquarium with lights of different wavelength, so that different sectors were of different colour. The subjects, Gasterosteus spirachia, tended to migrate to the blue-illuminated areas, and would especially avoid those areas which were

/illuminated

illuminated with red light. Using just red and blue lights, Graber then increased the intensity of the blue light, to find the equilibrium value. When its intensity was twenty times that of the red light, there was equal distribution of fish in the red and blue illuminated sectors. He concluded that either there was colour vision or that there was a difference in the subjective intensities of the colours. On the basis of the light preference experiments described above, he concluded that the latter was more likely. Zolotnitsky (1901) fed fish Chironomus larvae, which are red. When he had established a good feeding response to these, he put cards outside the aquarium, visible to the fish through the glass. On these cards were painted representations of Chironomus larvae, in different colours on the same grey background. Fish darted at the red paintings in preference to the others. Washburn and Bently (1906) trained specimens of Semotilus atromaculatus to feed from one of two pairs of forceps presented simultaneously, the red pair being baited, the green unbaited. When the stimuli were presented without bait, fish went to the red pair, and continued to do so even when the brightness of the red was varied considerably. These workers considered that there was sufficient variation in intensity of the colours to allow only the explanation of colour vision. Reighard (1908) performed an interesting experiment, reminiscent of Zolotnitsky's, but conducted at sea. He was studying the effects on fish distribution of the colours of the nearby coral reefs, and at one point dropped bait of different colours into the sea. He observed that fish took blue-coloured food, but would shy away from any that was coloured red. He changed the brightnesses of both

/colours,

colours, but this did not affect the preference of the animals.

Hess (1909, 1911, 1912, 1913) performed a series of experiments in which he attempted to show that the results obtained by these and other workers were capable of explanation on the basis of brightness alone. In view of the difficulties outlined above, his criticisms have for long gone unanswered. One of his first experiments was to repeat the work of Graber, using a tank illuminated by red and blue light. His fish, Atherina hepestus, went immediately to the blue area, and, like Graber, he had to increase the intensity of the blue light many times before there was an equal distribution of fish in both halves. On the basis of his work on brightness discrimination in fish, Hess concluded that the cue operative was that of intensity, and that there was no need to introduce the additional complication of colour vision. He next arranged the projection of a prism into a tank, so that there was a spectrum along its length, covering the whole area of the floor. Fish aggregated in the yellow/green portion of this; Hess blanked out this part of the spectrum, and reflected the remaining portions to fill the space. Animals went to the blue segment. When this and the purple areas were removed, fish migrated through the orange bands into the red end. In a subsequent experiment, he placed fish in a tank, where there were two areas of illumination, one of white light, the other of variable monochromatic light. The intensity of the white light was altered until there was an equal distribution of fish in both halves, the assumption was then that the white light had attained the same subjective intensity as the monochromatic light. He found that the yellow/green area was the brightest, that is, it required the least illumination from the white light to promote mixing in the two sections, and that, increasing through blue and orange, it was red that needed the most illumination to force fish to leave the white to go to the monochromatic area. (In the case of the yellow/green light, fish tended to be in the coloured area, in the case of red light, they tended to be in the white-illuminated area). Hess considered that these results were conclusively in support of the theory of brightness discrimination of colour. The alternative explanation was of course, quite as valid, that fish were responding on the basis both of intensity and of colour preference, and that considerable increases in white light, which would normally cause a prompt aversive action, were tolerated rather than enter an area of disagreeable monochromatic light.

Bauer (1910) put light-adapted <u>Charox</u> and <u>Atherina</u> into a tank illuminated with red and blue lights in separate areas, and found that all the animals went to the blue section. When a spectrum was placed across the tank, fish sought out the yellow/green area. If a red light was then flashed into the area, all the animals showed flight reactions, which were not manifested to other colours at varying intensities. Bauer suggested that some of Hess's negative results were due to the use of dark adapted fish, and when Hess (1911) re-examined this, he found that sensitivity to light increased thousandfold when fish were left in darkness for 15 minutes. Fish showed no reaction to the red/white situation when they had been dark-adapted, but as their degree of light-adaptation increased, they tended to migrate into the blue area. Hess also repeated Zolotnitsky's <u>Chironomus</u> experiment with dark and light adapted

/fish,

fish, and found that the latter fish responded more to colour.

Von Frisch (1911) placed fish in a tank with boxes of various colours, and found that they hid in the yellow/green ones in preference to the others. He next trained animals to feed from a coloured tube, placed horizontally on the floor of the aquarium. When they made this response well, he introduced a series of grey tubes, painted all shades from white to black. Fish immediately went to the coloured tube, and persisted in doing so even though the position of this was varied, and if there was no bait in it. The introduction of other tubes, of different colours, had no effect on the response. In a later experiment (1913) he fed fish yellow-coloured bait, then used scraps of grey and coloured paper dropped through the water. Fish dived only at the yellow scraps. He also trained fish to feed from a coloured funnel held in mid-water. When the response was well established, he put in a number of other funnels of different colours and shades of grey. The animals went only to the original coloured funnel.

Hess (1912) duplicated part of this work of von Frisch before it was published, and introduced his own modification. Instead of dropping the bait, then changing to the paper, he used the two simultaneously, and found that there appeared to be no difference in takes of bait and paper. This whole experiment is rather peculiar; one would imagine that since paper sinks more slowly than worms, it would have been quite easy for fish to tell the difference on that basis alone. On the other hand, if the two did fall at the same speed, the situation might rest merely on which of the two pieces was nearer the fish. Hess does not appear to have controlled for this. In a subsequent paper (1913) Hess

/reported

reported that he had observed a red disc at various depths in a lake, and found that it soon lost its colour. Whilst this was of interest, it contributed nothing to the question of interpretation of the laboratory experiments. Hess concluded from his series of experiments that since the curve of "subjective intensity" of light against wavelength resembled that for colour blind humans, there was no colour sense in fish.

Freytag (1914) initiated a new approach to the problem by trying to adapt minnows to backgrounds of various colours. He achieved a measure of light adaptation to grey backgrounds in periods of a day, but there were no responses to coloured backgrounds within this period. The times allowed for these changes were so short that it is not surprising that he did not attain much of a response to the greys, for complete adaptation often takes a matter of weeks. Mast (1916) made a more reasonable attempt using this method. He used fish which could make colour, rather than darkness responses, the flatfish _Paralichthys_ and _Amylopsetia_. Fish were given six weeks to adapt to floors of one colour, and were then transferred to tanks which had areas of floor of two different colours, one of which was the original hue. Blue-adapted fish went to blue on 88% of choices, green-adapted fish went to that colour 70% of choices, but red-adapted fish went to red backgrounds only 26% of the time.

Goldsmith (1914) used photographic plates to equate the intensity of her colour sources, and trained fish to feed from areas illuminated with either red or blue lights. Reeves (1919) trained fish to feed at one of two coloured patches, the intensities of which were initially very different. At the same time she gained some measure of brightness discrimination by training dace (Semotilus atromaculatus) to go to the dimmer of two lights. This was not easily learned, so she provided a red light as one of the stimuli, keeping the other white. The fish preferred the white light, and though she changed the intensity of both lights considerably, persisted in going to that light. Reeves thus gained some measure of brightness discrimination, and appliedthis to the colour situation. The very disparate brightnesses were slowly equated, until discrimination broke down; fish were then re-trained. She assumed that discrimination was at first on the basis of the brightness cue, but after the cue became insufficient, when the responses broke down, fish learned using colour as the cue. As McCleary and Bernstein (1959) have remarked, if colour can be used as a cue, and if the discrimination of brightness is so bad (a point which is not accepted in view of evidence above) why did the discrimination break down at all? In addition to this, the re-training could as well be improving performance on brightness discrimination as starting training on colour, so that the experiment, though suggestive, is not at all conclusive. Reeves also made a number of observations on unconditioned responses to lights of various wavelengths. In Umbra limi, the mud minnow, she found that a basal respiratory rate of 60 per minute was elevated to 85 in the presence of white light, but that red light caused a massive increase to 150 per minute. Other lights, of varying wavelegth and intensity, did not have this effect.

White (1919) attempted to train dogfish to discriminate between different colours, but, as in so many of these experiments, there does

/not

not seem to have been adequate control for brightness cues. Schiemenz (1924) used Goldsmith's method of equating intensities photometrically, and found evidence for discrimination of red and blue. Wolff (1925) used a spectral method, but both of these experiments, though there were attempts at control, were open to Hess's criticism that although it might be possible to make objective physical standardisations, it was very difficult to know what the subjective experience of the fish made of them.

Bull (1928, 1935a) in his series of experiments using the feeding-bottle method mentioned above trained a specimen of Crenilabrus melops to swim to the feeding bottle when a white light was presented, and to inhibit this reaction when a yellow light was shown. A second animal was trained with a green light as the neutral stimulus, a third with a red light. All three animals quickly formed associations of some durability, but Bull did not control for brightness to any very great extent. On the other hand, since he had found using this method, that brightness discrimination was poor, he was probably quite justified in assuming that the performance was due to the use of colour cues. Froloff (1928) found that Tinca vulgaris could form a differential response to conditioned light stimuli. The presentation of a white light was reinforced with shock after a 2-3 second CS/UCS interval, but the presentation of a red light was unreinforced. After about 100 trials, there was developed a strong reaction to the white light, but none to the red light. He also produced differential conditioning to red and green lights. Janzen (1933) and Nolte (1933) both trained fish to discriminate between various colours, the former using paper targets,

/the latter,

the latter, the projection of a spectrum.

One of the first workers to develop a good method for assessing colour vision in fish was Herter. In his (1950) paper dealing with the subject, he described the use of a new technique of "simultaneous colour contrast". This relied on the fact that if a grey square be placed on a background of a colour, the grey tends to appear to humans to be coloured, the hue being determined by that of the background. The rule of complementary lights applies, so that, for example, a yellow background makes the target appear blue. Herter trained his animals to discriminate red from blue, and did not take elaborate precautions against the possibility of brightness cues. He roughly equated the brightnesses by personal estimation. When fish had attained 90% correctness, they were transferred to the second stage of the problem. Large yellow and green squares were presented side by side, with grey squares of equal area and brightness in their centres. To colour-visioned animals, it would appear that the grey square on the yellow was blue, and that on the green, red. No brightness cues could indicate which was which, and only animals with colour vision could "see" the colours in the grey squares. All his fish went to the previous positive shape, and there were no difficultiss experienced. Controls for the detection of bait appear to have been adequate. The only criticism that can be made is that there might have been a difference in brightness of the green and yellow which fortuitously matched that between the red and blue. Hess's results for the subjective "brightnesses" of colours, mentioned above, would not support this, since blue/red would be the most extreme brightness discrimination, and yellow/green the very least.

/Thus

Thus this work gives very good grounds for supposing that fish can see colour.

Hurst (1953) trained Lepomis macrochirus in the following manner. A box was let down into the water of the training tank, and a light switched on. If a red light were presented, it signified that there was food in the box; if a green light were presented, there was none. He introduced a white light also, so that he could vary the intensity and gain some measure of brightness cues. Very quickly, fish learned to go to the red-illuminated box, but would also go to the green box, although they would generally avoid the white situation. After 100 trials without much improvement in discrimination, Hurst transferred these fish to a simultaneous discrimination problem. Two boxes were presented, and were illuminated with the colours used above, sides being alternated in a Gellerman sequence. When fish were consistently entering the red box for food, the intensities of illumination of the boxes were changed, the greatest level of illumination from one light being 40 times its lowest. Over 101 trials, with this variation, 94 responses were made to the red box, only 7 to the green box. From time to time, the colour filters were removed from the lights, and fish tested with lights of different brightness. No rewards were given. There were 12 responses to the brighter light, 16 to the dimmer light, and 2 failures to respond. In view of the known preference of fish for dimmer situations, this slight bias in favour of the dim light can be discounted, and it would seem that there were no brightness cues being used.

Zunini (1954) trained minnows to feed from funnels of different colour, but though he made some attempt to control brightness, this was not very conclusive. Similarly Privol'nev (1956) used an Ono-type choice

/situation

situation with marine fish, and found that most animals went to the blue-illuminated boxes, but he did not prove that this reaction was on the basis of colour.

McCleary and Bernstein (1959) made perhaps the most conclusive test of colour vision, using goldfish as subjects, and the heart-conditioning technique of Otis, Cerf and Thomas (1957). Fish were immobilised by rods passed transversely through the dorsal musculature, which were clamped to the sides of the tank. Shocking electrodes were placed on either side of the animal, and leads running through the body wall into the pericardial cavity, and exposed at their distal ends, served to allow the electrical activity of the heart to be recorded. Stimuli, coloured squares, bobbed up and down at the end of thin plastic rods, were presented laterally. The positive (punished) stimulus was presented for 5 seconds, and a 2 second shock was given 3 seconds after the presentation of the shape, so that shape and shock were removed simultaneously. The neutral stimulus, whose presentation period was 10 seconds, was unreinforced.

In the first stage of the experiment, fish were trained to discriminate between dark red and light green, or vice versa. Unreinforced test trials were run every ten trials, and it was found that there was a differential response to the colours within 15-35 trials. Presentation of the punished colour caused a drop in heart rate, presentation of the neutral colour might cause some drop, but not nearly as much. After this, fish were tested by unreinforced presentation of two grey squares, differing markedly in their brightness. One was a dark grey, the other a light grey, both shades being extreme enough to be easily discriminable from the grey wall of the tank. Fish reacted to these shapes on the

/basis

basis of the brightness difference given in their previous training, that is, a fish trained with light red positive would show a deceleration of the heart when presented with light grey. This indicated that there was a brightness difference between the two greys which was comparable with that experienced by fish presented with the two colours of disparate brightness.

A second group of fish was trained to discriminate between coloured squares having equal brightness as seen by human observers. Once a constant level of performance had been achieved, they were presented with both of the pairs of coloured squares used in the first experiment. Thus, for one pair of presentations, there would be reversal of brightness and positivity as compared with the other, and if brightness cues only were operative, it would be expected that there would be a different response to one pair of shapes than to the other. (The previous tests had established that there was such a brightness difference). In fact, all fish showed a deceleration to the positive colour, irrespective of its brightness. Bernstein (1961a & b and 1962) went on to use this technique further, but his findings were directed more towards investigation of interocular transfer and forebrain function, and will be dealt with below.

Arora and Sperry (1963) trained <u>Astronotus ocellatus</u> to jump out of the water to reach food suspended just beneath stimulus floats. A variety of colours was used, but in the absence of controls for brightness, this experiment, like so many others, was only partial proof for colour vision. Ingle's (1964b, 1965c) and Shapiro's (1965) papers on colour vision in goldfish will be referred to in later sections.

Muntz and Cronly-Dillon (1966) quoted electrophysiological evidence for the existence of three types of colour receptor in the fish retina (for example Marks (1963)) but concluded that this was not proof for colour vision, since the information might be discarded centrally. They used a simultaneous discrimination method similar to that devised by Meesters (1940) which will be described in more detail below. Coloured squares were placed on the far wall of the box, and feeding troughs were placed at the centre of each square. (Fish were pre-trained to feed from these troughs). Shades of blue, green and red were so selected that their reflectivities throughout the visible spectrum were different, so that, for example, dark red was darker, or less reflective than light green whatever the wavelength considered. Three shades of each colour were used, dark, medium and light. Fish were trained with various combinations of the pairs of colours, so that at one point the brightness bias would be to one colour, at the next trial period, the other colour would be "brighter". If brightness were the only cue operative, fish should not be able to perform the discrimination. Within 50 trials, at 10 trials per day, fish attained 90% criterion on their initial problem, and change of brightness did not affect this. Muntz and Cronly-Dillon also controlled for olfactory cues by baiting both shapes; in actual trials food had been placed in the positive colour's holder, and a small similarly-coloured pebble in the other. Thus, generally, there has been much work on this problem which has not answered the question. The work of Herter, McCleary and Bernstein, and Muntz and Cronly-Dillon shows that this perception is present; it remains to find the limits of acuity.

(iii) Shape discrimination.

There has never been much doubt that fish can respond differentially to different shapes, indeed, the first observations on this topic must lie long ago, since anglers have used lures for many years. Nevertheless, some schools tended to ignore this sense, and to concentrate on that of smell as the main perception of fish. That modality will be dealt with below, but evidence from the behaviour of fish in maze situations should suggest that there is much accurate use of visual information. The first part of this section will deal with the discrimination of planar shapes, a second and much shorter section will deal with the small amount of work done on stereometric shapes.

Whilst most of the workers reported here used the same shapes in different orientations, or shapes of equal area, one or two did use shapes of unequal area, so that the discrimination became one of size as well as shape. Schaller's (1926) work with the shapes mentioned, and 'Maes' (1929) work with doors of different shape are open to this criticism. Most of the work using stripe patterns has used shapes of the same size, irrespective of density of lines, so this would seem satisfactory. Some of Meesters' (1940) stimuli would not be of equal area, but his generalisation tests were of such magnitude of difference from originals that it seems hardly likely that area cues can have been operative at those stages, if they were initially. The same remarks apply to Herter's (1949) observations.

Goldsmith (1914) trained fish to feed from pointed forceps, and when this habit was well-established, she presented them with pairs of glass probes, with ends of different shape. Fish responded to rods with pointed ends, either abrupt or tapering points, but did not go to

/rods

rods with square, round, or two-pointed ends. She concluded from this that since trials were unreinforced, fish were responding on the basis of similarity to the feeding forceps, and that the cue was visual. Schaller (1926) performed a series of experiments with minnows. Food was placed under one shape, and a piece of food soaked in a bitter solution under the other. The paper is unfortunately rather rambling, and it is not clear that he made the appropriate controls for positioning, and for olfactory cues. Though no numbers of trials are given, he obtained discrimination between circles and triangles, circles and five-pointed stars, and between rectangles and squares, apparently of equal height. These were all learned to a high percentage accuracy.

Herter (1929) trained minnows, swordtails and dogfish in an extensive series of tests of fish's visual acuity. All three species could discriminate between a square and a circle, but the speed of learning was greatest in the minnow, least in the dogfish. Gobies took about 120 trials to learn to discriminate between  and  , reaching 100% correctness after this period. Perch learned to discriminate a black "R" from a white "L", reaching 100% in 100 trials. Minnows took about 140 trials to reach 100% on the discrimination of a circle from an ellipse with its major axis horizontal. Herter's simultaneous discrimination method was good, and his controls for olfactory cues emanating from the bait were negative.

Thus it had been proved that there was quite acute vision in fish, and Herter (1930) set out to investigate its limits and its possible method of working. He trained minnows to feed at the longer of two horizontal rectangles, which were of equal width. Fish were then

/presented

presented with two rectangles of equal length, placed over a perspective drawing of radiating lines in such a way that to human observers the rectangle nearer the focus of the lines appeared longer than that further away. All fish went to the rectangle nearer the focus, even though neither shape was rewarded. Other fish trained with the same rectangle problem were transferred to the Müller-Lyer illusion, where two lines of identical length appear of different lengths on account of inward- or outward-pointing lines at their angles. Again, the fish responded in the same way as humans, responding to the figure with the out-turned lines as being the longer. In a third experiment of this type, testing the similarities of vision in fish and higher vertebrates, Herter trained minnows to feed at the larger of two black circles, painted on a white background. Test shapes were two black circles of identical diameter, one surrounded by five large open circles, the other by nine small open circles. The solid circle in the latter situation seems to humans to be the larger, and it was found that this was also the case with fish.

Maes (1929) first made tests to determine whether her fish could discriminate between bait, *Arenicola*, and models of the worm. The fish, *Gasterosteus* and *Epiroches*, were unable to do so, and she concluded that since this was so for stimuli placed at some distance from the fish, such bait could be used in trials without the fear of visual or olfactory cues interfering with the discrimination of shapes. When, on the other hand, fish were presented with the bait, and also with shapes such as squares, diamonds, triangles and circles, they made no mistakes, and ignored thest last stimuli. Maes also performed an experiment similar to that of Goldsmith. Fish were fed from a pair of blunt-nosed

/forceps,

forceps, then presented simultaneously with these and with a series of test shapes, all approximating to the forceps, but having differently-shaped ends. Animals went to the forceps and to the two shapes which represented respectively front and side views of the forceps. Square-ended, thinner, or pointed models were generally ignored. The discrimination was still present if shapes were presented successively. She then went on to more elaborate trials, and found that fish could learn rectangle versus circle or triangle within 100 trials, and that diamond versus cross and triangle versus rectangle, both white on a black background could be learned within 80 trials.

Maes next used a maze situation for shape training. Fish were presented with a barrier, through which they had to swim to be rewarded with food on the other side. There were two holes in the barrier, and fish received food for swimming through one of these. Epiroches took 200 trials to learn to pass through a hole of U-shaped cross-section, and to ignore a hole of the same sort of shape, but with a triangular piece taken out of the upper curve of the base of the U. After this had been learned, one fish passed 7,191 times through the positive door, and only 13 times through the negative one. She used other pairs of holes, such as square versus U on its side, triangle versus square, and square versus diamond. All these were learned more easily than the first discrimination. In one aquarium, there were swimming several specimens of Julis vulgaris. Maes placed two blocks in the tank, which had holes of different cross section running through them, one rectangular, the other triangular. In the centre of the triangular tunnel, she placed a blade, so that fish swimming through might tend to prick themselves. The rates of movement through the two tunnels soon became very different, and very

/quickly

quickly fish learned to pass through the square tunnel, and to avoid the triangular one. Maes gives no indication of the length of the tunnels, so it is quite possible that fish could see the blade, and make avoidance on this basis. She experimented with a large number of barriers using the maze and feeding method outlined above, and found that her fish had a surprisingly good ability both to learn and to retain.

Rowley (1934) trained goldfish to go to one side or other of a T maze in response to a simultaneously-presented pair of shapes. Fish were rewarded with food when they had made the turn. They were initially trained to a criterion of 24/25 on a problem involving the discrimination of two sets of stripes, the separation of whose lines differed by 1 cm. When this easy discrimination had been learned, she transferred various fish to problems involving separations of 8, 7, 6, 5, 4, 3 and 2 mm. In the easier problems, there was almost no fall-off of response, but as the separation of the lines became less, the loss of performance became greater, and the time for re-learning longer. In almost all cases re-learning could occur, and the fact that fish could re-learn the most difficult problem whilst others could not re-learn the 4 and 3 mm. problems suggests that individual differences were playing a large part.

Rowley next trained fish to swim to the larger of two illuminated circles, differing in diameter by 2 cm; she used food reward and Gellerman alternation of stimuli as before, and trained fish to a criterion of 24/25. Animals were then transferred to discriminations involving differences in diameter of 1, 0.5, 0.3, and 0.2 cm. In all cases except the last one fish performed at 100% after various amounts

/of re-training.

of re-training. In the 0.2 cm. problem, out of nine fish trained, only two managed to reach a criterion of 12/12, and Rowley concluded that this sort of size difference was on the limit of the fish's visual acuity. Since in this last case the stimuli were illuminated discs, she tested for brightness cues, and found that altering the brightness of the shapes had little effect, except in the poorly-discriminating 0.2 cm. group.

Hager (1938) performed a series of experiments with minnows, training them on problems involving fine discriminations, such as 3 versus 4 vertical stripes. This was learned to 85% in 120 trials. Fish also managed to learn 5 versus 6 vertical stripes, but this took longer, 200 trials to attain 80-90%. Problems involving horizontally-arranged stripes were much more difficult, and it took fish on average 90 more trials to learn 3 versus 4 horizontal stripes than it took to learn the same problem with vertical stripes. A complete circle was discriminated from a broken circle without difficulty; mention has already been made of Hager's experiments showing that a discrimination, once formed, could be transferred through a contrast change without loss of performance.

Meesters (1940) was the first worker in the field of fish behaviour to introduce really precise and repeatable methods into the study of discrimination. All previous methods involved the introduction of shapes into the water, and allowed fish to see the shapes an unspecified time before they could make responses. Meesters' discrimination box dispensed with these methods, and allowed fish to be restrained whilst shapes were changed. A retention chamber gave access, through a small

/door

door, to the discrimination box, in which the two shapes were displayed, on the far wall, with food either in troughs at the base, or attached to the shapes. The latter method was more generally used, since it gave more contiguity of response and reward. Meesters conducted an extensive series of investigations into generalisation and discrimination which were too considerable to allow of full treatment here, and can in any case be summarised by consideration of a few cases. For example, he trained minnows to feed at the larger of two white outline squares on a black background. When contrast was reversed, the percentage perfomance dropped from 95 to 83. Returning to the original configuration, he progressively removed various parts of the squares, to try to find the essential areas. If only the LHS vertical side of each figure were left, the level of performance was hardly affected, but if only the upper horizontal lines were left, there was more considerable impairment, although fish could still discriminate between the two shapes. This phenomenon of greater acuity in vertical than in horizontal directions had been noticed by Hager, in the work quoted above. In other series of discrimination trials, Meesters used a triangle versus a square, and a black diamond versus a black cross. He manipulated these shapes as before, and rotated them. Generally, the level of discrimination was good, and animals could still distinguish between the shapes even when quite gross changes were made to the figures, providing that some essential feature, such as difference in length, were left intact.

Schreiner (1941) used a simultaneous discrimination method to train minnows to discriminate between red squares of side 0.5 and 2 cm., placed on 4 cm. squares of white paper. A Gellerman sequence was used

/to control

to control position cues, and food was given only after a response had been made, so that olfactory cues could not be present. Correction was allowed. In view of these precautions, it is surprising that his fish took so long to learn this very simple discrimination; they took 500 trials to reach 88% correctness, which suggests that there must have been other factors operative. Schreiner's work is interesting from the point of view of effect of external conditions on learning. So often animals are trained, with the assumption that time of day, or weather conditions have no effect, and it is rarely that the former, especially, is tested, though in view of rhythms this ought to be done if animals are trained at irregular times during the day. Schreiner showed that in minnows at least, the time of day appeared to have no effect on the learning or retention of a discrimination, and that weather, including thunderstorms, wind, and rain, appeared to be equally ineffective.

Herter (1949) trained fighting fish (Betta splendens) to discriminate between a cross and a circle; they took 60-90 trials to reach 100%. He then used the following generalisation situation: fish were presented with two shapes; a circle, whose diameter equalled that of the cross, which diameter was gradually decreased, exposing the four arms of a cross, until only the cross was visible; and a cross, from the centre of which there was gradually built up a circle, till only a circle was visible. From results from these tests, Herter concluded that the fish were paying attention to the edges of the cross, since they would discriminate between a circle and a circle with the smallest extension of the four arms of the cross. As soon as these vanished, the level of performance fell to chance percentages. Kettner (1949) trained fish

/using

using stripe discriminations, and found the same limits of acuity as had Rowley, in the paper mentioned above. Sperry and Clark (1949) in investigating interocular transfer in Bathygobius soporator, trained fish to take the higher and smaller of two stimuli, the upper one being food, the lower, a rubber lure. Whilst this work is of importance because of the findings on transfer, there were so many possible cues that its importance as regards visual acuity is rather doubtful.

Schulte (1957) in an extensive series of experiments investigating generalisation and interocular transfer, used fish trained both monocularly and binocularly. His figures for trials to criterion are high compared with those of workers such as Herter, and it may be supposed that this is due to his technique, and also to the fact that minnows are superior to goldfish in their learning ability (Savage, unpublished observations). For example, fish took 625 trials to attain an 80%+ level of performance on a problem of cross versus circle, which minnows learned in about 60 trials in Herter's experiments. Fish trained to discriminate between different numbers of stripes transferred successfully to problems involving more or less stripes, as long as there was a similar difference between the numbers of lines. Animals trained with a diamond and a square responded satisfactorily if the edges of the shapes were rounded off, if the shapes were made thick outlines instead of solids, if they were changed in size, or if only the bottom half of each shape were shown. On the other hand, if one shape were painted bright green, if both were represented as thin outlines, or if both were rotated through 45°, the discrimination broke down. Similar observations were made for stripe patterns; for example, as noted above, there was no effect from changes

/in number

in number of stripes, but if one series were kept vertical, and the other made horizontal, the discrimination tended to break down. Introduction of irregularities over the stripes, such as crosses, or squares, or the use of wavy stripes, had no effect. Very similar results were obtained for an outline circle versus a similar circle with a cross across its centre.

Wodinsky and Bitterman (1957) whose work will be dealt with in more detail below, trained _Tilapia macrocephala_ to press bars to obtain food; the bars were painted with horizontal or vertical stripes. Fish very quickly learned to press the correct bar. McCleary (1960) trained goldfish using the heart deceleration and avoidance methods outlined above. He found that fish could show a conditioned heart deceleration to one of two fishing lures within an unspecified number of trials. Using the avoidance method, he trained animals to avoid one shape, the CAS/UCAS interval being 5 seconds, and to remain still for the other shape, this being presented for 10 seconds. His fish learned this to a 9/10 criterion within about 110 trials. Botsch (1960) repeated most of Schulte's experiments, and confirmed them. He also used a problem involving four dots in a vertical line versus four dots in an horizontal line, and showed that there was good transfer to other problems, as long as there was some semblence of linearity. This paper will be dealt with in more detail in a later section.

Hemmings and Matthews (1963) trained _Tilapia macrocephala_ in a simultaneous discrimination situation where fish had to pass through holes of different shape to obtain food. In the first case, square versus circular holes was used, and over 250 trials with six animals,

/no signs

no signs of learning were seen. It has been suggested (Noble, personal communication) that the difficulty was in analysing the shapes in their entirety, but whilst this may be true to some extent, it is doubtful if it is the whole explanation, since, as seen above, Maes managed to train fish to swim through holes of different shape. In a second experiment, they used an operant technique which will be described in more detail below. They trained the fish with food as reward for bar-pressing, but could obtain no sign of discrimination with the square/circle problem, except in one animal. The five animals used in this experiment were then transferred to other problems, such as rectangle versus circle or square versus triangle, and these fish attained a high percentage correctness within 60-70 trials. This evidence would suggest that the square versus circle problem has a peculiar difficulty, but on the other hand, minnows have been trained with this discrimination without too much difficulty (Savage, unpublished results). Also, in view of the fine discriminations shown by fish in the experiments quoted in this section, it would seem unlikely that there should be this inability.

Mackintosh and Sutherland (1963) trained goldfish to discriminate between horizontal and vertical rectangles, using a box very similar to that devised by Meesters. Using a criterion of 18/20 correct trials, they found that fish took 60 trials to reach this if the vertical were the positive shape, and 80 trials if the horizontal were positive. Fish were transferred to larger and smaller shapes, and it was found that performance was better to larger than to smaller shapes. Fish trained with black shapes on a white background transferred to white shapes on a black background, but fish trained with this latter situa-

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tion could not transfer as well. Other animals were trained on the original problem, then were presented with shapes rotated through 5, 10, 15, 20, 25, 30, 35, 40 and 45°. Discrimination remained above 80%, with negligible deviations from 0° rotated fish, up to a rotation of 35°. The performance figures for rotations of 40 and 45° were near chance level. Further animals were trained ab initio with two rectangles at 45°, and the rate of learning was much inferior to that of the previous 90° problem. Animals which learned this were given tests where only one shape was presented at a time, the other side of the apparatus having only a feeding trough. Fish responded to the positive shape, and not to the negative, which showed that fish were not responding to the overall configuration of "V" versus an inverted V. There was some initial difficulty, which was probably due to the loss of this cue, and to the tendency to go to a shape, rather than to the trough.

Matthews (1963) used the operant technique mentioned briefly above to train fish to discriminate between a triangle and a circle, to a criterion of 80%. He then presented various modifications of these shapes, such as a triangle with a rounded apex, and a triangular shape with a rounded base. No reinforcement was given on these trials, and all combinations of these and the original shapes were presented each day. There were significant differences between responses to all shapes except the circle and the "pear shape". This suggested that the point of analysis was the base of the triangle. In the next experiment, the circle was omitted after circle/triangle training, and 180° rotations of the other three shapes were used, as well as those shapes in their original configuration. Pairing of an inverted triangle with either of

/the other

the other two shapes in either of their rotations produced a deficit in performance. Thus the suggestion that the base of the triangle was the important factor was supported by the data for upright shapes, but not by that for inverted shapes. In a third experiment, the pre-training was as before, but the number of trials was increased from 100 to 150. Test stimuli were a triangle, a pentagon, a hexagon with 90° angles at top and bottom, a square and a circle. Again, no reinforcement was given during test trials, but fish were fed immediately afterwards. Triangle was discriminated from all other shapes, but all other combinations except square versus hexagon gave near-chance results. These animals were transferred to angle-discrimination problems, and it was found that an inverted V with an 80° angle was not discriminated from a straight line. This does seem rather unlikely, in view of other workers' results, but perhaps the animals used and/or the training method were not suitable. Matthews concluded from his work that these findings could not easily be reconciled with Sutherland's hypothesis of analysis of vertical and horizontal extents.

Ingle (1964b) performed a most interesting experiment studying the transfer capabilities of goldfish. He used McCleary's avoidance shuttlebox, and trained some fish to avoid broad versus narrow black on white stripes, other fish to avoid red versus green squares. When all fish had attained a high level of accuracy, they were presented with broad red on white versus narrow green on white stripes, such that the positive stimuli were on the same plate. Fish had 5-6 trials with these stimuli, and were then transferred, so that stripe-trained fish went to the colour discrimination, and vice versa. Four animals trained on each of these problems showed nearly perfect transfer. In addition to this, Ingle trained fish with a black dots on grey versus grey dots on black problem, gave 5-6 training trials with black vertical stripes versus grey horizontal stripes, and found that there was near-perfect transfer to vertical versus horizontal black stripes. Ingle supposed that fish had not evolved a mechanism for selective attention, so that they tended to analyse a situation as a whole. Thus "irrelevant" data could be used very quickly, since it was already in the memory. This suggestion would not really seem fair, since it has been seen that not only do fish respond when small parts of the shape are all that is visible, but that when these parts are removed, the ability to discriminate is often impaired, even though much of the shape may be left. It would be interesting to see if this speed of transfer could occur in non-avoidance situations, since it may well be the speed of decision needed in the situation which facilitates such rapid learning. Aronson and Herberman (in preparation) used an operant method to train <u>Tilapia</u>, and found that there was good discrimination between vertical and horizontal stripes. Shapiro (1965) used a very similar situation with goldfish. Fish took about 120 trials to learn the problem when the shapes were presented successively, and 80 trials if the situation were a simultaneous one.

Mackintosh, Mackintosh, Safriel-Jorne and Sutherland (1966) investigated the effects of overtraining on reversal and extinction in goldfish, using the well-tried simultaneous vertical versus horizontal problem. These experiments were designed to test Sutherland's (1964) model of discrimination learning. In this, discrimination was seen as a two-part process, analysis of the shape, and association of this with a response

/ponse

ponse. In initial learning, switching-in of the response occurs faster than switching-in of analyser, so that learning should extinguish fast on account of the inadequacy of the analyser. Reversal should take a long time because the analyser is not sufficiently switched-in to allow change of response with any ease. If animals are over-trained, the analyser is switched-in, and extinction is lengthy. On the other hand, since the analyser mechanism is now well established, responses can be changed without affecting the stability of the analyser.

Mackintosh et al. set out to test this hypothesis. In their first experiment, they trained fish to discriminate between a benzene ring shape and one like a butterfly; some fish were taken to an 18/20 criterion, then reversed, others were given 100 trials' overtraining, then reversed. It was found that the overtrained fish did not reverse with more ease than the trained animals. They explained this by supposing that there was not the assumed disparity in strengthening of analyser and response mechanisms, so that "noise" in the response systems of overtrained animals prevented any speeding-up of the reversal as compared with trained fish. They performed a second experiment to test this idea. Fish were trained to discriminate between vertical and horizontal rectangles, which were both white at one presentation, both black at another. Some animals were trained to criterion of 80-90% over 65-85 trials, others were given 100 trials' overtraining. All animals were then transferred to a problem where a black/white choice had to be made, and where the vertical and horizontal properties were present but irrelevant. Finally, all fish received twenty test trials, for ten trials, black, for ten white, horizontal versus vertical rect-

/angles

angles, and any choice was rewarded. Overtrained fish made significantly fewer responses than trained fish to the original positive shape when both groups were transferred to the black/white problem, and this was explained by assuming that since the different tasks used different analysers and the analysers of the overtrained group were very strong, this group would find it more difficult to make the necessary changes. In the final stage of the experiment, both groups showed a significant tendency to choose the original positive shape, the vertical. If there had been a disparity between analyser and response strengths, so that the response extinguished faster in the second stage of the experiment, there would have been no preference in the third stage. On the other hand, if the analyser tended to extinguish faster than the response, the preference could be shown.

Ingle (1967) using goldfish and the avoidance technique mentioned before, trained fish to respond differentially to patterns of vertical and horizontal stripes. Animals were then transferred to problems which involved vertical versus oblique stripes, starting at an inclination of 52°, and progressing to 38 and 23°. All animals transferred this well; the part of this work dealing with inter-ocular transfer will be dealt with below.

Very few authors have used stereometric shapes. A number, such as Sutherland and Mackintosh, have used thick flat shapes against a discrimination screen, but there have been few uses of actual cubes, or spheres. The first to use this sort of stimulus was Herter (1930) who trained <u>Idus melanotus</u> to discriminate a cube from a pyramid, presented simultaneously. Fish learned this discrimination to 100% within 40

/trials

trials, which suggests that the use of these stimuli renders the problem more easy (for example Meester's minnows took about 80 trials to learn square/triangle). Maes (1929) trained her fish to feed at a prism rather than at a cube, and obtained rapid learning. Forbes (personal communication) trained goldfish to discriminate between spheres, cubes and pyramids, and found that the learning of these discriminations was faster than that of the equivalent two dimensional shapes. She also found that fish would show good transfer from flat to stereometric shapes, but that the opposite transfer was with difficulty. There seems much to be said for the use of these sorts of shape in learning studies.

Although there has been much work on the discriminative abilities of fish as far as shape is concerned, there has yet to be proposed any very comprehensive hypothesis of <u>how</u> the analytical part of such discrimination occurs. Sutherland (1964) has modified his original ideas of analysis in terms of horizontal and vertical projections, and supposed that Hubel and Wiesel's (1965) physiological findings may be incorporated into psychology. These workers found that they could obtain recordings from cells in areas 18 and 19 of the cat's cerebral cortex, when the eye was stimulated by various patterns of light. They found an hierarchical system of cells, responding to increasingly more complex visual stimuli. "Simple" cells responded to point light stimuli by giving "on" or "off" responses. "Complex" cells responded to slit light stimuli, such as bars with straight edges, and showed greatest responses for certain orientations of these. Two degrees of "hypercomplex" cells were found, which responded only to more definite shapes, such as lines of definite length, and in preferred orientations. Sutherland supposed that this data, derived for every aspect of a shape, was put into a "store", so that it could be used, in association with reward values, to determine subsequent responses to that type of shape.

Sutherland's model has considerable value in that it emphasises the two aspects of the average training situation; learning of stimulus/reward association, and learning of response. The latter factor is too often ignored. On the other hand, it does not allow predictions to be made as to how analysis will occur, and what factors in the stimulus will be of importance to the animal. For example, it has been seen several times, whilst dealing with the results of various workers, that quite gross changes can be made to the shapes of stimuli, as long as certain characteristics are preserved. Thus, for example, Meesters (1940) found that fish trained to go to the larger of two outline squares for food would choose the correct shape even if only one side of each square were left, and this could be any of the four. This would suggest that only part of the stimulus was necessary, and the work of Herter, and Hemmings and Matthews would support this. Unfortunately, Sutherland's hypothesis allows no predictions as to which aspects of a shape will prove most useful <u>to the animal</u>. The poor best that we have to accept is that fish analyse shapes, and make use of certain of their features. The work of Hemmings and Matthews suggests that there may be a surprising degree of discarding of analysed information.

In the absence of electrophysiological evidence, it would seem difficult to hypothesise as to the mechanisms involved, and, even if this data were available, it would not tell how much the fish used in the formation of discriminative memories.

(iv) Interocular transfer.

As in the field of interocular transfer in higher vertebrates, the study of the phenomenon in fish has only recently come into prominence, on account of the possibilities of investigating brain function using the same animal for operate and control. The first observation that such a capability existed in fish was by Sperry and Clark (1949) working with Bathygobius soporator. Since gobies tend to spend much of their time resting on the base of their tanks, it was decided that a definite response would consist of swimming upwards. Two stimuli were set one above the other on a metal rod; the higher of the two was crab meat, the lower, and larger, a red rubber lure, with cotton wool attached. Thus fish had to learn a response, then attach to it a discrimination. The stimuli were set at the base of the tank in early trials, but were gradually raised as training progressed. Experimental fish were anaesthetised, and one eye was covered with a thin cup of foil. When they had recovered, they were trained, being given 30 trials per day. The position in the tank, and the height of the stimuli were varied, and only if the fish made an unequivocal approach to the food was the rod allowed to remain in the water. When fish were well-trained, cotton wool was placed at the top of the rod, in place of the food, and fish went consistently to this, there were thus no olfactory cues operative at this stage of the experiment. (A fairer trial would have been with both shapes baited, or with baiting reversed).

Fish reached a criterion of 17/20 after 90-200 trials and were given 120 trials' overtraining, after which they were anaesthetised, and the position of the blinders changed to expose the untrained eye. In subsequent trials, of 16 fish, three showed total transfer, six showed good transfer (i.e. greater than 60%) and seven showed poor transfer (i.e. less than 20%). Several of these poorly-performing animals were trained again to the initially-trained eye, and then showed good transfer. Sperry and Clark also tested to see if learning were confined to one eye in binocularly-learning animals, by training fish with the above problem, then covering one eye and testing the other. There was no evidence of lateralisation of learning.

With the wisdom of hindsight, it is interesting to examine these findings, since in many ways they summarise the findings and theories of subsequent workers. The first observation is that to some extent interocular transfer _did_ occur, and it was in this sense total. _No_ fish failed to show a response. Without any further pre-training, all the animals went to the rod and attempted to feed; some took longer to do this than others, and it was observed that the fish which made the most prompt responses were those which showed the most accurate discrimination. Thus, even if the discrimination ability was not easily transferred, the response was. The second important point is that re-training on the initial side could facilitate transfer, and thirdly, binocularly-trained fish could perform equally well with both eyes.

Schulte (1957) investigated interocular transfer in goldfish, and it is with this animal that all subsequent psychological investigations have been made. Shapes were presented one above the other, the positive with a _Tubifex_ attached, the negative with a dummy bait. He trained fish both binocularly and monocularly, and found that for any discrimination, the former were superior to the latter in rate of learning. Out of a total of 21 fish trained, 18 transferred well, and Schulte suggested that the reason for poor transfer in the other three cases was that the fish had become too excited because of the change of eye. (Schulte used a cloth cover, which was wrapped round the head leaving mouth and eye holes; this would seem to be a less traumatic method than that of Sperry and Clark). Cross versus circle, horizontal rectangle versus inverted triangle, vertical and horizontal stripe patterns of three and four bars, rectangle versus diamond, and circle versus circle with superimposed cross were all discriminated and transferred well. In many cases, the percentage of trials correct was greater for the transfer eye than for the trained eye before transfer, i.e. when it had been well-trained. Schulte found that if he tried to obtain generalisations of the shapes on transfer, there was a breakdown of discrimination, although fish showed good discrimination of the same shapes on the trained side. For example, fish trained with 7 versus 4 vertical stripes failed to show transfer when presented with 7 versus 17 stripes on the naive side, although they responded to the greater number of stripes with the trained eye. Animals trained with diamond versus rectangle showed transfer with the naive eye to shapes with their edges rounded, but only the trained eye showed transfer to the upper and lower halves of these shapes alone. Transfer to the naive eye also failed when thin outline shapes were presented, or when shapes were rotated through 45°. He obtained very similar results for the other discriminations mentioned above. He also trained fish binocularly, and gave them the same transfer problems; there were very similar results to those for monocular fish. It was not possible to train fish to opposing discriminations in each eye, and he concluded that this was further evidence for interocular transfer. Schulte concluded that since binocularly-trained fish showed faster

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learning and better transfer than monocularly-trained fish, the difference was due to the mass of nervous tissue available to mediate the discriminations.

McCleary (1960) reported a series of negative experiments, in which he and his co-workers had tried to investigate interocular transfer in goldfish, using a number of techniques, such as T mazes, circular mazes, multiple-choice boxes, and avoidance situations. Contrary to earlier findings, transfer did not occur if fish were blinded in the naive eye during training of the contralateral side, when the response involved was one of any complexity, such as maze-running, or making rapid avoidances, through a swing door. It was often noted that on transfer trials, the presentation of the aversive stimulus might not produce a response, but it would often produce a startle reaction. It was supposed that this might be due to failure of the visuo-motor systems of the animal, which had not learned the response through the naive eye.

In his first experiment, McCleary used the conditioned heart deceleration method described above. Fish were trained to one eye with green versus brown fishing lures, of different shape. After an unmentioned number of trials, there was a clear differential response to the stimuli, and interocular transfer tests were conducted. There was immediate and complete transfer. He then trained fish with situations involving skeletal responses, and used a method where both eyes could be kept open during training. Fish were confined in a narrow box, so that they could not turn to see lateral stimuli with both eyes. In this way, one eye could receive discrimination training, whilst both received visuo-motor training. The stimuli used were as before, but one was neutral, the other aversive, and fish could avoid a shock by swimming

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into the goal box. Animals took variable numbers of trials to reach a 90% criterion, the average was about 140 trials. They were immediately given tests on the naive side, and there was complete transfer. Tests with blinders for reflection proved negative.

Fish were next fitted with bilateral translucent blinders, and trained in the same box as used in the previous experiment. The CAS was a thin beam of light, which was projected on to the blinder of one eye. The CAS/UCAS interval was 5 seconds, as in the previous experiment. Fish reached a 90% criterion within 70 trials, but on transfer tests, only 5/50 correct responses were made. McCleary concluded that this was further evidence for the need for visuo-motor experience on the naive side. He then repeated both these avoidance training stimuli with the use of the heart-rate method, and the use of blinders over the naive eye. In both cases there was good transfer; this seemed to prove that there must be some unilateral restriction of the response mechanisms, whilst transfer of discriminations can be accomplished. In a subsequent paper, McCleary and Longfellow (1961) trained fish to avoid one of two shapes, either a white square with six black circles arranged randomly inside it, or a white triangle, with six pairs of randomly-arranged semi-circles. This was easily learned, and interocular transfer was good. They pointed out that Hebb (1949) had suggested that interocular transfer could occur only if there had been prior binocular experience of the discriminanda, but that their results did not support such a suggestion.

Botsch (1960) used goldfish with a Meesters-type discrimination box, with feeding boxes attached to the shapes. Most of the shapes he used were those used by Schulte, and his results for transfer and

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for generalisation were very similar to that worker's. Botsch's findings in relation to tectal function will be discussed below. Shapiro (1965) also used goldfish, with an operant technique which involved bar-press responses by the fish. In the first experiment, she used successive presentation, with fish pre-trained to press for food. Blinders were fitted before discrimination trials, and fish were trained to press for food when a green light illuminated the bar, and to ignore the bar when a red light came on; other animals were trained with the reverse problem. After one eye had learned this, fish were again given bar-press training, and the blinded side was changed daily. This ensured that there was a well-established response present on both sides, so that if transfer did fail, it would be due to a failure of the systems mediating transfer of visual data or commands, rather than a failure of motor systems.

After this preliminary training, fish were given a successive vertical versus horizontal rectangle discrimination, and were trained on alternate days with each eye. The learning for one day was transferred perfectly to the naive eye, so that a smooth curve was obtained; if there had been no transfer, it would have been expected that there would have been separate curves for each eye, so that the overall rate of learning, taking both eyes into account, would have been half that of animals trained only to one eye. In a second experiment, she used simultaneous presentation of the same stimuli; fish were pre-trained as before, but both the red/green discrimination and the shape discrimination were given simultaneously. Transfer was again complete. This experiment, with its extremely good controls, is the most decisive

/demonstration

demonstration of interocular transfer in fish.

Ingle (1965a & b, and 1967) has investigated interocular transfer in goldfish, using McCleary's avoidance method, and has tried to find the limits of discrimination at which transfer can occur. Mention has been made above of Schulte's finding that generalisation trials impaired transfer, and Ingle set out to find if more difficult problems were transferred less easily than more simple ones. One of his most surprising findings was that although interocular transfer was generally good in fish, opposing responses could be taught to each eye. Thus one eye could be taught "go" to red, whilst the other was taught "no go" to this colour. In view of the findings of a number of workers, including Ingle himself, that position sense is good in fish, it may be that this is not quite as surprising as it seems, since the fish may have learned red/left/go, versus red/right/no go. The problem needs further examination.

In his early study (1965a) of the relative difficulties of transfer of different discriminations, Ingle trained fish using stimuli which had both shape (three horizontal stripes versus random dots) and colour (green versus red). In training trials, these were paired in a constant fashion. When fish had attained a high level of discrimination with the trained eye, the naive eye was tested with stimuli where the pairings had been reversed, i.e. if fish had had green and stripe positive, their shapes were green/dots versus red/stripes. No reinforcement was given in tests with the naive eye, and it was found that all animals responded to the previous positive colour, rather than to the shapes. Thus it seemed that colour information was transferred with more ease than shape information. In his most recent paper Ingle (1967)

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trained fish to avoid a disc with vertical stripes, but not one with stripes at 52° from the vertical. All fish learned this easily, and showed good interocular transfer (81% correct). Fish trained with a 23° difference of the oblique stripes learned at the same rate as the previous group (34 trials to 9/10 criterion), but showed little sign of transfer (53% correct). Two groups of fish were then trained, one with the 52° problem, the other with the 23° problem. When they had both attained criterion, they were both given transfer tests with a 38° problem, which was more difficult for the former group, easier for the second group. Despite this, the 52° group showed good transfer, the 23° group did not. Ingle concluded that information as to difficult discriminations did not transfer, and that on this basis it should be possible to train opposing difficult discriminations to opposite eyes. Black/white and stripe discriminations were used, and fish were trained, for example, black/left/positive and white/right/positive. When both sides had achieved criterion, each side was tested with its positive shape alone, and showed good performance. These results are peculiar, since Schulte had found that it was impossible to train fish to opposing discriminations with each eye, and Ingle's own suggestion on this possibility was limited to difficult problems, which black/white and stripe discriminations were certainly not. Ingle repeated the experiment, with trials on each side alternated, which should have made independent learning more difficult, but in fact animals learned faster. In a final experiment of the type, fish were presented simultaneously with opposing shapes, one to each eye, and learned well. Perhaps the position cue suggested above was operating here.

/Consideration

Consideration of these data led Ingle to suppose that one eye was supressing information transferred from the other. Fish were trained with discs with vertical or horizontal stripes, and whilst these were presented to the one eye, the other was presented with an irrelevant pattern of horizontal stripes, presented on all occasions. A novel stimulus was then introduced, a pattern of red horizontal stripes. If this were presented to the trained eye, it caused avoidances to be made. Presented to the naive eye, it had the same effect. Ingle argued that since both sides behaved in this way, there was no supression. Two groups of fish were trained with a vertical/horizontal problem, one group with an irrelevant stimulus presented to the untrained eye. On transfer trials, there was good transfer in the group without the other stimulus, but none in the group with the irrelevant stimulus. This suggested that interference could occur, and prevent the bilateral use of memory, either by preventing the laying-down of memories bilaterally, or by interfering with transfer. The difference between this and the previous experiment are perhaps explained by supposing that in the first case animals were excitable, and any new stimulus would have made them move, and in the second, the fact that two different shapes were presented, which were not "scaring" to the trained side, and did not cause avoidances.

Since these experiments tested for dissociation in the visual system, Ingle investigated to what extent there was integration in this system. Fish were given shapes presented to both eyes simultaneously, and had to treat unlike shapes, i.e. shape A to the right, shape B to the left, as aversive and similar shapes as non-aversive. Using red/green and red/white stimuli, he found that there was good performance

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of this task. Thus fish seemed to have a good capacity for selective attention with one eye; this might perhaps explain the lack of transfer with difficult problems. Ingle's work does tend to pose more problems than it solves; for example, if there is transfer of easy in preference to difficult problems, as both his and Schulte's work would suggest, it is difficult to see how easy problems such as black/white can stay lateralised enough to be trained with opposing rewards in both hemispheres. Schulte was unable to train his goldfish with opposing responses in each eye, even using very simple situations. Nevertheless, this work suggests that there may be more lateralisation of memory in fish than previously thought and that use of the appropriate unilateral lesion techniques may yield most useful information.

More recently, Mark (1966) trained specimens of Astronotus ocellatus to jump a small way out of the water to reach food held in the centre of a floating ring. When fish performed well, he covered one eye, and trained fish with triangle versus circle, the shapes being held on the floating rings. The positive shape was baited with blue-coloured liver, the negative shape with blue-coloured formalin-fixed liver. Since both baits were held out of the water, there were no olfactory cues. Fish took 40-100 trials to learn the discrimination to a 19/20 criterion, and the blinders were then transferred. All fish showed good transfer of the discrimination, and Mark concluded that there was good interocular transfer as long as fish had experience with the naive eye. Thus fish, contrary to early beliefs, have very acute vision. It has been seen that they can show good discrimination of brightness, of colour, and of shape. Entailed in the demonstration of this has been the fact that fish can learn well. They show classical conditioned reflexes with surprising rapidity, run mazes, learn avoidance problems, and can solve easily visual discrimination problems which are not beyond their limits of acuity. It remains to review briefly some of their capabilities in other senses, as far as are relevant to the study, and to touch on one other method of training which is conveniently treated in a section of its own.

4. Auditory discrimination.

A large number of early workers tested fish with sounds made in air, and many concluded that there was no auditory sense. Kreidl (1895, 1896) used goldfish, and observed that they gave no response to a bell whistle, pistol shot, in air, or the vibration of metal rods in water. He further showed that fish which were ostensibly being attracted to feed by the sounding of a bell were in fact being attracted by the sight of the feeder. There are many other references of this sort, conveniently summarised in Warden, Jenkins and Warner (1936).

Despite reports to the contrary, it has generally been assumed that fish can hear sound. Much of the literature relating to the perception of air-borne sounds was anecdotal, and the first objective observations on this were those of McDonald (1922) using Pimephales notatus. Fish were trained to come to one part of the tank when a 96cps. note was sounded, by the vibration of a string. After some time, fish would go to the feeding-place as soon as the feeding note was sounded. She controlled the experiment well, in that she remained hidden throughout the training, so that there were no extraneous visual cues. Westerfield (1922) was able to condition Umbra limi to feed on snail meat when a 288 cps note was sounded, but to inhibit a feeding reaction when a

/426 cps

426 cps note was given. The bait in this case was grey paper soaked in camphor; fish found this offensive, but could not discriminate it from the snail meat until they had actually tried to feed on it. Froloff (1925) trained fish with the conditioned response method outlined above, and noted that submerged buzzers gave much more rapid and long-lasting conditioning than buzzers in air.

Bull (1928) trained wrasse (Crenilabrus melops) to enter a feeding bottle when an electrically-operated tuning fork was sounded 5 seconds before the introduction of food. A response was well-established within about 40 trials. In a later experiment, Bull used a small immersed buzzer as CS, and shock, given as before, as UCS. With a CS/UCS interval of 5 seconds, his subject in this case, an eel, rapidly acquired a CR. Other animals, such as Gasterosteus aculeatus and Cottus bubalis, failed to show any reaction in this training, and Bull concluded that this was due to a species difference.

Stetter (1929) trained goldfish, minnows and catfish to discriminate between notes produced by whistles, strings, and humans, on the one hand, and by tuning forks on the other. Fish had to swim from the base of the tank to the surface to receive food, for the positive note, and to inhibit this response for the negative note; failure to do this was rewarded by administration of a noxious piece of food. (Quinine was added to the meat). Stetter found that minnows could easily discriminate these notes, presumably on account of their overtones as much as their differences in fundamental. He also found that they could discriminate pure tones with differences of several octaves, and he gradually fined this difference down until he found that the smallest dif-

/ference

ference which was discriminable was a minor third. In one or two cases, really extensive training, over many hundred trials, produced discrimination between notes with an interval of a second (i.e. notes adjacent on the chromatic scale).

Wohlfahrt (1939) trained minnows with a situation in which a single repeated note on a flute was the food signal, and an alternation of two notes was a warning signal; if fish went towards the feeding place, they received an electric shock. Fish easily discriminated notes 2-3 tones different, and he found that the auditory limits stretched from 40-to 4-800 cps. Dijkgraaf and Verheijn (1950) repeated Wohlfahrt's experiments, and obtained very similar results. They then changed their method, so that instead of using shock for the negative sound, there was no reinforcement, the sound was neutral. Fish soon habituated to the neutral stimulus, and it was found that it was possible to obtain discrimination in the 400 cps+ region, which was not found before. In one case, a fish showed good discrimination up to 3,200 cps. There was a rather peculiar finding in this series of experiments, that destruction of the system of Weberian ossicles did not affect the discrimination. It is difficult to imagine how vibrations could have been analysed in the absence of this obvious sound-detection system.

5. Other sensory modalities.

(a) Chemoreception.

Of all possible senses, those of chemoreception have been most freely attributed to fish by scientific observers, although laymen have for long realised that the visual sense is as often used in finding food. Nevertheless, it has been very difficult to discriminate between olfaction,

/mediated

mediated by the olfactory organs of the snout, with input to the forebrain, and gustation, confined in higher forms to the input from sense organs in the mouth, but in fish presumably spread over the surface of the body, in the cutaneous extensions of the Vth, Vllth and Xth cranial nerves. For this reason, many of the early workers failed to make definite assessments of one or the other sense.

Sheldon (1909) used dogfish, and pipetted small amounts of various solutions over different parts of the animals' bodies. Sulphuric, nitric and hydrochloric acids, and sodium hydroxide, caused movement away from the pipette in whatever concentration they were used. Sodium and ammonium chlorides, and picric acid were also disturbing, but there seemed to be little response to sugars. G. H. Parker (1910 et seq.) performed a large series of experiments on the chemoreceptive capabilities of fish. He put two cloth bags into an aquarium, one containing minced earthworm, the other cotton wool. Catfish approached the food-containing packet, but ignored the dummy. He found the same sensitivity of skin as Sheldon had found. In a later paper (1922) he found that Ameiurus sp. would attack the same lures as used above, and similarly ignore the dummy, but that if the olfactory tracts were cut, the response vanished. This suggested that there could be some distinction between long-range olfactory and shorter-range gustatory senses.

Strieck (1925) trained minnows to feed on grated beef which had been immersed in one of three solutions, musk, cumarin, or skatol. The fish were then presented with the food adulterant alone, absorbed on cotton wool, and were also given wads of wool with the other two substances on. They reacted only to the training smell. He repeated this work with blinded fish, using glucose, acetic acid, quinine and salt as stimuli, put into solution with grated beef as before. Fish were given food immersed in one of the substances, then tested with wads of wool which they were allowed to put into their mouths. They made no attempt to take in any except the one impregnated with the food-adulterating smell. Although fish were allowed to ingest the wads to some extent, and the solutions were more "taste" than "smell" in nature, the criticism still remained that animals could have been using olfaction. Strieck's work in this connection will be dealt with in the section on forebrain ablation.

Von Schiller (1933) whose experiment with the light-discrimination of minnows has been referred to in an earlier section, transferred his fish from a problem involving dim and bright lights to one which involved dim and bright smells. Hornböstel (1931) put forward the thesis that saturated carbon ring compounds were "brighter" than unsaturated ones. The suggestion presumably was that one caused a greater dicharge in the sensory system than did the other. Von Schiller found that fish which had discriminated the light difference well showed a preference for one of the two smells; dark-trained fish chose the dark smell, and light-trained animals went to the light smell. Whatever the physiological meaning of the "dark" and "light" analogies, von Schiller's experiment showed that fish could distinguish between indol (dark) and musk ketone (light), and that there existed a capability for generalisation not only within one sensory modality, but between these.

Bull (1937) using the simple inclined maze described above, trained Pleuronectes flesus to swim into the food box when the salinity of the surrounding water was lowered. This was a beautifully-controlled

experiment

experiment; the temperature of the diluted water was equated with that of the normal circulation by condensers, all switches and taps were mounted on rubber so as to be soundproof, the flow of water through the discrimination chamber was calibrated to be smooth and not turbulent, and the constant head apparatus ruled out pressure fluctuations. Some animals learned very quickly, taking as little as eight trials for there to be an initiation of activity by the introduction of the more dilute water. The delicacy of the perception was surprising - a 4% change in salinity was noticed with ease, and even with 0.5% changes, the discrimination was hardly affected. A change of 0.2% seemed to be the limit, equivalent to the addition of 75 cc's fresh water to 15,000 cc's of sea water. Bull found that two individuals of *Gobius flavescens Fab*. showed consistent responses to salinity changes of 0.06%.

Neurath (1949) found that minnows could discriminate between eugenol and phenylethyl alcohol, even at concentrations of 6 x 10-8 parts per million for the former, and at 4.3 x 10-10 for the latter. Hasler and Wisby (1950) used bluenose minnows (*Hyborhynchus notatus*) and found that they could detect phenol in water at a concentration of 0.01 parts per million, which was far below the threshold for human detection. Van Sommers (1962) whose operant technique will be described in more detail below, trained goldfish to regulate their oxygen supply, to obtain water containing dissolved oxygen, as opposed to the normal flow, which in this experiment was of de-oxygenated water.

(b) Temperature.

The first well-controlled experiment on discrimination of temperature changes was that of Bull (1936) who used a variety of marine fish, such as *Gadus callarias*, *merlangus*, and *virens*, and *Raniceps raninus*.

/The maze

The maze used was that just described in the experiments on salinity discrimination. Water was added to the main inflow, with the same controls for pressure, vibration and salinity which had been used above. This water flowed down tubes with wire windings, so that current flow through these could cause transient and accurately-controllable changes in temperature. A series of thermocouples was arranged in the tank, so that the passage of the temperature wave could be assessed, and it was found that the maximum rise in temperature was found in the fish's normal position at about two minutes after the onset of the heating current. Bull found that within about thirty trials there was a response to temperature alone, before food was introduced, and that the minimum effective stimulus was in the region of $0.05^{\circ}C$, an extremely fine difference. Some animals were capable of responding consistently to differences of as little as $0.01^{\circ}C$. The great temperature sensitivity of fish was shown physiologically by Sand (1938) working with elasmobranchs. He was investigating the functions of the ampullae of Lorenzini, jelly-filled pits on the snout, and used Raia as subjects. He initially used pressure stimuli, but soon found that the rate of firing of the neurons changed markedly when temperature fluctuations occurred. Changes of $0.3^{\circ}C$ produced changes in firing rate of several hundred percent, and there was a rather paradoxical effect in that increases in temperature lowered the rate of discharge.

Dijkgraaf (1940 & 1943) trained a number of freshwater fish to feed in response to a change in temperature of the water flowing into the tank, and found that a change of $0.5^{\circ}C$ was sufficient to produce a rapid feeding reaction within about thirty trials. Berwin (1941)

/used

used an 11°C difference when training minnows to feed when a jet of cold water was projected, and to flee when a jet of warm water was used. The learning took longer than in previous experiments on account of the technique used; this will be discussed in more detail when dealing with the effect of forebrain lesions on learning. Rozin and Mayer (1961) tested the temperature selection ability of fish using an operant technique. Goldfish were placed in a small container, the temperature of which was raised slowly to 40°C, very near the death temperature. In first trials, fish were given small amounts of cold water, whose introduction caused a 0.3°C drop in overall temperature. In later trials, these fish were trained to push a bar to get this colder water. Fish maintained the temperature between 36.5 and 33.5°C, rarely allowing it to change outside these limits. As Rozin and Mayer suggest, 36.5°C is probably the lowest temperature where there is sufficient discomfort to promote aversive activity. They performed the obvious controls of testing for increased activity due to the rise in temperature, and for the increased oxygen lack as temperature increased, but concluded that these were not very important factors.

(c) Water currents.

Bull (1935b) is one of the few workers to have investigated the sensitivity of fish to currents, and to use the onset or change of currents as stimuli in a learning situation. Using Blennius pholis, he placed his fish in the centre of a ring of outlets, with an outflow on one side. This approximated the dwelling of the blenny, and animals would live quite satisfactorily in the entrance of the outflow pipe. A single constant outflow was used to provide the neutral stimulus, and when this was changed, and current allowed to flow down another pipe,

/food

food was given some 15 seconds later. One of the few criticisms that can be made of Bull's work is that he should have controlled more for the effect of stopping the neutral current, the effect might be due as much to the cessation of one stimulus as to the onset of a new one. In initial trials, the signal current was directed at right angles, to the side of the fish, the neutral stimulus was usually directed at the snout, by the way the animal lay in its box. Fish took about 20 trials to show a response to the 90° change in direction of current. Some animals would not learn with this angle of change, and required as much as 112 - 157° change; no animal was found to be able to form a consistent reaction with a change of less than 90°. This lack of ability is surprising in view of the proven sensitivity of the lateral line organs (see, for example, Lowenstein, 1957). It may be that since fish moved, the new stimulus would not fall on the same portion of the body each time, but generalisation has been seen to be good in fish, so it would hardly seem likely that this is a valid explanation.

(d) Sensitivity to electric fields.

In view of the fairly common use of electric shock reinforcement in a number of the laboratories investigating fish behaviour, it is surprising that there should be so little data as to the sensitivity to shock of these animals. Hermann and Matthias (1894) found that salmon would orient to the anode of a D.C. field at an unspecified voltage. Scheminsky (1924) showed that this anodal orientation reflex occurred at low amperages, but that at higher current densities fish finally rolled over and died. Regnart (1931) in one of the few quantitative studies, found that fields of 2 mu a./sq. cm. were effective in pro-

/ducing

ducing an orientation reflex.

Perhaps the most interesting example of all fish sensory modalities is that of the electrical-field sensitivity of some gymnotids and mormyrids, most interesting, because it lies outside the human experience. Such senses as sight, smell and pressure-reception are to some extent understandable on the basis of shared experience, but we have no appreciation of electric field distribution. This makes the study of the electric fish a fascinating one; the study of dwellers in others of Plato's caves, whose impressions of reality differ from our own on account of their sense impressions of the outside world.

Lissman and Machin (1958) found that if magnets or electrically-charged insulators were held near tanks containing Gymnarchus niloticus, these fish showed definite reactions. They trained fish to feed when a magnet was placed near the tank, and not when this was removed. (They took adequate precautions to avoid other cues, such as those caused by visual and vibratory disturbances). They found that these fish emitted a low-voltage electric current, which ran from head to tail in the water surrounding the animals. Thus equipotential lines could be assumed to run in circles with centres at head and tail. Since there were receptors in the skin, which were jelly-filled and connected with the surface, and were found in profusion in these fish, Lissmann supposed that these were detectors of changes of potential.

He and Machin trained Gymnarchus, using a simultaneous discrimination situation. Fish were presented with two porous pots, one empty, one with a glass rod in its centre. Water permeated the pots, which were identical, so that their distortion of an electric field was very

/low

low. Fish were presented with both pots, and behind each was food. If they went to one pot, they were allowed to feed, if they went to the other, they were banged on the snout. Fish quickly learned to go to the correct pot, and this could only have been accomplished on the basis of electrical detection of the non-conducting glass. They found that fish could discriminate between glass tubes differing in diameter by 0.6 cm. These data caused them to conclude that the fish orientated in their normal habitat by the appreciation of field difference, and Lissmann's subsequent observations would support this. It remains to be seen how peculiar to these fish is such a sense, since Bullock (1966) reported the following experiment. Fish, both "electric" and "non-electric" were trained to pass through one of 8 holes to receive food. Above each hole was a wire, and the wires were joined, so that, for example 1, 3, 5 and 7, and 2, 4, 6 and 8 would be connected. If the connections were changed, the electric fish were disturbed, and the accuracy of their responses fell, on account of the change of electrical environment. Unfortunately, so did that of the "normal" fish, and it may be that there are senses in normal fish which have yet to be investigated. For example, it could be that "electric" fish use an "active" detector system, actually emitting electricity, whilst "normal" fish use a passive system, detecting very small changes in local currents. This remains to be seen.

(e) Operant conditioning.

In this type of training, animals are required to manipulate part of their environment to produce the required reinforcement, without themselves moving bodily from one part of the apparatus to another. This has generally meant that animals have pressed bars to obtain food

/or other

or other reinforcement.

Bitterman, who has led in the development of automatic apparatus for training fish, published the first paper on the use of operant techniques, with Haralson in 1950. Goldfish were presented with a wall in which was set a round paddle, almost flush with the surface. The paddle was connected to a delicate lever system, which allowed a make/break circuit to be connected, which in turn gave reinforcement for bar presses, via an automatic food dispenser. Initially, food was put on a spike on the paddle, and fish learned to feed from this. The amount of food was gradually reduced, and food was allowed to fall into the tank from above, when the bar was pressed. After some trials of this sort, fish learned to press the bar for food, and feeding on the press was discontinued. The rate of bar-pressing rose, and a consistent response was obtained after 50 trials.

Wodinsky and Bitterman (1957) used Tilapia macrocephala in a simultaneous discrimination situation. Two discs were presented, one with vertical, one with horizontal stripes. Fish were pre-trained with a grey disc in the way outlined above, and were then transferred to the discrimination; the side of the positive shape was alternated in a Gellerman sequence. Fish learned rapidly. Bitterman and his collegues have used this technique to test a number of discriminations and learning situations in fish.

Rozin and Mayer (1961) in their study of thermoregulation mentioned above, used a bar-press which allowed fish to introduce small amounts of cold water into their immediate surroundings. In initial trials, the temperature of the surrounding water was increased to 40°C, which

/fish

fish found uncomfortable. Bursts of 2-3 cc's of colder water were then given, and each administration was signalled by the onset of a small light overhead. After 50 such experiences, the lever assembly was introduced, and a method of successive approximation used as in Bitterman's experiments, to make the fish push the bar. For example, in early trials, fish would be reinforced for going near the bar, then for touching it, and finally, only when they pressed it. The temperature was then raised to 40°C, and the fish was allowed to control its own temperature, with results which have been discussed above. Van Sommers (1962) also used an operant technique in the experiment on oxygen-motivated behaviour discussed above. Goldfish had to swim through a door, and break a light beam, which action switched a photo-relay, and admitted a burst of oxygen-containing water, for 15-20 seconds. A red light came on during admission of oxygenated water, and a green light came on when de-oxygenated water was flowing.

Hemmings and Matthews (1963) and Matthews (1963) used bar press techniques very similar to those described for Bitterman, and found that fish learned well, using food reward. Shapiro (1965) also used this technique, as did Aronson and Heberman (unpublished) in a paper which will be discussed in more detail below.

A number of other workers, such as Geller (1963 & 1964), and Prazdnitkova (1964) have used operant techniques, but they have little to add to the discussion of the possibilities of such training outlined in this section.

6. Effects of damage to the optic tectum.

Early reports of damage to the tectum tended to be rather contra-

/dictory.

dictory. Ferrier (1886) stated that there was loss of equilibrium and disturbance of locomotion following partial bilateral removal in carp. If the removal was more was more extensive, locomotion became impossible, and animals became hyperactive. These results did not agree with those of Baudelot (1864) who removed the tectum from sticklebacks, and observed that there were no changes in locomotion, but that reaction to visual stimuli was considerably reduced. More recent work has tended to confirm these observations, and to attribute irregularity of movement to damage to underlying structures, such as the tori semicirculares, which lie beneath the tectum, and have connections with the cerebellum. Rizzolo (1929b) found that bilateral tectum ablation in Galeus canis caused transitory aberrance in movement, but that these were generally reduced or absent within a day of the operation. Similarly Sears (1934) and Sanders (1940) whose work is considered in more detail below, observed that goldfish with quite extensive tectal lesions could swim and feed quite satisfactorily. Dijkgraaf (1949a) working with minnows, found that animals with both unilateral and bilateral lesions could swim quite normally, whereas those with damaged tori semicirculares showed circus movements towards the undamaged side.

Many of the early accounts of disruption of normal locomotion by tectal lesions can be discounted on the basis of the work of these observers, but that there may be deficits in certain situations will be shown later, though this may not be entirely due to damage to motor systems. Kirsche (1960) has shown that unilateral lesions may cause bending to the undamaged side, and that bilateral lesions may cause irregularities in movement.

/7. Stimulation

7. Stimulation of the tectum.

Chauchard and Chauchard (1927a) electrically stimulated the surface of the tectum of Mugil auratus, and found that there was a complex representation of body movements over its area, that specific areas of tectum were responsible for the activation of different parts of the body. For example, stimulation of the antero-median surface of one side of the tectum caused movements of the tail to the opposite side, and a spreading of the caudal fin. As the point of stimulation was taken more posteriorly, still remaining in the median area of the tectum, anal, pelvic, dorsal and pectoral fins were operated in sequence. Stimulation of the lateral areas of the tectum caused rotation of the ipsilateral pectoral fin; stimulation of the innermost sector of the lobe caused eye movements. Similar results were obtained by stimulation of the optic tecta of various elasmobranchs.

Ten Cate and ten Cate (1931) curarised small lesioned areas on the superficial layers of the tectum in dogfish, and found that these localised lesions affected specific muscles and body areas. Unilateral lesions of this sort tended to disarrange co-ordination of animals, so that deficits occurred in posture and movement. Akert (1949a) investigated the visuo-motor functions of the tectum, using as his theoretical basis the "visual grasp reflex" hypothesis. This suggested that when a new object was perceived on the periphery of the visual field, there was a special involuntary movement of head and eye to bring this object into the centre of the field, so that it could be more accurately analysed. Akert examined salmon, where, he stated, the grasp reflex was particularly well-developed. He first plotted eye movements produced by tectal

/stimulation,

stimulation, finding that there were areas whose stimulation produced constant responses of the contralateral eye. When slightly higher voltages were used, Akert found that he could elicit movements of the whole animal, turning movements which were directed away from the stimulated side. These beautiful experiments went far towards explaining the role of the tectum in the co-ordination of movements, and also explained why there were motor deficits in some animals. An object appearing in the peripheral visual field of one eye would cause stimulation of the contralateral tectum, and initiate eye movements towards that shape, and also body movements which would turn the fish towards this new stimulus. It might even be supposed that since greater voltages were needed to cause the body movements, more "significant" visual stimuli would cause bending of the body, and approach, whilst less important stimuli would merely cause eye movements. The results of Boyd and Gardner (1963) involved tectal stimulation, but they are related more to learning, and will be considered in a later section.

8. Electrical activity of the tectum.

Buser and his associates, in a series of papers on work with the catfish and other teleosts, found that photic stimulation of one eye led to the recording of evoked potentials from the contralateral tectum lobe, though none could be recorded from the ipsilateral lobe. (Buser (1949a, b & c, 1950, 1951 & 1955), Buser and Scherrer (1950), Buser and Dussardier (1953)). (This observation alone was of importance, since it implied that there was no ipsilateral projection of optic input, and confirmed the anatomical observations that optic fibres crossed completely. There were also obvious implications when interocular transfer was considered; for example, there would seem little possibility that the transfer was mediated by a direct projection of the stimulus on both hemispheres, and this made it more likely that analysis might be performed on one side, and information as to this transmitted to the other side, for the appropriate response to be selected. On the other hand, it might be that transfer of information occurred at a lower level; perhaps this is less likely on the basis of Mark's work, to be considered below. (He found that splitting of the tectal commissure interrupted transfer). In view of the work of Schulte and Ingle, it would seem reasonable to make this assumption of a certain degree of lateralisation of memory).

A single flash of light produced a series of changes in the tectum. The first observable potential change was a series of small rapid increases of negative polarity, due to firing in the terminal fibres of the optic nerve as they ran over the surface of the tectum. After this, slow potentials were recorded, which were due to intra-tectal fibres; these showed a reversal of polarity within the tectum, being negative on the outside, and positive on the inside. Both the slow and fast components of the response had two peaks, and Buser found that if light-adapted fish were used, rather than dark-adapted, as in these experiments, one of the slow peaks would vanish. He concluded that there were separate rod and cone outputs giving slow and fast responses respectively, and that light adaptation had caused the rod response to wane. Responses of the tectum to coloured lights were very similar to those for white light.

In later experiments, Buser and Dussardier (1953) showed that there was a representation of particular retinal quadrants on particular areas of tectum. For example, stimulation of the superior nasal quadrant of

/the retina

the retina produced an evoked potential in the postero-lateral sector of the tectum. These results were in good agreement with those of histological workers, outlined in a later section.

Schadé and Weiler (1959) found that they could record similar optic potentials from the tectum of goldfish when photic stimulation was applied to the contralateral eye, and also that in lightly-anaesthetised fish which were also dark-adapted, such flashes called forth an arousal reaction in the tectum. In the resting state, there was a regular intra-tectal rhythm of 8-13 cps., which was interspersed with short bursts of higher frequency; both rhythms were synchronised on both sides of the tectum. The higher frequency bursts (18-24 cps.) became more frequent as the amount of external stimulation increased. This was seen especially when a light was suddenly shown to one eye (a stroboscopic stimulation of 50-100 cps. was used) even in light-adapted fish. The responses of the tectum to light were very similar to those observed by Buser, that is, a fast component succeeded by a slow one. Schadé and Weiler also found a close retino-tectal relationship, but since this has been investigated in more detail by later workers, it will be described there.

Enger (1957) used free-swimming cod (*Gadus callarias*) without anaesthetic, and found that he could record rhythms similar in frequency to those found by the above workers, again, the higher-frequency components tended to increase when animals were exposed to increased disturbance. Under light anaesthesia, the lower-frequency waves preponderated. Dark-adapted fish showed an arousal reaction to the onset of an 800 cps. stroboscopic light, but there was no detectable tectal response to acoustic stimulation.

/Schwassman

Schwassman and Kruger (1965) followed up and improved on the rather crude observations of previous workers on the retino-tectal projection. They used a number of species of fish, including goldfish, and found that there was a well-organised and very precise gridwork of point-to-point projection, which was organised in the way suggested by the work of a number of histologists, that is, with anterior and dorsal retinal sectors being represented in the posterior and ventral surface of the tectum. It is also of interest that they did not find any preferential representation of the central region of the retina, although a number of histological observers, including Akert (1949 a) had suggested that such existed, on account of degeneration studies, and consideration of the distribution of cones in the central region of the retina.

Prosser (1965) studied optic responses in the tectum of Leuciscus rutilis, and found very similar potential changes to those found by previous workers. He noted that there were very similar responses to both "on" and "off" stimulation. He concluded that the superficial component of the response was axo-dendritic, from optic fibres. The lower negative potential he supposed to be axo-somatic, and the layer of positivity in the centre of the tectum he supposed to be a "source" for the current on either side. His work is also of interest in that it was one of the few attempts to correlate learning and brain activity. Fish were given two second light stimulation, at the end of which there was administered an unspecified length of shock of unspecified voltage. Trials were given at ½ minute intervals, and 20-70 trials were given in all. Every 10 to 20 trials, recording was made from the tectum as the light alone was presented. These tests were negative in 8 fish, but 17

/animals

animals showed varying degrees of response. There were changes in the long component of the light-evoked potentials, and in some cases a new component appeared, an extra "off" response. In the absence of reinforcement, this extra component extinguished within 10 trials.

Hara, Ueda and Gorbman (1965) have recently investigated the effects of thyroxine on optically-evoked potentials in the tectum. They found much the same kind of phenomena in normal fish as previous workers, but when fish had been given large amounts of thyroxine over several days, there was an increase in sensitivity to optic stimulation, and response latency dropped, whilst amplitude of response rose. They compared their work to that of Hoar et. al (1952) who had shown that administration of thyroxine to free-swimming goldfish caused an increase in the amount of swimming shown by fish, and lowered the threshold of sensitivity to electric shocks. In view of the extremely high non-physiological doses employed, it is doubtful whether these results mean very much in terms of the action of the hormone in the fish's normal life. The thyroid of fish is generally rather inactive, and it is certain that it could never produce changes of this order.

9. Anatomical and behavioural evidence of tectal organisation.

Two main workers have investigated the anatomical side of the tectal organisation outlined above in physiological terms. Lubsen (1921) used the Marchi method, and traced degenerating axons into the tectum from damaged retinal areas. He found the projection of dorsal retinal areas was to the ventral part of the tectum, and that anterior retinal areas were represented in the posterior part of the tectum. Akert (1949a) plotted this distribution more accurately, and found that in trout there

/was a

was a very close relation between area of retina and area of tectum. Dijkgraaf (unpublished, reported by Healey (1957)) made unilateral tectal lesions of varying extent, and assessed the effects, the visual deficits, in terms of feeding reactions to stimuli presented to fish in various parts of the visual field. He was able to produce a map which correlated well with the findings both of the anatomists and the physiologists. Cronly-Dillon, Sutherland and Wolfe (1966) severed bilaterally one of the two main brachia of the optic nerve leading to the tectum, and found that, for example, if the lateral brachium were severed, there was blindness in the ventral visual field, and fish would not react to food stimuli placed there.

Meader (1934) carried out an extensive series of histological investigations into the distribution of optic fibres in the teleost _Holocentrus uexillarius_. There was firstly a medial fasciculus of the optic nerve, which ran to the dorsal and anterior sections of the tectum, and gave off collaterals to the lateral geniculate nucleus. A second main branch of the optic nerve ran ventrally and laterally, and supplied the posterior and ventral parts of the tectum, and this also gave off collaterals to the geniculate. A third branch, smaller than the other two, ran dorsomedially, and supplied the anterior edge of the tectum, and the dorsal diencephalon, whilst two smaller fasciculi ran to the anterior thalamus and to the lateral geniculate nucleus respectively. Although it has been shown in elasmobranchs that there is projection of olfactory, gustatory, and cutaneous information directly to the tectum, this has not been shown in teleosts (Kappers et al. (1936)). Nevertheless, in view of the presence of large numbers of these fibres in the dorsal thalamus of these fish, and the existence of large tecto-thalamic tracts, it is not unreasonable to suppose that such a connection may exist.

Tuge (1934b) demonstrated the existence of a cerebello-tectal pathway (it had been known for some time that the reverse path existed). He made cerebellar lesions, and used the Marchi method to trace degeneration into the tectum. He suggested that this connection, with the further involvement of the tori semicirculares, was concerned with visuo-motor co-ordination, and, in view of the work of such observers as Akert (1949a) it would seem likely that such integration might occur via this path. Although it is beyond the scope of the author and often beyond that of the literature to deal extensively with the further connections of fibres associated with the optic tract, it is worth giving a brief description of the connections of the lateral geniculate nucleus as observed by Meader (1934), in view of discussion of results obtained by the writer. Apart from the direct and collateral connections already mentioned, with parts of the optic nerve, there were connections with the tectum, the nucleus rotundus, nuclei of the ventral thalamic wall, and the hypothalamus, as well as a commissural connection with the contra-lateral nucleus. Thus, as would be expected, the visual input was distributed very widely, and this has important implications for the interpretation of some behavioural work.

Though it had been known for a long time that the optic tectum of many fish would regenerate, it was not until the careful studies of Kirsche (1960) that the details of this phenomenon were uncovered. Using carp, <u>Carassius carassius</u>, he showed that some regeneration had occurred 50 days after operation, if the basal layer of the tectum had been left intact in one area, but that total regeneration took over 100 days. Kirsche delineated three main areas concerned with regener-

/ation:

ation: a layer near the torus longitudinalis (a pair of longitudinally-running tracts in the centre of the tectum), a layer at the posterior edge of the tectum, and the basal layer of the tectum. These areas of cells decreased as fish grew, so that regenerative capacity decreased with age; fish 2-3 cm. long could show very rapid regeneration; fish 15-20 cm. long were much inferior, and in very large fish there was almost no regenerative capacity. Unfortunately, Kirsche undertook no behavioural studies, so that it was impossible to know at what stage physiological function was normal. The studies of Botsch (1960) which will be dealt with in detail below, suggest that attainment of function precedes "normal" histological re-organisation, and the work of Sperry (1949) supports this.

10. The functions of the optic tectum in learning and retention.

In view of the obvious importance of the tectum, in the co-ordination of eye and movement, and its multiplicity of input, it is not surprising, that in the absence of a true cortex in fish, the functions of this should have been attributed to this area. This concept, and the fact of cortical mediation of learning in higher forms, led a number of workers to examine the tectum to try to find if it had similar importance in either learning or retention.

The first paper dealing with the effect of tectal lesions on the retention and formation of associations was that of Sears (1934). His experimental animals were goldfish. He first examined normal fish, placed in a confined cage, and noted their activity during periods of about an hour, then their reactions to sudden increases of illumination. There were two classes of reaction, eye movement, and a "startle" reaction of the whole body; the former was much more common than the latter.

Sears had intended to use shock as UCS in his training, but he decided against this because fish generalised in the following manner: fish which had received several shocks, without any other stimulus associated with them, showed a "CR" to light, even though they had never experienced shock in any association with this stimulus. He therefore used as UCS a jet of water, which was directed into the cage from above, impinging on the fish's back. This stimulus operated for 0.5-1 seconds. Sears classified responses to this stimulus in categories of decreasing intensity, all of which were directed at keeping the fish at a constant level in the water, and reducing the downward push of the UCS current.

He then gave fish associations of light as CS, with water-jet as UCS, with a CS/UCS interval of four seconds. The mean intertrial interval was one minute. Fish reached a criterion of 8/10 correct responses within 50 trials, and would extinguish the response within 20 trials. Animals which were trained to this criterion, then operated, with removal of most of the dorsal tectum, and some lateral showed complete retention of the habit, but Sears noted that extinction tended to occur in these fish with more rapidity than it did in normals. Naive operated fish showed a slightly more rapid acquisition of the task than did normals, taking 40.2 as opposed to 46.8 trials to reach criterion. Thus it seemed, that for simple tasks at least, the tectum was not as important as had been thought.

Sanders (1940) used goldfish, and a much more complex learning situation. His apparatus consisted of three tanks, interconnected by small doors. The centre tank, which constituted the start box in early trials, opened into a goal box, which was dimly illuminated. The stimulus to enter the goal box was the onset of an illuminated disc over the start

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box, and fish were rewarded for entering the goal box by being given ants' eggs. Fish initially took 13 minutes to enter and feed, but over four trials, their reaction times dropped to less than three minutes, then stayed level at about 1½ minutes. Sanders' next experiment involved placing fish in the third compartment, which gave access to the original start box. An olfactory stimulus, amyl acetate, was introduced, and the disc over the former start box was illuminated. When fish swam through this box, and into the goal box, they were rewarded with food as before. When reaction times for this whole sequence had fallen to about 100 seconds, the olfactory stimulus was omitted, and reaction times rose sharply, even though the light stimulus was still present. The same happened if the visual stimulus was omitted, and Sanders concluded that both stimuli were necessary for the efficient performance of the task.

A third series of fish was trained with the initial two-box situation, then transferred to a modified second situation, where an olfactory cue was used to initiate movement into the light stimulus box, but fish were not allowed to pass into the goal box to receive food. The reward in this case was the vision of the illuminated disc, which, by its previous association with feeding in the goal box, attained the status of a positively-reinforcing factor. Fish learned both situations well, and their reaction times in the latter case fell to about 100 seconds in 15 trials, comparable with those of fish being rewarded in the goal box. When responses had attained some degree of constancy, four fish were lesioned, and one other used as an operated control. In subsequent trials, only the operated control showed the pre-operative level of performance, the lesioned fish either failed to respond, or took a

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long time to do so. Sanders concluded that the tectum was mediating the retention of this second order conditioning, and compared it with the cerebral cortex, putting forward a "reverbatory circuit" hypothesis to explain the memory.

Whilst this was a very well-controlled experiment, the conclusions and interpretation are open to some dispute. Firstly, some of the fish did show reactions after the operation, and, in the absence of reinforcement, these events would be sufficiently unlikely to be significant. Secondly, Sanders made no tests to see if his fish were capable (a) of normal vision, and (b) of normal visuo-motor co-ordination. These factors can hardly have been normal, since it has been seen fromthe work of a number of observers that tectal lesions impair vision, and also affect the ability of fish to make directed movements, or at least, movements of the complexity involved here. Without the proper controls, it is simpler to assume that the deficits seen here were of this nature, rather than to suggest that the tectum is a memory centre; it has already been noted that Sears' work would not support this concept.

Visual analysis must occur in the tectum, as well as in the retina, and since this is so, it is difficult to distinguish between failures due to loss of retention and those due to loss of the analysis centre. The critical experiment would seem to be one where fish were trained, then the tectum was removed, and allowed to regenerate. If the memory were in the tectum, it would be expectated that no retention would occur. If the memory were at a lower level, and the tectum were analysing visual stimuli and transmitting analysed data to this, it might be expected that there would be retention.

Dijkgraaf (1949a) trained minnows to feed when a 1650 cps. note was

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emitted from an electric tuning fork set in the wall of the tank. A feeding response soon developed, and this was not abolished by bilateral removal of the tectum, so that auditory memories were not located in the tectum.

Botsch (1960) published an extensive series of experiments dealing with the effects of tectal lesions on discrimination and generalisation of visual stimuli. Goldfish were presented with two shapes, below each of which was a food trough, whose contents were not visible to the fish. Shapes were alternated from side to side in a semi-random fashion. (The shapes used, and the findings for normal fish, have been described above in summaries of the work of Schulte and Botsch). Botsch found that learning of discriminations did not generally survive bilateral tectum removal, and the amount of post-operative deficit in discrimination and generalisation was roughly proportional to the difficulty of the discrimination, and to the amount of tectum removed. The ability to re-learn the problem was also proportional to these factors. Botsch found that operated fish with about half the tectum remaining were superior to normal fish in their ability to generalise stripe patterns, but inferior in ability to generalise dot patterns.

One result, which, if accurate, is of interest, was that regeneration of the tectum was advanced and organised within four weeks of operation. The work of Kirsche quoted above suggested that much longer periods were needed, but certainly there does seem to have been some reorganisation. Perhaps this difference reflects the greater regenerative ability of goldfish. If Botsch's figures are accurate, there is a nice correlation in one or two cases between discriminative performance, and

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time elapsed, which might suggest that the greater ability was from recovering visual input, as much as from learning. In other cases, the postoperative results follow the pattern that the greater the lesion, the greater the effect, and, although Botsch interpreted these facts in terms of Lashley's mass action principle, it would seem a more economical explanation to cite visual deficit as the cause of apparent failure to retain or learn.

Boyd and Gardner (1963) whose interesting results with stimulation of the forebrain will be described below, also tried the effect of electrodes implanted in the anterior edge of the tectum, just dorsal to the habenular nuclei. Fish were placed in a tank, with the electrodes implanted, and leads held so that they were not impeded, and were stimulated if they entered one half of the tank, not if they remained in the other. (Controls with fish with implanted electrodes and no stimulation showed that there was no initial preference for side). For one tectal placing, there was a 50% distribution of side, that is the stimulation had no effect. When the current was increased to 120 mu a., the stimulation became more positively-reinforcing, and, as current increased, there was more and more choice of the stimulated side. Such fish, when confronted with a bar-pressing situation, would radidly learn to press for stimulation when a signal light was on, and their pressing curves were of the strongly negatively-accelerated type seen in Olds' positive electrode placings in rats. Just what the nature of the reinforcement was to these fish is highly speculative, possibly there was stimulation of tectal-hypothalamic tracts; one can hardly imagine that flashes of light would be pleasurable. One possible explanation is that it has already been seen that stimulation of the anterior edge of the tectum causes activation of the tail muscles, this might cause fish to swim into the stimulated zone in the large tank experiment, but it is difficult to see how this could operate in a bar pressing situation where fish themselves determined the stimulation, when this stimulation was supplied only for short intervals following pressing, and when the fact that stimulation would follow bar pressing was signalled by an overhead light.

The importance of the tectum for vision was shown incidentally by Cronly-Dillon, Sutherland and Wolfe (1966) whose work on blinding various segments of the visual field by section of optic nerves projecting to the tectum has been mentioned above. They also trained fish with the same brachium of the nerve severed on both sides, so that they were blind in one hemisphere, dorsal or ventral. The discrimination was a simple vertical/horizontal one, which animals learned rapidly. The severed optic nerves were allowed to regenerate, then the other fascicles of the nerve were cut. Thus, when fish were trained again, completely different tectal segments to those which had been used in the previous training were receiving the input. Nevertheless, there was good transfer, which showed that although there might be projection of shapes on the tectum, this did not have to be in the same segment for recognition to occur.

Mark (1966) has recently investigated the functions of the optic tectum in interocular transfer. In some unpublished observations, Sperry, Arora and Mark had found that if the tectum were damaged on the trained side, there was transfer when the fish was tested using the intact tectum. Using Astronotus ocellatus, Mark split the tectal commissure (a thin band of fibres joining the two halves of the tectum in the basal layer) and

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allowed up to three months for recovery. He observed that whilst there was regeneration of the tectum, the tectal commissure did not regenerate. Both normal and split fish were trained monocularly (with blinders over the naive eye) to discriminate between a circle and a triangle, using the response method outlined in the section on interocular transfer. (All animals were pre-trained binocularly, to feed from the food holders). When blinders were changed, it was found that there was transfer in the normals, but not in the lesioned fish. Mark noted that he had tried to cut the posterior commissure in both Astronotus and goldfish, which Ingle (1965a) had cited as the site of transfer but had found that there was far too much interference with movement to allow any assessment of transfer. The present author has confirmed this using goldfish.

11. General effects of removal of the telencephalon.

Since the telencephalon represents the evolutionary precursor of the highly-developed cerebral area of higher vertebrates, it is at once natural and surprising that many of the early workers should have removed it. Natural, in that they wished to see what functions this organ was serving in an animal without a cerebral cortex, surprising, in that they often expected to find changes in behaviour similar to those seen in higher vertebrates lacking a cortex, or with partial cortical damage.

Numerous writers have dealt with the effects of removal of the telencephalon from a variety of elasmobranchs and teleosts. Desmoulins (cited by ten Cate (1935)) is generally accepted as having been the first to remove this part of the brain in fish, in 1825. He found that there were no obvious changes in locomotion or orientation after damage in a variety of marine teleosts, and a large number of workers have confirmed this. (See, for example, the review by Healey (1957)). Ferrier's (1886) results are some of the few to disagree with this; he found that carp,

/once

once operated, could swim naturally, but were virtually unable to stop swimming. They would cease this motion only when apparently exhausted, or when prevented from swimming further by the walls of the tank. Ferrier also stated that fish with this lesion could not feed. In view of the lack of effect found by many workers since, Ferrier's findings must be attributed to damage to parts of the brain other than the forebrain; for example, it has been seen that in some cases, tectal lesions may cause hyperactivity. A more probable explanation, in view of the author's experience with this operation, is that excess bleeding occurred, and that the clot so formed pressed on the midbrain, and caused the motor irregularities.

12. Other general effects of forebrain removal.

Whilst the previous section catalogued the lack of effect of removal of this part of the brain, it came to be accepted that there were certain parts of fish's behaviour which were affected.

The most obvious function of the telencephalon, and that which has been most generally accepted, is that of smell. Steiner (1888) demonstrated that after removal of the forebrain, Squalius cephalas located food more by sight than by smell. Strieck (1925) used blinded minnows, and trained them to go to a variety of olfactory cues, such as musk and skatol, to obtain food rewards. Fish were given their food impregnated with one of these substances, and it was found that after a time, they would react to presentation of the odoriferous substance alone, absorbed on cotton wool. When these fish were tested with a variety of other substances, similarly presented, they showed no reaction. Other fish were trained as mentioned above, with "taste" substances, and they performed well also. When the forebrain was removed, the former ("Smell")

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group showed complete loss of discrimination, the latter ("Taste") group showed no deficit. This experiment not only showed that the forebrain was concerned with olfaction as the entry of the olfactory nerves would suggest, but showed that the rather tentative distinction of smell from taste was in fact a valid one.

Janzen (1933) published a precise study of other effects of forebrain removal and used goldfish as subjects. Like other workers, he showed that there were no changes in movement, and that his fishes swam normally after operation. Removal of the pelvic and pectoral fins had no effect on locomotion over and above that seen for the same damage in normal animals. The amount of food, and the number of feeding times, were very similar in operated and normal fish. One of the most obvious differences in the fish was that the rate of gill movement was much more constant in operated than in normal fish. This was in direct contrast to the results of Springer (1928) who had observed in Mustelus and Squalus there were spasmodic respiatory movements after operation. Again, it is likely that some more posterior parts of the brain had been damaged, or that there was excessive bleeding. So few of these early workers controlled for the amounts of brain removed that it is difficult to assess their results. Janzen also observed that the rate of eye movements was much more variable in normal than in operated fish.

He then subjected lesioned fish to optokinetic tests, allowing fish to swim freely on the inside of a vertically-striped rotating drum. Both normal and operated fish tended to follow the stripes, but the operated fish appeared to be more "bound" to the stimulus, and were more unable to disregard it. If the forebrain were removed on one side only, it was noticed that there was much less effect, that these animals were

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intermediate between normals and fish with bilateral removals. It is unfortunate that Janzen did not notice if there was any difference in performance between intact and lesioned sides. One of his most interesting experiments was as follows: animals were placed singly in tanks across the centre of which were situated grids of vertical bars, with the same separation. This was in some cases 1.8 c.m., in others 2.9 cm., and in a third group 4 cm. Normal fish seemed to be attracted by this arrangement, and would swim through the bars in preference to remaining in one half of the tank; indeed, some of them would swim a criss-cross pattern in and out of the spaces. Operated fish were far less willing to cross, and the difference between operated and normal fish was greatest for the smallest bar separation, and least for the 4 cm. separation. In all cases, the animals with unilateral removal of the brain were intermediate between the normals and the operated fish in their rate of crossing. Fish with unilateral removals of the forebrain tended, when presented with a L/R choice, to go always to one side, but this tendency was also found in intact animals. He did not specify if there was a tendency to go the lesioned or to the unlesioned side in these animals. Janzen performed further experiments with L/R choices and unequal light intensities in the two halves, and concluded that there were two tendencies involved, a tendency to remain in whatever light intensity was present in the immediate surroundings and to seek out this intensity in preference trials, and a tendency to seek out a particular light intensity. The first of these abilities was unimpaired by forebrain removal, the second was abolished. Thus, there were some very definite effects of forebrain removal, which in Janzen's opinion represented a loss of ability to react towards new stimuli in a variable manner; initiative.

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Nevertheless, there remained an ability to swim and make spontaneous movements, and to carry on the usual functions without impairment; spontaneity.

Hosch (1936) confirmed Janzen's observations as to the lack of effect of forebrain lesions on movement and feeding. Using minnows as experimental subjects, he also found that there was a marked drop in the frequency of eye movements; these dropped to about half that of intact animals. In gobies, forebrain removal brought about a change in background preference. For example, before operation, 58.4% of time in a choice box was spent on a dark grey background, 16.8% on black, and the rest of the time on the other greys, the white surface was shunned almost completely (2.4%). After operation, 34.8% of animals' time was spent in the white area, and only 17.7% of time was spent in the previously-preferred dark grey area. There was also a much more marked scatter of times in the various areas, with approximately equal times being spent in the rest of the greys. Although Hosch interpreted this as a change of preference, it is quite possible that it was rather the reverse, a lack of response to preference. The original preference may have been present, but on account of the removal of the forebrain, had insufficient motivational value to cause movements to be made.

He also found that nystagmus and optokinetic responses were more stereotyped in lesioned than in normal fish. Hosch noted that removal of the anterior part of the forebrain did not produce these effects, but he did not show the extent of his lesions. He found that reactions to such stimuli as banging on the tank, and unfamiliar shapes could be elicited only with difficulty in lesioned fish, but that once they were shown, they tended to be more violent than those shown in normal fish.

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In one of his tanks he placed minnows, and a shelf under which fish could hide. When he banged the wall of the tank, fish would seek cover, and would re-emerge only after some minutes, and much careful survey of the immediate surroundings; they would emerge with every sign of caution. Lesioned animals, on the other hand, would rarely show this escape reaction, and in several cases it was necessary to actually strike the fish with a glass rod before they would hide. The time taken to re-emerge was much less than for normals, and these fish would emerge from hiding without any sign of fear. These results supported those of Janzen in suggesting that there might be motivational effects from the forebrain, although this supposition was not made at the time.

Wiebalck (1937) removed the telencephalon from specimens of Box salpa and Smaris alcedo, but was unable to get his fish to survive for any great length of time. Some of his observations suggest the sorts of damage mentioned above, over-enthusiastic removal damaging other brain areas, or pressure of clots. He placed fish in a bowl, one half of which was illuminated with blue light, the other with red, and recorded the time spent in each half. It is unfortunate that he did not record the number of crossings from one sector to the other; in view of work just quoted, there might have been a considerable difference. Although he stated that there were no differences between the groups of normal and lesioned fish, a Mann-Whitney test by the author suggests that his results show a significant difference (P nearly 0.05). On the other hand, Wiebalck attributed this difference to the fact that his lesioned fish tended to get more fungus infection than normals, and stated that fish with fungus tended to go more to the red section, in contrast to

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the normal preference, which, as has been seen in the section on colour vision, is to go to blue, and to avoid red. In view of this, it is very difficult to evaluate these results.

Little general work of this sort has been done in recent years. Ingle (1965a) has made the following report: goldfish were placed in a vertical cylinder, of 12" diameter. There was a 2" wide x 3" high hole in one part, giving access to a large 30 gallon tank. Fish were placed in this cylinder, and times for escape were measured. Most normal animals would escape from the cylinder within two minutes on their first trial, and times rapidly dropped to about 10 seconds. Fish lesioned 3-5 days before training would take much longer to escape, and some would not escape in the time given. If fish were left for 10 days after operation, the escape ability appeared, and these animals were not very much inferior to normals.

13. Stimulation of the forebrain.

Ferrier (1886) stimulated the brain of a carp using an unspecified electrical method, and found that there were movements of the whole animal, generally of the muscles of the contralateral side. Polimanti (1912b) found that stimulation had no effect on the musculature of various marine teleosts. Chauchard and Chauchard (1927a & b) stimulated the forebrain of Mugil auratus and Trigla gurnardus, using condenser discharges, but were unable to elicit any movements. If the stimulating current was increased, it was found that there were body movements, but the value of the current in these cases was so great that it was impossible that it was stimulating only that area immediately beneath the electrodes. They assumed that the movements were due to the breakdown of insulation, and the stimulation of both tectum and tegmentum.

Ferrier's work is usually explained on this basis. The work of Boyd and Gardner (1963) although involving stimulation of the forebrain, will be dealt with in the section on forebrain and learning.

14. Electrical activity of the forebrain.

Enger (1957) recorded potentials from the telencephalon of free-swimming cod (Gadus callarias). With bilaterally-placed wick electrodes on the forebrain, he found an 8-13 cps. rhythm, which was interspersed with amounts of higher frequency material, the amount of this increasing as the amount of ambient noise increased, irrespective of the modality of that noise. If noise were reduced; or anaesthesia were applied, the 8-13 cps. rhythm increased in amplitude, and the higher frequency components faded out. This suggested that both forebrain and tectum (which also showed these responses) were very sensitive to the level of outside activity. Enger confirmed this; dark-adapted fish were shown an 800 cps. stroboscopic light, and the 8-13 cps. rhythm was suppressed and replaced by an 18-32 cps. rhythm. Anaesthesia had the effect of reducing the frequency of the arousal reaction waves to 14-18 cps., whilst preventing the inhibition of the slower wave. There was no telencephalic arousal to sound stimuli.

Schadé and Weiler (1959) obtained somewhat different results using curarised goldfish, with various levels of anaesthesia. There was a 9-14 cps. rhythm in the telencephalon, which was interspersed with varying amounts of higher frequency components, this depending, like Enger's results, on the amount of general noise. Sudden flashes of light, which, as has been seen, produced arousal reactions in the tectum, had no constant effect on forebrain activity. In some cases, there was a reaction, in others, none. They concluded that the forebrain was an

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electrically uninteresting area, and that its functions were mainly olfactory. Aronson (1963) quoted two Russian papers which have not been available in translation. Maliukina and Flerova (1960) recorded from the forebrain of goldfish, and found that there was an arousal reaction to light. Voronin and Gusel'nikov (1959) found that they could not demonstrate differential responses from different areas of the forebrain of unspecified fish, but could show arousal reactions to visual, auditory and tactile stimuli. Kandel (1964) put forward the interesting suggestion that part of the forebrain was concerned with osmoregulation. He found that salt water introduced into the olfactory organ of goldfish gave rise to sustained inhibition of activity in the neurosecretory axons of the magnocellular part of the pre-optic nucleus, which axons discharge products into the distal part of the pituitary. Fresh water did not have this effect. It is known that the neurohypophsis of teleosts contains a factor which can cause anti-diuresis in higher vertebrates, but its function in fish is not understood. (See, for example, Barrington (1963)). On the other hand, Yagi and Bern (1963) have put forward evidence to suggest that the caudal neurosecretory system of the urophysis may be involved; similar treatments with fresh and salt water produced depression and activation of electrical activity in this organ, and it has been suggested that two types of neurohormones are operative. In view of this, it is rather difficult to evaluate Kandel's work. Certainly, fish with the forebrain removed do not show any sign of difficulty even over very extended periods, but perhaps this would better be shown if they were subjected to osmotic stress.

More recently, Oshima and Gorbman (1966) have recorded potentials from the olfactory bulb and from the telencephalon of goldfish, using curarised preparations, whose CNS has been destroyed posterior to the middle of the tectum. (Such a procedure would seem rather dangerous, in view of the likelyhood of generation of injury potentials). They used a standard salt stimulus applied to the olfactory bulb, and found that a three-phase burst of firing could be recorded from the olfactory bulb, and that after section of the olfactory tract, that is, isolation of the pedunculate olfactory bulb, the third component of the response was eliminated. This was interpreted as being due to lack of central (telencephalic) facilitation of olfactory responses. There was also a drop in amplitude of the other responses. Application of thyroxine produced a similar effect, in animals with tracts intact, inhibiting central effects, and preventing the secondary response of the bulb. Thyroxine also had the effect of exalting the primary olfactory response. This work is interesting in that, along with the observations of Enger, and Schadé and Weiler, it supplies evidence of forebrain function, but the thyroid effect is of doubtful significance, since, as in the case of the tectum work, the quantity used was so far above that possible in the natural course of the animal's life. Oshima and Gorbman also recorded from the telencephalon, and found that maximal olfactory responses were obtainable from the medial part, and that these faded if electrodes were placed more laterally.

15. Effects of forebrain lesions on social and reproductive behaviour.

The first definite effects of forebrain lesions were noted by Kumakura (1927) who removed the telencephalon of goldfish. Although half an hour after the operation animals swam normally, and showed no other obvious signs of discomfort, they would not form shoals or aggregate in the normal fashion. They tended to show more spontaneous swimming on the day after operation, but it was between five and fourteen days before they would shoal as pre-operatively. There were no operated controls, and the usefulness of this work is diminished to that extent. Hosch (1936) showed that in minnows the times spent shoaling by normal and operated fish were very different. Operated fish tended to spend longer in solitary conditions, and could break away from a shoal with greater ease than could normals.

In a series of investigations Noble (1936 & 1937) and Noble and Borne (1941) investigated the effects of removing the forebrain from various teleosts. Their results were published only as brief communications, so that much of the value is lost. Hemichromis maculatus with unspecified partial forebrain lesions could breed, but the brooding of the eggs was interfered with in an unspecified way; this deficit lasted for over a year, during which one fish bred twelve times, the other twenty eight times. Other aspects of the reproductive process did not seem to be affected, since the lesioned fish could mate without difficulty. Unilateral removal of the forebrain did not totally abolish courtship behaviour, but bilateral removal eliminated mating completely. Forebrainless fish could show aggressive behaviour, but this was not consistent, and was by no means always elicited in correct situations. Essentially similar results were obtained with Betta splendens. In Xiphophorus helleri, sexual behaviour persisted after operation, but fighting was inhibited.

Wiebalck (1937) in the paper quoted above, found that shoaling in Box and Smaris was inhibited after lesion. Berwein (1941) performed a

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long-needed experiment, that of severing the olfactory tracts. She found that normal, tract-sectioned, and forebrainless minnows all shoaled, and that if a new fish were introduced into the aquarium, it would not be allowed to join the shoal, but would be driven off. Forebrainless fish were much more rapid than either tract-sectioned or normal fish in accepting newcomers. In a subsequent experiment, Berwein found that lesioned fish were much faster than normals in becoming accustomed to life in a new tank, and to taking food under these strange circumstances. Again, tract-sectioned animals were much more similar to normals than to lesioned fish, so Berwein concluded that the effect had other than olfactory bases.

Kamrin and Aronson (1954) found that in Xiphophorus maculatus, the platyfish, all sexual acts except gonopodial swinging (the actual act of copulation) were impaired and decreased in frequency after removal of the forebrain. There was no actual elimination of any act, so Aronson concluded that the forebrain facilitated acts whose neural organisation was represented lower in the brain. In a subsequent paper, Aronson (1948) found that the various stages of courtship of the teleost Tilapia macrocephala were unaffected by forebrain lesions, but that such acts as hole-making, oviposition, and fertilisation were. For example, only 8% of lesioned males made fertilisation movements in the presence of freshly-shed eggs.

Hale (1956b) using Lepomis cyanellus, made a series of lesions of varying extent to investigate the effect of the forebrain in governing aggressive behaviour. He found that unilateral removal rarely if ever had any effect. Removal of the lateral and dorsal edges of the brain

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reduced the probability of the animal starting a fight, that is, lowered the position of the fish in the social hierarchy. Ventral lesions had an even more pronounced effect, possibly because of the severance of the forebrain bundles. The amount of "driving", or aggressive behaviour decreased as the size of the lesion increased, and in animals with total removal, there was very little spontaneous display of aggression. If these fish were stimulated, they would show well-organised behaviour, and Hale concluded that this was in favour of Aronson's facilitatory hypothesis.

In a series of papers, Segaar and Nieuwenhuys (Segaar (1956, 1960 & 1961), Segaar and Nieuwenhuys (1963)) have investigated forebrain function in reproduction in the stickleback, using van Iersel's (1953) ethological analysis as a basis for comparison. They divided post-fertilisation behaviour of the male into three components: a tendency to display aggression, a sexual tendency, and a parental one. These were expressed as percentage of total time spent in that activity. The sexual tendency was initially high, but this decreased over the seven days till the hatching of the eggs. The aggressive tendency fluctuated, being generally high, but low at the time of hatching, whilst the parental tendency rose until hatching, then fell rapidly. Anterior or total lesions reduced aggression and sexual activity, but tended to elevate parental activity. Lesions in the posterior median dorsal area increased these former activities, whilst depressing the latter one. Segaar and Nieuwenhuys went on to investigate the effects of smaller, electrolytic lesions, and found that lesions in the last-mentioned region could give different results for very small differences of lesion. If the lesion were in the posterior part of this region, parental activity was elevated, if in the anterior part, parental activity was depressed. In neither case was either of the two other variables affected. Although these workers have drawn very few conclusions from this work, it does suggest a degree of localisation which had been foreseen in Hale's work, and which would hardly support Aronson's facilitatory hypothesis.

16. Effects of removal of the telencephalon on learning and retention.

In view of the fact that the forebrain was the evolutionary precursor of the elaborate cortical development of mammals, a number of workers attacked it with the supposition that they would find the same sorts of deficit as were found from cortical lesions in the higher forms, they tended to forget, as has been seen in the introduction, that it would be much more realistic to look for effects analogous to those found from basal lesions in higher vertebrates, from libic or similar lesions.

The first experiments were those of Scharrer (1928) who was not even concerned with memory. He was, as has been described, investigating the location of the site of light discrimination which was extra-ocular, and used the dorsal light technique described in an earlier section. Blinded fish were fed when a light came on, and after some trials, they showed a feeding response to the light alone. Removal of large amounts of the forebrain did not abolish the response.

Subsequent workers have adopted a rather peculiar attitude to the effect of forebrain lesions. As will be seen, there has in fact been evidence for forebrain involvement in learning from very early experiments, but since deficits were not absolute, the authors have tended to state that there were no effects, and reviewers, reading only summaries, have tended to promulgate the idea of total lack of effect.

/Such

Such criticism does not apply to Janzen (1933) although his work is often quoted as evidence for lack of effect. He trained lesioned and normal fish to discriminate between red and blue shapes, using food as reward. The operated animals were at all stages of learning except the first day inferior to the normals. Operated fish made on average 10% more errors than normals, and were more variable in their responses. Janzen concluded that the operation did affect learning, but he is rarely given credit for having made this observation.

Nolte's (1933) results were rather peculiar. He trained minnows to discriminate between various parts of spectra, and they were rewarded for going to the correct sections. Fish learned this well, and performance did not seem to be affected after operation, although Nolte expressed these results as totals; they would have been more informative if they had been expressed in graphical form, in view of his next results. He trained a number of fish on a + versus - problem. They learned very well, as would be expected from data on visual discrimination given above. After operation, length of survival was in all cases except one very short, and only twenty to thirty trials were obtained. One animal was trained over about 100 trials, and it is perhaps significant that its performance was much more variable than before operation, when it had rarely dropped below 80%. After operation, it dropped to 50%, and fluctuated considerably, even though the performance remained on the 100% side of the chance line. It would have been interesting to see what type of curve Nolte obtained for his long-term survival colour discrimination animals. Hosch (1936) working with minnows, made much more definite observations on the functions of the forebrain in learning. He trained normal and lesioned fish to discriminate between a four-pointed

/star

star and a circle, both of which were provided with feeding troughs on their faces. The rates of learning of normal and operated fish were very similar. Similarly, he trained fish to go to one or other side of a T maze to receive food, and there were again no differences; reversal trials yielded the same result.

Berwein (1941) trained blinded minnows to respond to changes in temperature of the surrounding water, using the technique of cold/food and hot/shock which was outlined in an earlier section. Water below 11°C was naturally considered aversive, and hotter water attractive, so the bias of the discrimination was in the correct direction. Berwein stated that there were no differences in learning between normal and lesioned fish. If she was implying by this that the asymptotes after 11 days' training were not different, this alone would be disputable, but the rates of learning were much impaired in lesioned fish. These animals took 11 days or more to reach the level of response which normals had reached within three days. In spite of this, little is made of the fact in the literature.

Zunini (1954) summarised his large series of experiments, mainly on minnows, and stated that some forms of learning were affected, and that others were not. Animals trained with a simultaneous colour discrimination showed no deficit after operation, though he gave no details of these results. He found that animals trained with simple maze or detour problems, such as Russell's food jar, or Beniuc's screen problem, showed an almost total deficit after operation, and their reaction times rose to those of naive animals, but did not fall in the rapid fashion of normals. Naive lesioned animals were similarly inferior, taking much longer than their intact counterparts at all stages of the training.

Baru (1955) and Karamyan (1956), both quoted from Segaar (1965) trained a variety of marine fish to show conditioned responses to light and sound associations with shock as UCS. In dogfish, skates and some teleosts there was no effect of total forebrain removal, and naive lesioned fish showed no learning deficits. These experiments were designed to test the effects of forebrain removal on time-dependent paradigms, a very reasonable supposition, in view of the increased latencies seen by other workers, and it is rather surprising that there was such a lack of effect. Kholodov (1961), also quoted from Segaar (1965) trained both normal and forebrainless carp. Fish were initially trained to snap at a hanging bead when a light came on, and they were rewarded with food. Next, a second bead was introduced, and a differently-coloured light presented; fish had to inhibit reactions in this case. There was no difference between normal and lesioned fish in either retention or learning. Increases in complexity of such tasks produced no difference between the normal and operated fish.

Hale, in his first 1956 paper, used a Welty-type maze, where fish had to swim from one box to another for food when the connecting door was opened. He investigated the phenomenon of social facilitation, whereby groups of fish learned more rapidly than single animals. Single animals took about 8 minutes at first, dropping to about 4 minutes, whereas groups of three animals took 6 and ½ minutes respectively. Single operated fish took 12 and 11 minutes, grouped operated fish took 12 and 4 minutes. Thus there was a social effect in the forebrainless fish, but the deficit in performance was very large even in these cases. If single normals were lesioned when they had learned, their reaction times went up to 11 minutes, a very nice correlation with the results for single lesioned

/fish

fish trained only after lesion. In his paper, Hale quoted two pieces of unpublished thesis work, which supported these results. Mussey (1942) and Hillowitz (1945) both found that goldfish lacking forebrain took longer to run Welty-type mazes than did normals.

Aronson and Herberman (1960) in a very short abstract, reported that forebrain lesion in Tilapia caused fall-off of a previously-learned bar-pressing response; food was the reward. The latency of pushing was decreased, and the number of pushes was reduced. Warren (1961) trained fish in the maze situation described above. His fish were specimens of Macropodus opercularis, and both normal and lesioned animals were trained with a variety of maze problems, then with an L/R T-maze problem, and reversals of this. The operated fish were significantly inferior to normals on the first maze problems, and showed an oscillation when given reversal tests which suggested that the original problem was never forgotten. Operated fish took twice as long to learn reversal of the original task as they did to reverse this reversal. Normal fish took about the same number of trials to accomplish each reversal. This result is reminiscent of Hosch's observation that these animals took longer to acquire a reaction, but were more violent in it when they had learned.

Bernstein (1961a & b, 1962) used the conditioned heart deceleration technique outlined above, and initially stated that pre-operatively-trained hue and brightness discriminations were affected in goldfish. In later papers, he modified his views, allowing that his previous recovery time of a few minutes before the start of training was insufficient. When some hours' recovery were allowed, fish showed good discrimination of both colour and brightness, generalised to these, and showed

/interocular

interocular transfer of the differential response. Thus, like the work of the Russians quoted above, this work with simple conditioned responses does not seem to have shown up any functions of the forebrain.

Boyd and Gardner (1963) whose stimulation work with the optic tectum has been described, used exactly the same technique with fish with telencephalic electrodes. An electrode in the dorsal region of the forebrain had no effect up to values of 120 mu a., but after this, the fish spent 80% of its time on the unstimulated side. Another placing, more anterior, was aversive from 20 mu a. upwards (figures for time on the stimulated side dropped from 40% at this current to 5-10% at 120-160 mu a.). If these fish were presented with a bar press situation at the termination of this training, such that they could avoid stimulation when a light came on by pressing the bar, the rate of pressing rose rapidly. This would seem to be very strong evidence for the forebrain's functioning in some sort of reward-addressing capacity.

Ingle (1965b) trained goldfish in a continuous Y maze, of the type described above. Fish were trained with two schedules, a "go to one particular side", and an alternation schedule. Operated fish were inferior to normals in learning the latter task, but were superior to normals in learning a task which involved going to the same side each time. Ingle suggested that this was due to the greater ease with which lesioned fish could learn the more "reflex" tasks.

17. Anatomical data on the telencephalon.

In view of the subsequent interpretation of the behavioural results of the present work, a brief review of the homologies and structure of the forebrain will be made. Obviously, there is no development of higher cortical structures in these animals, and the areas present are those

/which

which in higher Vertebrates would be classed as the basal forebrain areas of the limbic system and its associated nuclei. To what extent this can be taken to mean that these nuclei and tracts <u>are</u> represented in fish is doubtful. Crosby's school (see for example Schnitzlein (1964)) have produced a scheme which identifies a series of primordia, which are supposed to be the evolutionary homologues of the mammalian structures. On the other hand, consideration of evolutionary changes would lead to caution on this point, since the teleost forebrain, in common with the rest of teleost structure, has evolved divergently from the main amphibian/reptile/mammal stock over about 400 million years, and can be expected to have developed its own peculiarities.

Furthermore, Nieuwenhuys (1966 and other papers) has put forward evidence to show that the tela of the neural tube in fish, a thin non-nervous sheet, extends considerably during development. The dorso-lateral walls of the tube move laterally and their area of attachment to the tela bends ventrally, describing a semicircle, meeting and fusing with the ventrolateral part of the tube. The extra-cerebral space enclosed by the meeting of the fold and base of tube is seen in <u>Polypterus</u>, but is obliterated in higher fish. Thus parts morphologically dorsal come to lie laterally. Unfortunately, Schnitzlein and his co-workers have not incorporated these data into their anatomical system, so that their nomenclature would in certain cases seem erroneous. For example, the medio-dorsal area of the brain, their primordium hippocampi, would <u>not</u> represent the dorsal area of the brain if Nieuwenhuys were correct, since the original dorsal area of the brain would lie laterally. These differences have yet to be resolved, and it is perhaps better to adhere to the non-homologising system of Nieuwenhuys than to use rather doubtful homologies of the Crosby type.

The forebrain of goldfish consists of two symmetrical hemispheres, joined ventro-posteriorly to the diencephalon, and sending a pair of olfactory tracts anteriorly to the pedunculate olfactory bulbs in the snout. The olfactory tracts divide into two main portions before they enter the telencephalon, the lateral and medial olfactory tracts. The lateral tract runs just dorsal to the entorhinal fissure, the point of attachment of the thin roof of the brain. The medial tract runs more directly into the brain, entering medially and ventrally.

The telencephalon can be divided roughly into two main parts, the dorsal and ventral halves. The dorsal part consists of a small number of regularly-arranged cell areas, without any very striking nuclear masses, but distinguishable from one another by cell size. The ventral segment contains the most obvious tracts and nuclear areas. The medial olfactory tract, running ventrally, gives off fibres to the medial portion of brain possibly including the medial dorsal area. It also contributes a decussating component to the anterior commissure, which joins the two hemispheres ventrally about two thirds of the way from their anterior end. The medial olfactory tract runs posteriorly to terminate in the medial extreme posterior part of the dorsal area. Other fibres terminate in the supra-commissural part of the medial area of the brain, at the junction of drsal and ventral areas. Fibres from the lateral olfactory tract run into the ventral part of the lateral dorsal area, and it would appear that a number of these decussate in the anterior commissure.

Two main tracts connect the forebrain with lower brain areas, the medial and lateral forebrain bundles. The medial bundle derives much of its input from the medio-dorsal part of the ventral area, and also /from

from the pre-optic nucleus. (The magnocellular part of this nucleus sends neurosecretory axons to the pars nervosa of the pituitary). The main part of the median forebrain bundle terminates in the hypothalamus, in the region of the nucleus subrotundus, which in turn has connections with the nucleus rotundus and the tectum. There is no evidence as yet for the existence of ascending fibres in this bundle.

The lateral forebrain bundle derives input from most areas of the dorsal segment of the brain. The extreme ventral portion of the lateral area is, according to Nieuwenhuys, the only area not sending a complement of fibres. These fibres associate in a large strap-like tract running just dorsal to the entorhinal fissure in the lateral edge of the lateral dorsal area. Some fibres decussate in the anterior commissure, but many cross to the lateral area of the contralateral side, forming a true commissure. The lateral forebrain bundle enters the diencephalon, and terminates in the inferior lobe of the hypothalamus; some fibres, however, terminate in the nuclei subrotundus and rotundus. Johnson (1911) considered that there were afferent fibres to the dorsal part of the dorsal area in this bundle, coming from the inferior lobe of the hypothalamus, and Sheldon (1912) agreed that there was afference to the forebrain in this bundle, although he did not specify the origin of the fibres. Herrick (1924) considered that there were afferent tracts which ran from the hypothalamus to the dorsal and central areas of the dorsal segment. Nieuwenhuys has described ascending fibres in the telencephalon of the stickleback, terminating in these areas. In view of the electrophysiological findings of arousal reactions, it would seem most likely that there were such tracts.

Other main tracts in the forebrain are as follows: a tract from the extreme posterior segment of the central area of the dorsal segment, running to the habenular nucleus, and a tract from the magnocellular part of the pre-optic nucleus, with the same termination.

Nieuwenhuys (1959) considered that the main olfactory input was to the medial part of the dorsal area, and extreme posterior part of the central area, of the dorsal segment. The pre-optic nucleus also received olfactory input. These observations would agree with those of Kandel, and of Oshima and Gorbman, using electrical recording methods. The dorsal area of the forebrain, whilst receiving a small olfactory input, receives most of its fibres from the lower areas of the brain.

18. Lesions to other parts of the brain, and their effects on learning.

Lesion techniques have only just reached the fineness necessary for attacks on thalamic and hypothalamic nuclei, so there is no data on these areas. Nolte (1933) using the colour discrimination technique outlined above, found that damage to the cerebellum had no effect on previously-trained discriminations. Kaplan and Aronson (1963 and 1965) found that using an avoidance technique, of light/shock association, cerebellum-ablated *Tilapias* could not learn. The lesion, given to pre-operatively trained fish, abolished the learning. They gave no indication of the extent of lesions, and this lesion is one which, *par excellence*, needs controls to assess the amount of motor impairment caused.

19. Non-lesion techniques of affecting memory.

Agranoff and his associates have made a number of experiments investigating the effects on retention of electroconvulsive shock (ECS) and puromycin (an inhibitor of protein synthesis). They trained goldfish to avoid a light stimulus by swimming into a goal box, and used a CAS/UCAS interval of 20 seconds. Davis, Bright and Agranoff (1965)

/trained

trained fish with 20 learning trials on one day, and retention trials (10) three days later. ECS, or administration of puromycin within 90 minutes of training, produced significant deficits in retention three days later. Injection of a puromycin derivative (puromycin aminonucleoside) or of saline, had no effect. The amount of deficit was also proportional to the amount of puromycin administered (Agranoff, Davis and Brink (1966)). They observed (Agranoff, Davis and Brink (1965)) that administration of puromycin before training seemed to facilitate learning, and concluded that the consolidation of long-term memory was being affected, but that short-term memory was relatively untouched. They also showed that there was an inhibition of protein synthesis in the brains of their fish following injection of puromycin, but that this inhibition lasted longer than the memory deficit. Unfortunately, they did not assess the effects of the drug on general mobility. It has been shown recently by Barondes and Cohen (1967) that in avoidance-trained rats, puromycin has these effects, but that other protein inhibitors, even more potent in some cases, do not. This suggests that there was some specific effect of the substance other than on protein synthesis, and also there may have been some slowing of the fish, since it has been observed that in <u>Octopus vulgaris</u> there is a "doping" effect (Young, personal communication).

20. From these data, it seemed reasonable to suppose that the forebrain might be involved in learning in the capacity of a system which mediated the correct addressing of the emotional results of various actions. As has been seen, there has been a tendency to minimise its importance, and to attribute its olfactory functions alone. It will have become obvious from the discussion that this is not fair, since much of its

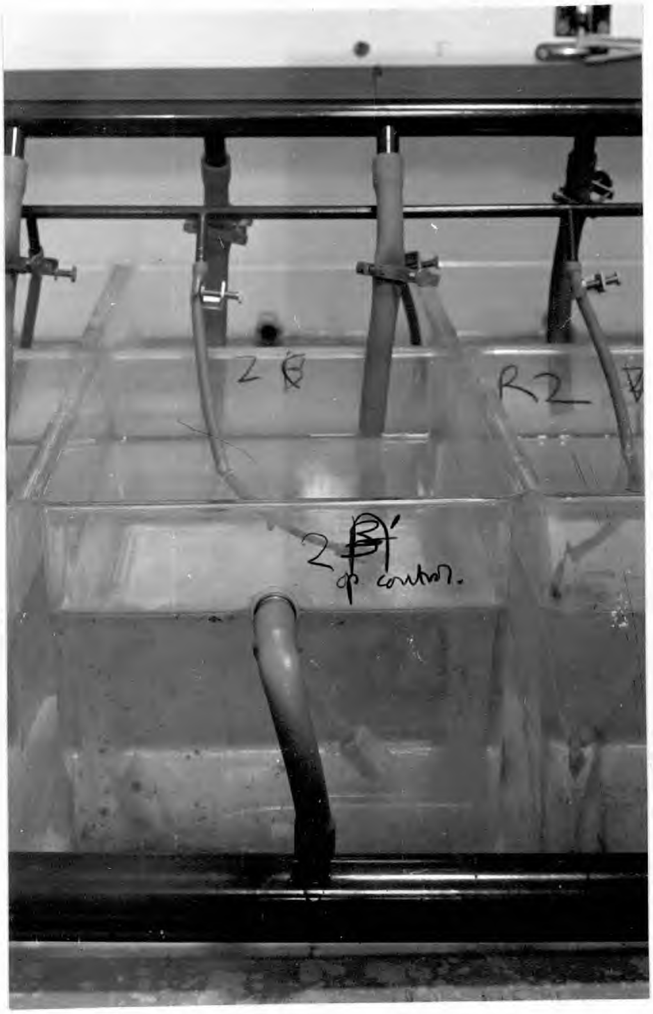
/dorsal

dorsal area is without olfactory input, and this has been observed both anatomically and physiologically. Bitterman's and McClary's avoidance situations seemed to provide useful methods for studying the effect of the lesions on negatively-reinforced trials, and Boyd and Gardner's work suggested that it might be as well to investigate this polarity of reinforcement in preference to the positive side. In addition to this, the simple methods of Bitterman gave an opportunity to investigate the optic tectum, using stimuli and responses which could be appreciated by fish with visual deficits, and promised to allow some unravelling of the old confusion between "memory" and lack of acuity.

(When the early work on simple avoidance was finished, Segaar's article in Progress in Brain Research 14 was published, and made mention of Aronson's work, reported only as brief abstracts. Prof. Aronson sent the author details, and stated that he and Kaplan had found that removal of the forebrain in <u>Tilapia</u> interfered with avoidance learning, and abolished such learning in previously-trained animals. Section of the olfactory tracts did not. Whilst this thesis was being typed, Hainsworth, Overmier and Snowdon published a paper dealing with the effects of forebrain lesions in goldfish, and replicating one of the situations already used by the author, that of light stimulus and 15 second CAS/UCAS interval. Results were very similar to those obtained already by the author).

Figs. 1 a and b: Tanks for experimental animals.

1(a) General view.



1(b) Individual tank.

METHODS

1. Maintenance of animals.

(a) Initial treatment.

Goldfish (Carassius auratus), 4-5" in overall length, were obtained from a regular supplier, and placed in large stock tanks for at least a week. During this period, any obviously moribund animals were discarded. Sodium chloride was added, at 10-15 grm. per litre, to relieve any fish in poor condition. The stock tanks were continuously aerated and the water in them was changed regularly every three weeks. In the intervening period, the water was continuously filtered over activated charcoal and spun glass. The stock tanks were so positioned that direct sunlight rarely fell on them, but were otherwise allowed to attain room temperature. All animals were fed regularly (see below).

(b) Tanks for experimental animals.

At the end of the quarantine period, or when fish had attained suitable fitness, certain animals were selected for training, an attempt being made to choose those individuals which showed the greatest flight reaction to movements outside the tank, bangs on the wall, and the greatest persistence in evading the net. Selected animals were placed in the experimental tanks, details of which are shown in Figs. 1 a & b.

An overhead pipe, with offsets over each tank (14 in all) allowed the flow of water to each tank to be separately controlled. This had the advantage that diseased fish could be isolated whilst receiving an uninterrupted water supply. Each tank had its own outflow pipe, emptying into a common ring drain which emptied into the sink. Aeration was supplied in a similar manner, a common inflow pipe giving a branch to each

/tank.

tank. The tanks used were of polystyrene, 9 x 9 x 12" containing 9 litres when filled to the level of the overflow. The aeration was maintained continuously, the water was generally run for 6 hours each day, the rate for each tank being in the order of 500 - 700 cc's per minute. Waste materials were washed out, and the rate of change of water was such as to prevent large temperature fluctuations, even in summer. No specific measures were taken to remove chlorine in the incoming tap water, but it is generally accepted (van Duijn 1956) that if the rate of flow is not too great, constant aeration removes most of the gas. The number of animals in any tank never exceeded three.

(c) Feeding.

In early experiments, fish were fed on commercial dry fish foods, but the increased incidence of fin-rot, and of general poor health, prompted a change to fresh food. Daphnia and Tubifex, the most commonly used live foods, had the disadvantage that they frequently carry the spores of Ichthyopthyrius multifiliis, the protozoan causing the skin disease known as whitespot. All fish contributing to the results below were fed on either heart or liver, the former being preferable on account of its greater integrity. Fish fed on this food alone survived indefinitely. Every two days all animals were fed as much chopped meat as they would eat. It was found that more frequent feeding was not necessary; fish would not feed.

(d) Disease.

Since the condition of fish from the supplier varied considerably, especially in early 1965, resistance to disease was not constant. Many fish developed fin-rot, a condition of general debility, characterised

/by the

by the development of blood streaks between the fin rays of the dorsal and caudal fins, followed by the disintegration of the interstitial membrane. A pronounced humping of the anterior dorsal surface of the fish, and a drooping of the anal region were also noted. The first sign of this, or of any other disease, was the drooping of the dorsal fin. Fish affected by fin rot would often succumb to other diseases. The condition seemed to be caused by underfeeding, constipation due to feeding on dry foods, and lack of adequate aeration or of filtration.

Fish suffering from this disease were at once isolated, and left in well aerated tanks in a 1-2% solution of sodium chloride. In some cases an 0.01% solution of methylene blue was used, to kill bacteria on the skins of the fish. With regular feeding, fish generally recovered within a fortnight, though the regeneration of lost areas of fin took longer.

Other diseases were less frequent. One or two cases of white spot (Ichthyopthyrius) were successfully treated by immersion for ¾ hour in 0.3% picric acid, followed by a few days in 0.01% methylene blue. Two cases of dropsy (an accumulation of fluid in the coelomic cavity) were encountered, but it was not found possible to save the animals. The method quoted by van Duijn (1956) was tried, namely hypodermic draining of the fluid followed by immersion in 3% hydroquinone, but this proved ineffective. Post-mortem examination failed to determine the cause of death; van Duijn has suggested that the disease is viral; fortunately its occurance was rare.

The only other infection encountered was the infection of fish by Lernocera, an ectoparasitic copepod, whose hooked holdfast made a considerable wound in the body wall, and caused a great weakening of the host.

/host.

All fish were examined for Lernocera when they were initially brought in, and were isolated if infected. Animals were anaesthetised, and a small swab, soaked in concentrated potassium permanganate, was applied to the distal end of the parasite. After a number of such treatments, the parasite released its hold, or could be gently removed by forceps, and the fish was allowed to recover in a 5% solution of sodium chloride.

2. Operations.

(a) Anaesthetics.

A number of anaesthetics was tried, including chloroform, ether, and urethane. The first two were rejected on account of the difficulty of obtaining aqueous solutions, and speed of recovery. Urethane, as a 2% solution, gave good anaesthesia, but the carcinogenic properties of this substance induced the choice of MS222 Sandoz (tricaine methane sulphonate). This was used in concentrations of approximately 1:5,000 in tap water. This figure was arrived at after attempts to find some relation of either temperature or body weight to the necessary concentration of anaesthetic. No relation could be drawn between these quantities, the rate of anaesthesia seeming to be more dependent on the general state of the fish than on size, or concentration of MS222. With the concentration of 1:5,000, most animals took about 1-2 minutes to roll on to their sides, and to cease opercular movements. The cessation of opercular movements was taken as indication that anaesthesia had reached a point suitable for the commencement of operative procedures. Animals could be held in this condition for upwards of 1 hour, though in practise a period of 20 minutes was rarely exceeded. Flushing

/of fresh

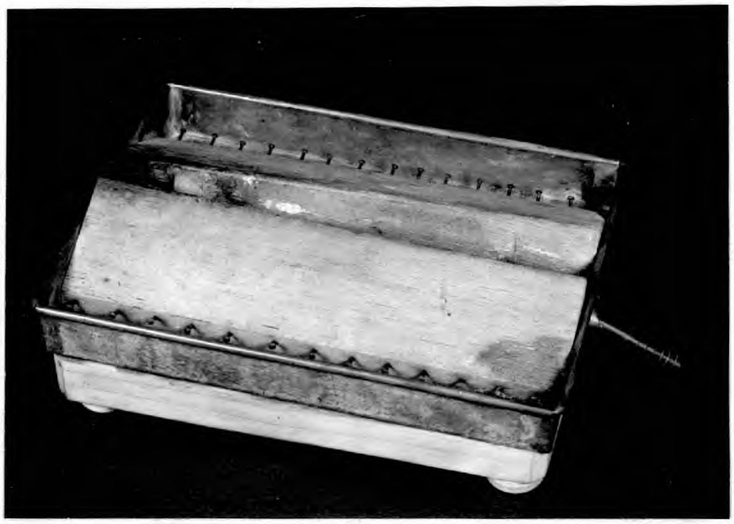


Fig. 2a: Dissection dish used in all operations save electrolytic ones. Fish's head rested at LHS. N.B. waste pipe for used water at RHS.

Fig. 2b: Dish under dissection microscope. N.B. water pipe (supplying animal's respiratory needs during operation) running round base of apparatus.

of fresh tap water over the gills produced first opercular movements about 5 minutes after removal from MS222, and 5 minutes after these movements, the animal was generally swimming reasonably normally. In all cases except those where serious damage had been done to the brain, fish were able to feed and were fully recovered within 20 minutes of the operation.

(b) Treatment after operation.

Operated animals were placed in the experimental tanks, in which a small amount of MS222 had been dissolved. This served to quieten the fish during the recovery period; some animals tended to show extremely energetic movements when they were fully recovered, and damage themselves. The flow of water through the tank over the next few days soon lowered the level of anaesthetic to near zero. On the day after operation, the animals were fed, and only if animals fed properly and regularly were they allowed to go forward for training. Despite the apparent lack of effect of operations even minutes after recovery, ALL operated fish were left for five clear days before training was commenced. This gave animals a chance to recover from any post-operative shock, for healing of the skull wound to occur, and for the animals to regain their strength.

(c) Operating trays.

Two operating trays were used, which were basically similar to that described by Healey (1940). One of these is shown in Figs.2a and b, the other in Figs.2d and e.

Fish were wrapped in wet tissue paper, with the opercular region

/exposed

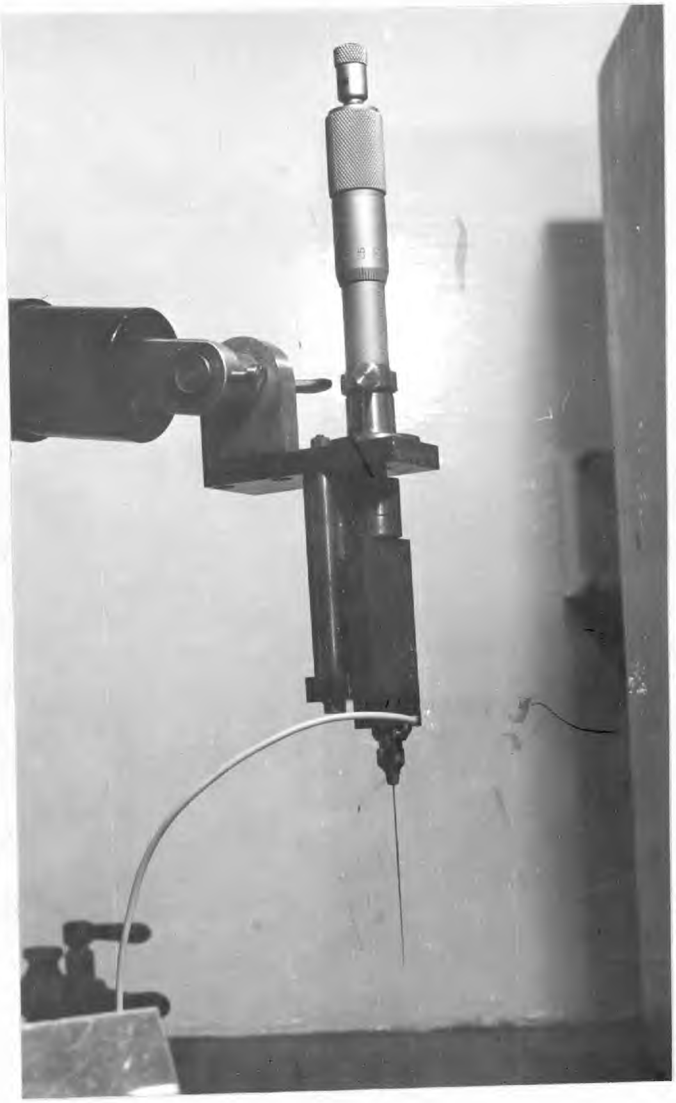


Fig. 2c: First configuration of electrolytic lesion unit.

exposed, and placed in the deep groove in the wooden block; the taper in this groove allowed the fitting of fish of different sizes. A thin polythene tube was placed in the mouth, through which was passed water from one of two bottles, one containing a 1:5,000 solution of MS222, the other tap water. Water draining from the animal flowed away in the pipe at the base of the tray, and suction was used to facilitate this. (The average rate of flow of liquid over the gills was 20 cc's. per minute).

(d) Instruments.

Operations were performed under a Zeiss binocular dissection microscope (Figs. 2b and e). A 4 mm. trephine was used to remove a circle of skull, and excess fluid over the brain was removed by gentle swabbing. In some early cases, suction was used, but it was found to be insufficiently controllable. Jeweller's forceps and fine scissors were used for most operations, whilst specially sharpened corneal knives were used for very fine surgery. All instruments were boiled regularly, and were cleaned after use.

(e) Electrolytic lesions.

In the later stages of the project, an apparatus was developed for the placing of electrolytic lesions. The basic unit is as shown in Fig. 2c.

I am indebted to Dr. B. G. Cragg for help in the design of this instrument. It consisted of a micrometer screw which drove a slide, at whose end the needle was located. The slide was magnetically attached to the baseplate, and moved on ball-bearings; this gave an extremely smooth movement. Initially, this unit was used attached to a universal

/joint

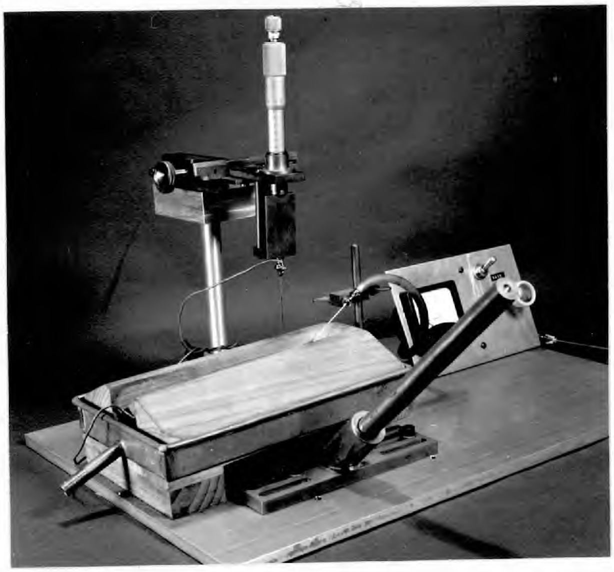


Fig.2d: Final configuration of unit. Note water tube at RHS end of dish.

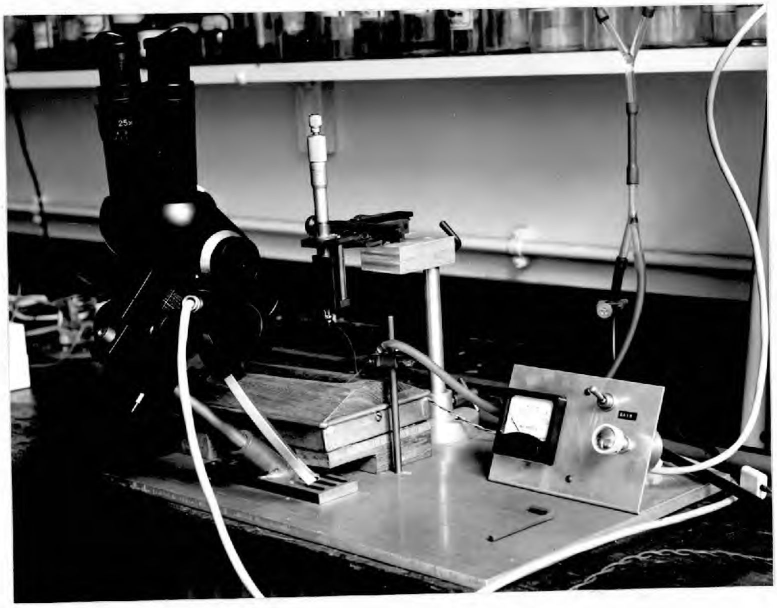


Fig. 2 e: Electrolytic lesion unit with dissection microscope in place.

joint on a clamp stand, but the whip in the system was such that it was impossible to move the micrometer screw without causing oscillation of the needle; in one case it was necessary to kill the animal being treated, on account of the amount of other damage caused. The apparatus was therefore modified to the configuration shown in Figs. 2d and e.

The dissection tray, similar to that described above, was rigidly fixed to a heavy aluminium baseplate. In the base of the wooden groove was located a copper plate, which served to earth the animal. The micrometer head was attached to the slide stage of a microscope, so that accurate, steady movement could be achieved in the horizontal planes. The whole assembly could be raised and lowered, and turned sideways, to facilitate placing of the animal. The dissection microscope mentioned above could be placed on the oblique bar in front of the dish, and used to study the placing of the needle. On a small panel at the side were located controls, an on/off switch, and a potentiometer, and a milliammeter. The intensity of the lesion could be controlled by varying time or amperage. The animal, lying in its wet wrappings on the copper plate, was made negative, whilst the needle was positive. Thus the optimum condition of small electrode and large earth areas was approached. The needle liberated Fe^{++} and Fe^{+++} ions at the site of the lesion, and these could be stained in the manner indicated below.

Needles for use with this unit were prepared in the following manner. No. 1 beading needles were obtained, and sharpened in the acid mixture shown below:

/Distilled water

Distilled water 15%

Conc. H_2SO_4 .... 45%

Sat. H_3PO_4 .... 40%

A 24v. DC variable voltage source was used. The acid mixture was at first heated before use, but it was found that the action was too violent, and thereafter cold acid was used. A large copper baseplate was placed in the bottom of the acid bath, being the anode. To begin with, a high current density was used, and this was reduced as the sharpening proceeded. The needle was moved slowly up and down, to preserve a smooth taper. Immediately a needle was finished, it was dropped into a bath of absolute ethyl alcohol; this prevented the rusting which would have developed if the needle had been left in air for any time. A number of needles were then pinned, blunt end fist, into a cork, which was placed so that the needles dipped into a bottle of warm thin varnish. This bottle floated in a reservoir of water, whose level was then allowed to fall by siphoning. Thus a smooth deposition of varnish was achieved. The cork was then left for a day in an oven at 60°C, to harden the varnish, and the process was repeated. The insulation at the tip of each needle was broken by touching the tip, connected to the + pole of the power source, to the above acid solution, with the minimum current. If this did not break the insulation, voltage was slowly increased until small bubbles were seen at the tip of the needle, indicating that the end had been exposed.

In operations, the animal was anaesthetised as indicated above, and placed in the tray with water flowing over its gills as before. The

/skull

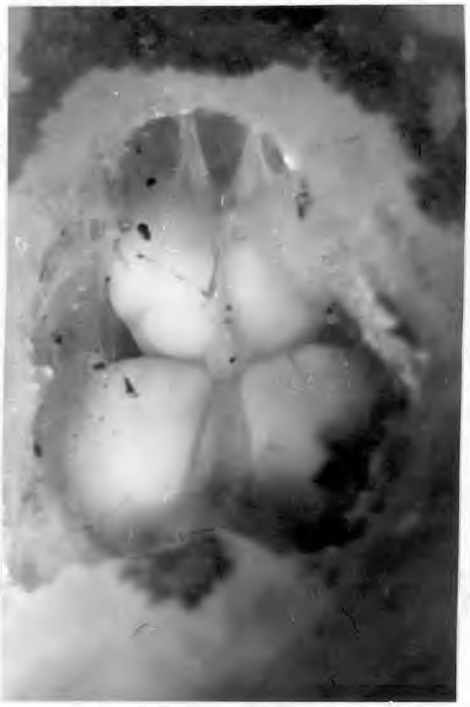


Fig. 3a: Forebrain (anterior) and optic tectum (posterior) exposed during operation. Note paired olfactory tracts running anteriorly.

skull was trephined, and the micrometer head swung into position and locked. Using the microscope, the needle was moved into the correct position over the brain, and then lowered by the micrometer movement. An attempt was made to draw up a map of the co-ordinates of various brain areas using easily-measured external features, such as length or distance between the eyes, but there was found to be too much variation in the relations to make this approach worthwhile. The tip of the needle was therefore aligned by reference to the few observable sulci and areas, and the general distances from edges of the brain. Results obtained using this crude technique were quite good. Certainly, much of the bleeding and excess damage sometimes associated with usual surgical methods could be largely avoided.

Once the needle had reached the required depth, the current was switched on, and adjusted to a value of about 0.5 mA, for between 15 and 30 seconds.

(f) Types of lesion.

(i) Control operation and general procedure.

All operations commenced with the removal, by means of a trephine, of a circle of bone from the dorsal surface of the skull. The thin cartilaginous bar underlying this was then removed with forceps, and the surface of the brain swabbed with a small piece of tissue paper. Fig. 3a shows this stage of operation. After surgery, the circle of bone was replaced, and the wound was sealed by application of Eastman 910, an extremely powerful adhesive, which set on coming into contact with water. The cap so formed lasted long enough to allow the bone

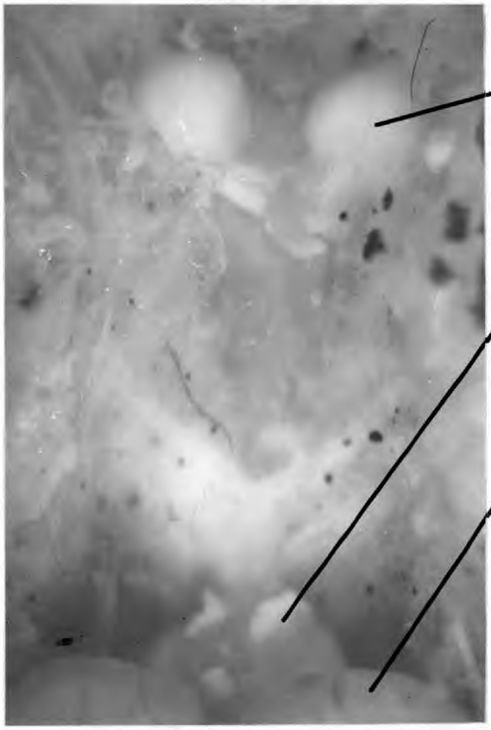


Fig.4 a: View of brain at rather higher magnification than in Fig.3 a, with forebrain largely removed.

circle to heal into place, after which it would fall off. This constituted the control operation. In cases where there was excessive bleeding, a small pad of sterile gelfoam was laid over the surface of the brain before closing the skull. Other recommended methods of closing the skull, such as the use of dental cement, mixtures of glycerine and wax, and latex, were tried, but without any great degree of success. The method used by Ingle (personal communication) involving the placing of plug-in latex caps on the skulls was rejected as being unnecessarily complicated.

One or two other types of lesion were made, but the major types used are listed below; the others will be mentioned in the results section.

(ii) Bilaterial removal of the forebrain.

This was a fairly easy operation, though inclined to be rather bloody, on account of the cutting of the major arteries of the forebrain. The olfactory tracts were cut, and held with fine forceps so that the forebrain could be lifted, and the position of the diencephalon and optic tracts noted before severing the narrower posterior end of the telencephalon. Fig.4 a shows the operation. In no case did any damage occur to the optic tracts or the diencephalon, or to lower areas of the brain.

(iii) Unilateral removal of the forebrain.

A rather more difficult operation, since, in addition to avoiding the optic tract on one side, care had to be taken to avoid damaging the central area of the remaining hemisphere when severing the commissural area. Generally, a small area of the commissural bed was left intact on

/the damaged

Fig.4 b.

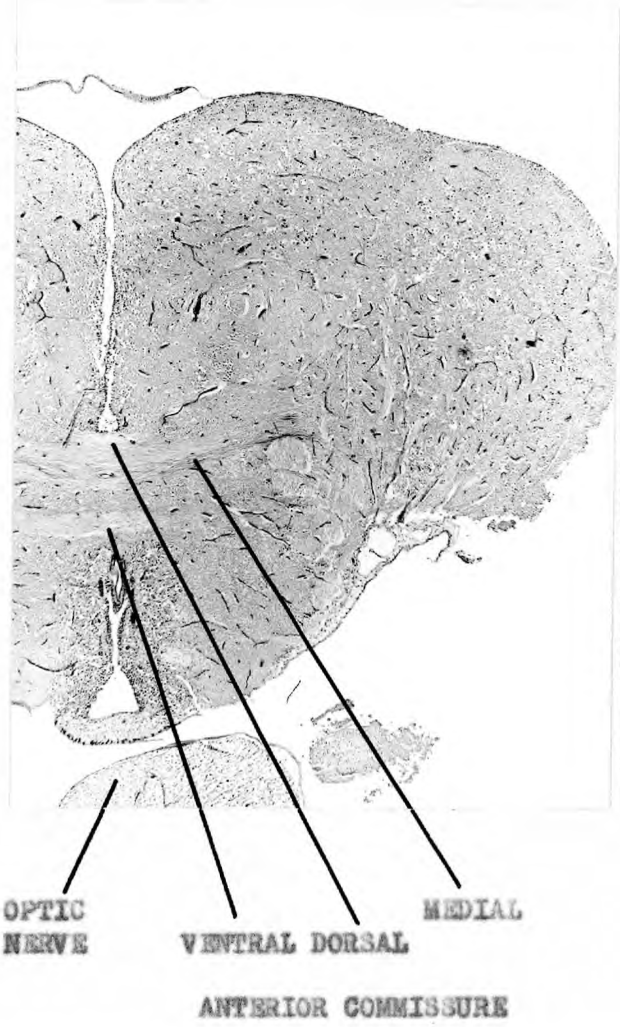


Fig.4 c.

Fig.4 b: Transverse section across forebrain, through anterior commissure.

Fig.4 c: Sagittal section through anterior commissure, near midline.

Both Holmes stained, sectioned at 12 mu.

the damaged side, so that the commissural blood vessel was not prevented from supplying blood to the intact hemisphere. (See below for details of this circulation).

(iv) Removal of the lateral area of the forebrain.

This was a rather difficult to achieve in entirety, on account of the large extent of the area concerned, and of the ventral component and its containing of the main veins draining the forebrain. No obvious landmarks are present to indicate the area, but it was usually possible to achive a fairly complete removal of the more dorsal components.

(v) Removal of the dorsal area of the forebrain.

Removal of this area was much easier than that of the previous area. Comparatively little bleeding resulted, but the same criticism applies to it with regard to the judgement of extent.

(vi) Severence of the forebrain commissures.

The commissures lie deep between the halves of the forebrain, as shown in Fig.4b. They were difficult to locate; the thin tela covering the brain had to be cut away, and the hemispheres gently pulled apart, until the thin white transverse line of the commissures could be seen. It was found that severance of the commissures was comparatively easy, but that the operation often involved the cutting of the blood supply to one or both halves of the forebrain. Fig.4c shows the arrangement of the artery to the forebrain; it can be seen that the operation is by no means easy. The photograph shows the blood vessels around the commissure. Ischemia resulted when the input was stopped; details of this will be given in the section dealing with results.

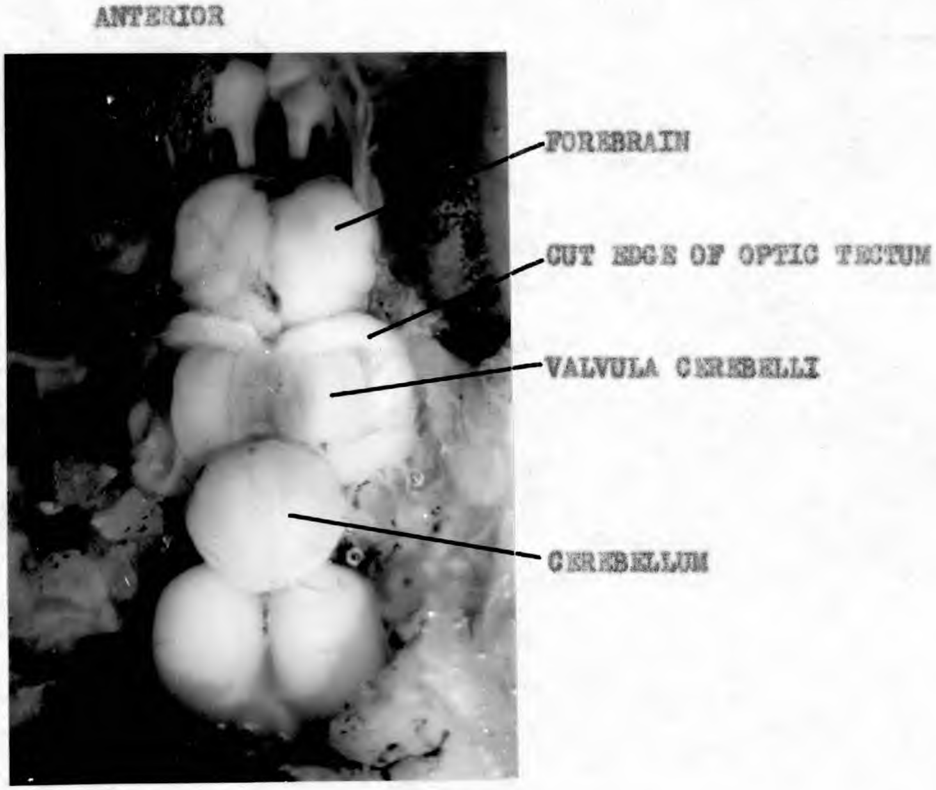


Fig. 5a: Dorsal view of brain after bilateral removal of the optic tectum.

(vii) Electrolytic lesions to the forebrain.

The advantage of this method was that it was possible to make lesions to deeper areas of brain whilst leaving the superficial areas relatively intact. In addition to this, the damage caused by surgical manipulations was not incurred. Lesions were made to the lateral area of the forebrain, and also to the central area; in both cases the lesions were unilateral. The central area was certainly inaccessible by other techniques.

(viii) Bilateral removal of the optic tectum.

Areas of optic tectum were removed by pinching a fold of median tectum in forceps, care being taken to avoid touching the underlying valvula cerebelli. The tectum was lifted and parted from the torus longitudinalis, fine scissors being used to extend the cut laterally and anteriorly. Fig.5a shows the completed operation. The anterior edge of the tectum was generally left intact on account of the presence of the habenular nuclei, the entry of the optic tracts, the forebrain bundles, and, most important, of the presence of large arteries in the border. (The bleeding caused by bilateral tectum removal was, in those cases where these vessels were damaged, so severe, that in one or two cases huge clots formed over the surface of the valvulae, and even extended over the forebrain. In these examples, fish would on recovering from anaesthetic, display peculiar types of locomotion, such as looping, swimming on one side, and rolling about the longitudinal axis whilst looping; a sort of "barrel roll". Such animals were re-anaesthetised, the skull removed and the clot carefully picked away from the surface of

/the brain.

Fig.5b.

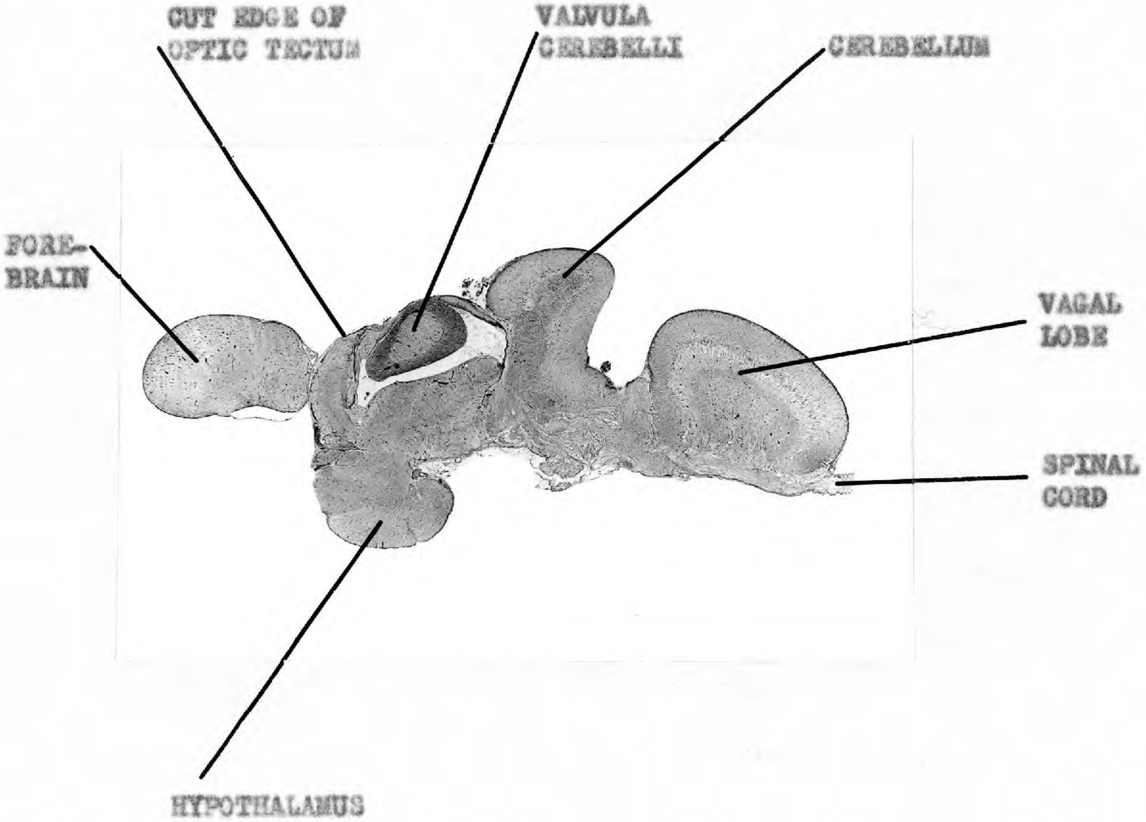


Fig. 5b: Sagittal section of goldfish brain after removal of optic tectum.
(Holmes stained, 15 mu section).

the brain. When they recovered from the operation, they displayed no obvious motor deficits.)

It was generally not found possible to remove the tectum very completely on its lateral aspects, not only because of manipulative difficulties, but also because of the danger of damaging the underlying ventral thalamus. Fig.5b shows a sagittal section through a lesioned brain; note that the anterior edge has been left relatively intact.

3. Histology.

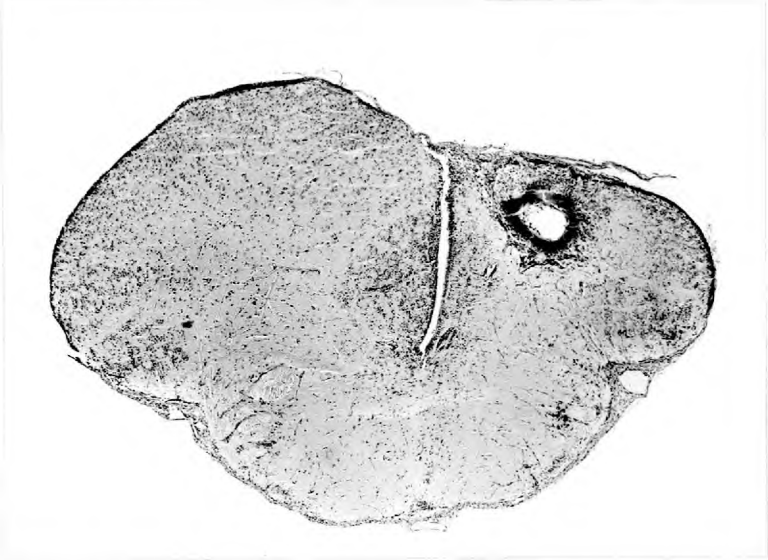
(a) Fixation and staining.

Fish were killed by a few minutes' immersion in 1:2-3,000 MS222. The top of the skull was removed, and the brain washed in 4% neutral formalin from a pipette. The brain was removed as rapidly as possible, with frequent washings of formalin, and was placed in 30 cc's neutral formalin (Lillie's formula). Brains could be kept for some time in this fixative. Specimens were embedded in paraffin wax at 56°C and sectioned at 10-20 mu. Various stains were tried; Cajal, Bodian, Holmes, Thionin, Nauta, Haematoxylin, Haematoxylin and eosin, Bielchowsky, and modifications of these. The difficulty of obtaining a good all-round staining of the forebrain is well-known; the two methods selected as giving the best results were Holmes and Haematoxylin and eosin.

Brains with electrolytic lesions were fixed as indicated above, and allowed to remain in the solution for a few days. They were then immersed for 2-3 days in a 3% solution of potassium ferrocyanide, which stained the iron deposited in the process of making the lesion. They were then treated in exactly the same manner as normal lesioned brains.

/In the

c



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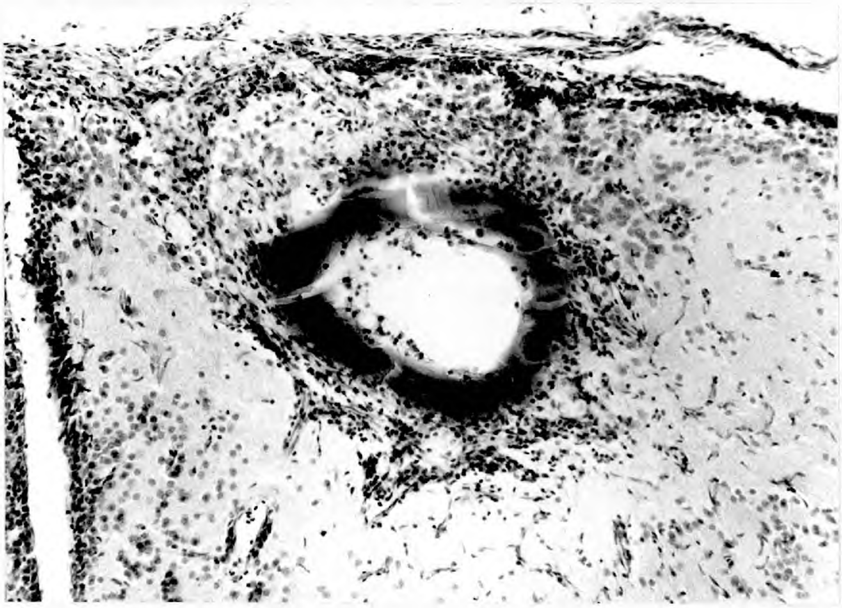


Fig. 5c: Transverse section through brain showing unilateral electrolytic lesion in central area, which has destroyed the dorsal and most of the lateral areas.

Fig. 5d: Higher power of lesion site, showing deposited $Fe^{++(+)}$ stained with potassium ferrocyanide

(Both Haematoxylin and eosin stained 20 mu sections).

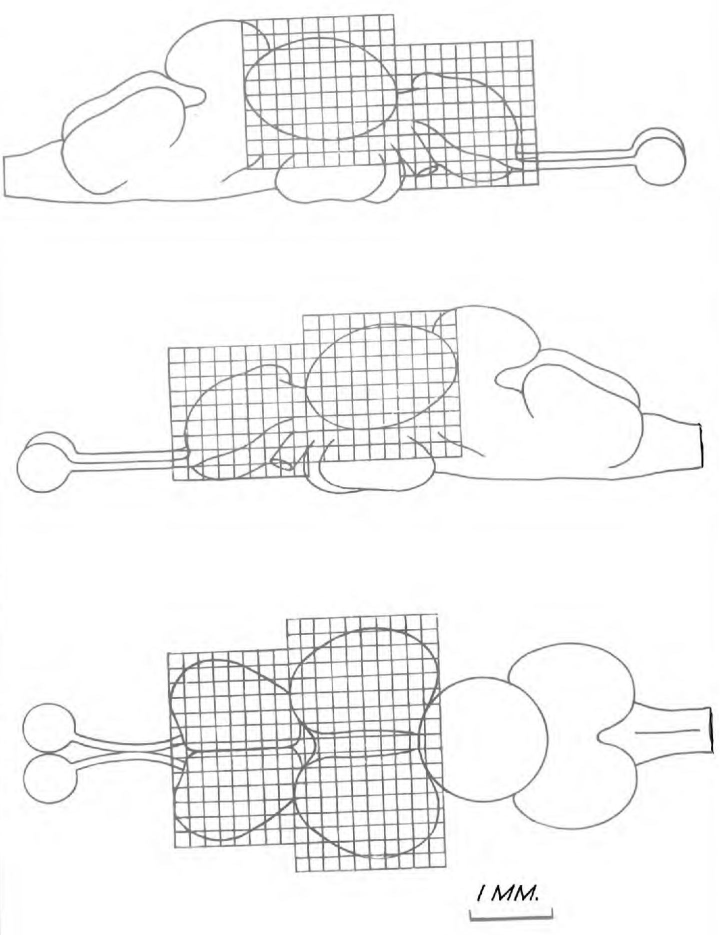


Fig. 6 a: Grid drawn up for plotting of extent of large forebrain and tectal lesions.

In the sections, the area of the lesion was neatly marked by a dark blue ring; it was found that greater contrast was produced by the use of Haematoxylin and eosin than by Holmes. Figs.5c and d show a brain damaged in this manner.

(b) Examination of lesions.

Three standard sheets were prepared for plotting the extent of damage to the brains. The first, shown below, was constructed from photographs made of the brain of CA 134, during and after dissection. No fixative was used in this operation, to preclude the possibility of distortion. A regular grid was drawn over the figure, to allow accurate plotting of the areas damaged (Fig.6a).

These sheets were used to assess the grosser lesions, such as the bilateral removals of forebrain or tectum. Brains so lesioned were sectioned sagittally, and examined under a projection microscope, which was calibrated by use of a micrometer slide. Reconstructions of the RHS and LHS side views were made, and transposed on to the appropriate drawings. For the dorsal reconstruction, the number of sections containing the area in question was counted, and divided by the number of lines of grid covering that area on the drawing. Then slides were examined at intervals of this number, or, where large changes occurred, half this number.

Such a technique, whilst satisfactory for the complete removals, was insufficiently informative when more discrete lesions were concerned. A series of drawings of transverse sections of CAII was prepared, the total length of the brain being divided into nine, and sections drawn at these intervals to correspond roughly to the vertical divisions of the

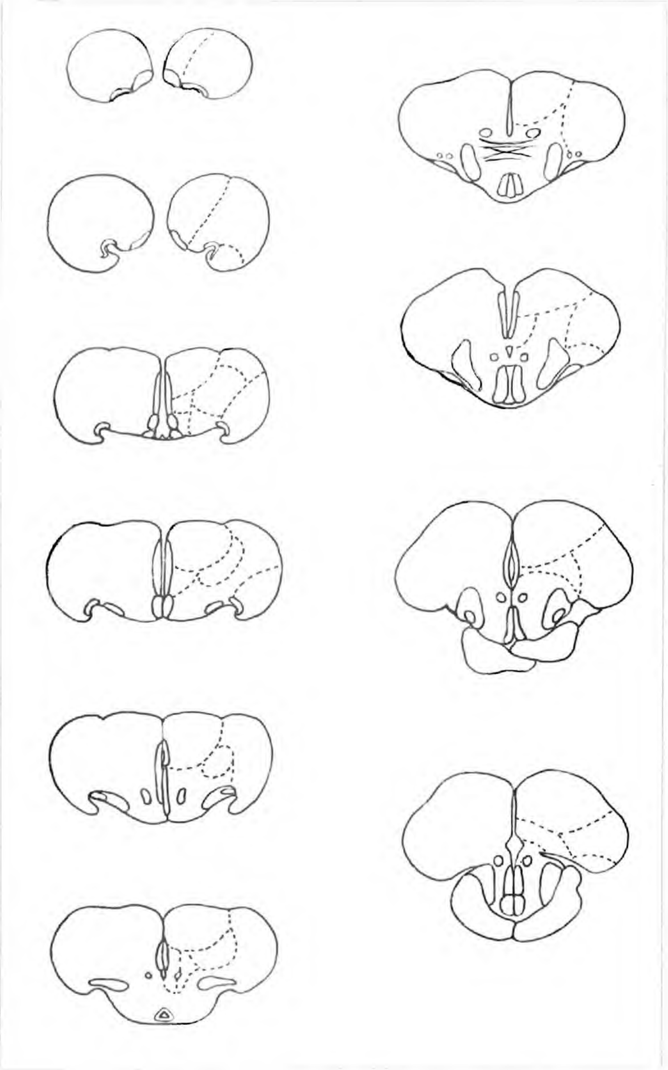
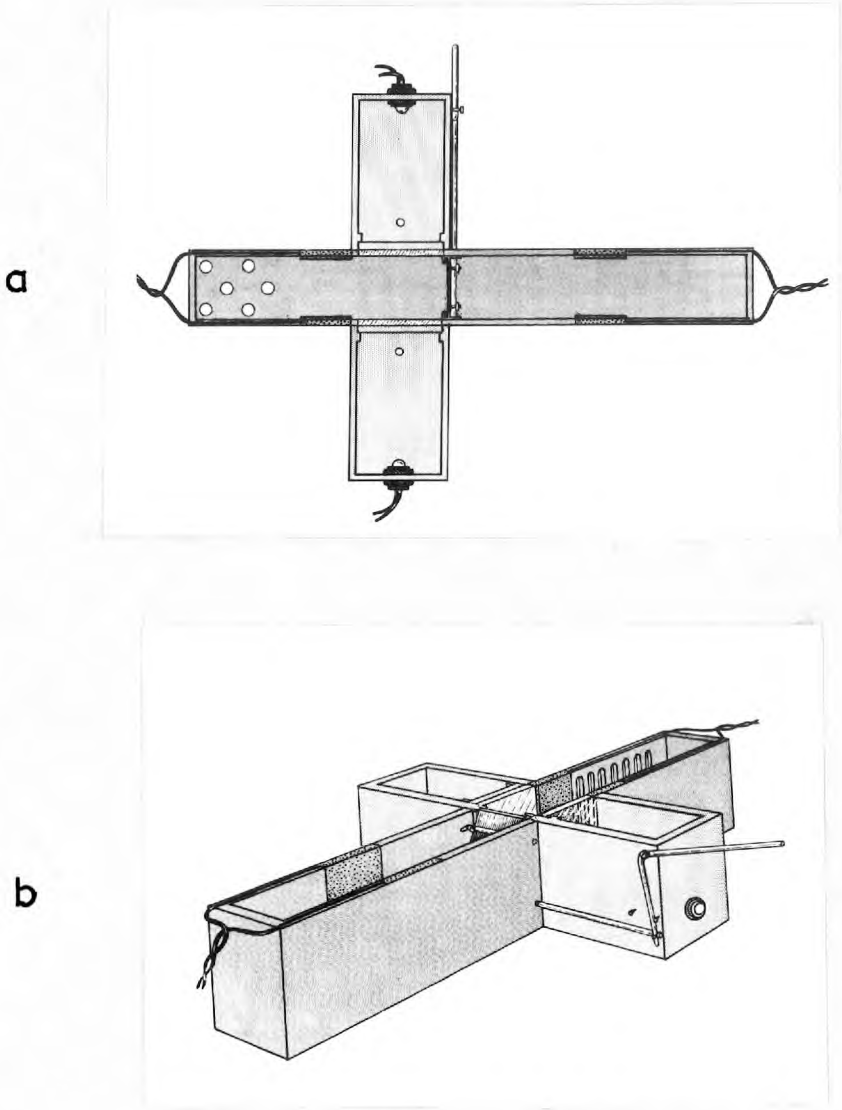


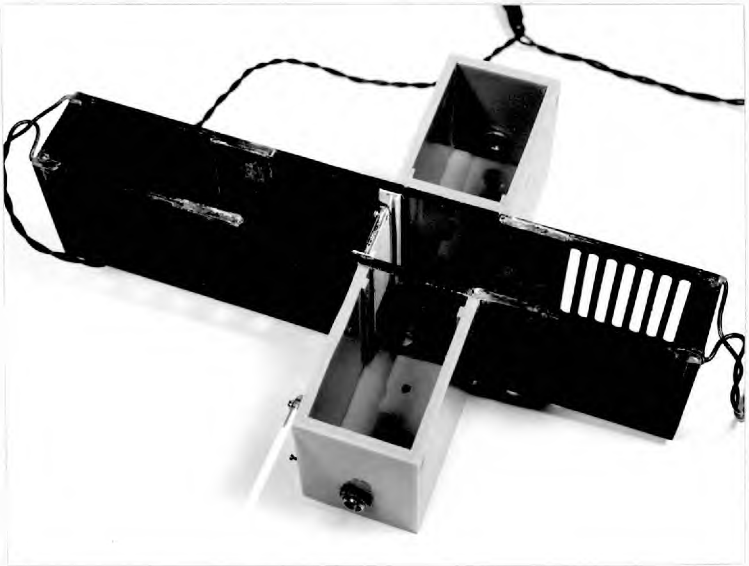
Fig.6b: Standard series of 10 cross sections of brain for assessing partial forebrain lesions. Sections 1-6 and 8-10 correspond to levels of cross lines on previous figure. Section 7 shows the region of the anterior commissure.



Figs. 7 a and b: Diagrams of B I.

7(a) from above.
7(b) from side.

c



d

Fig. 7 c: B I.

Fig. 7 d: B I in tank. Note overhead light and dark glass screen (at LHS).

drawings above. (The tenth section, that of the anterior commissure, was put in to allow damage to this to be assessed). One side of the drawing was left blank for marking the lesion, the other was marked to show the areas present at that point. Such a sheet is shown in Fig.6b.

In addition to these sheets, which gave indication of the areas removed, a series of sheets was used which listed all the areas and the main observable tracts in the forebrain, included such areas as the optic tectum and the thalamus, and allowed separate treatment of right and left sides.

4. Apparatus.

Classical conditioning and avoidance situations using electric shock as the UCS or aversive stimulus were used throughout the project, for reasons outlined in the introduction. All experiments, except the earliest few, were conducted in a room totally blacked out by a light-tight blind. A small 30 watt light, situated over the bench where the experiments were conducted, gave sufficient light to allow the taking of notes, and manipulation of fish.

(a) Training box I.

This apparatus is shown in Figs. 7 a, b, c, and d. It will be referred to hereafter as B I. It proved to be a most useful and adaptable design. There were three main components; a start box, a goal box, and two lateral windows opening into the start box. The start and goal boxes were connected by a door; this could either be a simple oval hole, with a vertically-sliding door to close it, or a swing door, needing to be pushed at the base, and being locked by a lever at the side of the

/apparatus.

apparatus. The dimensions of the apparatus were as follows: width of start and goal boxes: 1¼"; length of start box: 5¼"; length of goal box: 5¾" (the slightly greater length of the goal box was to allow fish to decelerate once they had entered the box); depth of water in the apparatus: 2¼"; width of transparent side-windows: 1⅜"; distance from windows to back of side box: 2¾"; dimensions of oval door: 1-7/16" x 1"; height of base of door above floor of apparatus: ⅜"; height above floor of small lights in side boxes: ¾" (these were positioned mid-way along the far wall of the box); electrodes (1" x 2¼") were positioned immediately behind the windows in the walls of the start box, and 2¼" in front of the door in the goal box. The position of the electrodes was such that fish of the size used in these experiments were shocked irrespective of their position in the start or goal boxes. The width of the apparatus was that based on series of trials in which fish were confined between moveable plates in a long tank. The width chosen was that in which most fish whilst not being too confined, were unlikely to turn around. Obviously the variation in size and degree of activity of fish would affect the tendency to do this, but the arrangement was generally satisfactory.

The floor of both start and goal boxes was perforated with ⅛" diameter holes to allow waste to fall through. The entire apparatus was suspended in a polystyrene tank 9" x 9" x 12", which provided a large circulation of water; it was regularly aerated, and the water was changed every week. The vertical grill in the right side of the start box served to transmit animals' movements to the paddle of an activity detector.

/Initially,

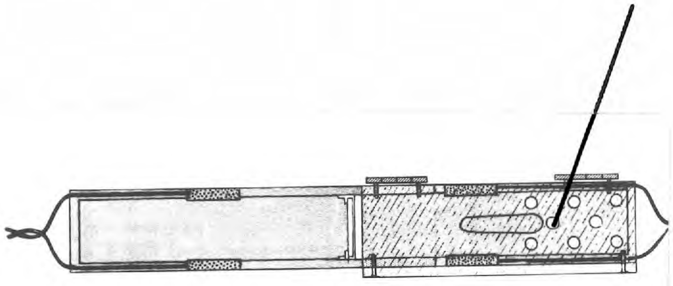
Initially, the side boxes were to have been air filled, so that the movement of shapes in them would not cause water vibrations to reach the fish. It was found in the first series of trials, however, that animals splashed water into these boxes, and that the apparatus tended to float because of the displacement of the water. Small holes in the base of each box allowed water to enter; there did not seem to be any great effect from vibration of shapes, and in any case, the animals paid far more attention to the shapes than to their disturbances.

The whole apparatus was made of non-toxic perspex; the start and goal boxes were of black perspex, both doors were white, the windows were of transparent perspex, and the doors to these and the walls of the side boxes were of medium grey perspex, to allow the use of both black and white shapes in discrimination situations. A small 6v light was fixed in the wall of each lateral box, to serve as a unilateral stimulus. (Fish could see into each lateral box with one eye only; evidence for this will be given later).

The box, inside its tank, was mounted on 1½" thick foam rubber, to reduce vibration, and was positioned behind a 18¼ x 13-3/16" sheet of darkened glass, as shown in Fig. 7 d, so that when illuminated from above, no sign of movement could be detected on the far side of the glass. Thus the experimenter could work unseen. Lighting, apart from the small general light mentioned abcve, was provided by a 60 watt lamp 17 cm over the box, the lamp being long enough (10") to illuminate both chambers equally well.

/ (b) Training box II.

a



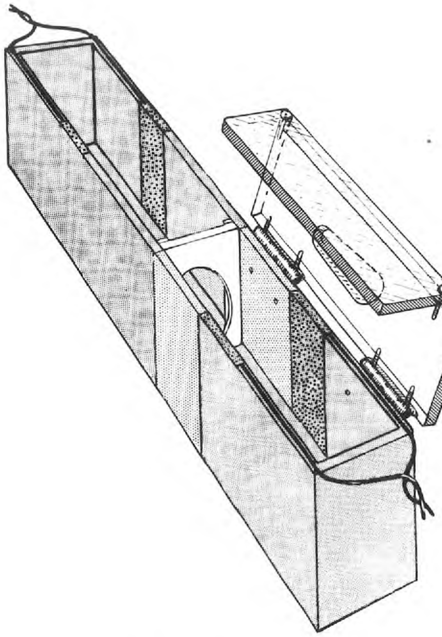


Fig. b.

Figs. 8 a and b: Diagrams of B II.

8(a) from above (Arrow marks position of photocell).

8(b) from side.

c

d

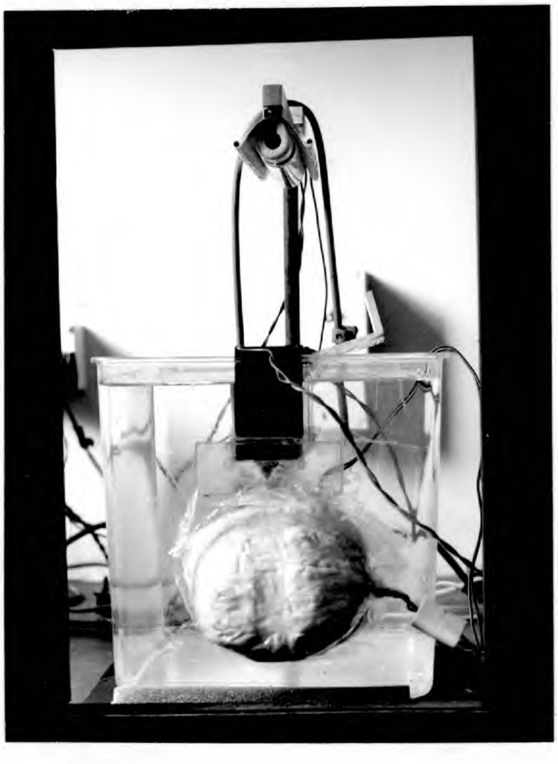


Fig. 8 c: B II

Fig. 8 d: B II in tank. Note overhead light, and buzzer (padded attachment to tank). Dark screen removed to show apparatus.

(b) Training box II.

This is shown in Figs. 8 a-d, and will henceforth be referred to as B II.

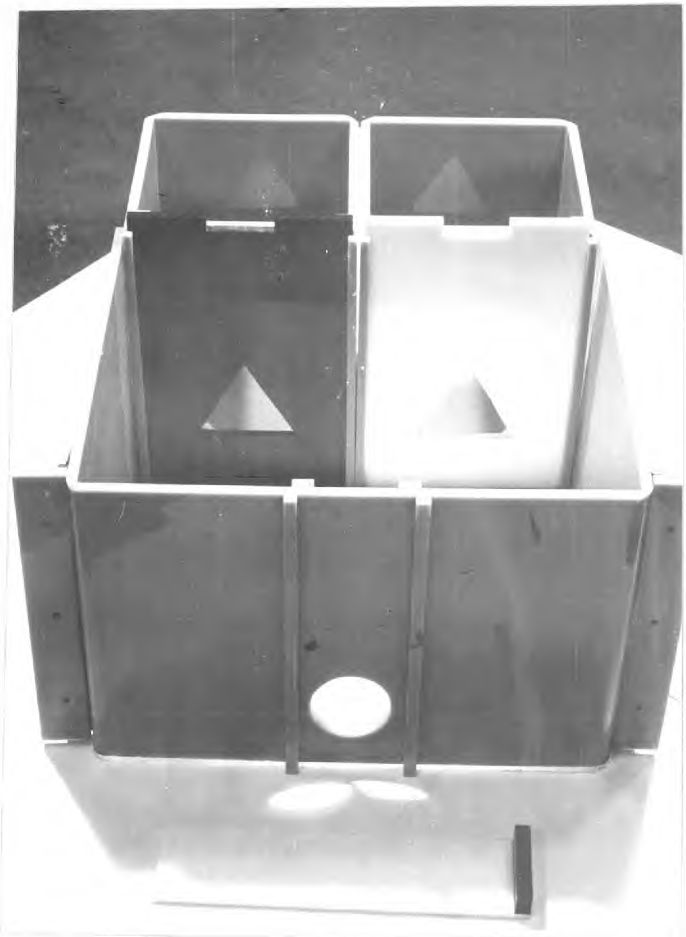
The apparatus was arranged in a tank, on rubber and behind a screen, in a manner identical to that described for B I. The start and goal boxes were constructed of the same perspex as B I, and were black, with grey insets in the front wall of the start box to resemble the condition in B I with both side boxes occluded. A phototransistor was fitted in the base of the start box in the position indicated (Fig. 8 a), and was used, in conjunction with circuits described below, to measure the activity of animals. A transparent cover of perspex (5-3/16" long) could be folded over the start box when confining animals over long periods without supervision, as in experiments lasting overnight.

A buzzer, operating on a 1½ volt battery, emitting a note of 440 cps, at 0.345 watts, was fixed in the outside wall of the tank (see Fig. 8 d) in a padded container to reduce noise in the room, whilst transmitting the sound to the subject in its box. Its intensity could be modulated by a potentiometer; it was controlled by a foot-switch.

The dimensions of the box were as follows: width of start and goal boxes: 1¼"; length of start box: 5¼"; length of goal box: 5¾"; width of grey insets in start box: 1⅞"; dimensions of door hole: 1-7/16 x 1"; distance of electrodes in goal box from door: 2¼"; (electrodes in start box ⅛" in front of grey insets); electrodes: 2¼" x 1"; depth of water: 2¼".

/ (c) Training box III

e



f

Figs. 8 e and f: B III.

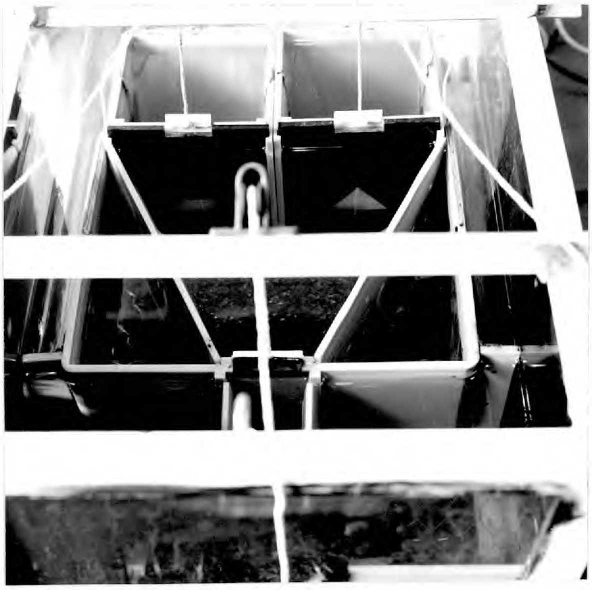


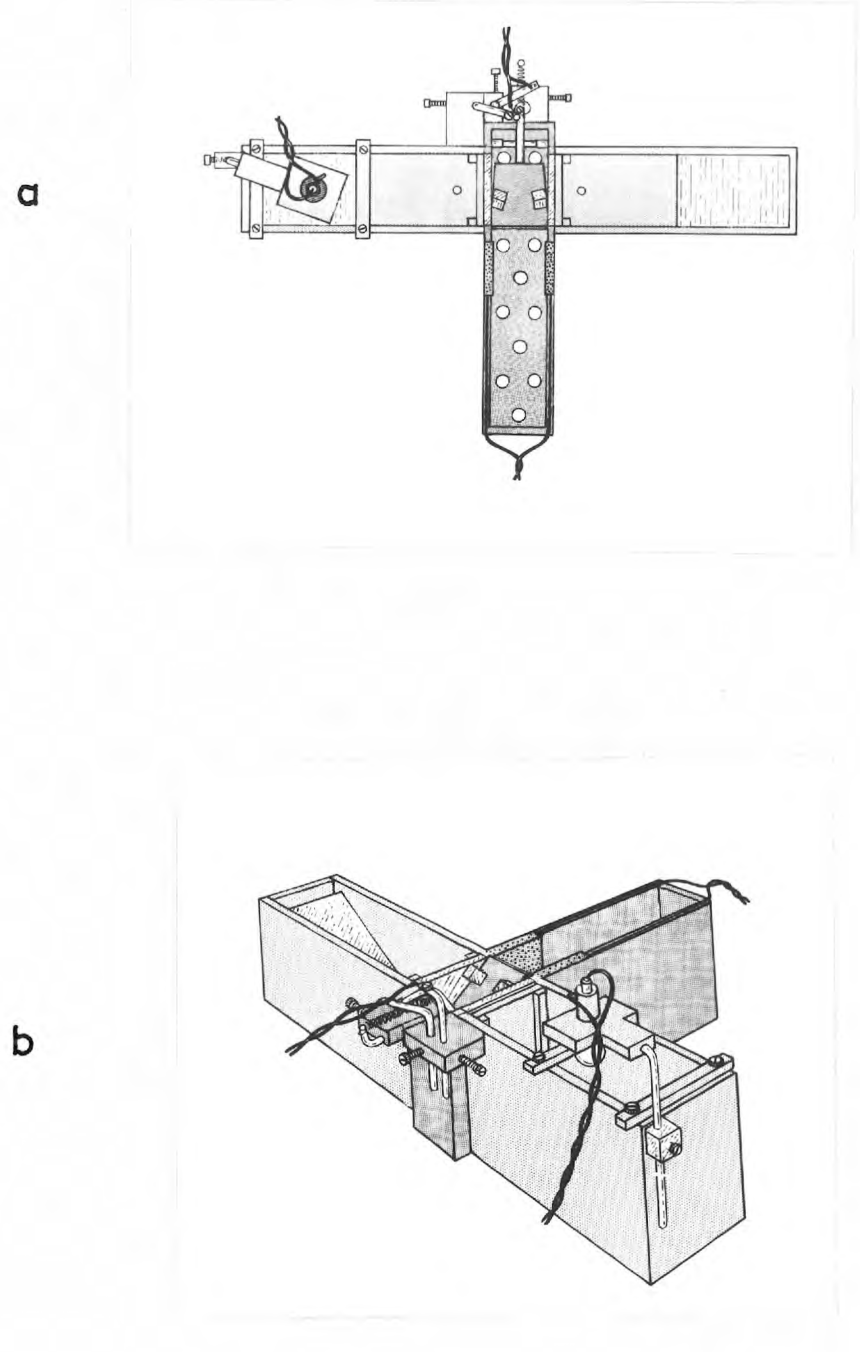
Fig. 8 g: B III in tank. Note strings to allow remote operation of doors.

(c) Training box III.

This apparatus, similar to that used by Hemmings and Matthews (1963) was made for another worker, and modified by the author for use in avoidance training situations. The final configuration of B III is shown in the photographs Figs. 8 e-g. It was made of non-toxic perspex, and was, with the exception of parts mentioned below, grey. There were three main compartments; the first being a narrow antechamber which gave access, through a circular door, whose vertically-raising partition was white, into the discrimination chamber. Two grey diagonal pieces induced animals to concentrate on the two doors at the far end; it was found that if these were not present fish tended to swim to the near corners of the box and avoid the far end. The doors, through which fish had to pass in order to reach the goal boxes, were either black or white, and had holes of equal area (10.9 sq. cm.) cut in the shapes of triangles or squares. Behind each door was a white vertical partition, which could be lowered to prevent the escape of animals. All doors were worked by overhead pulleys. Electrodes were fitted to the walls of the discrimination chamber and to the walls of each goal box. These were 4-7/16" x 5" in the case of the former, and 3" x 2" in the latter. (These values were calculated to give equal current densities in the three boxes). If it was wished to use one goal box only, a grey plate was fitted in front of the door, in place of the plate with a hole.

The apparatus was fixed on a transparent perspex base which held it steady in the bottom of a 24 x 12 x 12" glass tank. The water

/level



Figs. 9 a and b: Drawings of B IV.

(a) from above.

(b) from side.

c

d

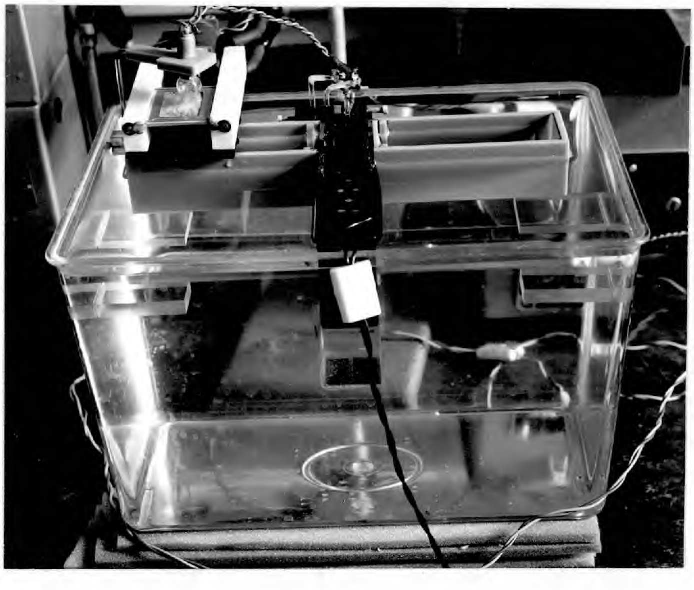


Fig. 9 c: B IV.

Fig. 9 d: B IV in tank. Note shape-changing unit at LHS.

level was kept constant, being 5½" inside the apparatus. Illumination was provided by a 60 watt lamp overhead. No screen was used, but the design of the apparatus, the fact that the experiment could be conducted from some distance, and the general level of illumination, minimised the dangers of disturbance. Water was changed every week, and was aerated except during training.

The dimensions of the box were as follows: height of apparatus: 6-1/16"; length and width of antechamber: 4½" x 1¾"; diameter of hole into discrimination box: 1⅜"; height of this above floor: ¾"; length of discrimination box: 7"; width of same at far end: 8½"; at near end: 2⅛"; width of doors of goal boxes: 4"; inside dimensions of goal boxes: 5⅞" x 4-1/16"; height of discrimination holes above floor: 1-7/16"; diameter of holes in walls of goal boxes (behind discrimination holes): 5.1 cm.

(d) Training box IV.

B IV is shown in diagrams and photographs in Figs. 9 a-d.

This apparatus represented an attempt to produce a completely automatic training situation, using a bar-press response in place of the avoidance movement used in other paradigms.

The box consisted of one chamber containing the subject, flanked by two lateral boxes in which the stimuli could be presented; stimuli were shown from above each box, and reflected into the fish's eye by mirrors set at 45°. The transparent windows to these boxes could be covered by grey perspex doors. The fish chamber was made of black perspex, and electrodes (1" x 2¼") were fitted ⅛" in front of the

/windows.

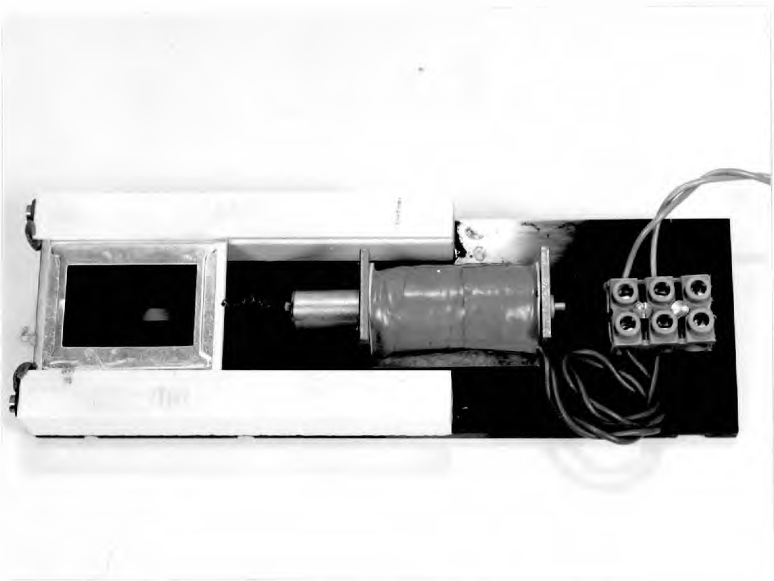


Fig. 9 e: Shape-changing unit. Solenoid at RHS, slide at LHS. Note horizontal bar visible in front of base hole.

windows. Directly in front of the fish was a paddle of white perspex in the form of an ellipse, attached to an overhead electrical contact by a thin transparent rod. The black oblique overhead sheet induced fish to collide with the bar. Thus a push of the bar would make circuit; the layout of the entire circuit is given below. Two types of discrimination were used; a brightness discrimination, lights of 0.515 and 1.3 log foot candles being used, and a shape discrimination, using horizontal and vertical rectangular holes cut in thin ebonite and mounted on a 35mm slide holder, so that an overhead light shone through one of the two holes. Shapes were changed automatically by means of a solenoid, which pulled a new shape into place when current passed; a spring returned the original shape when the current ceased. The shape-changing unit is shown in Figs. 9 d and e.

The box was mounted in a 9 x 9 x 12" tank, filled with water to a depth of 2¼", and mounted on 1½" foam rubber.

The dimensions of BIV were as follows: length of fish chamber: 5¼"; width of same: 1"; dimensions of paddle: 1" x 1½"; height of same above floor: ¼"; width of windows: 1½"; distance from windows to base of mirror: 2¼"; to top of mirror: 4½"; height of light above floor: 3¼", 4½" for shape discrimination; (light positioned centrally above mirror); electrodes (positioned directly ⅛" in front of the windows) 1 x 2½".

(e) Electrical circuits associated with the training units.

(i) B I

The apparatus was illuminated by a 60 watt lamp, which was

/controlled

controlled by a switch at the side of the tank. ALL switches were mounted on foam rubber, to reduce vibrations caused by switching. A similar switch, in parallel with a foot-press on the floor, controlled the primary coils of a transformer, delivering a shock through both pairs of electrodes simultaneously; at the same time, a small neon, fitted to the top of the lamp, lit to indicate the duration of shock. The small lights fitted in the lateral boxes were fed by the 6v tapping from a mains transformer, through a dimming circuit, which could be shorted to give illumination. Either of the two lights could be turned on; a reheostat was used initially to equate photometrically the intensities of the lights, so that when the side to be used was changed, the light intensity would be the same. The dimmer arrangement was chosen in preference to simple switching off in order to prolong the life of the bulbs, which were rather difficult to obtain and replace. The two bulbs initially installed lasted throughout the experiment.

(ii) B II.

The arrangements for light and shock were identical with those for B I described above. The buzzer was an old one of the type used in horns, and was run on a 1½v cell in series with a 1 Meg ohm potentiometer and a foot-press. The phototransistor-recording attachment used with B II is described below.

(iii) B III.

Shock and light switching were the same as in the previous two boxes.

(iv) B IV.

The method of shocking was the same as for the boxes above,

/except

except that the switching was done automatically. The light intensity was lower; the circuit is described below.

(f) Integrator-amplifier circuit.

This unit was based on a circuit design by Bitterman (1965), to whom the writer is indebted for information concerning it. Bitterman used a gramophone crystal cartridge, with a paddle attached, to transduce water vibrations caused by fish when manifesting CR responses. After a series of trials with this attachment, it was found that only fairly gross disturbances were detectable, such as those seen when fish received shocks. The normal gentle activity of fish, moving up and down the box, was absolutely undetectable. The circuit was modified but this too was insufficiently sensitive. It was then decided to use phototransduction. Two types of cell were used, cadmium sulphide and phototransistor (Mullard OCP 71). The former was used in conjunction with a Schmitt trigger circuit; this was found to be sensitive, but its response latency was too great. The OCP 71, with the modified Bitterman circuit, gave extremely good results. It was possible to adjust the gain to such an extent that the smallest flicks of the caudal fin could be detected. The switching of the relay was sufficiently fast to allow the most rapid movements of animals to be accurately recorded. The whole layout, including the power pack and Rustrak 4-channel recorder, was generally used in conjunction with B II, where the OCP 71 fitted in the hole indicated in the digram (Fig. 8 a).

One disadvantage of the circuit was that at high rates of operation, there tended to be oscillation, so that, for example, in the

/conditioned

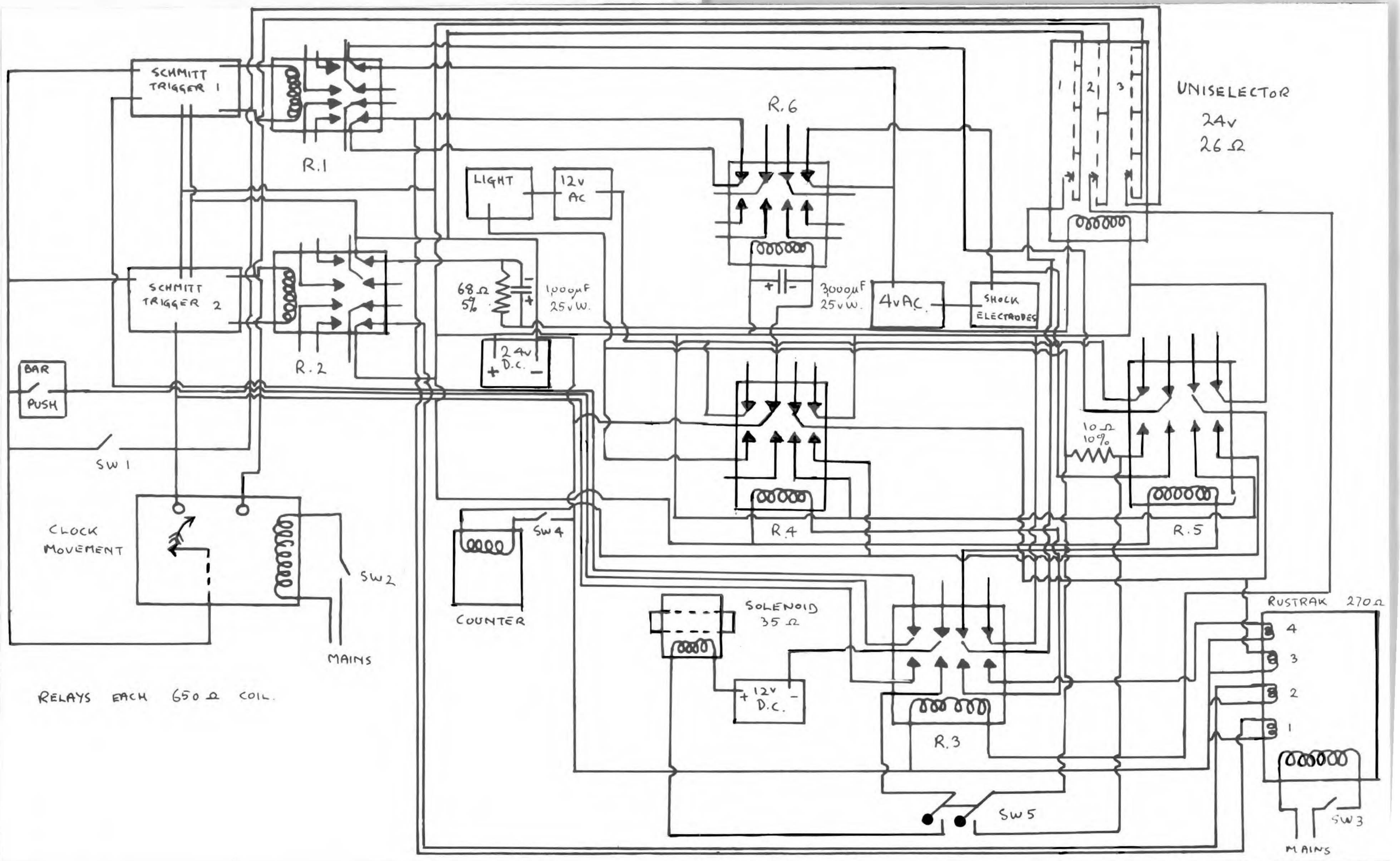


Fig.10a: Circuit diagram of automatic apparatus. R 1 - 6; relays
SW 1 - 5; switches.

conditioned reflex training, figures are not accurate counts of numbers of passes of the tail, but are proportional to these. Since this was the same for all animals, no attempt was made to rectify it - this would have been extremely difficult. In slower-moving activity in trials, there was an equivalence of pulses on the chart and passes of the tail over the phototransistor.

(g) Circuit of automatic apparatus.

The overall circuit is shown in Fig.10a.

Before describing the functioning of the apparatus, it is perhaps convenient to present the training scheme of each of the two discriminations used.

(i) Light discrimination.

Positive trial.

A. Bright light on.

Interval of 7 seconds, if bar pushed, trial terminates by light going off. A period of 1 second was allowed after the light onset, when bar pressing had no effect, to allow animals time to get used to the light, otherwise they tended to react immediately.

B. If no bar push, 3 v AC shock on.

Interval of 3 seconds, during which, if bar is pressed, trial is terminated as above.

C. If bar is not pushed in this interval, trial terminates automatically at its end.

Initially, a number of trials was run using the bright light alone, switched on automatically every minute, so that each trial needed a bar press to terminate it, either to prevent being shocked, or to terminate

/the shock.

the shock.

Negative trial.

A. Dim light on.

Interval of 10 seconds, during which, if bar is pressed, fish receives shock, shock persisting as long as the bar remains depressed.

B. At the end of this period, trial terminated automatically. Trials were alternated, and given at 1 minute intervals.

(ii) Shape discrimination.

The shapes used were horizontal and vertical rectangles, moved into position by the shape changer mentioned above. The light came on for both trials, but at the same intensity as that used in the previous positive trial. The vertical rectangle was used in the positive trial, the horizontal rectangle in the negative trial. Since only the learning capability of fish in this situation was being assessed, no attempt was made to test for vertical/horizontal preferences.

Bearing these operations in mind, the functioning of the apparatus in light discrimination situations will be described.

Trials were initiated by the completion, through the clock mechanism, of the circuit through Schmitt trigger 2. This switched off relay R2, sending a pulse through the coil of the uniselector, stepping its contacts on one space. The 1,000 mfd. condenser in parallel with the 68 ohm resistor was to smooth switching, and prevent double-stepping of the uniselector. Channel 1 of the uniselector passed current from the 24 v DC source to one of two relays, R4 or R5; channel 2 passed current to another relay, R3, which determined which of the relays above should be

/activated.

activated.

If R4 were switched on, the following events occurred:

(i) The 12 v AC source was connected to the stimulus light, at the bright setting.

(ii) R6 was switched off, but, on account of the 3,000 mfd. condenser across its coil, the actual switching occurred 7 seconds later. As the relay went off, it closed a 4 v AC circuit leading to the shock electrodes.

(iii) If R6 did go off, the fish would generally press the bar push, thereby activating Schmitt trigger 2. This in turn would switch off R2, and step the uniselector on, terminating the trial. If fish pushed the press in the 7 second period before R6 opened, they would re-energise it, avoid a shock, and at the same time cause the events just outlined to terminate the trial. Note that the bar push circuit could only terminate trials for the positive "bright" trials, since it was connected via R2, which completed circuit for ST 2 only in positive trials; there would be no circuit in negative trials.

(iv) If the fish did not press the bar in the entire 10 second trial period, the spoke of the clock mechanism would reach the second contact. Circuit would be made through channel 3 of the uniselector, and ST 2 would open R2, stepping on the uniselector, and ending the trial irrespective of the fish's performance. If the fish had pushed the bar, the uniselector would have stepped on, and channel 3 would no longer be open to allow this action of the clock mechanism.

If R5 were switched on, the following events would occur:

(i) The 12 v AC

(i) The 12 v AC source was connected to the light via a 10 ohm resistor, dropping the light's intensity by half, 1.3 to 0.515 log foot lambert.

(ii) The bar press circuit was connected, via R3, to Schmitt trigger I, so that pushes of the bar would not activate the uniselector.

(iii) If the fish pressed the bar, ST I would operate, and R I would go into the open position, closing the 4 v AC circuit leading to the shocking electrodes. The shock would remain on as long as the bar was depressed.

(iv) If the fish did not press the bar, after 10 seconds, the spoke of the clock mechanism would make circuit via the uniselector as in (iv) above, and terminate the trial.

Shape discrimination trials. Switch 5 was closed, which shorted out the 10 ohm resistor in the light circuit, and connected up the solenoid. Activation of R 3 caused the solenoid to operate, moving a vertical rectangle into position. After the trial, the spring would return the slide, and for the next trial, the horizontal rectangle would appear, at the same intensity as the vertical.

These arrangements allowed the trials to be made automatically, but the following circuits had to be added to allow recording. A Rustrak 4-channel 24 v DC recorder, with 15 rpm motor and variable gearbox, was used for the purpose.

(i) Counter. This was connected in series with R4 and R5 in parallel, so that, when SW 4 was closed, the closure of either relay and its subsequent opening stepped the counter on one. Thus the total number of

/trials

trials given could be determined.

Circuits for Rustrak:

(ii) Channel I: A 24 v DC supply to R I and R 6, which closed when either was opened. This indicated, for R 6, that is in bright or vertical trials, the onset and finish of a shock, and, for R I, for dim light or horizontal, bar presses (= shocks) during the trials. Note that since ST I and R I were operative during intertrial periods, pushes in these periods could be recorded on channel I also.

(iii) Channel 2: This circuit made via R 2, if that relay opened, hence indicating the beginning and end of trials, and, for positive trials, the bar push that terminated the trial.

(iv) Channel 3: Connected in series with R 4 and R 5 in parallel, this channel indicated the onset of the lights.

(v) Channel 4: This circuit made via R 3 and indicated which of the two relays, R 4 or R 5, was being energised, hence, which type of trial was in progress. The channel was energised for positive trials.

For these recordings, the Rustrak was run with a N^{o}6 gearbox, giving a chart speed of ¾" per minute. The use of four channels may seem excessive, and indeed the recording could have been done on two, but the unit served as much for telemetry as for recording in the early stages, allowing faults to be traced with ease. A strip of metal, with sections cut in its side accurately reproducing the important time relations (in inches) of the apparatus, was made up, and could be placed over charts

/to allow

to allow rapid reading of the results.

5. Electrodes and shocking.

Since shock reinforcement had been chosen as the sole method of reinforcement, it was important to devise repeatable methods of administering shocks.

(a) Polarity. It was decided from the outset that only AC would be used for shocking. A number of workers have shown that aquatic animals, including fish, tend to orientate with head towards the positive pole of an underwater field; this would prove a considerable source of error in experiments requiring animals to make directed responses, as opposed to simple CR's. (See, for example, Scheminsky, 1924).

(b) Source of shock. Many early workers used induction coils and accumulators as source of shock, but it was considered that it was easier to rely on the use of transformed mains voltages. In B's I, II and IV, the source of the shock was a Douglas MT3AT 2 amp transformer, with the 230 v primaries connected. The 4 v tapping was used. In B III, with the greater distances between the electrodes, a more powerful transformer was used, giving 6 v at the secondaries; the area of the electrodes was calculated to give a current density equal to that in the other three boxes.

(c) Method of administering shock. Froloff (1925, 1928) used a thin wire embedded in the dorsal musculature of the fish, and a large base plate fixed to the base of the tank, so that the animal could be shocked by induction coil irrespective of position. Whilst obviating the difficulties of corrosion inherent in totally exposed electrodes in

/salt water

salt water, this system, as Bull (1928) has pointed out, is one which would seem to place the fish under considerable restraint, and prove uncomfortable. Bull (1928) using salt water fish, employed the same method of induction coil shocking, but used a free-swimming fish. He connected the terminals of his induction coil to large plates of zinc, which were immersed in a bath of saturated zinc sulphate. Bridges of agar jelly, dipping at one end into this, and at their other into the water containing the fish, transmitted the shock. The system developed by Longo, Holland and Bitterman (1961) for use with fresh water fish has the advantages of Bull's arrangement without the rather complicated electrodes. They used plate electrodes painted on to the sides of the apparatus. This eliminated the difficulty of corrosion of the electrode causing a fall-off of shock intensity, and allowed the animal to be shocked if any part of it lay between the two electrodes.

The type of electrode employed was a modification of the Bitterman type, and the writer is indebted to Prof. M. E. Bitterman for details of formation. Thin brass sheets, of correct dimensions were sandpapered to provide a rough surface. Connecting wires were soldered on to the tops, and they were araldited in position in the training boxes. A solution of the electrode material was prepared by dissolving expanded polystyrene in methyl ethyl ketone, until a fairly viscous solution was produced, then adding powdered graphite until a thick but paintable solution was obtained. This was applied to the brass in two thin coats, as it was found that a single thick coat tended to crack and peel. Electrodes produced in this way could be washed in hot water, scrubbed

/and dried

and dried without damage, and all lasted the length of the project without need of renewal.

(d) Intensity of shock. Values for resistance per inch and for voltage and current for the boxes are given in Table 2 . A series of animals was subjected to ¼-½ second shocks of varying intensity. A table of the effects noted is given below:

Table 1

Effects of shock on fish. Administered in box I with oval door closed. Duration of shock in all cases 0.25 - 0.5 seconds.

Voltage used	Milli Amperage at centre	No. of fish	Mean time to resume normal behaviour (secs.)	Remarks
30	17	3	120+	Fish were stunned by this voltage, losing balance and rolling on to their sides. Recovery was prolonged.
24	13	2	120	Results were much the same as for 30V.
20	11	2	60 - 90	The main difference was in length of recovery.
18	9	3	60 - 90	As for 20V.
15	7.5	2	60	Fish still showed a tetanus, and there was a considerable lag before activity due to shock could be observed.
12	5.8	3	40	The first voltage at which a definite escape movement could be observed during the shock period.
10	4.1	3	15	No sign of tetanus; high activity caused by shock, then cessation of movement until the 15 second period had elapsed.
9	3.9	3	10 - 15	High activity from shock, and rapid recovery.
8	3	3	1 - 1½	As for 9V.
6	2	3	-	No sign of any tetanus or after effect.
5	1.75	7	-	Fish showed no tetanising by shock, and showed no change after shocking.
4	1	10	-	Activity from shocks high, and maintained high for some 15 seconds after its termination.
3	0.75	5	-	Shock very little effect first time, but response high on second stimulation.

/From this

Table 2

Data on Shocking:

Voltage used on boxes I, II, and III.

4.0 volts across. Volts for edge to centre 0.7 v
Amps at centre 1.m.A.

Across 7.5 cm. of resistive sheet 200 Ω
425 Ω

Mean of boxes 105.83[redacted] Ω per inch

From this list, it can be seen that the high voltage shocks, even at $\frac{1}{4}$-$\frac{1}{2}$ second duration, were such as to immobilise, rather than to activate fish. The tetanus lasted some time. The value arrived at, and used throughout the series of experiments, was 3-4v AC, which gave consistent activation of fish, without giving any after effects; the subjects were able to maintain movement even during shocking.

Bitterman (1965) used a 40 v AC square-pulse shock; even allowing for the 0.25 sec. pulse every 1.5 sec. this seems to have been unnecessarily high in view of the results given above. In view of the possible damage to the CNS and other systems from shocks, it was perhaps best to use low values with animals not only undergoing lengthy training, as opposed to c. 10 days with Bitterman, but also being operated upon to varying degrees of severity. (Evidence will be given below as to the lack of effect of shocking on normal and operated animals).

6. Timing.

The 5 and 15 second trials were timed on a 10 second sweep stopwatch, and measured to the nearest quarter second; longer trials were timed by a stopclock, to the nearest second; this was also used to measure inter-trial intervals. In the automatic habituation trials, a 24v DC supply was fed via the sec. hand of the clock, on to the Rustrak, to give minute marking. The inter-trial interval was 1-2 minutes.

7. Training methods.

(a) Selection of fish.

Fish were selected on the basis of general health and rapid avoidance movements, as mentioned above. After the first ten trials, they were again assessed, and if reaction times were excessive, and persisted

/so over

so over the next ten trials, the fish were rejected. Another phenomenon noted was the peculiar susceptibility to shock seen in a few animals. These animals were to other intents normal, would eat, avoid the net and generally behave like the other fish, but when they were shocked for the first time they rolled on to their sides and took some minutes to regain the normal posture. When tested again after recovery was apparently complete, or the next day, they manifested exactly the same signs. It was not found possible to correlate this behaviour with any illness, despite the fact that the majority of animals behaving in this way died not long after the testing, in the order of a week or so later. Needless to say, such animals were not trained. Some fish, after the first day's training, were found to be very weak, and would often die. It was generally the rule that if fish did survive the first day, they would survive the entire period of training.

Fish were fed every two days, after training, so that food could be sufficiently digested before the next training session. If fish were trained too soon after feeding, they tended to regurgitate their food when shocked or making avoidances.

(b) Identification of individuals.

Small cuts were made in the dorsal, caudal, and anal fins to allow identification, though in many cases the colouring was as good a guide. Only one such cut was made per fish, and all such cutting was done under anaesthetic; there was no loss of mobility due to these cuts. From time to time, as the cut areas regenerated, the area of cut was again lesioned. Other methods of marking, such as branding with dyes or heat (too

/ephemeral),

ephemeral), tagging with beads on nylon (likely to cause skin tumours) and identification by natural colouring (too changeable) were abandoned for the reasons given.

(c) Fitting of blinders.

Although the experiments were in the main designed to avoid the use of blinders, a number of control experiments were performed in which blinders were necessary. Methods for uni- or bilateral blinding have varied. Sperry and Clarke (1949) used cups of tantalum foil which were inserted under the external cornea. This was a good method, since the blinder was not too discommoding. Schulte (1957) used a cotton bag tied over the animal's head, but this was considered to be too hampering, and would probably be lost in avoidance situations. McCleary and Longfellow (1961) used cups of black vinylite placed under the sclerotic ring, and Shapiro (1965) used a similar method.

In the present series of experiments, opaque black polythene was moulded into cups by a hot ¼" diameter probe, and cut to size. Fish were anaesthetised, and the cups were placed under the sclerotic ring, and sealed into place with a little Eastman 910. Blinders remained in place throughout the training periods, which were generally in the order of five days, the maximum being ten days.

(d) Initial habituation of fish.

Naive fish were caught in a small net and transferred from their home tanks to the training apparatus. They were left there, without overhead illumination, for ten minutes, followed by a further period of five minutes with the light on, during which period they were observed. If the animal lay quietly, training was initiated, if it showed signs of

/agitation,

agitation, it was allowed a further period of rest, until it had settled. Thereafter, animals were allowed 2-5 minutes' quiet before the start of training on any given day. Fish very soon habituated to the net and training boxes.

(e) Training.

In all learning experiments, fish were given no more than 10 trials per day. This was to avoid dangers of overtraining, and of excess shocking on one day, and conformed with the numbers given by workers such as Behrend and Bitterman (1962), Schulte (1957) and Mackintosh and Sutherland (1963). Results obtained by Savage (1964 unpublished) showed that both goldfish and minnows learned food-rewarded discrimination situations quite fast when given this number of trials daily, as opposed to results obtained by West (1964, unpublished) who used mass-training methods, and found almost total lack of retention on some days following massed trials.

The inter-trial interval was of the order of 1-2 minutes, but this was lengthened in cases where the subjects were extremely excitable. No trial was initiated until fish had remained still for at least ten seconds after the door had been opened, though this interval was varied to ensure that it would not become the stimulus for avoidance.

Shocks were administered by a hand-switch or a foot-press, and averaged 0.25 - 0.5 sec. in duration. Reaction times were measured from the onset of the stimulus, light or shape, till the passage of the base of the caudal fin over or under the door. Responses tended to be

/all-or-nothing,

all-or-nothing, and whilst this was not a highly accurate method, it at least gave a repeatable criterion. Fish were given 1 minute in the goal box before being transferred back to the start box in a small net. They were then given time to settle down before the next trial. Training was done in the mornings, though as numbers of animals increased, it tended to spread over the day, and was performed every day, to eliminate discrepancies due to days missed. A criterion of 80%$^{+}$ correct performance over 5 days, or 90 trials was adopted in simple avoidance situations. A criterion of 70%$^{+}$ over 5 days was adopted in later experiments.

(f) Shape discrimination using B I.

All trials were made using the Gellerman sequences, which ensured that there was no alternation of the shapes, and that there were never more than three successive presentations of the same stimulus. Animals were trained to avoid one shape, and to remain in the start box for the other. Details of the results obtained are reported in the results section.

(i) Times.

Stimulus/shock intervals of 15 or 5 seconds were used in these trials.

(ii) Door.

Initially, the oval hole and vertically-sliding door were used; later trials were conducted using the swing door.

(iii) Shapes.

The main shapes used were rectangles and squares. Their dimensions were as follows:

Rectangles: 2 x 0.4 x 0.4 cm.

/Squares:

Squares: 2 x 2 x 0.4 cm.

The oblique rectangles were mounted at 45°. The shapes were first attached to grey perspex clips, which slid over the far edge of the lateral box; these were later replaced by hooked transparent perspex rods which fixed either on this edge, or on to the edge of the windows. When it was found that shapes had to be moved the shapes were mounted on thin transparent rods, $2\frac{1}{2}$" long, $\frac{1}{8}$" in diameter.

(iv) Contrast.

The medium grey used in the lateral boxes provided a background suitable for the discrimination of both black and white shapes. The shapes shown abve were used in both black and white preparations.

(v) Presentation of shapes.

In early experiments, shapes were fixed in place, or set moving, (by mechanical or manual methods) in the dark, and a light was switched on to begin the trial. Later, the light was kept on, and the shapes introduced. Moving shapes were used in all later experiments. Shapes were moved manually, at the ends of thin transparent perspex rods. An obviously valid objection to this method was that it allowed cues to be given by variation in movement. An automatic shape mover was tried, but it caused more disturbance than it saved. It was found later, experimenting with control groups, that as long as the shape moved, it did not seem to make very much difference if small differences in movement occurred. In the actual trials, however, the following scheme was adopted, that as soon as the stop watch was started, and the shape

/introduced,

introduced, the experimenter started to count up to ten, moving the shape to its lowest point at each number, and achieving a quite constant rate of 2 bobs per second.

(g) Light discrimination using B I.

The small lights placed in the far walls of the lateral boxes of B I were used. As explained above, a dimmer circuit was employed, so that a faint glow of the filament was visible, but the increase in light when the dimmer was shorted out was very large. The swing door was used, and only one of the windows was opened. The initial light/shock interval was 15 seconds, but this was soon changed to 5 seconds. Animals had to swim through the swing door in this period to achieve a correct trial.

(h) Simple conditioned responses.

A light/shock association was formed, using B II and CS/UCS intervals of 5 seconds. The activity produced was recorded on the Rustrak, running with the N°60 gearbox, giving a chart speed of 7½" per minute. The phototransistor/amplifier circuit was used to measure the degree of movement. The first nine trials were with CS/UCS intervals of 5 sec., the tenth trial was with a CS/UCS interval of 10 sec., so that reading could be taken.

(i) Light avoidance.

Both B I and B II could be used for these trials. For B I, the oval door and vertical slider were placed in position, and the windows were occluded; the situation was thus exactly the same in both boxes. Fish were placed in the start box in the dark, and allowed to quieten. The vertical door was gently removed, and the overhead light switched on after a suitable interval. Animals received shocks after 15 or 5

/sec.

sec. for failure to avoid.

(j) Sound avoidance.

A buzzer fitted to the wall of the tank containing B II could be activated by a foot-press. Except for a continuous overhead light, conditions and training were exactly the same as just described for (i).

(k) Trials with B III.

Relatively few trials were made using this box, on account of the length of trial necessary, and the fact that, as explained above, it was designed for another worker, and was not entirely satisfactory. Fish were pre-trained for both situations used in the manner following:

A fish was placed in the small antechamber; the first door was opened, allowing it to pass through into the discrimination chamber; if the animal did not pass through within a minute, it was encouraged with a small probe, with a pair of electrodes in the tip carrying 6v DC. As soon as the tail of the fish was past the door, the stop clock was started and the door closed. The animal was confronted with only one open door, a plain one when position training was desired, and a shaped one in the case of discriminations. After 15 seconds, if the animal had not moved into the goal box, it received a shock. (As soon as the goal box was reached, its door was closed, and the overhead light extinguished. The light was switched on after the animal had been transferred back to the start box in a small net.) The same side each trial was used for position training, sides were alternated in a Gellerman order for shape discrimination pre-training.

In discrimination trials, both doors were open; being identical in the position trials, different shapes in the square/triangle trials.

Animals were punished for entering the wrong box, but were allowed to emerge to enter the correct box, which they generally did quite quickly. The dimension of the shapes were as follows: area of both: 10.9 sq. cm.; side of triangle: 5 cm. (regular 60° triangle); side of square: 3.3 cm..

(l) Trials with B IV.

The sequence of events for this box has been described above. The dimensions of the two rectangles used were identical, being: 1.5 x 0.3 cm.. Animals were allowed some minutes in the box before trials were started; trials were initiated every minute, and after the counter had reached twelve, that is the animal had had twelve trials that day, the clock mechanism was stopped, and the subject removed.

(m) Activity recording.

The phototransistor/amplifier/Rustrak setup was used, in conjunction with B II, to gain a measure of the activity of animals over various periods.

(i) The activity in CR's, as dealt with in section (h).

(ii) Recording the degree of activity caused by shocks, and other stimuli. (The gearbox used in these trials was generally N°12 or 60, to allow easy reading of the considerable amount of activity).

(iii) Recording activity over long periods for studies of habituation. Initial periods were 10 minutes, with a N°6 gearbox, to assess the amount of fall-off of activity in perids comparable to those before training. Later, trials were run with a N°$\frac{1}{4}$ gearbox, over 24 hours, in one case, over 48 hours, to measure habituation. In these long trials, the animal was put into the apparatus in the dark, and the light was switched on, the stopclock started, and the automatic recording apparatus

/set in

set in motion after 30 seconds. The blind was kept down throughout the period, and nobody was allowed to enter. Aeration was kept constant, and checks with permanganate marking showed that a satisfactory circulation of water was maintained, even in the box. With an overhead light on for 24 hours, there was obviously danger of overheating; one check over this period, in a dummy run, showed that there was a 10°C rise in temperature. Bearing in mind Q^{10} for biological reactions, this was too great a rise, and a condenser water-cooling system was fitted to the tank. In similar runs, it was found that the rise could be kept to within 2°C at the maximum.

(n) Number of shocks given per trial.

Behrend and Bitterman (1962) showed that for conditioned avoidance learning, the paradigms of Hunter and Warner were equally effective, i.e. a single shock, terminating in a given time, or a shock persisting as long as the animal failed to avoid. In view of the possible dangers inherent in the Warner method, a modified form was adopted, that of giving well-spaced shocks up to a certain arbitrarily-determined time. For 15 second trials in B I and B II, shocks were given at 15 seconds, and at 5 second intervals thereafter up to one minute. If an animal had not avoided by this time, it was gently propelled through the door. In 5 second trials, the shock was given at 5 seconds, and at 5 second intervals up to 30 seconds. In trials with B III, shocks were given at 15 seconds, and at 15 second intervals up to 5 minutes, in both positional and discrimination trials. B IV trials used the Warner method of shocking, the shock being terminated by the animal to bring action and reinforcement into close contiguity.

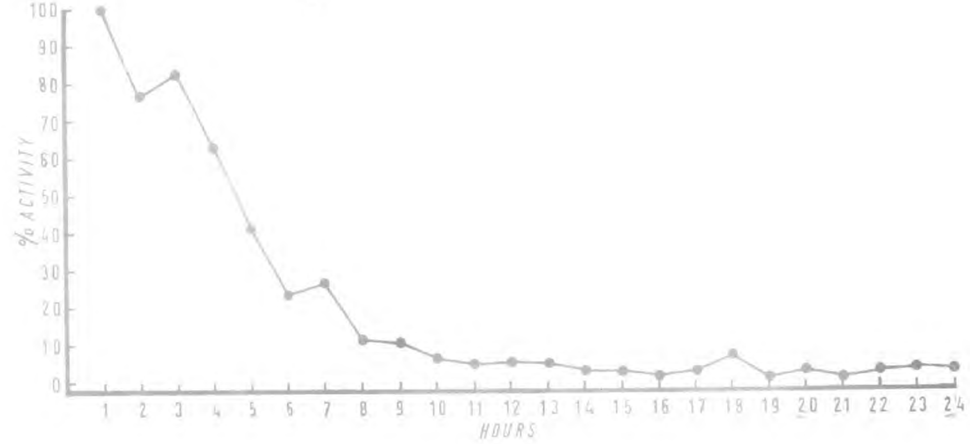


Fig. 11a: Habituation in constant conditions in BII, measured over 24 hours. 9 animals. Total activity over first hour taken as 100%, activity in succeeding hours expressed as percentages of this.

Ordinate: activity per hour, as percentage of that in first hour.

Abcissa: hour periods.

RESULTS.

A. Normal and operated control animals.

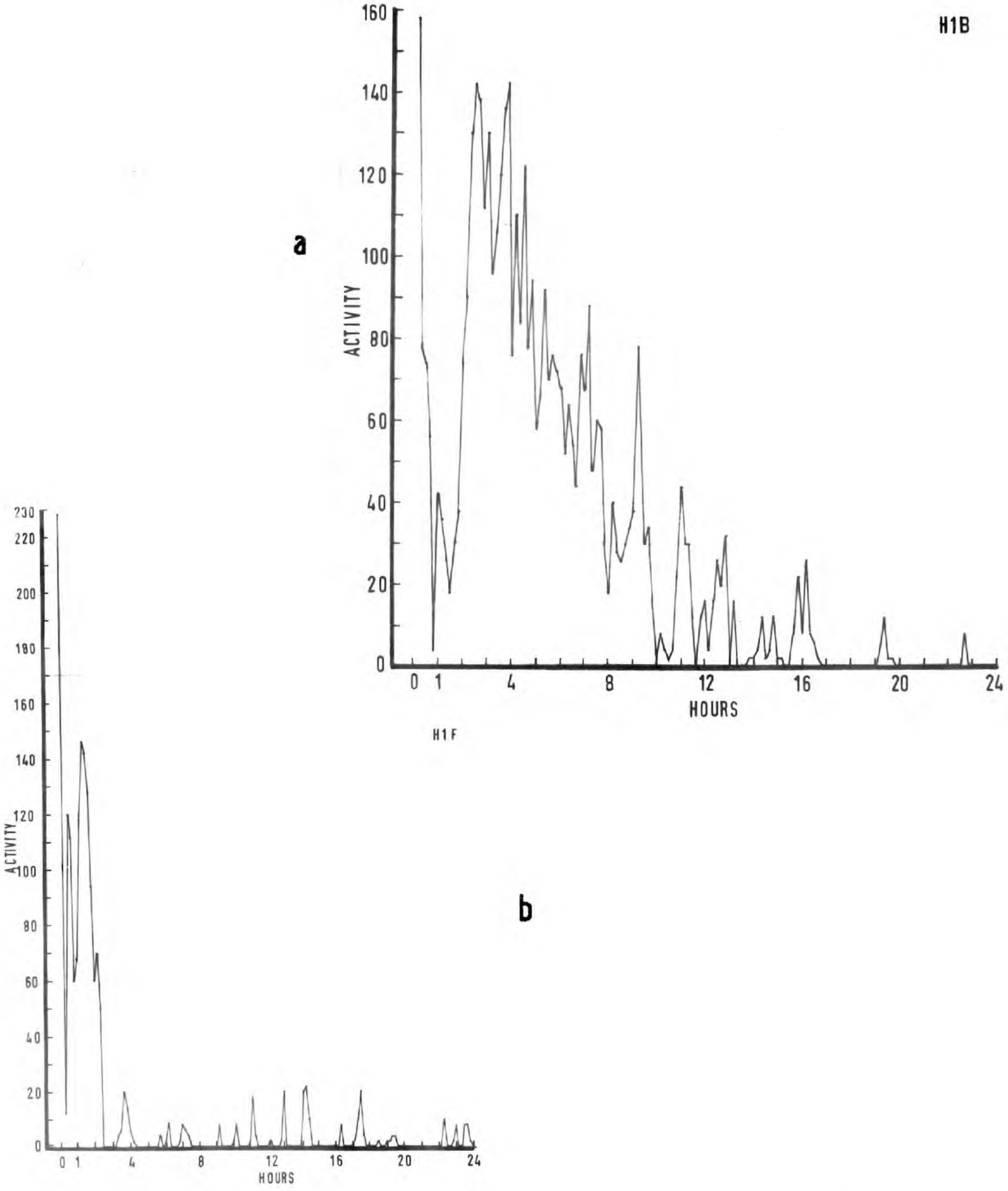
1. Effects of control operations.

The control operation, as described in the methods section, had very little effect on the behaviour of fish. Animals would swim normally within minutes of recovery, and soon after were feeding as before. In one or two cases, the skull circle was damaged, and secondary fungus infection developed, rendering the animal unfit; such animals were discarded. Generally, however, animals recovered rapidly and completely. Even if the skull roof came off, the fish was generally unharmed, and a plug of connective tissue would soon form to close the wound. There were no signs of damage to the brain, the few brains taken for histological examination were wholly normal.

2. Habituation.

Nine fish were kept in BII for 24 hours under conditions outlined in the method, and one animal was kept so for 48 hours. Whereas the times of placing in the box were kept as constant as possible for the training groups, variation was introduced into this group to show up any sign of diurnal rhythm which might persist in spite of the constant conditions. Fish HIC, for example, was started in the morning, fish HID in the evening. Fig.11 a, shows the mean activity curve obtained for 9 of these animals.

The fall of activity can be seen clearly (activity was summed for each fish for 10 minute periods) and the curves for animals started at various times show little sign of any diurnal rhythm. The rate of habituation was very variable, for example, fish HIB (Fig.12 a) took 13

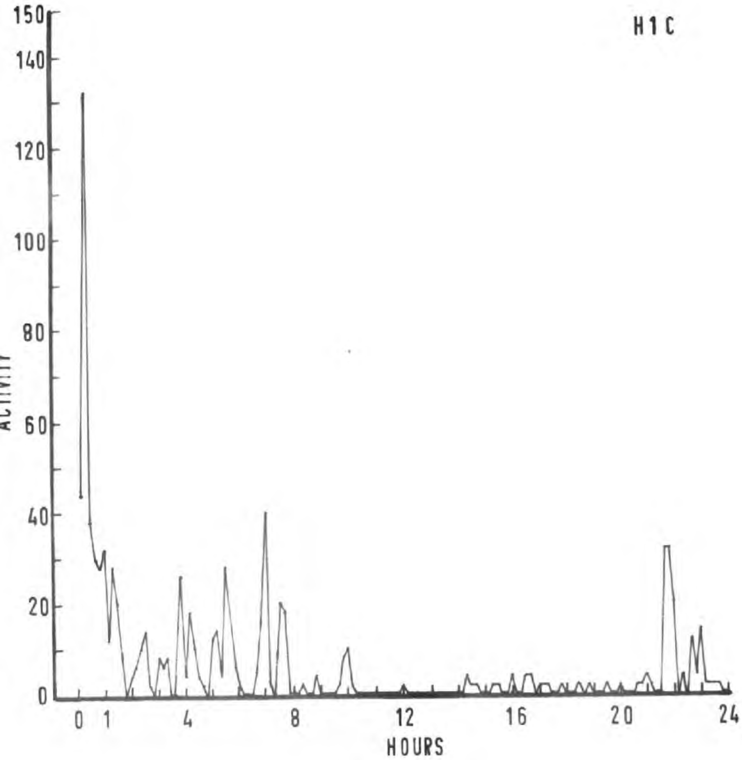


Figs.12 a - e: Habituation results for five individual fish.

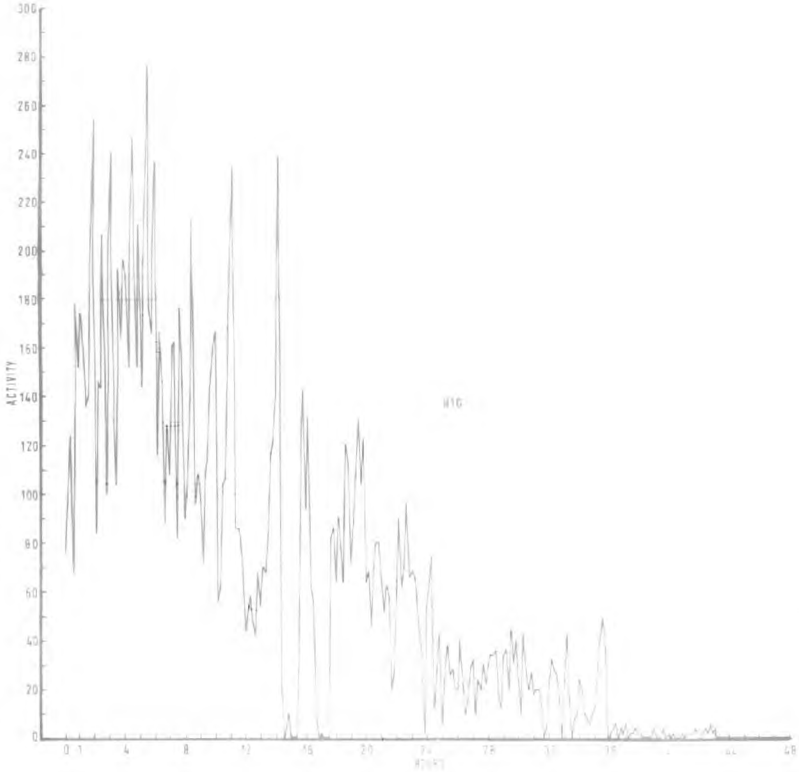
12a: Fish HIB Start 1.07 p.m. 24 hours' recording.
12b: Fish HIF Start 1.03 p.m. 24 hours' recording.

Ordinate: activity (pulses on the recorder) in ten minute intervals.

Abcissa: ten minute intervals.



c



d

Fig.12c: Fish HIC Start 10.29 a.m. 24 hours' recording

12d: Fish HIG Start 12.43 p.m. 48 hours' recording

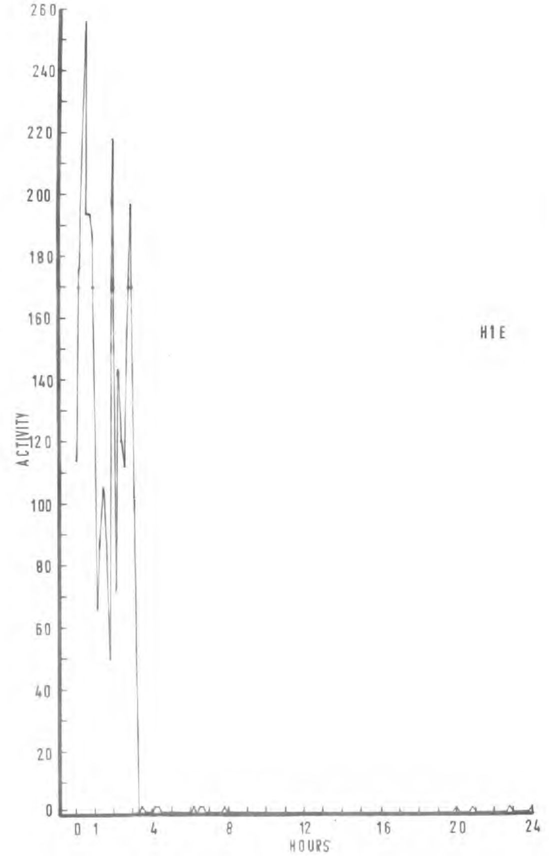


Fig.12e: Fish HIE Start 12.24 p.m. 24 hours' recording.

Table 3 a

Activity of 5 animals (meaned) measured over 10 minute periods daily for 14 days before and after control operation.

Day	Mean Activity in 10 min.	Day	Mean Activity in 10 min.
1	237	15	89
2	351	16	201
3	181	17	185
4	100	18	26
5	69	19	222
6	250	20	574
7	276	21	583
8	537	22	478
9	133	23	250
10	469	24	43
11	67	25	202
12	134	26	60
13	536	27	540
14	332	28	481

→
op.

Mean
Average: 262.4 281.0

Total activity after operation 107.1% of total before.

hours to achieve quiesence for $\frac{1}{2}$ hour, whereas fish HIF (Fig.12b) took $2\frac{1}{2}$ hours to achieve this state, and fish HIC (Fig.12c) took 9 hours. The level of activity achieved by these last two animals even after 24 hours did not remain at zero. One animal, HIG (Fig.12d), showed no sign of quietening down when the chart was examined after 24 hours, so it was left for a further 24 hour period. After this period, it had settled considerably, and for the last 5 hours had hardly moved. The rather abrupt cessations of activity seen in such individuals as HIE (Fig.12e) suggested that the recording circuit might have been at fault, but the system was checked at the end of these experiments, and was rarely found to be changed in sensitivity. The few points above zero on the curves after the large fall show that there was some small activity, but that the fish were otherwise inactive.

These results are of interest in showing the rather variable times taken to habituate by individual fish. In contrast to these results were those for a group of five fish whose activity is summarised in Tables 3a and 6b. These animals were placed in the start box of BII for 10 minute periods daily, for 14 days. They were then subjected to the control operation, and, after the recovery period, given 14 days' further testing. Although the total time in the box was 4 hours, 40 minutes, there was no sign of the fall of activity seen in the fish of group HI. (The mean activity for these fish at 4 hours, 40 minutes was about 40% of that shown in the first hour). That for the 10 minute group varied considerably over the test periods, and showed no such overall fall.

Thus habituation occurs faster in continuous constant conditions,

/rather

rather than in periods of such given daily. The fact that it occurred at all supports the suggestion made in the introduction, that failure to find cessation of activity was due to the irritation of the recorder (see, for example, Bull (1928) and Spencer (1927)). Even in the presence of light, 9 of the fish used showed a mean of 50% of initial activity within 4½ hours, and within 10 hours showed less than 10% of this. It was frequently observed that the rate of habituation was greater in the relative darkness of the training situation, without the overhead light. In this condition, it was found that nearly all fish would remain still for long periods (30 sec.+) after about 10-15 minutes. Bull's (1928) observations for similar conditions agree with this. Unfortunately, as was stated in the method, the Bitterman paddle system was far too insensitive for this sort of measurement, and quantitative observations had to be confined to quite highly illuminated situations (intensity of light at position of fish 2.4 log foot lambert). A possibility for further investigation would be the use of a vinylite screen over the lamp, this would cut out visible and U-V light, but allow 1-R to pass. The phototransistor apparently responds to 1-R, and would switch when fish interrupted the beam. On the other hand, 1-R might be detectable by the animal.

The results are interesting also for comparison with those of Welker & Welker (1958), outlined in the introduction. In most of the graphs shown here, and the others obtained, there was a drop in activity during the first 30 minutes, which was large compared with subsequent drops (see, for example, Figs. 12a, b, c & e). The activity mostly rose again, then declined more slowly. This sudden drop in

/activity

activity may be the initial reaction recorded by Welker & Welker, whose observations extended only over 10 minutes. These workers observed that fish would move round the tank, "exploring" it, after an initial period of quiescence, and they considered that the movements were a sign of habituation. It is unfortunate that their observations should have terminated at this point, since most authors (see Thorpe 1963) have considered habituation to general experimental conditions as cessation or diminution of exploratory activity, due to familiarity with the surroundings. The results reported here support that hypothesis; fish showed an initial burst of activity, probably due to the onset of the light, then quiescence, followed by increased activity due to lessened fear of surroundings. This activity waned as conditions remained constant, and the amount of "fear" due to unfamiliarity, lessened. This habituation was terminated by very reduced activity. If fish had been allowed an even longer period in the box, it is possible that other factors, such as hunger, would have increased, and promoted activity. (All fish were fed some hours before the experiment, so that differences in hunger-motivated activities were not involved).

3. Reaction to shock.

The effects of shocks of varying intensity have been noticed above. It remained to see if the repetition of shocks would have any injurious effect on the animals, or affect the threshold of effectiveness. Five animals were given sessions of II minutes in BII for 15 days, during which sessions they received 10 shocks at minute intervals. The activity throughout the period was measured by the photocell circuit; time marks on the Rustrak chart indicated the onset of shocks.

/Table

Table 3b

Activity produced in 5 normal fish by 10 shocks each day. (Shocks at minute intervals). Amount of activity on first day taken as 1, and that on succeeding days expressed as a fraction of first days' activity.

DAY	ACTIVITY
1	1.0
2	3.7
3	4.4
4	6.3
5	5.5
6	5.9
7	6.2
8	5.7
9	7.0
10	5.4
11	5.3
12	4.7
13	6.1
14	5.3
15	4.9

Table 4 a

Conditioned reflex formation in 5 normal fish. (CS/UCS interval 5 sec. Figures indicate mean number of pulses to light alone recorded by activity detector. Arrow indicates time of control operation). Test CS/UCS interval 10 seconds.

DAY	MEAN RESPONSE	DAY	MEAN RESPONSE
1	1.8	11	19.2
2	8.6	12	13.8
3	4.6	13	30.2
4	7.0	14	29.4
5	12.2	15	43.6
6	12.6	16	30.4
7	16.0	17	26.6
8	17.2	18	21.8
9	17.0	19	39.6
10	33.6	20	35.6
→			

Table 3b shows the activity produced, with the overall activity shown in the first day's test being taken as 100%. If the effect of continued shocking were to raise the threshold to shock, it would be expected that the overall activity values would fall. In fact the reverse of this occurred, showing that animals if anything became more susceptible to shock, that it was an effective stimulus throughout the period.

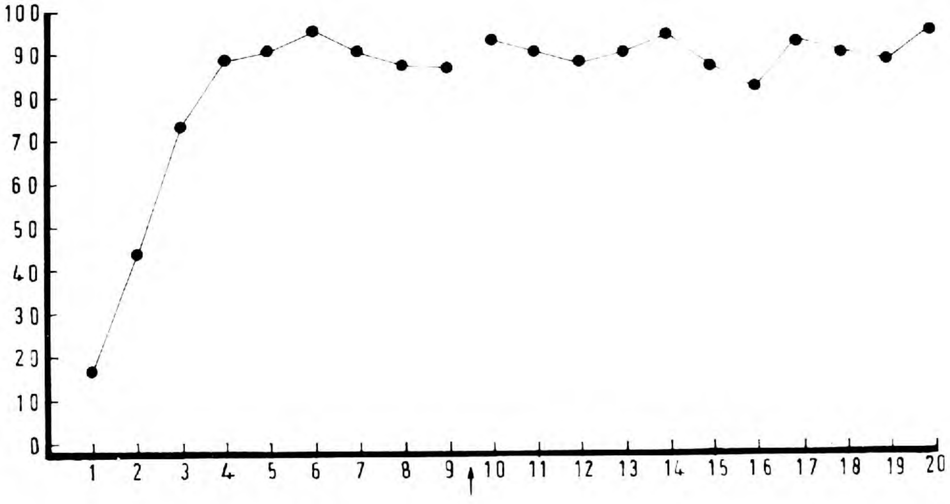
The continued administration of shocks had no observable effect on the subjects, their feeding was normal, and their behaviour in the tanks and in BII was not seen to change throughout the experiment. The fish survived for months after the shocking, and were finally used for the preparation of normal brains for histology. Examination of the bodies at operation showed no sign of abnormality. Thus it may be concluded that in the absence of any other factors, shocking of the magnitude and duration used was quite safe, and left no easily-observable after effects.

4. Formation of simple conditioned responses.

Table 4a shows results for a number of animals trained in BII with the light/shock schedule outlined in the method, using a CS/UCS interval of 5 seconds. The amount of CR produced in individual animals was very variable, perhaps on account of the rather lengthy CS/UCS interval employed. The arrow indicates the position of the control operation; subsequent performances were not affected by this, they were if anything improved.

The speed of learning the light/shock association was lower than that reported by some workers. For example Prosser and Fahri (1965) found a consistent deceleration of respiratory movements in the presence

/of the



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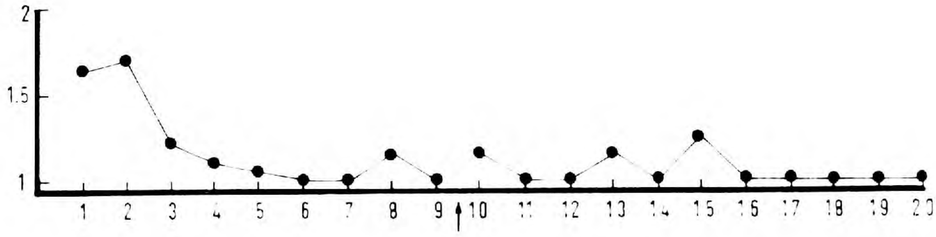


b

Fig.13a: Results for 6 animals trained to avoid light with a CAS/UCAS interval of 15 seconds. Control operation marked with an arrow.

Ordinate: percentage of avoidances each day.
Abcissa: days' trials (10 trials per day).

Fig.13b: Shock index for the above animals. Index = mean number of shocks given per escape. Control operation marked by an arrow.

Ordinate: shock index each day (shocks/escapes).
Abcissa: days' trials (10 trials per day).

of the CS (light) alone after 5 trials in some cases, 10 in others. A CS/UCS interval of 5 seconds was used. Froloff (1925) who also used light and shock, with a CS/UCS interval of 5 seconds, found that some animals would show a consistent response within 6 trials.

The results reported here resemble those of Bitterman (1965) in showing an increase of response with time. There is often a surprising lack of detail given on this point. For example, the mean activity for the test on the first day was 1.8, but this can hardly be said to show conditioning, since such a small response could equally well be due to "noise", the normal movement of the fish, or to startle reactions to the light. Sears (1934) was one of the few observers to examine this problem, and he set up a series of response-type criteria, to allow discrimination between the last-mentioned factors and real CR's. For example, in the results reported here, if the activities of untrained fish were measured for 10 seconds after a light came on for four days, means of 3.75, 0.5, 2.5 and 1.75 were obtained. (These data were taken from activity results for 8 animals, all given 10 minutes' activity testing daily). Thus it could be said that the CR had appeared by the end of 20 trials (Mann-Whitney U-test, $T = 15$, $R = 5, 8$, $P < 0.01$ for the second day's results). There was no significant difference between the groups on the first day.

5. Overhead light avoidance.

(a) Light/shock interval of 15 seconds.

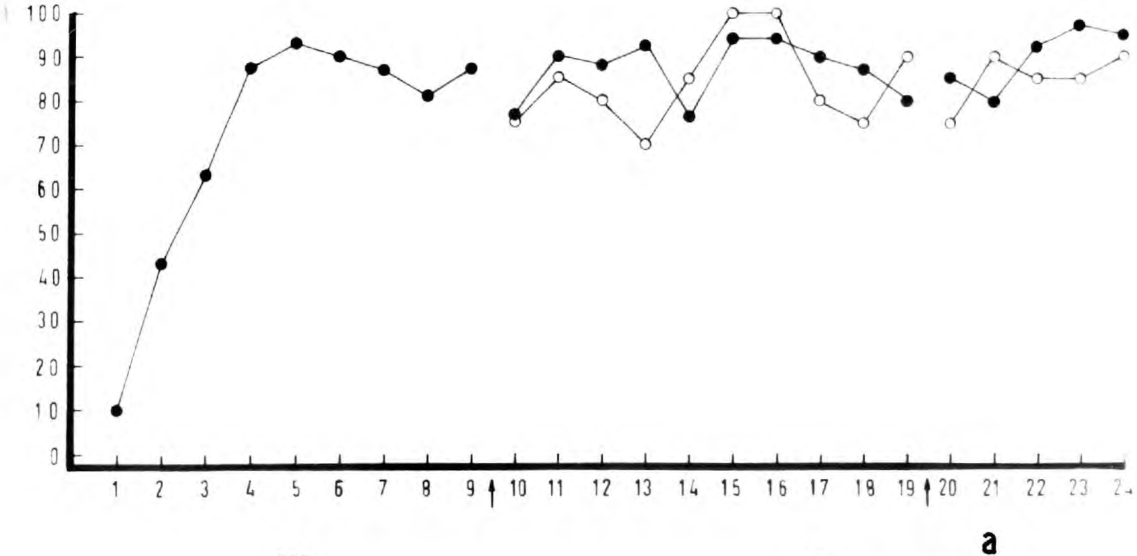
Fish were trained in either BI or BII as outlined above. Fig.13a shows results for six animals trained on this problem - the arrow indicates the position of the dummy operation; after 90 trials,

/where

where most animals had achieved a 90%+ avoidance level. As can be seen from the succeeding 110 trials, there was no sign of deficit following the operation, indeed, the first session of trials after operation was at a higher percentage correctness than that immediately preceding the operation.

Fig.13b shows an index plotted for each day's training. The usefulness of this index will, it is hoped, become apparent when used for comparative purposes with other groups, but it will suffice here to explain its derivation. As mentioned in the method, a shock was given at 15 seconds after the onset of the light if the fish had not avoided, and at 5 second intervals thereafter until it did avoid, up to an arbitrary maximum, 9 in this case. The total number of shocks given for one day could be derived from the recorded reaction times. This figure was then divided by the number of wrong trials, to give the mean number of shocks given in each incorrect trial. This was the figure plotted as the index. Thus, for example, if a fish beginning its trials received 15 shocks for 10 incorrect trials, the index for that day would be 1.5. Reference to Fig.13b will show that the index was initially "high" at 1.6, and that it fell rapidly to 1 or near 1, indicating that fish rarely needed more than one shock to promote avoidance. (If a fish made 10 out of 10 correct trials, this was counted as 1, since the purpose of these index curves was to demonstrate changes in facility of avoidance, and 1 represented the maximum ease, whether or not promoted by shock). The index was, of course, more accurate at the beginning of training, where large numbers of errors were being made, than at the end, when few errors were made, and a single lengthy trial might

/elevate



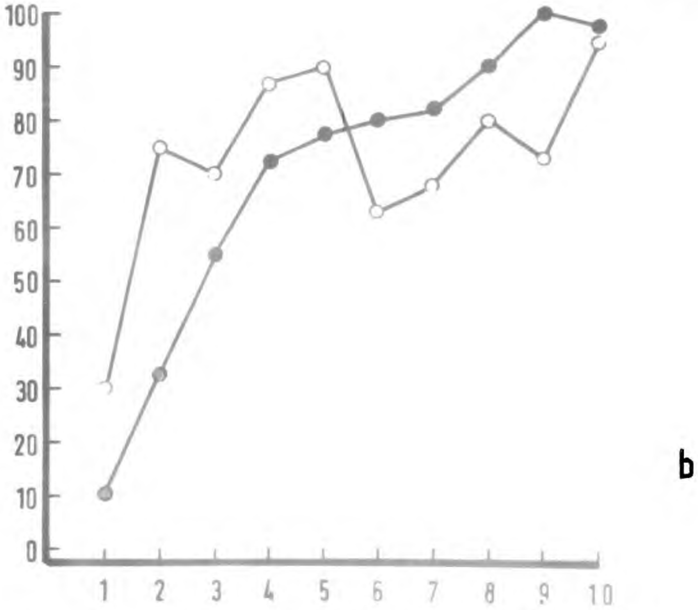


Fig.14 a: Results for 6 animals. First arrow indicates control operation. Closed circles: 4 animals trained after recovery from operation, then left untrained for seven days (second arrow). Open circles: 2 animals trained after control operation, then left untrained for ninety days (second arrow).

Ordinate: percentage of avoidances each day.
Abcissa: days' training (10 trials per day).

Fig.14 b: Results for two groups of 4 fish trained to 80-90% to avoid light (CAS/UCAS interval 15 sec.)
Closed circles: Reversal: shock if avoidance made.
Open circles: Extinction: no reinforcement for any response.

Ordinate: percentage <u>failures</u> to avoid per day.
Abcissa: days' training (10 trials per day).

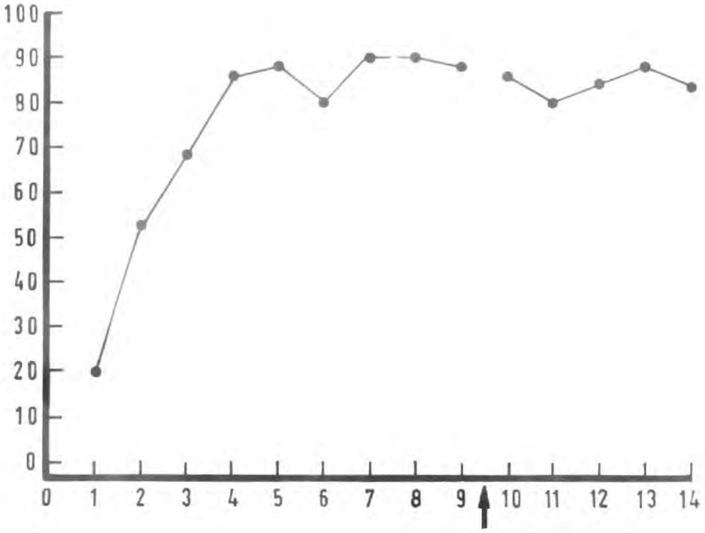


Fig.14c: Results for 5 animals trained to avoid light with a 15 sec. CAS/UCAS interval. Arrow indicates placing of bilateral eye covers.

Ordinate: percentage avoidance per day.
Abcissa: days' training (10 trials per day).

elevate the index considerably.

Note here that the index was, like the percentage avoidance, unaffected by the dummy operation.

(b) Retention in the memory.

Fig.14a shows the results for six animals trained as above. Dummy operations were performed at the point indicated by the first arrow, after 90 trials, and animals were then trained for a further 100 trials. Four animals were then left untrained for 7 days, two animals for 90 days. The second arrow indicates the resumption of training; the 7-day group, as might have been expected from the results for the 5-day gap operated controls above, showed no change, but the two long-term animals showed no appreciable deficit even after that period. (The two curves between the arrows show the groups separately, to give an idea of the differences in performance before the test interval).

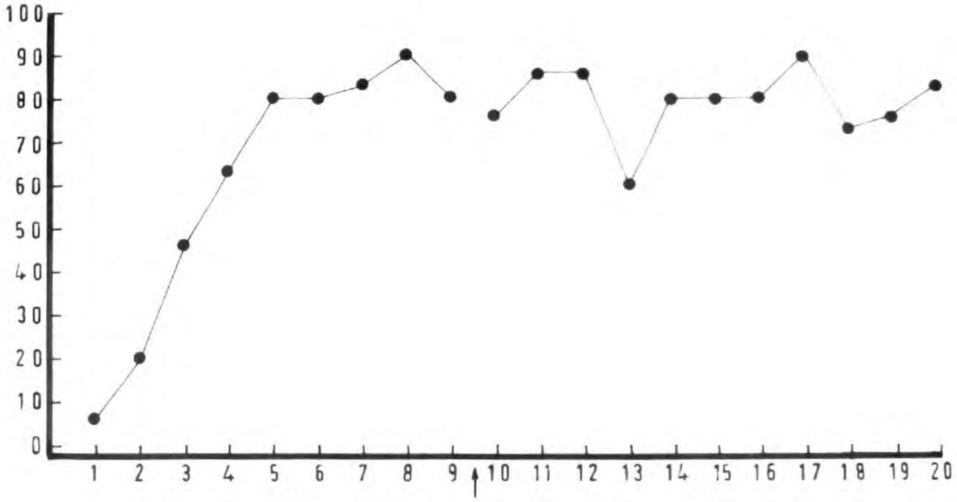
(c) Reversal and extinction.

Fig.14b shows results for 4 animals of the operated control group were subjected to reversal, 4 to extinction, after the completion of about 150 trials when the mean performance over the last 50 trials was 90%+. It can be seen that the reversal procedure tended to increase the avoidances as compared with the extinction group, whose avoidances fell off more rapidly. After 60-70 trials, however, animals would fail to avoid the light stimulus on 70% of trials.

(d) Effect of fitting blinders to trained fish.

A control group of 5 animals was trained with the 15 second light schedule. Results are shown in Fig.14c. Learning followed the normal rapid pattern, and, after 90 trials, the fish were anaesthetised,

/and blinders



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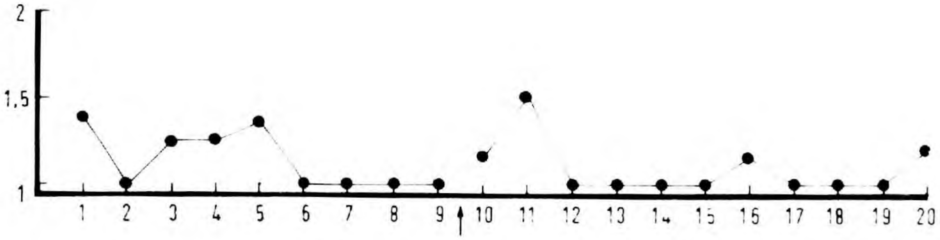


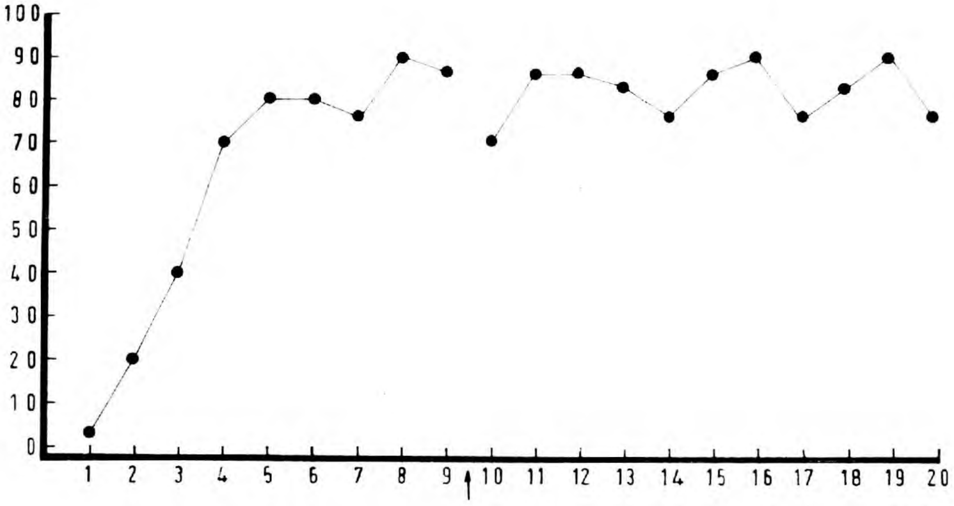
e

Fig.14d: Results for 3 animals trained to avoid light with a CAS/UCAS interval of 5 seconds. Arrow indicates the control operation.

Ordinate: percentage avoidance per day.
Abcissa: days' training (10 trials per day).

Fig.14e: Shock index results for the above animals

Ordinate: shock index per day (shocks/escapes).
Abcissa: days' training (10 trials per day).



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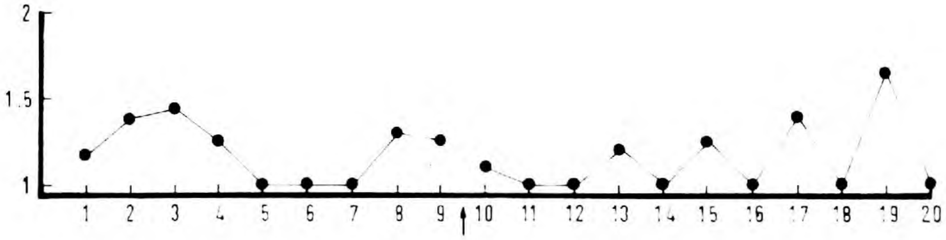


b

Fig.15a: Results for 3 animals trained to avoid sound stimulus (CAS/UCAS interval 5 seconds). Arrow indicates position of control operation.

Ordinate: percentage avoidance per day.
Abcissa: days' training (10 trials per day).

Fig.15b: Shock index results for above groups.

Ordinate: shock index per day (shocks/escapes).
Abcissa: days' training (10 trials per day).

and blinders placed over the eyes. During the five-day recovery period, they were observed, and were fed. No responses were made to food until it had fallen below the fish, and was presumably detected by smell; this would indicate blindness. No response was made to sudden movements, such as hand movements outside the glass. Fish were re-trained, and it can be seen that there were prompt avoidances, and that the avoidance memory was retained. This was presumably mediated mainly via the light-sensitive area of the diencephalon (see Scharrer (1928)) though of course the blinders may not have been completely light-tight. All fish showed prompt reactions to the light after blinding, but in some instances had difficulty in finding the door in time. Individual scores for the first ten trials after fitting of blinders were 10, 9, 8, 10 and 6.

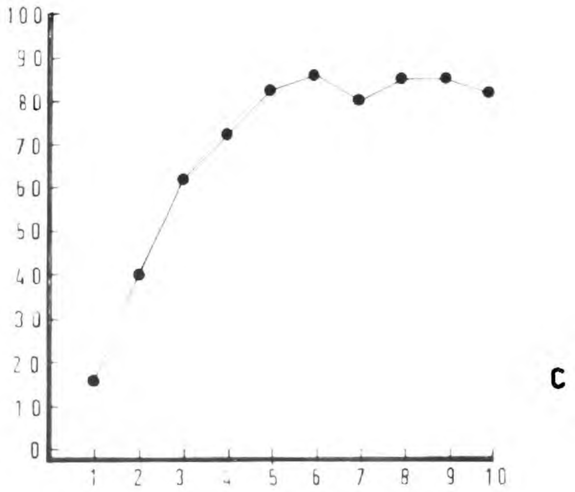
(e) Light/shock interval of 5 seconds.

Fig.14d shows the acquisition curve for three animals trained in BII using this interval; the graph below,14e shows the shock index for these animals. The rate of learning was rather slower than that for the 15 second group, as might have been expected, and the maximum level of performance was rather less. As in the previous case, the dummy operation had no effect on either the percentage avoidance or the shock index.

6. Sound avoidance.

Using BII and the buzzer fitted to the wall of its tank, a group of three fish was trained in an avoidance situation identical to those described above, using sound as the aversive stimulus; the overhead light was on all the time. Fig.15a shows the results obtained,

and Fig.15b



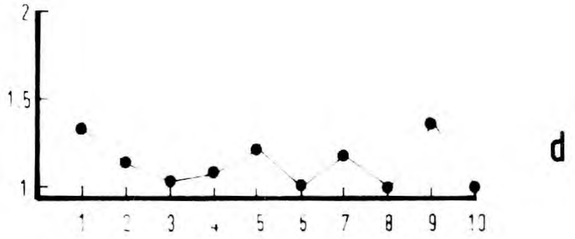


Fig.15 c: Results for 6 animals trained to avoid sound (CAS/UCAS interval 5 seconds) after being fitted with blinders.

Ordinate: percentage avoidance per day.
Abcissa: days' training (10 trials per day).

Fig.15d: Shock index results for the above animals.

Ordinate: shock index per day (shocks/escapes).
Abcissa: days' training (10 trials per day).

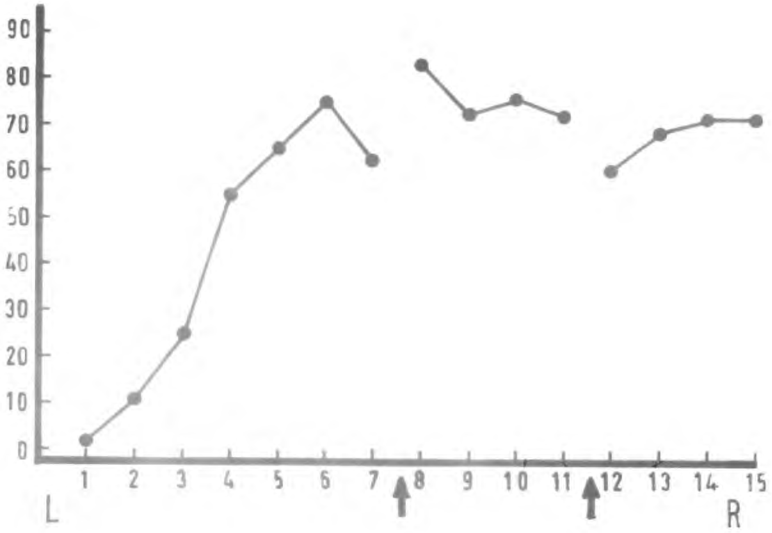


Fig.15 e: Results for 9 animals trained to avoid a small light, presented unilaterally. (CAS/UCAS interval 5 seconds).
First arrow; control operation.
Second arrow; transfer of training from LHS to RHS.

Ordinate: percentage avoidance per day.
Abcissa: days' training (10 trials per day).

of the CS (light) alone after 5 trials in some cases, 10 in others. A CS/UCS interval of 5 seconds was used. Froloff (1925) who also used light and shock, with a CS/UCS interval of 5 seconds, found that some animals would show a consistent response within 6 trials.

The results reported here resemble those of Bitterman (1965) in showing an increase of response with time. There is often a surprising lack of detail given on this point. For example, the mean activity for the test on the first day was 1.8, but this can hardly be said to show conditioning, since such a small response could equally well be due to "noise", the normal movement of the fish, or to startle reactions to the light. Sears (1934) was one of the few observers to examine this problem, and he set up a series of response-type criteria, to allow discrimination between the last-mentioned factors and real CR's. For example, in the results reported here, if the activities of untrained fish were measured for 10 seconds after a light came on for four days, means of 3.75, 0.5, 2.5 and 1.75 were obtained. (These data were taken from activity results for 8 animals, all given 10 minutes' activity testing daily). Thus it could be said that the CR had appeared by the end of 20 trials (Mann-Whitney U-test, T = 15, R = 5, 8, $P < 0.01$ for the second day's results). There was no significant difference between the groups on the first day.

5. Overhead light avoidance.

(a) Light/shock interval of 15 seconds.

Fish were trained in either BI or BII as outlined above. Fig.13a shows results for six animals trained on this problem - the arrow indicates the position of the dummy operation; after 90 trials,

/where

where most animals had achieved a 90%+ avoidance level. As can be seen from the succeeding 110 trials, there was no sign of deficit following the operation, indeed, the first session of trials after operation was at a higher percentage correctness than that immediately preceding the operation.

Fig.13b shows an index plotted for each day's training. The usefulness of this index will, it is hoped, become apparent when used for comparative purposes with other groups, but it will suffice here to explain its derivation. As mentioned in the method, a shock was given at 15 seconds after the onset of the light if the fish had not avoided, and at 5 second intervals thereafter until it did avoid, up to an arbitrary maximum, 9 in this case. The total number of shocks given for one day could be derived from the recorded reaction times. This figure was then divided by the number of wrong trials, to give the mean number of shocks given in each incorrect trial. This was the figure plotted as the index. Thus, for example, if a fish beginning its trials received 15 shocks for 10 incorrect trials, the index for that day would be 1.5. Reference to Fig.13b will show that the index was initially "high" at 1.6, and that it fell rapidly to 1 or near 1, indicating that fish rarely needed more than one shock to promote avoidance. (If a fish made 10 out of 10 correct trials, this was counted as 1, since the purpose of these index curves was to demonstrate changes in facility of avoidance, and 1 represented the maximum ease, whether or not promoted by shock). The index was, of course, more accurate at the beginning of training, where large numbers of errors were being made, than at the end, when few errors were made, and a single lengthy trial might

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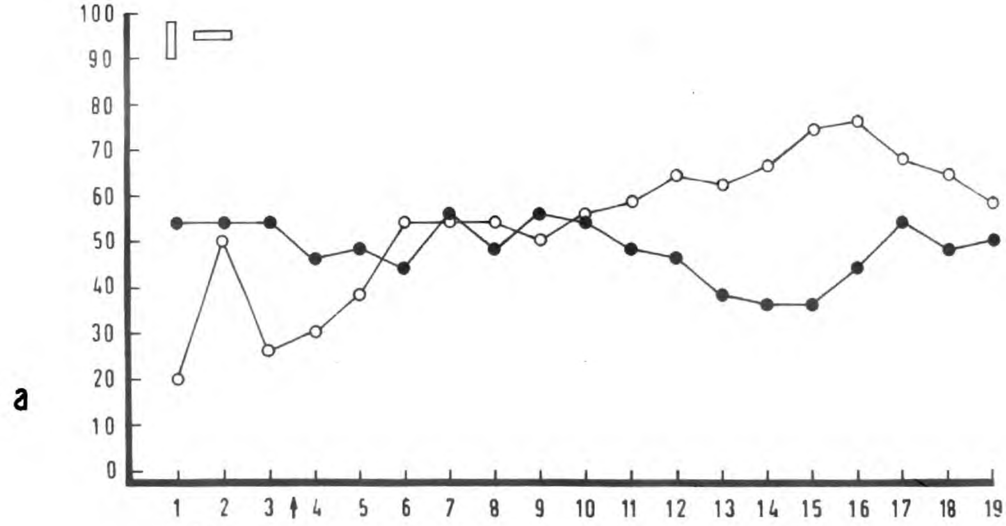


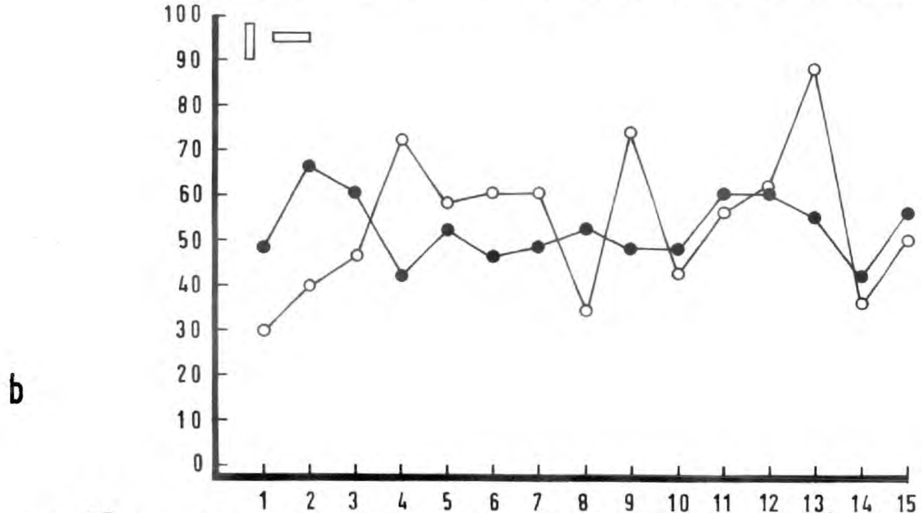


Fig.16a: Results for 5 animals trained to avoid |, not —, presented unilaterally. (CAS/UCAS interval initially 1 minute, arrow indicates change of this to 30 seconds). Trials initiated by switching on light overhead; shapes stationary. Only one shock given to cause avoidance.

Ordinate: percentage of trials: Closed circles: correct response
Open circles: avoidance
Abcissa: days' training (10 trials per day).

Fig.16b: Results for 5 animals trained to avoid |, not —. Conditions as for previous figure, except shocks given of 5 second intervals (up to one minute) until avoidance occurred.

Ordinate: percentage of trials: Closed circles: correct response
Open circles: avoidance
Abcissa: days' training (10 trials per day).

swing door, and this was made of sanded plastic and was hence matt. Chances of reflection would therefore seem to be small. (Similar tests were made with the horizontal/vertical avoidance situation described below, with the same negative results). The light differences involved were far too small to have been detected in the diencephalon. This needs direct bright light for perception to occur (see Scharrer 1928).

8. Factors in the formation of "go/no go" learning paradigms.

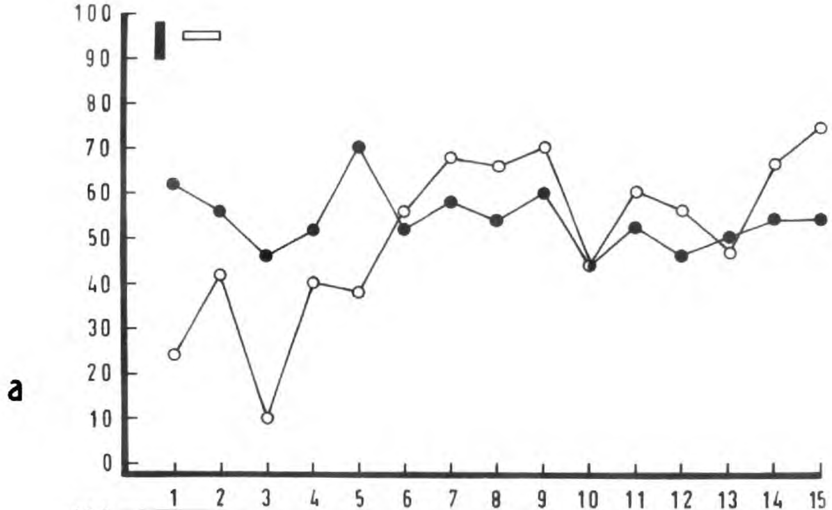
The first of these experiments were chronologically before those described above, but are dealt with here for the sake of continuity. A suitable method for training with discriminatory avoidances was evolved in the following manner:

(All experiments in this section were performed in BI).

In view of the possible extra cues involved in manually-moved shapes, it was initially decided to use stationary shapes. These were placed in the side boxes, and were hidden from the animal until just before the trial, when the door of the side box containing the shape and the door of the goal box were opened simultaneously. (The oval door to the goal box was used.) Initial shapes used were the white vertical rectangle as the aversive stimulus, the white horizontal rectangle as the non-aversive stimulus. These shapes were placed in position on the grey holders mentioned above. Trials were initiated by the onset of the overhead light, and the initial light/shock interval was 60 seconds, being changed to 30 seconds after 30 trials. In Fig.16 a, showing results for this experiment, the change of response time is indicated by the arrow.

A single shock to promote avoidance was given at the end of the light/shock interval, or, if a wrong avoidance had occurred, a single

/shock



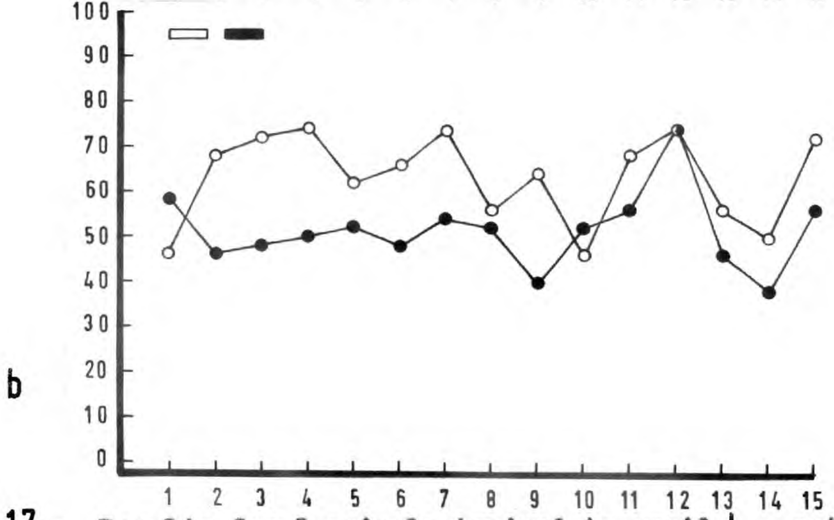


Fig.17a: Results for 5 animals trained to avoid | , not — . CAS/UCAS interval 15 seconds. Trials initiated by switching on light.
Closed circles: discrimination.
Open circles: avoidances.

Ordinate: percentage of trials.
Abcissa: days' training (10 trials per day).

Fig.17b: Results for 5 animals pre-trained to avoid ▭ (transferred to the problem when 5/5 avoidances). CAS/UCAS interval 15 seconds. Trials started by onset of light. Discrimination ▭ VS ▬
Closed circles: discrimination.
Open circles: avoidances.

Ordinate: percentage of trials.
Abcissa: days' training (10 trials per day).

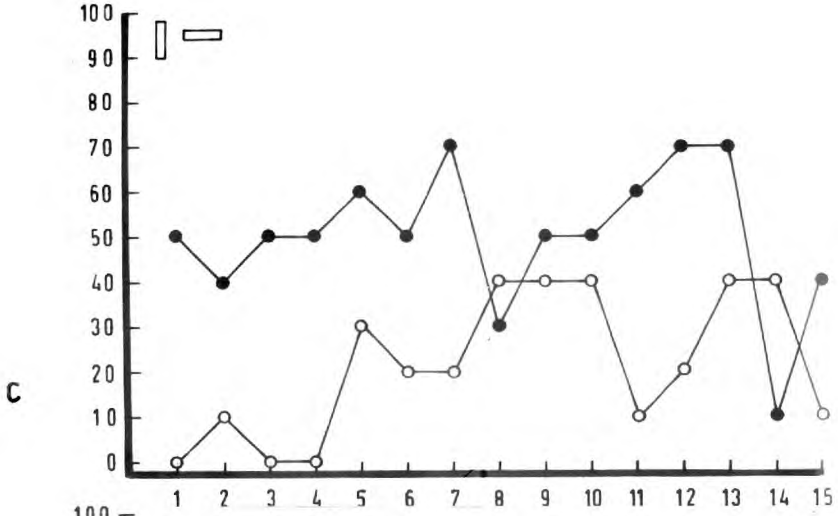
shock of similar duration was given in the goal box, as soon as the tip of the caudal fin had passed over the edge of the door. Fig.16a shows that no significant learning had occurred after 190 trials. The black points represent percentage of trials correct, the open circles represent avoidances, correct or incorrect, as a percentage of all trials. This terminology will be adhered to throughout the thesis.

Some of the animals in this group failed to escape after the one shock given in the previous experiment. As will have been seen in the earlier simple avoidance trials, referring to the shock index graphs, there was usually need for the animal to receive more than one shock to promote escape in early trials. (There was no manual guidance of the fish in the first experiment; if they did not avoid, they were left in the start box until the commencement of the next trial). It was decided to give shocks every 5 seconds up to one minute, to make escapes more certain, and Fig.16b shows the results for a group of five animals trained with this change; all other conditions were as in the previous experiment.

The results of these two experiments showed that the number of avoidances increased rapidly, but that they tended to bear increasingly little relation to the shape being displayed. In particular, animals failed to inhibit responses to the non-aversive stimulus.

In the next series of experiments, the stimulus/shock interval was reduced to 15 seconds, and the vertical rectangle was made black, in an attempt to make the discrimination easier. Results for these animals are shown in Fig.17a. Apart from two high percentages over the first few days, the curve for discrimination was not much above chance level,

/though



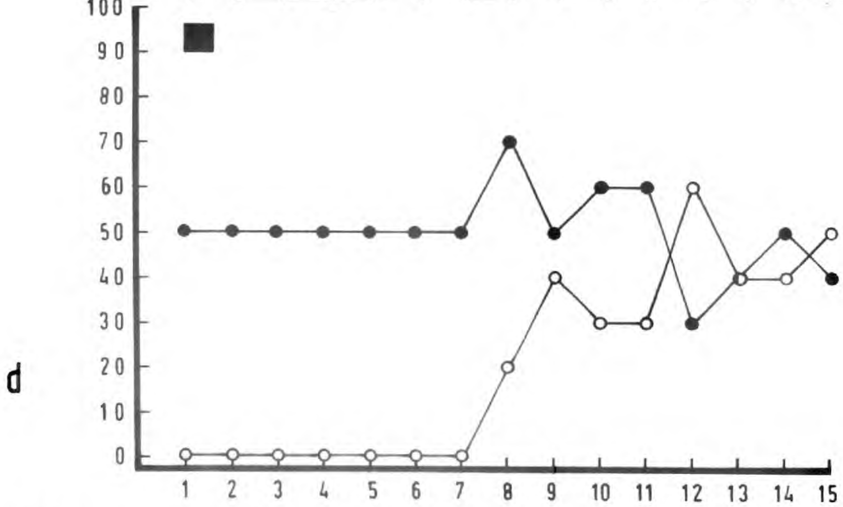


Fig.17 c: 1 animal. | vs — CAS/UCAS interval 5 seconds. Trials started by onset of light.
Closed circles: discrimination.
Open circles: avoidances.

Ordinate: percentage of trials.
Abcissa: days' training (10 trials per day).

Fig.17 d: 1 animal. □ vs nothing. Trials started by onset of light. CAS/UCAS interval 5 seconds.
Closed circles: discrimination.
Open circles: avoidances.

Ordinate: percentage of trials.
Abcissa: days' training (10 trials per day).

though the decreased stimulus/shock time did have the effect of reducing the number of avoidances made as compared with previous groups.

A further group of five animals was trained to test the effect of pre-training on the performance of this task. A black vs white horizontal rectangle discrimination was used, animals being pre-trained to avoid the white shape, and being transferred to the discrimination only when they had achieved a sequence of five out of five correct avoidances. Conditions were otherwise as in the previous experiment. This pre-training proved to be of little use, the avoidance level was higher than that of the previous group, whilst discrimination was close to chance. Results are given in Fig.17b.

Two animals were now trained, one with white vertical vs horizontal rectangles, the other with a black square vs nothing. The stimulus/shock interval for both these animals was 5 seconds. The rectangle animal (Fig.17c) showed some increase of accuracy for a few days, then fell sharply and showed no sign of discrimination. The square-trained animal showed very poor discrimination, the level both of avoidance and of discrimination was low for 50 trials, then avoidances increased rapidly, but were not directed towards the aversive square. (Fig.17d).

It was apparent that the animals were reacting to the light, and that the shapes were being ignored, or treated as irrelevant. Another animal was trained as follows: the overhead light was kept on throughout the training. The aversive stimulus was a black vertical rectangle, the non-aversive stimulus a white square. The shapes were moved regularly up and down at the ends of thin transparent perspex rods.

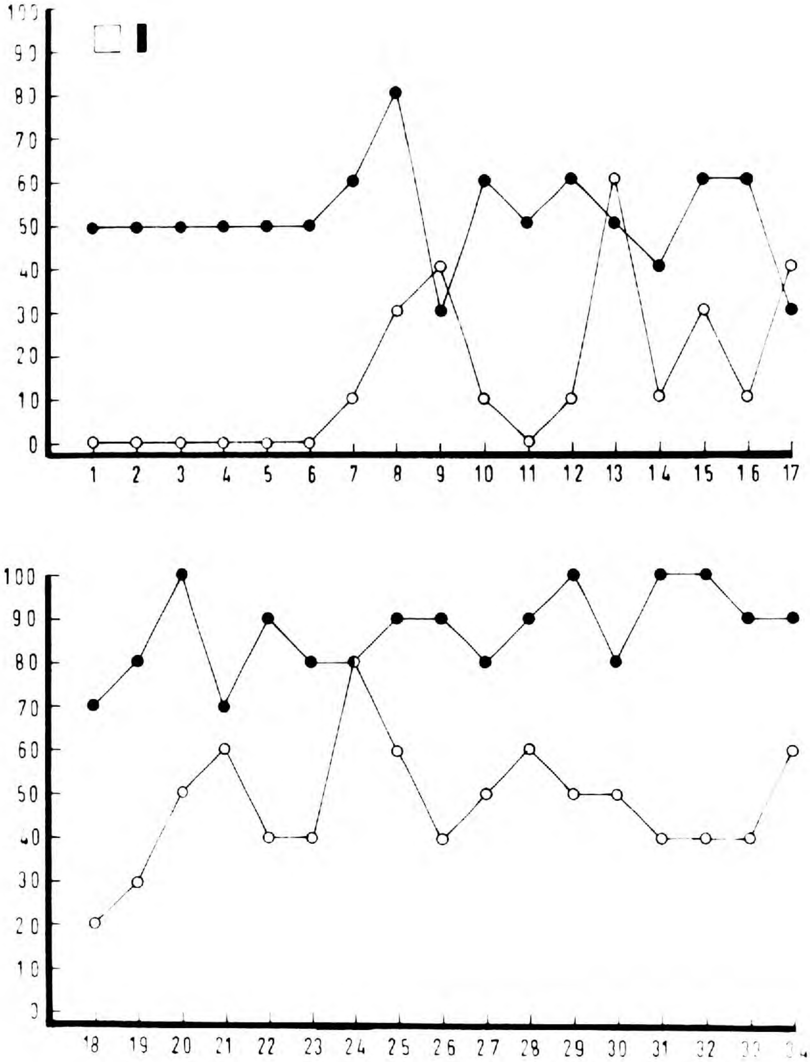


Fig.17e: 1 animal. Light on all time. Moving shapes (□ vs |)
CAS/UCAS interval 15 seconds.
Closed circles: discrimination.
Open circles: avoidances.

Ordinate: percentage of trials.
Abcissa: days' training (10 trials per day).

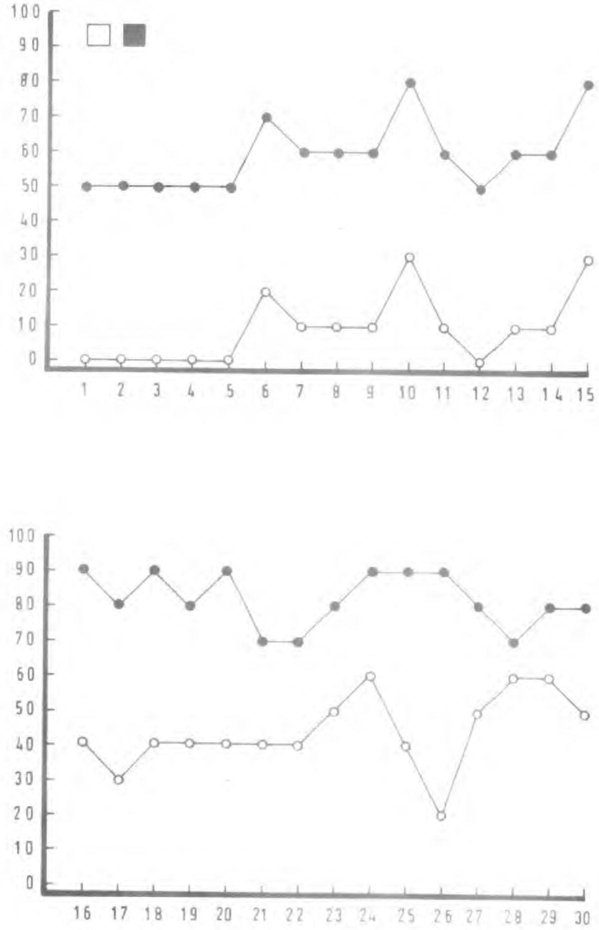


Fig.18 a: 1 animal. □ vs ■ CAS/UCAS interval 5 seconds. Light on all time. Shapes put into place for start of CAS interval.
Closed circles: discrimination.
Open circles: avoidance.

Ordinate: percentage of trials.
Abcissa: days' training (10 trials per day).

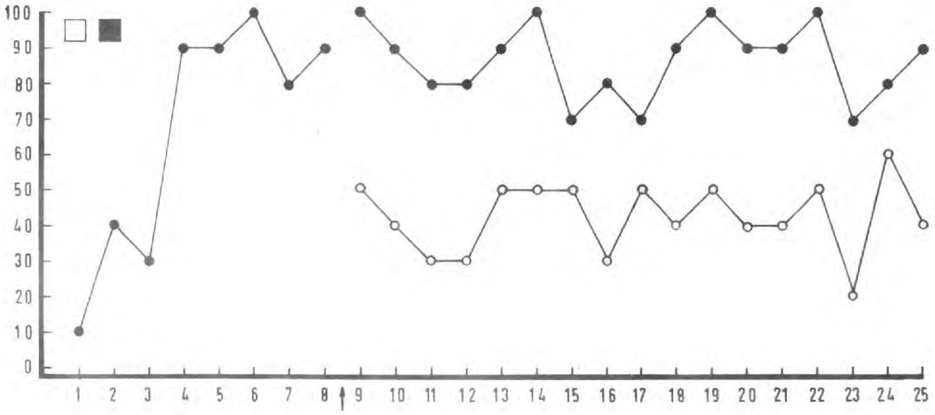


Fig.18b: 1 animal. 8 days' pre-training to avoid moving □ CAS/UCAS interval 15 seconds. Then □ vs ■, both moving.
Closed circles: discrimination.
Open circles: avoidance.

Ordinate: percentage of trials.
Abcissa: days' training (10 trials per day).

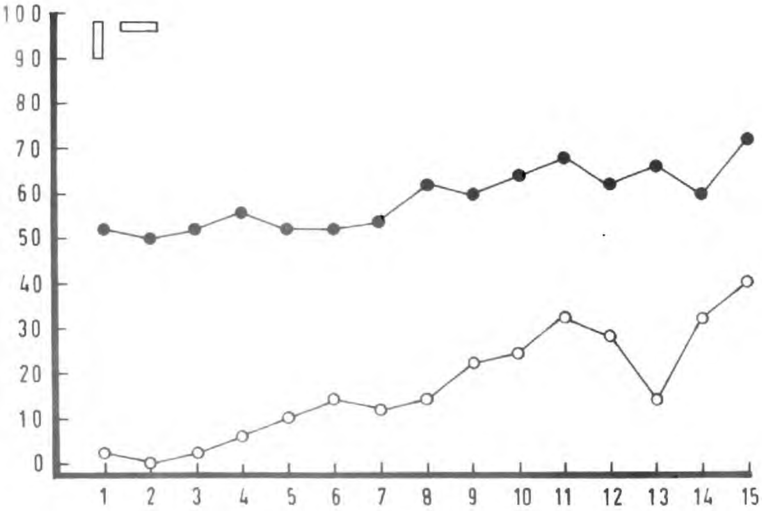
A 15 second interval was allowed for escape, and the oval door was used again. For some time (see Fig.17 e) it seemed that no learning would occur, but, just as trials were about to be terminated, a high level of performance was achieved and maintained. This animal tended to fail to inhibit responses on the non-aversive trials, and had to be left for considerable intervals between trials before it would settle sufficiently.

Since failures were generally the avoidances of the non-aversive shape, the stimulus/shock time was lowered to 5 seconds. Shapes used were black and white squares, which were put in place in view of the animal, the timing being started from this point. The shapes were not moved, this seemed to have a less disturbing effect on the animal used (Fig.18 a). In view of the slow rate of learning shown, and the failures to escape, it was decided to make use of moving shapes. (A group of animals was trained using small black and white circles which were hung on thin transparent perspex hooks, on the inside of the side window, so that they were very close to the fish. It was hoped that the fish would find these more obvious. A 5 second interval was used, and, despite some quite high percentage results at first, a near-random level was attained).

A single animal was trained to avoid a white moving square, with an interval of 15 seconds, and, after reaching a 90% correct performance for this, was transferred to a problem involving a black/white discrimination. Fig.18 b shows the results obtained. The animal performed well, but towards the end of its training, it failed to inhibit the avoidance response, and tended to avoid shapes as soon as they were introduced,

/rather

c



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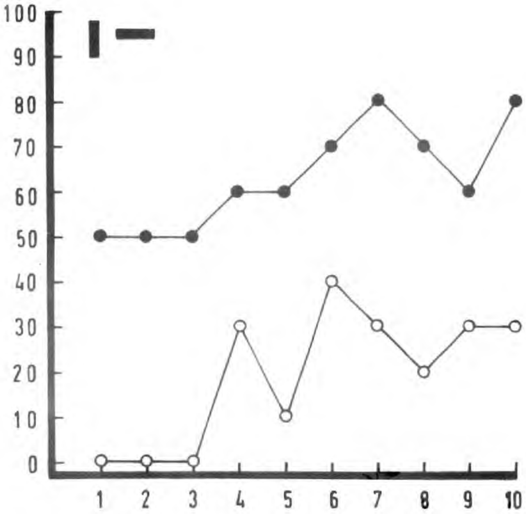
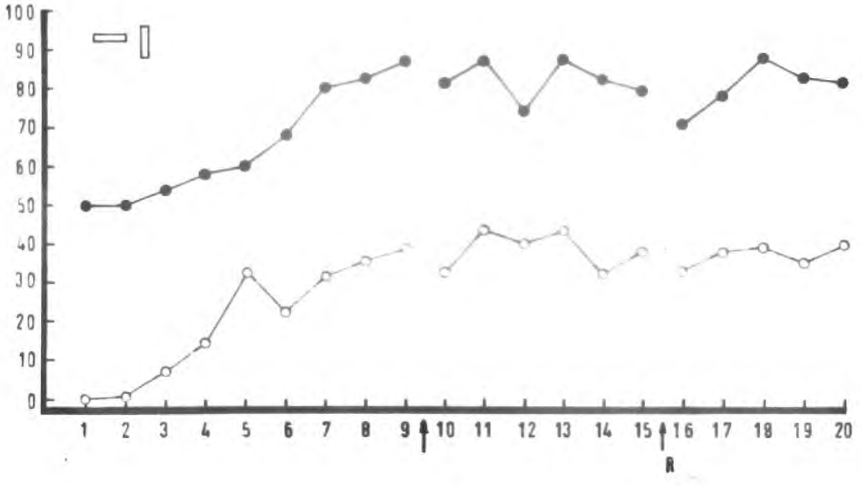


Fig.18c: Swing door introduced. 5 animals. CAS/UCAS interval 15 seconds. | vs — , moving.
Closed circles: discrimination.
Open circles: escapes.

Ordinate: percentage of trials.
Abcissa: days' training (10 trials per day).

Fig.18d: Swing door. 1 animal. CAS/UCAS interval 5 seconds. | vs — , moving.
Closed circles: discrimination.
Open circles: escapes.

Ordinate: percentage of trials.
Abcissa: days' training (10 trials per day).



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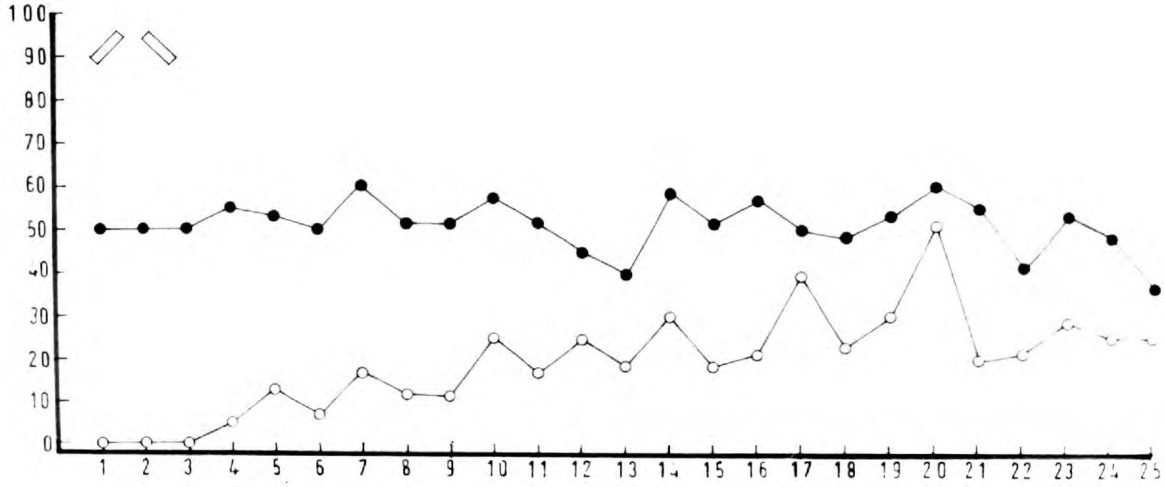


b

Fig.19 a: Results for 15 animals. | vs — . CAS/UCAS interval 5 seconds. Moving shapes. First arrow: control operation. Second arrow: transfer of training from LHS to RHS.
Closed circles: discrimination.
Open circles: avoidance.
Ordinate: percentage of trials.
Abcissa: days' training (10 trials per day).

Fig.19 b: Results for 6 animals. / vs \ moving. CAS/UCAS interval 5 seconds.
Closed circles: discrimination. Open circles: avoidance.
Ordinate: percentage of trials.
Abcissa: days' training (10 trials per day).

rather than to discriminate. This had been a problem in previous trials, and it was decided to use a swing door. BI was modified, and Fig.18c shows the results for five animals trained in this modified box using a horizontal vs vertical white rectangles with a 15 second interval. The percentage avoidance did not rise as fast as in previous experiments, but it rose consistently, and escapes to the non-aversive shape were rarer.

Results from the small light trained group dealt with above suggested that better learning might occur with a smaller time interval, and Fig.18d shows the results for a single animal trained with this interval and black rectangles. The animal learned well, and in under one hundred trials, so it was decided to standardise the procedure.

9. Learning of vertical vs. horizontal.

Fig. 19 a shows results for 15 animals trained as outlined above, on the discrimination of white rectangles. Virtually all animals made their first avoidance within the first 50 trials, and the group as a whole had achieved a level of 81% after 100 trials. (Points to the right of the first arrow show the results for these animals after recovery from the control operation; there was little deficit in postoperative performance). After 150 trials, over the last 80 of which a fairly consistent level of performance had been maintained, the animals were trained on the RHS, at the point indicated by the second arrow. There was not the same difficulty with fish positioning as seen in the small light group, perhaps on account of the more obvious nature of the stimuli.

10. Learning of a discrimination between oblique rectangles.

/There were

There were several reasons for investigating this problem. Results from animals split after training suggested that since there was a greater rate of learning on the untrained side than initially on the trained side, there had been transfer of information, at a lower level. On the other hand, it was possible that since fish had learned the responses with the naive eye, they could more rapidly attack the associated shapes, after analysis. This would suppose that there had been no transfer of discriminative information, and that the increased rate of learning was due solely to the existence on the untrained side of the appropriate response mechanisms. It was therefore decided to train animals to perform the responses of the situation, but without the ability to form very definite shape/response associations. Mirror image problems are difficult to learn for any animal (see for example Sutherland 1961) and it was decided to use oblique rectangles. Mackintosh and Sutherland (1963) showed that goldfish could learn to discriminate between 45° oblique rectangles presented simultaneously only with difficulty, so successive presentation should prove more difficult still to discriminate shapes, if not impossible.

Once animals were avoiding well, though not necessarily discriminating well, they could be transferred to the two other problems, and the rate of learning compared with that of naive fish.

A single white rectangle, fixed at 45° to the end of a transparent rod, could be rotated to present either / or \ . Fig.19 b shows the results for six animals trained for 250 trials on the LHS. The level of avoidance rose steadily, though more slowly than for the | vs — group dealt with above. The level of discrimination remained near

/chance.

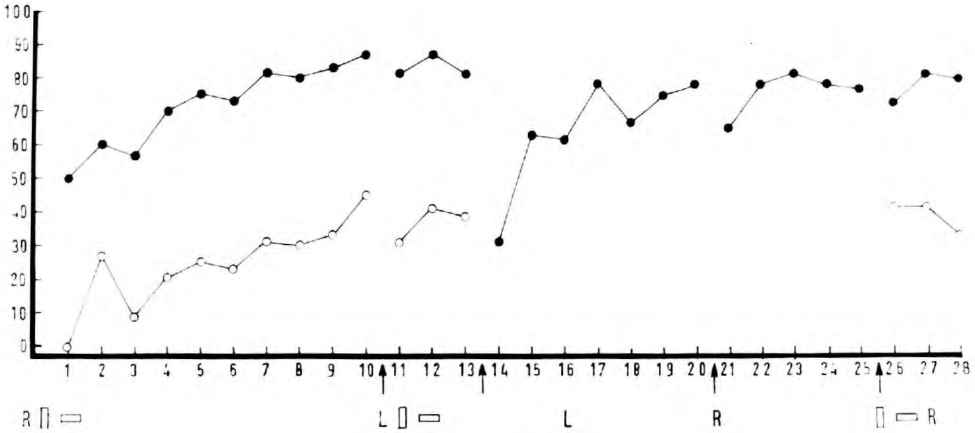


Fig. 20 a: Results for the 6 animals shown in Fig. 19 b. R | —: transfer from / vs \ LHS to | vs — RHS. L | —: transfer of training from R to LHS.
Second arrow: transfer from | vs — to small light avoidance, LHS.
Third arrow: transfer of this training from L to RHS.
| — R: final training of | vs — on RHS.
Closed circles: discrimination.
Open circles: avoidance.

Ordinate: percentage of trials.
Abcissa: days' training (10 trials per day).

chance. Some animals attained quite high levels of discrimination for single days, but this rarely persisted, and often fell as far the other side of the chance line the next day. In general, it seemed that the inability to discriminate the shapes also inhibited the level of avoidance as can be seen from the next section.

11. Transfer of problems.

The six animals trained in the previous section were next trained on the horizontal/vertical rectangle problem, using white rectangles. The initial training was to the contralateral eye to that used above, that is, the RHS eye. The acquisition curve is shown in Fig. 20 a. Reference to the curve for naive animals trained on this problem shows that the rate of learning, and of rise of avoidance, were greater in the transfer group. Inter-ocular transfer was again satisfactorily demonstrated.

After this inter-ocular transfer had been achieved, this group was transferred to the small light problem. The unmarked arrows (Fig. 20 a) indicate this transfer, and the inter-ocular transfer of the task. The rate of acquisition was again greater than that for naive animals, and the inter-ocular transfer was again positive. The final 30 trials for this group were used to test the retention of the rectangle problem after the small light training; retention was good.

12. Automatic apparatus results.

(a) Initial results.

Two animals were given 100 trials in B IV using only the circuit controlling the aversive stimulus, so that the light always

/presented

Table 5 a

Automatic apparatus: first results. CS/UCS interval 7 sec.

(A bar-press terminated the trial in all instances. 10 trials per day).

% Correct responses.

DAY	Animal 1	Animal 2
1	0	0
2	0	10
3	10	10
4	10	10
5	0	0
6	0	10
7	0	20
8	0	40
9	0	0
10	0	0

presented was the bright light, preceding shock unless the bar press was operated. The experiment was used to test the apparatus, and to see if learning could occur in these conditions. Results are shown in Table 5 a.

The acquisition of the response was slow, but this was due rather to deficiencies in the apparatus than failures to learn. For example, in early trials, there was a tendency for the uniselector to over-step at the end of the trial, so that even numbers of pushes might terminate the shock, then initiate the next trial. This extinguished the bar-pressing response. The fault was overcome by placing a 1,000 mfd condenser across the uniselector coil, with a time constant of about one second, so that rapid successive presses did not start a new trial. Fish never pressed the bar in the first second of a trial, so the innovation did not prevent a response from being effected.

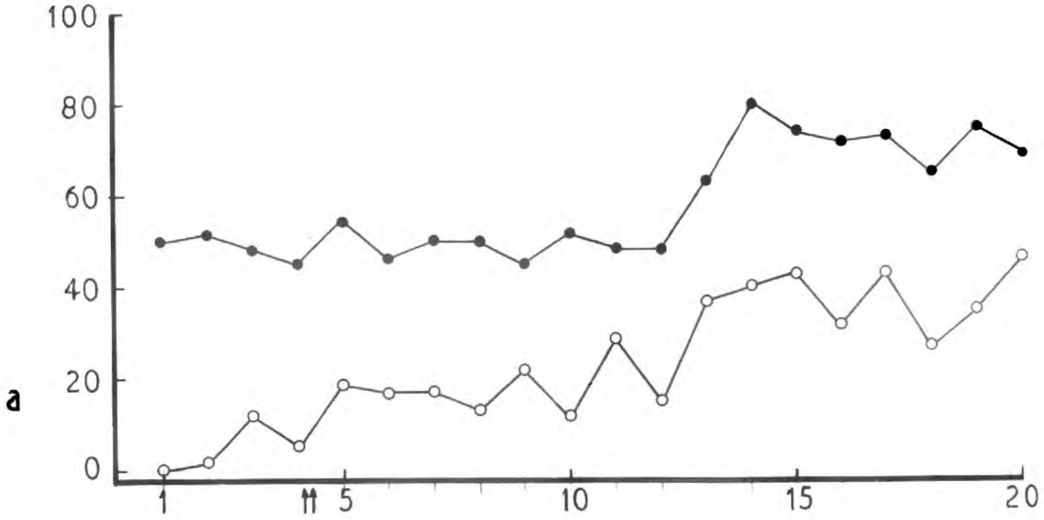
A second fault was that in some cases splashing by the fish caused shorting of the bar press mechanism at the contact above the press. This prevented cessation of trials, hence fish would receive continued shocks even if it pressed the bar. This, of course, extinguished the response. The contact was raised, and this solved the trouble.

Thirdly, fish tended to push the bar and to wedge themselves by it, and to be unable to make the next response. Another failure was that they tended to raise their heads so much that they missed the bar. A black oblique plate was fitted above the bar, and this directed the response satisfactorily, and prevented wedging.

(b) Brightness discrimination.

Five animals were trained with a bright light as the aversive

/stimulus,



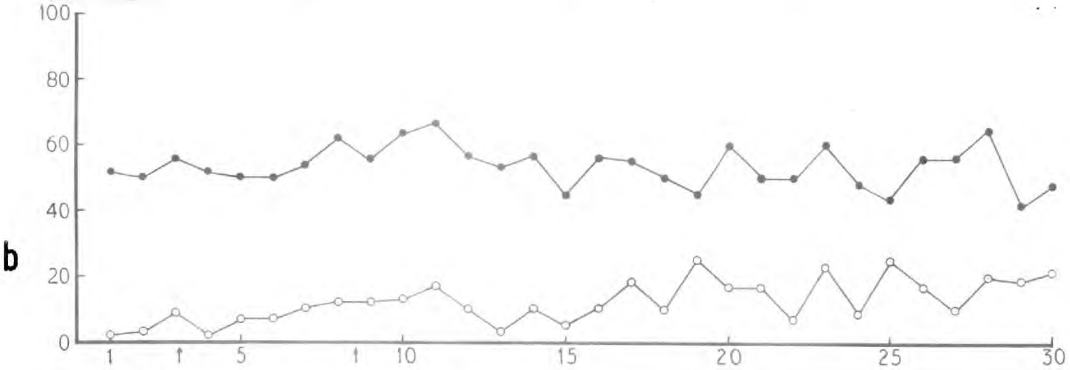


Fig. 21 a: Results for 5 animals. Automatic apparatus. CAS/UCAS interval 7 seconds. Bright vs dim lights.
First arrow: <u>mean</u> first incorrect non-aversive trial.
Second arrow: <u>mean</u> first correct aversive trial.
Closed circles: discrimination.
Open circles: avoidance.

Ordinate: percentage of trials.
Abcissa: days' training (12 trials per day).

Fig. 21 b: Automatic apparatus. 5 animals. | vs — , both stationary.
Trials started by illumination of shapes.
CAS/UCAS interval 7 seconds.
First arrow: <u>mean</u> first correct aversive trial.
Second arrow: <u>mean</u> first incorrect non-aversive trial.
Closed circles: discrimination.
Open circles: avoidance.

Ordinate: percentage of trials.
Abcissa: days' training (12 trials per day).

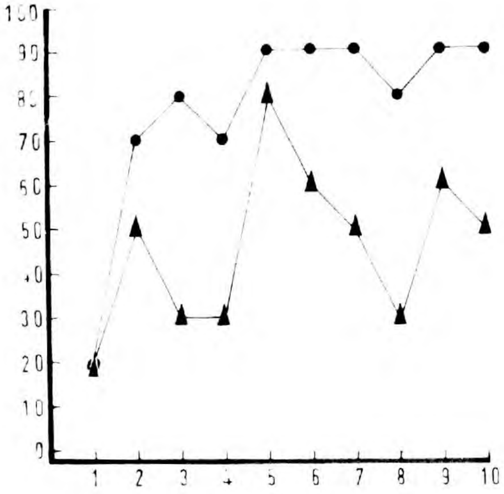
stimulus, and a dim light as the non-aversive stimulus. For technical reasons, 12 trials per day were given. Fig. 21 a shows the acquisition of this task, the open circles show percentage bar-presses. The time from the start of training to the first correct press was very variable, for example animal A took 13 trials, animal C took 66 trials. After 100 trials or so, however, all animals had started to press regularly, and the level of discrimination rose slowly. Fish showed rapid forward movements when the bright light came on, and tended to back away when the dim light came on.

(c) Horizontal vs vertical rectangle discrimination.

Fig. 21 b shows results for five animals trained with the rectangle discrimination, with the circuits outlined above, and 12 trials per day. These animals were given 360 trials, but, as can be seen from the figure, the level of discrimination did not rise above chance level, and the level of bar-pressing was not as high as in the previous group.

It is of interest to note here the parallel between results obtained in previous sections, and those reported here. In the section dealing with the methods of discriminatory avoidance training, it was noted that stationary shapes were not effective stimuli for formation of accurate discriminatory avoidance responses. The results here support that supposition - the animals could learn to discriminate differences of brightness, but were unable to discriminate rapidly between stationary illuminated shapes. The movement parameter is obviously one of considerable importance as far as this type of avoidance-learning is concerned.

/13. Position learning.



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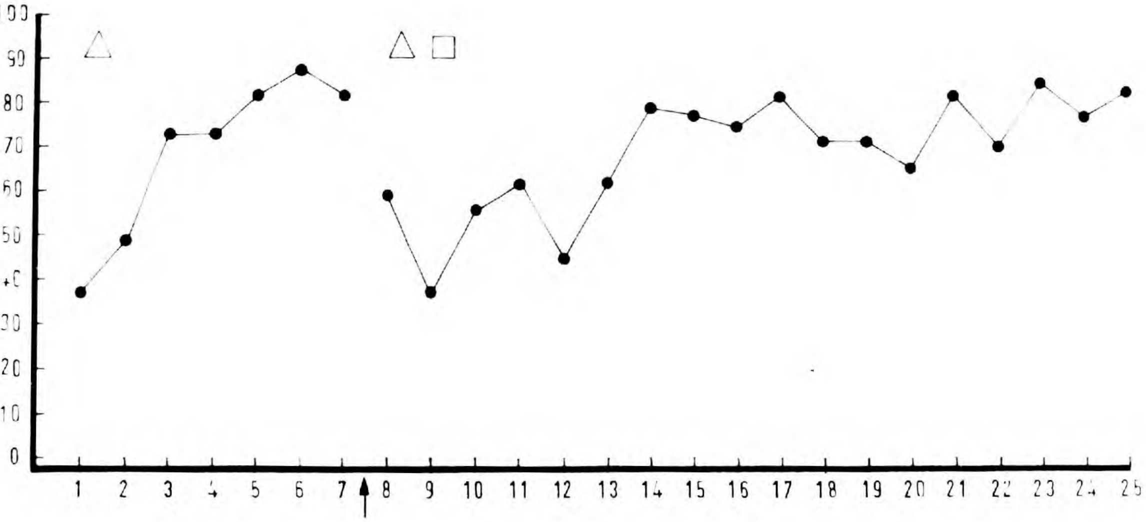

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Fig. 21 c: Results for 1 animal trained in B III. Training to L. 15 seconds in discrimination chamber before shock.

- ●: correct trials irrespective of shocks in discrimination box.
- ▲: correct trials before end of 15 second interval.

Ordinate: percentage of trials.
Abcissa: days' training (10 trials per day).

Fig. 21 d: Results for 7 animals trained in B III. First 70 trials, pre-training with only positive hole open. (△). Arrow indicates change to △ vs □ holes. (CAS/UCAS interval 15 seconds).

Ordinate: percentage correct choices.
Abcissa: days' training (10 trials per day).

13. Position learning.

One animal was trained in BIII to enter the LHS goal box. The grey outline holes were used, and the animal was allowed to swim through the apparatus for some hours before the start of training. Once training began, it was given shocks if it entered the RHS box. As can be seen from Fig. 21 c, it very quickly acquired the position habit. The circles represent choices correct irrespective of time spent in the discrimination box, the triangles represent the percentage of trials where the animal performed correctly in the 15 seconds before the first shock. It is obvious that quite large numbers of trials needed this reinforcement even when the position habit was developed to high percentages. Despite an initial preference to go right, the animal very quickly learned to make the left turn, and this speed of learning accords well with the results of previous workers, such as Beniuc (1934), in showing the importance and strength of such cues.

14. Triangle vs square discrimination.

Seven fish were pre-trained in BIII to enter the goal box with a triangle-shaped hole, the other hole being closed. The side of the open door was alternated in a Gellerman sequence, to avoid the positioning so easily developed as seen above. When levels of 80% correct in 15 seconds had been achieved, animals were presented with the triangle/square discrimination, the positioning of the shapes again being determined in a Gellerman sequence. After performing for some 60 trials around chance level, most animals achieved a fairly high level of discrimination, which was maintained over 100 trials. There were no effects on the discrimination when animals were subjected to a

/control

control operation. There will be mention in a later section of the effects of forebrain removal on the retention of this discriminative ability (Fig. 21d).

B. Effects of bilateral removal of the optic tectum.

1. General effects.

When allowance had been made for the somewhat aberrant forms of swimming indulged in by normal animals in the process of recovery from anaesthesia, it could still be maintained that there were forms of disturbance peculiar to animals whose optic tecta had been damaged. (See the introduction for a discussion of this). Most frequently noticed was a tendency to remain bent to one side during the early stages of recovery, followed by rapid circling to that side when movement became more active. These unusual movements had vanished by the time the fish were examined on the morning of the next day.

Some animals swam upwards, with their snouts breaking the surface of the water, others tended to perform loops in the vertical plane. These again had generally vanished within one day. On the day after operation, animals were swimming normally, and were indistinguishable from normals. In one or two cases, motor deficits persisted over a period of days to such an extent that the animals had to be killed; in these cases reference to notes made at the time of operation indicated that the tori semicirculares had been damaged. All other animals survived well, and after some initial difficulty, fed regularly. One impression gained from observing these animals was that they were more active and more easily aroused than normal fish (see below).

2. Activity of tectumless animals.

/It was

Table 6 a.

Activity of one tectumless and two normal fish. (10 min. periods).

DAY	Lesioned	Normal	Normal
1	384	269	681
2	765	149	540
3	790	65	195
4	675	285	384
5	1,740	222	122
Total	4,354	990	1,681

77.1% of tectum removed.

Table 6 b.

Activity of animals before and after operation. (Summed over 10 minute periods over 14 days). (5 fish).

Before	After	% change
3881	753.7	+ 197.5
324	3,086	+ 952.5
1472	8,338	+ 564.5
502	6,306	+ 1255
3308	12,000	+ 362.5

Mean : + 660.4

Mean for 5 normals : + 107.1

(activity measured before
and after control operation)

Mean amount of tectum removed : 76.26%

It was briefly noted above that the activity of the lesioned animals seemed to be greater than that of normal or operated control animals. This impression was confirmed in some of the avoidance training experiments described below. It was therefore decided to run a number of fish to gain a quantitative measure of this. Results from four initial animals, including two normal and one tectumless animal, suggested that fish lacking the tectum were more spontaneously active. Table 6 a shows the activity of this animal compared with the activity of two normals. Following this, 5 animals were put in BII for daily periods of 10 minutes for 14 days, and their activity measured. They were then operated, and their activity measured over the next 14 days. The results are shown in Table 6 b. Comparison of these results with those obtained for operated controls in the A section will show that there was a marked increase in activity of the tectumless animals compared with the operated controls. It was hoped to test the effects of lack of illumination on this activity, but, as mentioned in the methods section, the paddle method was far too insensitive, and there was not time to develop another method for recording.

The increased activity following tectal ablation was not due to the same causes as that immediately after operation, since, as mentioned above, that activity vanished soon, within one day. There also tended to be a reversal of the emphasis on fins in lesioned fish, the pectoral fins being used much more than the myotomes and caudal fin, except where directed movements, such as avoidances or escapes, were involved.

3. Reactivity.

A number of animals was placed in BII and activity records

/taken,

Table 6 c

Activity produced by shock. (5 fish, with a mean of 76.26% of the tectum removed. Fish given 10 shocks per day, and activity expressed as a fraction of the activity observed on the first day).

DAY	ACTIVITY
1	1.0
2	3.4
3	3.9
4	6.4
5	8.2
6	4.8
7	7.9
8	4.6
9	6.7
10	4.9
11	5.5
12	6.2
13	7.3
14	5.3
15	6.4

Table 6 d

Conditioned reflex training. (CS/UCS interval 5 sec., arrow indicates time of operation. 3 animals used, with a mean of 75.3% of the optic tectum removed). Response expressed as number of pulses on recorder.

DAY	MEAN RESPONSE	DAY	MEAN RESPONSE
1	0	11	24.0
2	6.7	12	19.3
3	0	13	33.3
4	3.3	14	39.0
5	51.3	15	30.0
6	16.0	16	34.3
7	16.0	17	33.7
8	18.7	18	31.0
9	46.0	19	28.7
10 →	59.3	20	29.0

Table 6 e

Conditioned reflex training. (CS/UCS interval 5 sec., 3 fish with a mean of 78.8% of the optic tectum removed).

DAY	MEAN RESPONSE
1	2.3
2	9.0
3	7.7
4	30.7
5	14.3
6	20.7
7	21.7
8	18.0
9	5.3
10	18.7

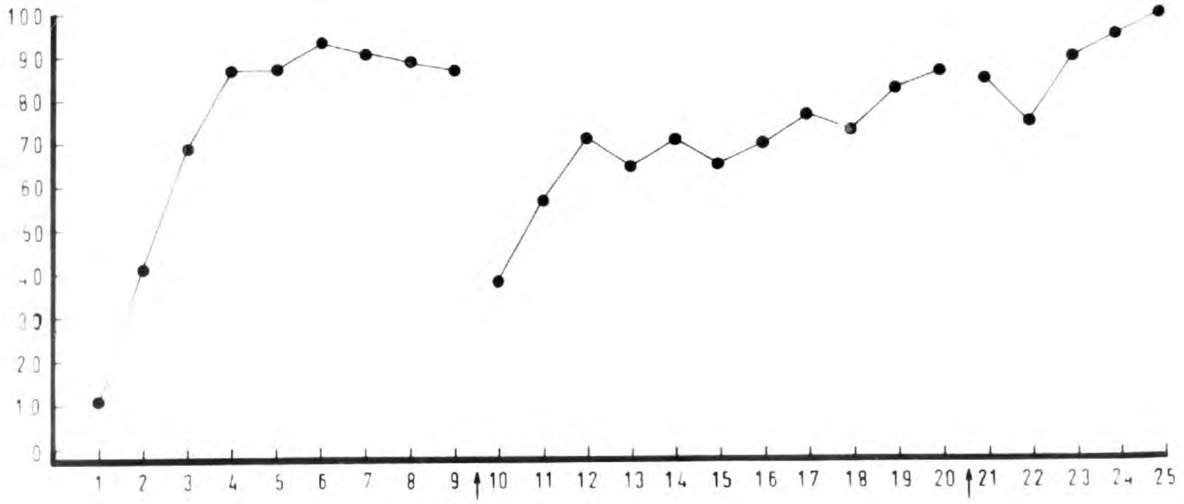
taken, whilst various stimuli were applied. Several animals were given the buzzer, for an interval of 1-2 seconds, but in no case was there any sign of change of activity following this stimulus. The same animals were given shocks at regular intervals, as described before, and the activity noted. Table 6 c shows results for effects of shock. The immediate effect of a shock was to elevate the level of activity beyond that at the resting level, but the motion so produced died away and was succeeded by a period of quiesence quite unusual for such animals, which inactivity was replaced by the usual irregular movement within 20-30 seconds. As in the case of the operated control animals, the process of shocking over a number of days seemed to elevate rather than depress the level of activity due to a shock. It may be concluded that shock was as effective a reinforcement in these fish as it was in normals, and that continued shocking at the voltage used was not deliterious.

4. Formation of simple CR's.

Table 6 d shows the results for 3 animals trained as outlined above, with a 5 second CS/UCS interval; the arrow indicates the time of the operation. Table 6 e shows results for 3 animals operated, then trained. Comparison of these results with those for control animals (Table 4 a) shows that neither learning nor retention was affected by tectal removal.

These results are in agreement with those of Sears (1934) who showed that tectal lesions did not interfere with learning or retention of simple conditioned responses to light/water current associations. Sears rejected the use of shock as UCS on account of the tendency of animals to over-react - this was not observed in the animals used in

/the present



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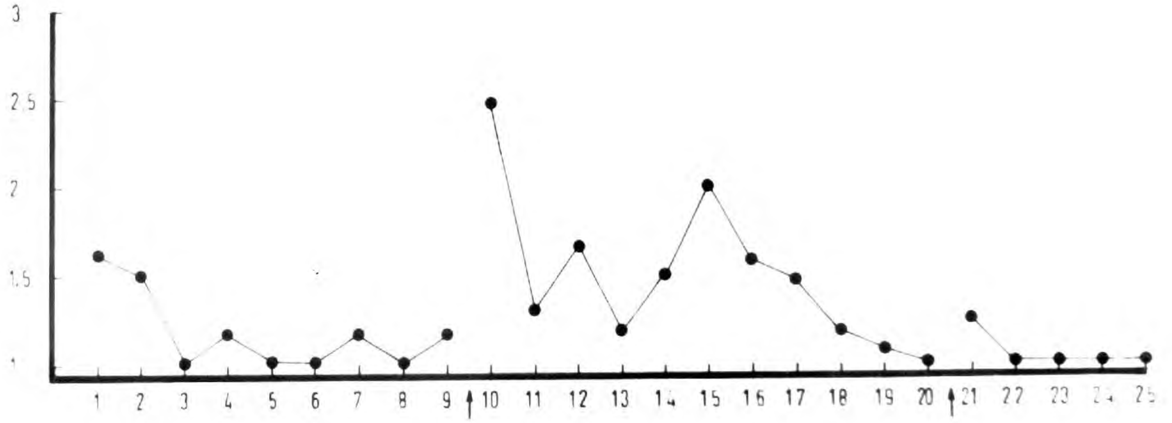
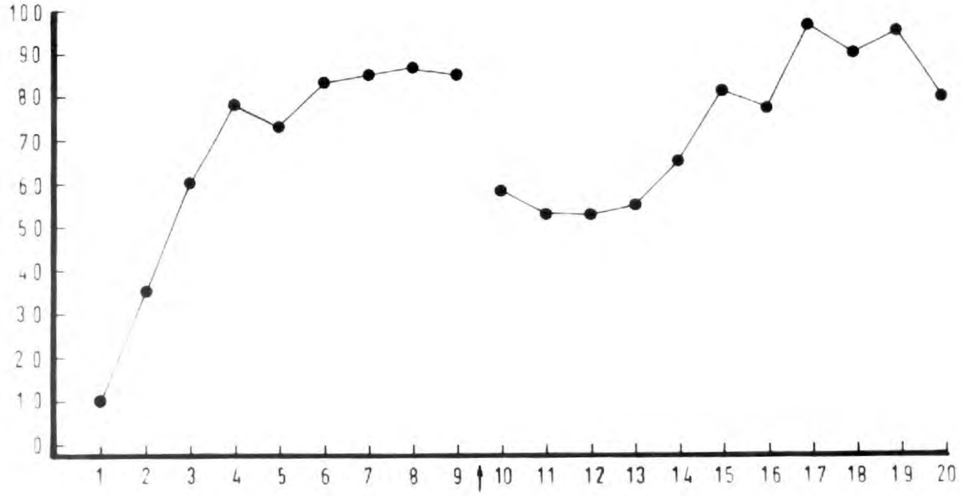


b

Fig.22 a: Results for 7 animals trained to avoid light (CAS/UCAS interval 15 seconds). First arrow indicates operation, removal of 72% of optic tectum. Second arrow indicates cessation of training for 5 animals, and continuation for 2 others.
Ordinate: percentage avoidance per day.
Abcissa: days' training (10 trials per day).

Fig.22 b: Shock index results for the above animals. (Mean number of shocks per incorrect trial). Arrows as above.
Ordinate: shock index per day (shocks/escapes).
Abcissa: days' training (10 trials per day).



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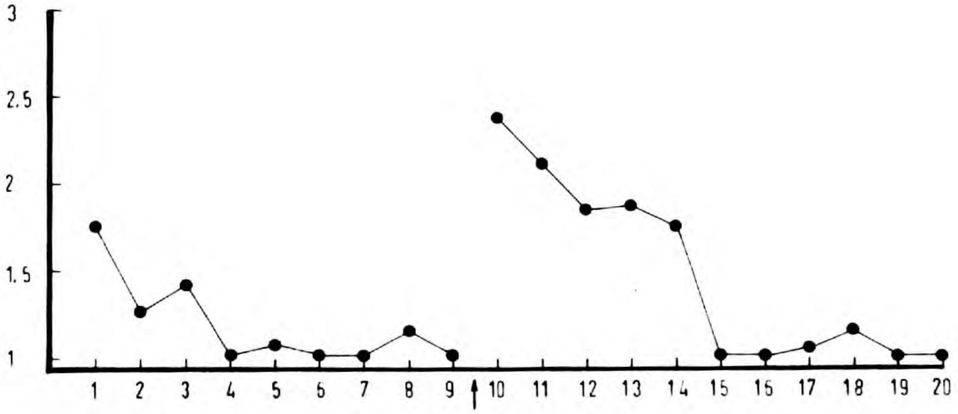


d

Fig. 22 c: Results for 4 animals trained to avoid light (CAS/UCAS interval 5 seconds). Arrow indicates removal of 78% of optic tectum.
Ordinate: percentage avoidance per day.
Abcissa: days' training (10 trials per day).

Fig. 22 d: Shock index results for the above animals.
Ordinate: shock index per day (shocks/escapes).
Abcissa: days' training (10 trials per day).

the present experiments. Thus it is possible to eliminate the optic tectum as a memory centre as far as these rather simple tasks are concerned. It remained to see what effect tectal removal had on learning and retention of the more complex tasks of avoidance-conditioning.

5. Overhead light avoidance.

These data are initially presented in a rather compressed form to give the overall picture, that tectal lesions <u>did</u> affect avoidance learning and retention. More detailed examination of the results showed that some additional useful information could be gained from consideration of individuals, or of smaller groups.

A. Lesions after training.

(a) Light/shock interval of 15 seconds.

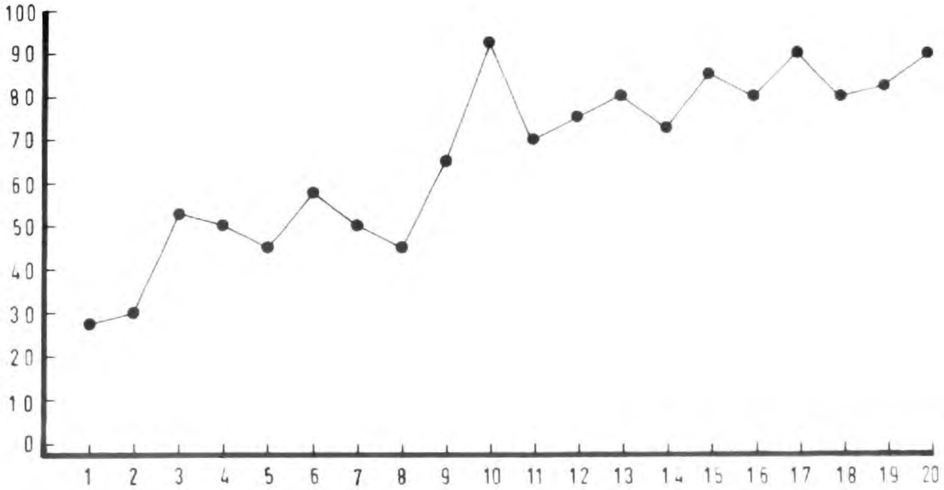
Fig. 22 a shows the results for seven animals trained for 90 trials before operation, and 110 trials (five animals) and 160 trials (two animals) after operation. Fig. 22 b shows the shock index for these animals.

The initial learning curve was very similar to that shown by normal animals, animals attaining up to 100% in some of the last trials before the operation. It can be seen that there was a deficit after operation, which slowly vanished over the next 100 trials. The shock index curve showed an increase after operation, which dropped to near unity over much the same period. A mean of 72.1% of tectum was removed from these animals, the extremes being 65.75 and 80%.

(b) Light/shock interval of 5 seconds.

Fig. 22 c shows results for four animals trained before operation, and it can be seen that there was a deficit in post-operative

/performance



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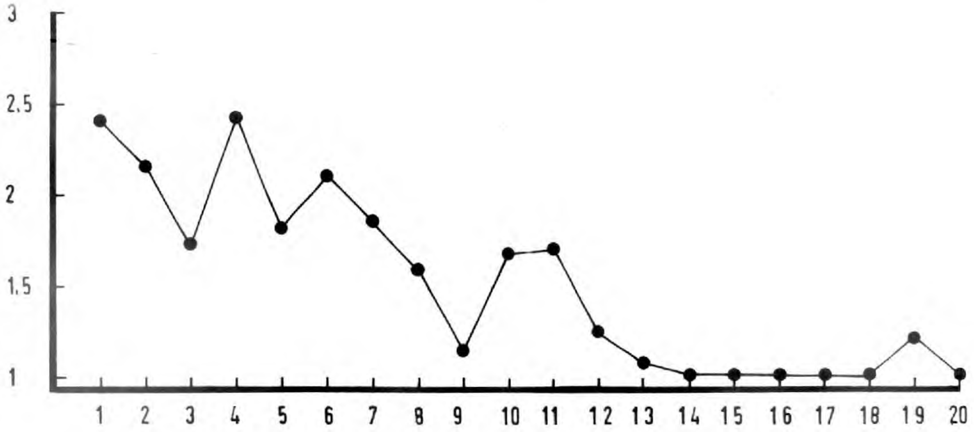
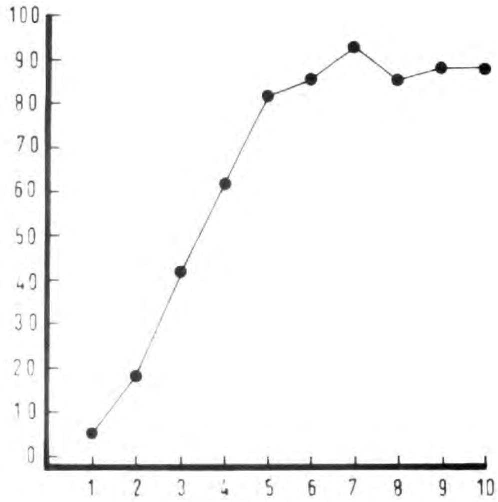


f

Fig. 22 e: Results for 4 animals trained after removal of 69% of optic tectum. Light avoidance (CAS/UCAS interval 15 seconds).
Ordinate: percentage avoidances per day.
Abcissa: days' training (10 trials per day).

Fig. 22 f: Shock index results for the above animals.
Ordinate: shock index per day (shocks/escapes).
Abcissa: days' training (10 trials per day).



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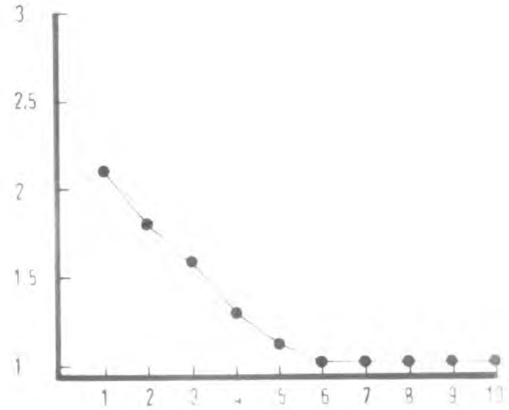


b

Fig.23 a: Results for 4 animals trained after removal of 75% of the optic tectum. Light avoidance, CAS/UCAS interval 5 seconds.

Ordinate: percentage avoidance per day.
Abcissa: days' training (10 trials per day).

Fig.23 b: Shock index results for the above animals.

Ordinate: shock index per day (shocks/escapes).
Abcissa: days' training (10 trials per day).

performance as compared with normals, and that the pre-operative level of performance was finally attained. Fig. 22d, giving the shock index, shows that there was an increase in this after operation, which fell over several days' trials. 78.4% of the tectum was removed from these fish. The lowest amount was 74.3%.

B. Lesions before training.

(a) Light/shock interval of 15 seconds.

Fig. 22 e shows results for four animals given 200 trials on this problem after operation. Fig. 22f shows the shock index for these animals. Comparison of these curves with those for normals shows that there was a great difference in both level of performance and index, the former being lower, the latter higher than those of normals. The average performance in both percentage and index attained the levels of normals only after about 100 trials. A mean of 69% of the tectum was removed - the lowest figure was 56.8%, the highest 72.1%.

(b) Light/shock interval of 5 seconds.

Four animals were used in this experiment, and Figs. 23 a and b show respectively the levels of performance and index for them. Although the rate of learning was not as obviously different from that of normals as that for the 15 second group, a statistical comparison shows that the difference in performance over the first thirty trials ($T = 10$, $P < 0.05$) was significant. A mean of 74.8% of the tectum was removed.

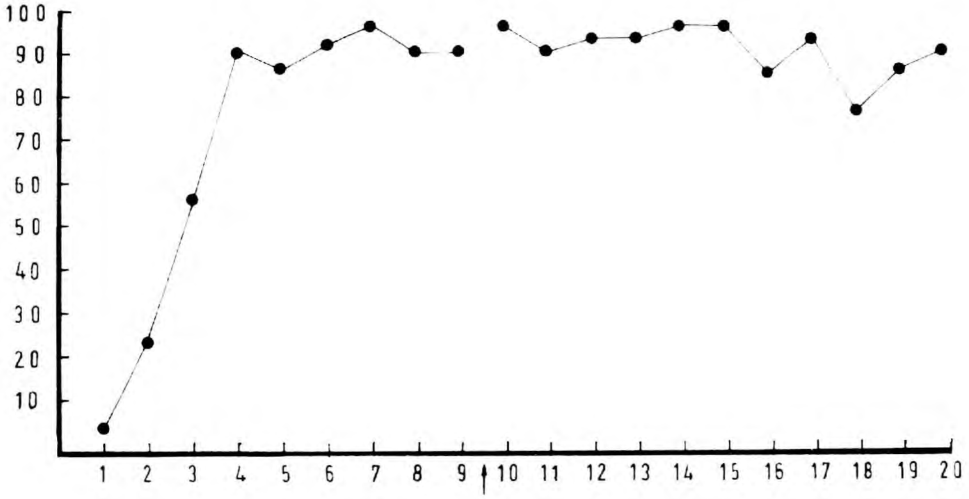
C. More detailed examination of results.

(a) Animals lesioned after training.

(i) 15 second group.

Three of these animals showed no deficit after operation, and

/Figs.



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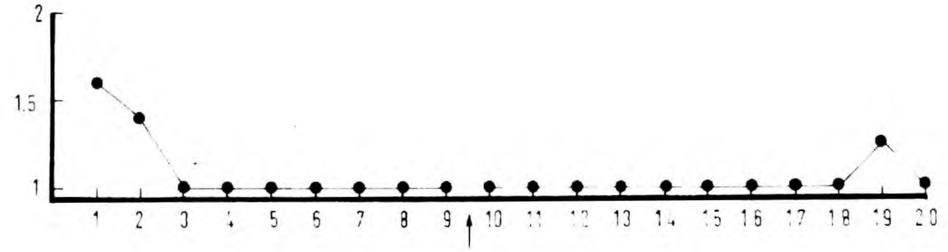
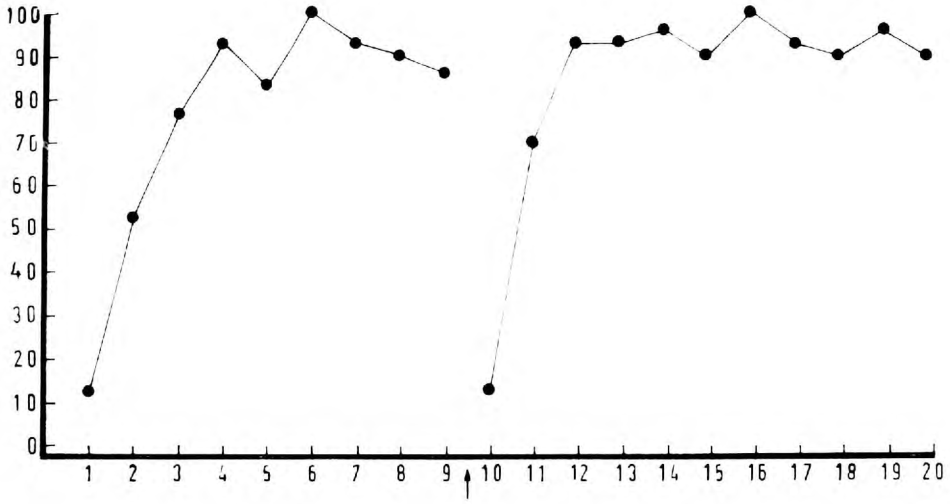


d

Fig. 23 c: Results for 3 animals, trained to avoid light. (CAS/UCAS interval 15 seconds.) 71% of optic tectum removed at point indicated by arrow.
Ordinate: percentage avoidance per day.
Abcissa: days' training (10 trials per day).

Fig. 23 d: Shock index results for these animals.
Ordinate: shock index per day (shocks/escapes).
Abcissa: days' training (10 trials per day).



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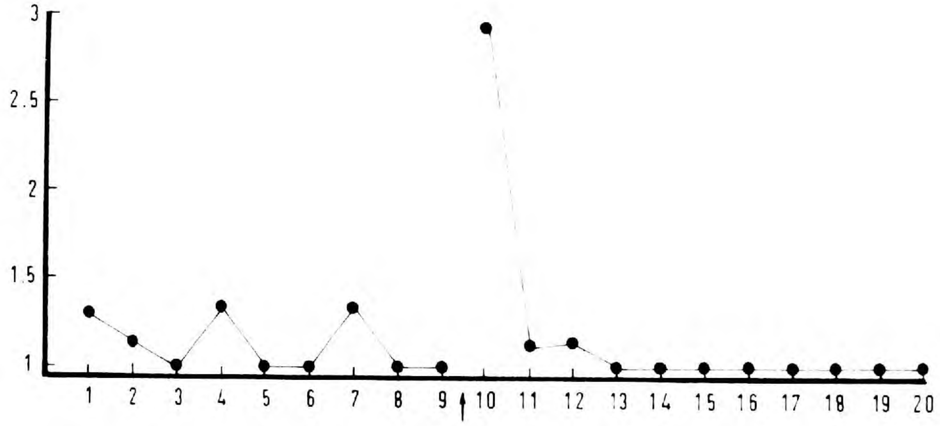
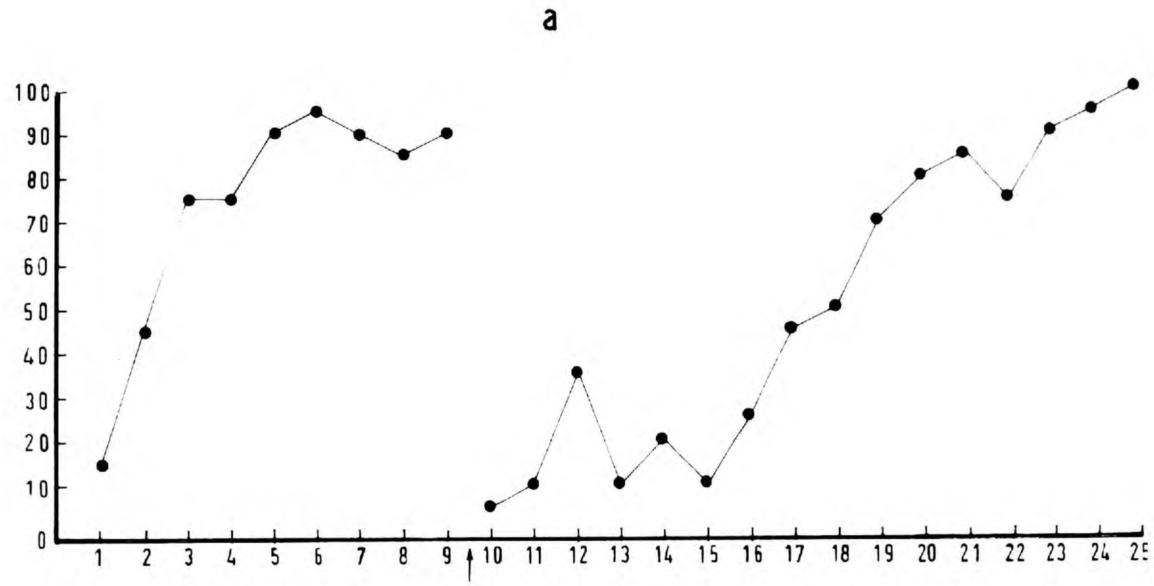


f

Fig. 23 e: Results for 2 animals trained to avoid light (CAS/UCAS interval 15 seconds). 73% of tectum removed at arrow.
Ordinate: percentage avoidance per day.
Abcissa: days' training (10 trials per day).

Fig. 23 f: Shock index results for the above animals.
Ordinate: shock index per day (shocks/escapes).
Abcissa: days' training (10 trials per day).



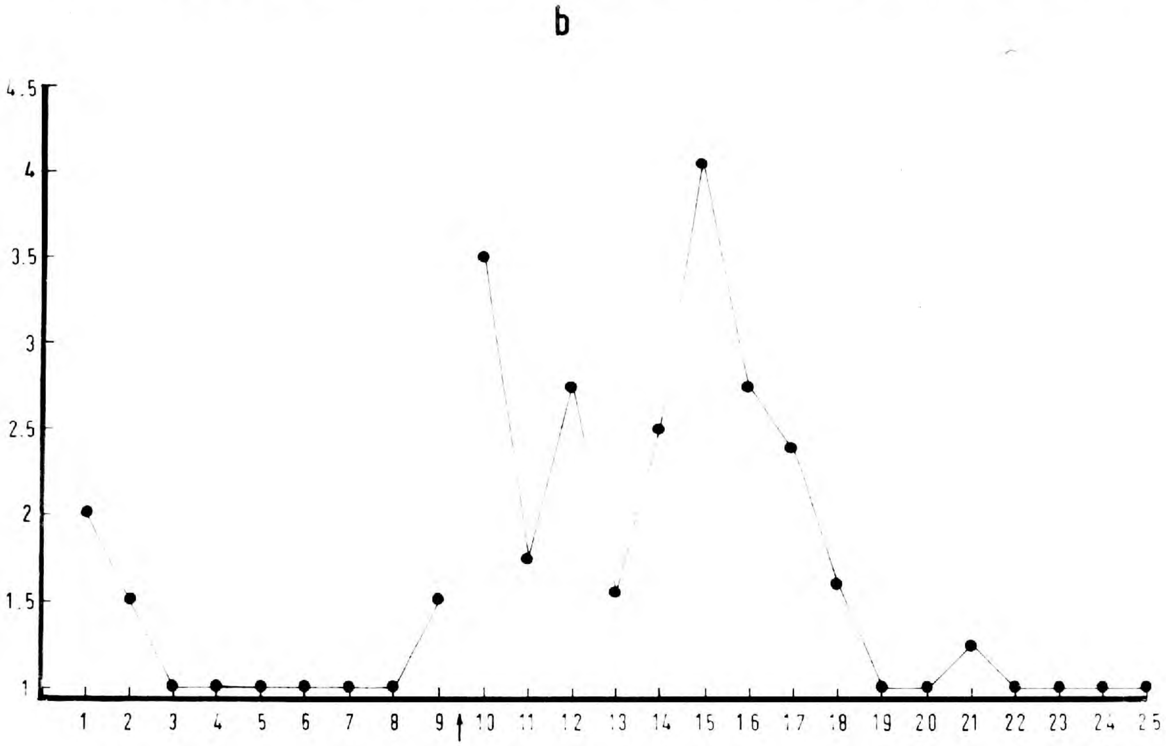


Fig. 24 a: Results for 2 animals, trained to avoid light (CAS/UCAS interval 15 seconds). 75% of tectum removed at arrow.
Ordinate: percentage avoidance per day.
Abcissa: days' training (10 trials per day).

Fig. 24 b: Shock index results for the above group.
Ordinate: shock index per day (shocks/escapes).
Abcissa: days' training (10 trials per day).

Figs. 23 c and d show the percentage avoidances and shock indices for these animals. It will be seen that there was no change in the level of the shock index after operation. 71.2% of the tectum was removed.

Two animals showed rather transitory deficits, which had, within the limits of variation, vanished after about 15 post-operative trials. It was noted at the time that as soon as the light came on in the first post-operative trials, both animals showed a considerable startle response, but seemed unable to "remember" what response to make. It was also noted that they experienced difficulty in performing the escape, and needed to receive several shocks before they would escape. This behaviour was much reduced on the next day's trials, and both avoidances and escapes were smoother, whilst the percentage of the former rose rapidly, so that after 20 trials, both animals were performing at about 80%. The increase in difficulty in achieving escapes can be seen from the shock index curve in Fig. 23 e. The index rose sharply after operation, but fell as rapidly. (Fig. 23 f shows the results for the percentage avoidance). (72.5% of the tectum was removed in these animals). These two animals were in fact the only two used from the first animals operated, the others being rejected on the basis of other damage, or death soon after the onset of post-operative training. In view of the activity shown after operation, and mentioned above, the photocell/Rustrak unit was connected to BI.

Results for two more animals are given in Fig. 24 a and b. There was a considerable long-lasting deficit, taking 100 trials to be removed; performance was thereafter normal. The increase in the shock index was huge, and its diminution to near unity took about 90 trials.

/Histology

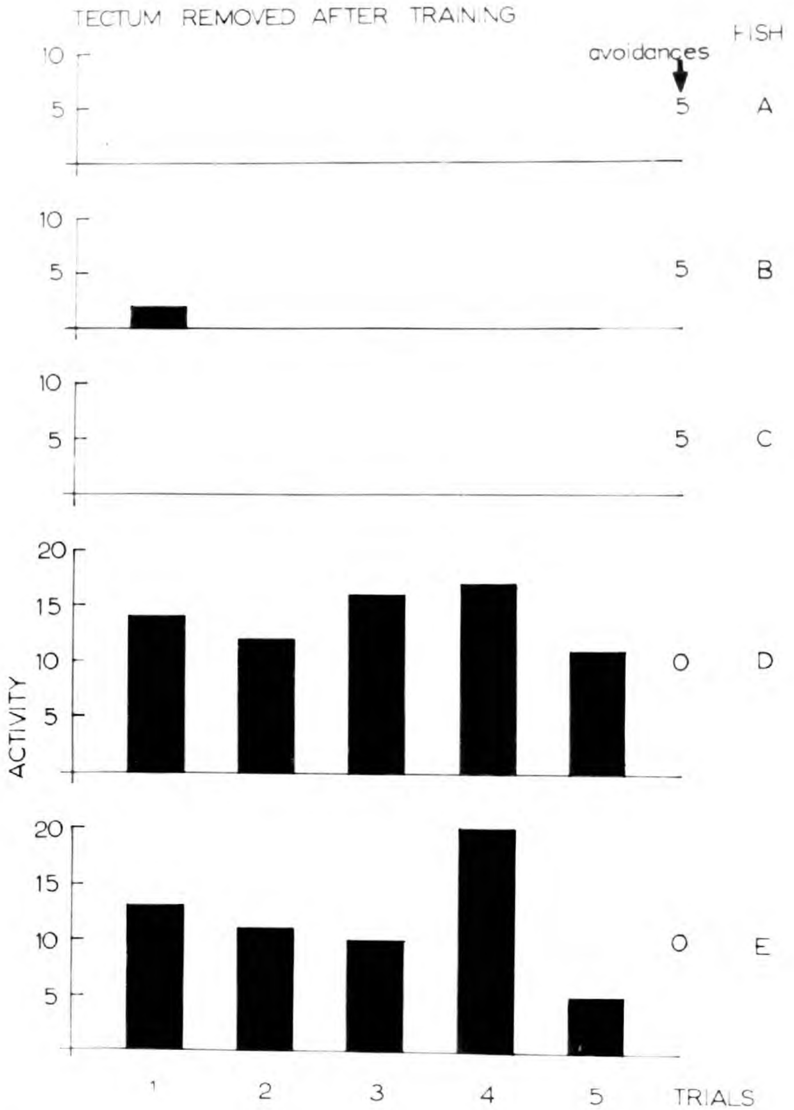


Fig. 24 e: Results for activity recording from 5 tectumless fish after operation. Trained pre-operatively to avoid light (CAS/UCAS interval 15 seconds).
Five trials, activity measured as number of pulses on recorder in the CAS/UCAS interval.

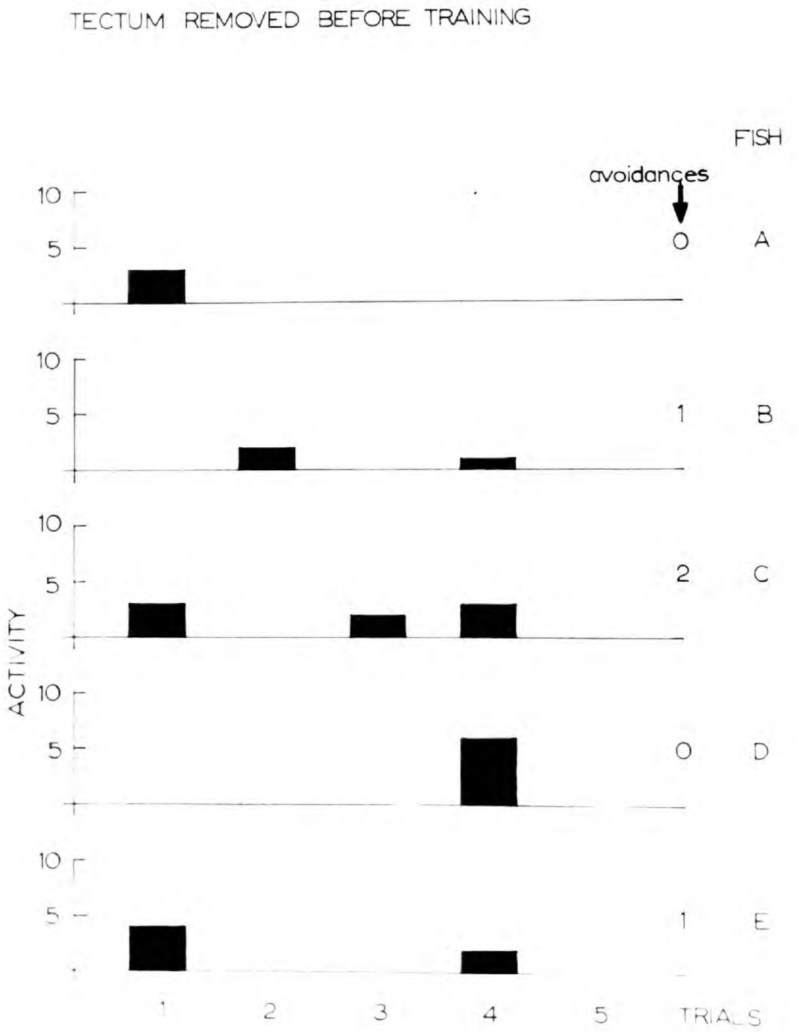


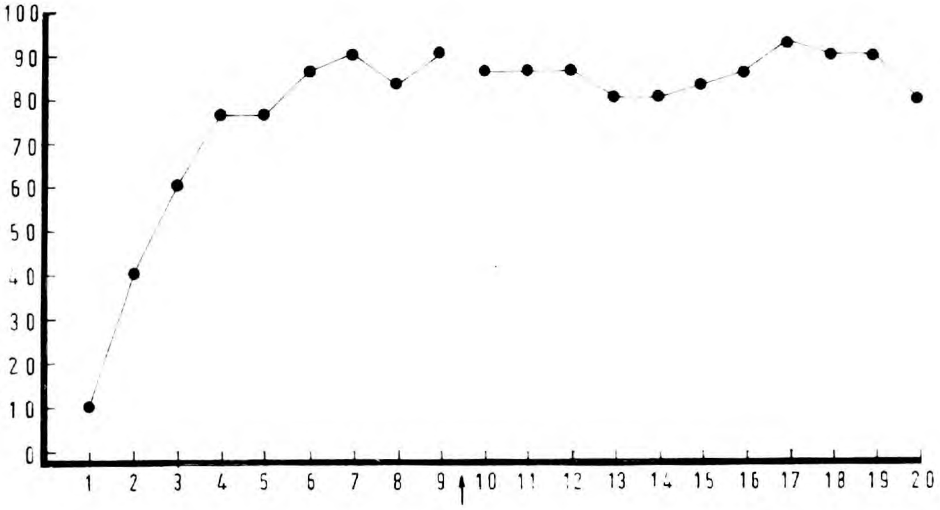
Fig. 24d: Results for activity recording from 5 naive tectumless fish on their first five trials.
(Light avoidance, CAS/UCAS interval 15 seconds).
Activity measured in CAS/UCAS interval.

Histology showed that 80 and 65.75% of the tectum had been removed, a mean of 72.9%.

It was again noted that the animals showed a startle response in the first post-operative trials, but in this case, this was measured quantitatively by the photocell circuit. Figs. 24 c and d show respectively the results for five animals trained pre-operatively, and five naive lesioned animals on their first five trials. Both groups of animals were allowed to avoid an escape, the recording was only in the CAS/UCAS interval.

The three animals showing total retention are those dealt with above; they avoided 5/5 times on the first trials, and there was not much activity in the start box. The two other animals in this figure are those mentioned immediately above; they showed no avoidances, but as can be seen from the activity histograms, they manifested considerable activity when the light came on. (It was impossible, on account of the lack of adequate dark recording apparatus, to show the change in absolute terms, but notes show that there was a very marked increase, and the activity records show that there was an interval of some seconds after the light came on before the start of the activity). The results for the five naive animals show that no such increase in activity occurred at the onset of the light in all those trials.

Figs. 24 a and b show that there was a correlation of the deficit in avoidance with the increase in the shock index, and that the increase in the level of avoidance occurred at approximately the same rate as the fall in the index. Animals became more able to perform the action of passing from start to goal boxes.



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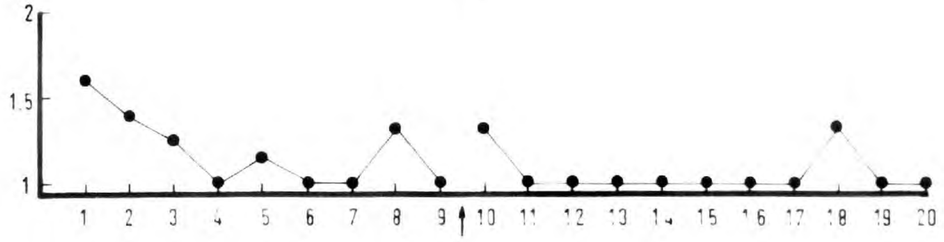
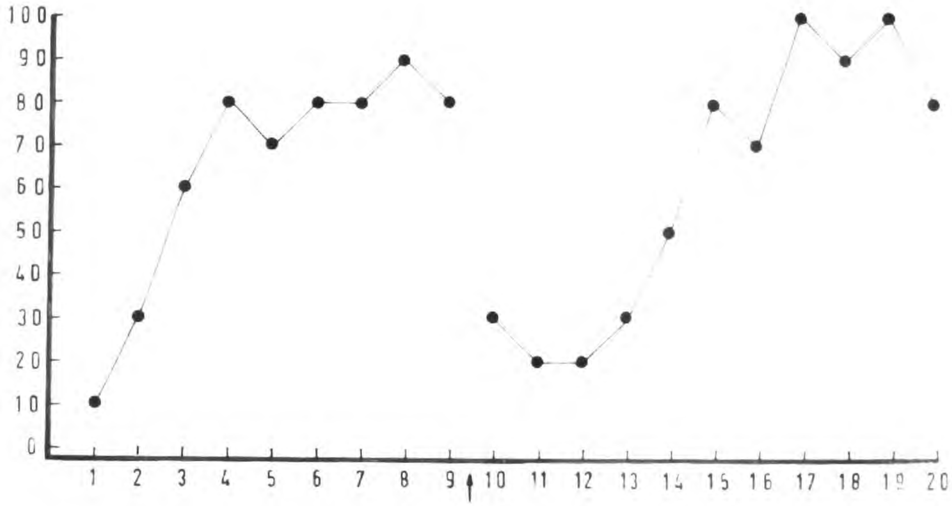


f

Fig. 24e: Results for 3 animals, trained to avoid light (CAS/UCAS interval 5 seconds). Arrow indicates removal of 79% of optic tectum.
Ordinate: percentage avoidance per day.
Abcissa: days' training (10 trials per day).

Fig. 24f: Shock index results for the above fish.
Ordinate: shock index per day (shocks/escapes).
Abcissa: days' training (10 trials per day).



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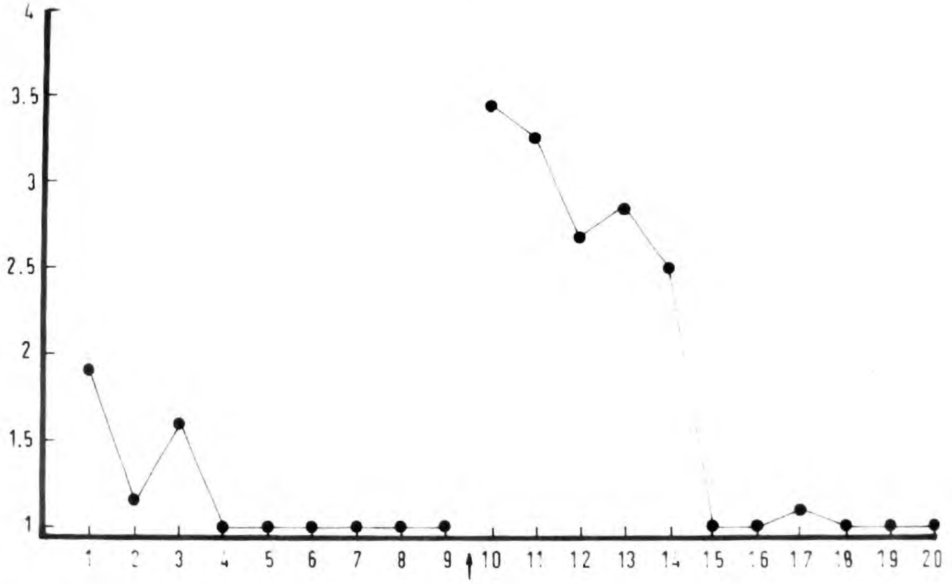

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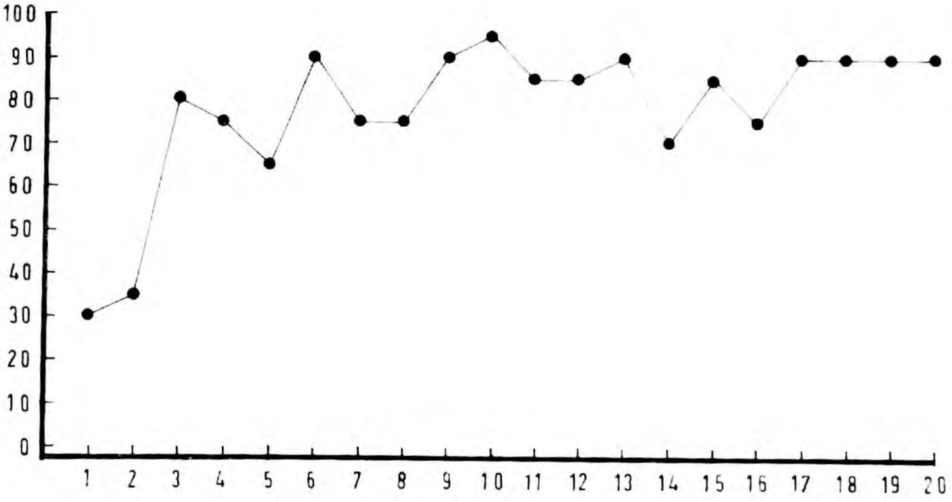
Fig. 24g: Results for 1 animal, trained to avoid light (CAS/UCAS interval 5 seconds). Arrow indicates removal of 77% of the tectum.
Ordinate: percentage avoidance per day.
Abcissa: days' training (10 trials per day).

Fig. 24h: Shock index results for the above animal.
Ordinate: shock index per day (shocks/escapes).
Abcissa: days' training (10 trials per day).



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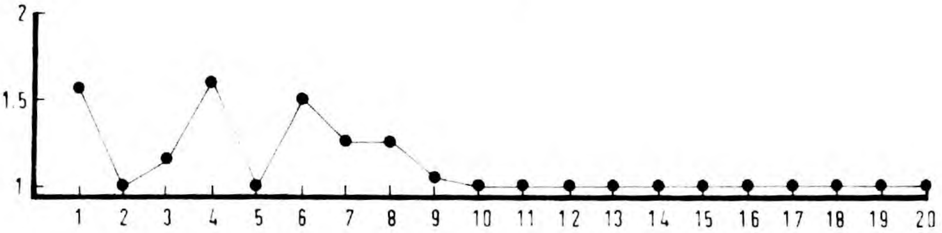
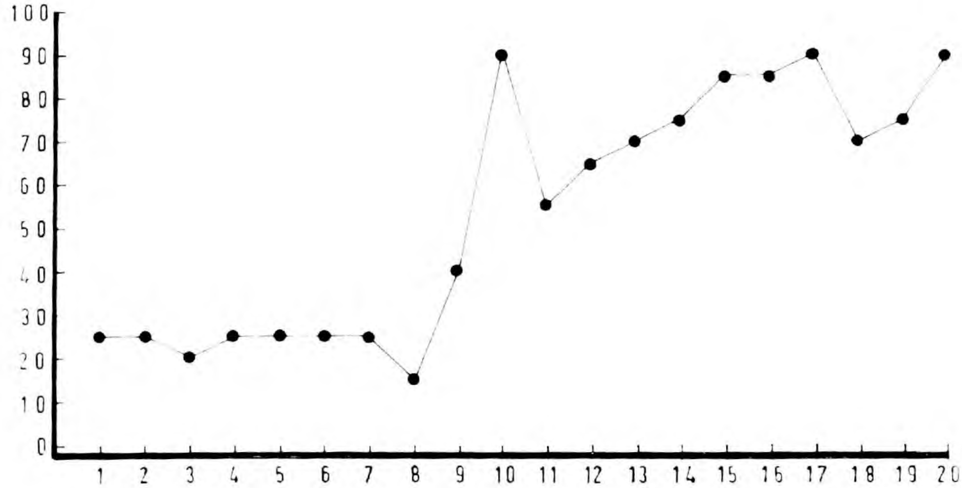


b

Fig. 25a: Results for 2 animals, trained to avoid light (CAS/UCAS interval of 15 seconds) after removal of 72% of the optic tectum.
Ordinate: percentage avoidance per day.
Abcissa: days' training (10 trials per day).

Fig. 25b: Shock index for above animals.
Ordinate: shock index per day (shocks/escapes).
Abcissa: days' training (10 trials per day).

c



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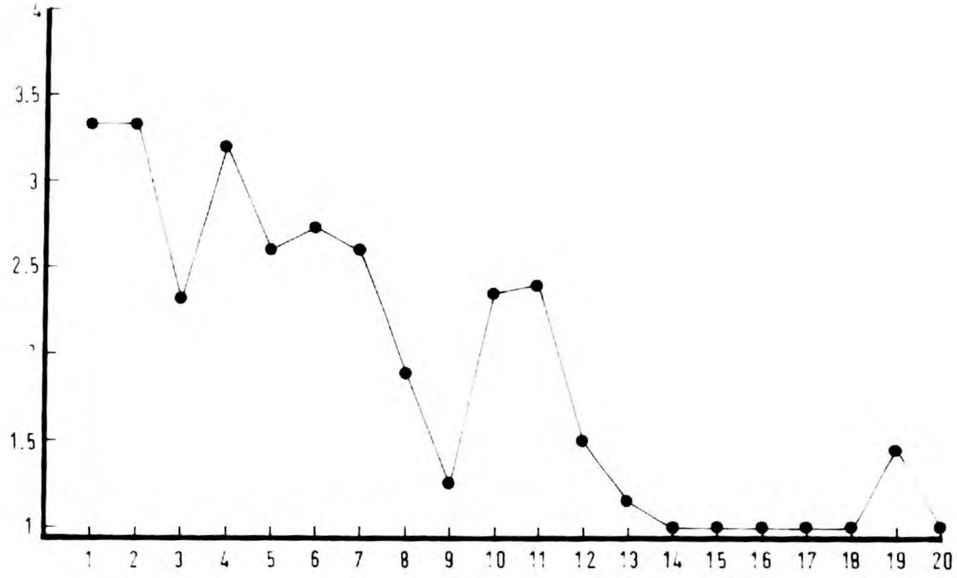


Fig. 25 c: Results for 2 animals trained to avoid light (CAS/UCAS interval 15 seconds) after removal of 66% of optic tectum.
Ordinate: percentage avoidance per day.
Abcissa: days' training (10 trials per day).

Fig. 25 d: Shock index results for the above animals.
Ordinate: shock index per day (shocks/escapes).
Abcissa: days' training (10 trials per day).

(ii) 5 second group.

Results for three of these animals are shown in Figs.24 e and f. There was total retention in these animals, and the level of the shock index was unaffected by the operation. 78.8% of the tectum had been removed. One animal, whose results are shown in Figs.24 g and h, showed a deficit, which was large at first, and gradually vanished.

Notes made at the time again show that there was an increase in the animal's activity when the light came on, and that it was uncoordinated in its actions, both before and after receiving shocks. Unfortunately, one of the valves of the amplifier had failed, and it was was not possible to obtain a replacement in time. The shock index shows this increase in time for escape did occur, and that the decrease of this time could be correlated with increase in percentage avoidance. 77.1% of the tectum had been removed from this animal.

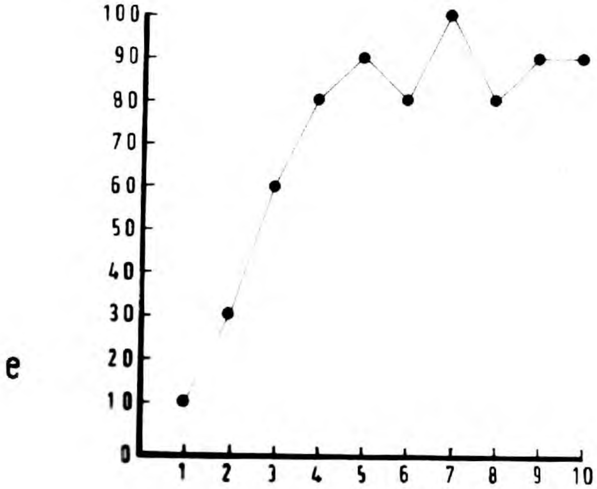
(b) Animals lesioned before training.

(i) 15 second group.

Two animals showed an approximately normal learning, and their percentage avoidance curves, whilst not very similar to those of normals (for example, the level attained on the first day was much higher than usual) attained equal levels within thirty trials. The shock index was low. These results are shown in Figs.25 a and b. (72.2% of optic tectum had been removed).

Two other animals showed poor learning over quite a long period (about 90 trials) then showed a rapid increase in performance at the 10th day, which persisted for the rest of the training. Figs.25 c and d show this and the shock index curve.

/The shock



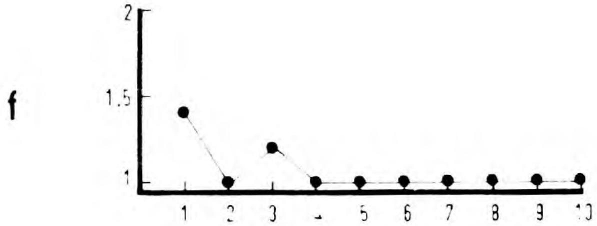
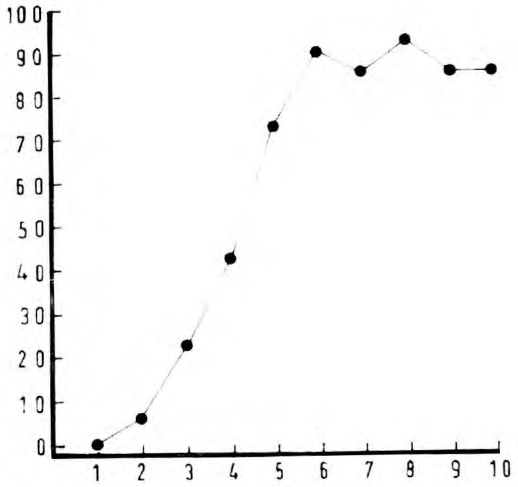


Fig. 25 e: Results for 1 animal trained to avoid light (CAS/UCAS interval of 5 seconds) after removal of 86% of the optic tectum.
Ordinate: percentage avoidance per day.
Abcissa: days' training (10 trials per day).

Fig. 25 f: Shock index results for this animal.
Ordinate: shock index per day (Shocks/escapes).
Abcissa: days' training (10 trials per day).

g



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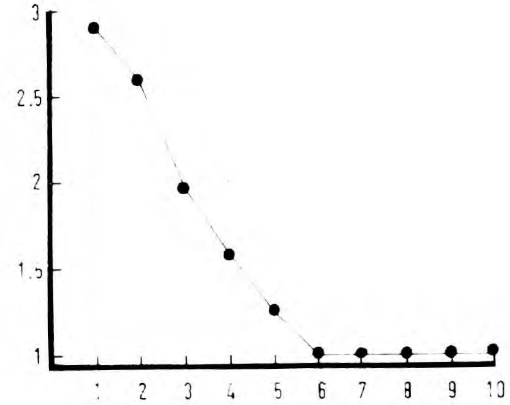


Fig. 25 g: Results for 3 animals with 71% of the optic tectum removed, trained to avoid light (CAS/UCAS interval 5 seconds).

Ordinate: percentage avoidance per day.
Abcissa: days' training (10 trials per day).

Fig. 25 h: Shock index results for the above group.

Ordinate: shock index per day (shocks/trials).
Abcissa: days' training (10 trials per day).

The shock index was initially high, at 3.35, and fell off but slowly over about one hundred trials, attaining an approximately normal level at the 120th trial. It was noted that a sharp response to the light developed within about twenty trials, but that animals rarely managed to co-ordinate their reaction in a sufficiently swift manner to bring about an avoidance, or even a rapid escape. (65.9% of the tectum had been removed).

(ii) 5 second group.

One of these animals with 86.4% of its tectum removed performed normally, and its avoidance and index curves are shown in Fig. 25e and f. Three other animals with a mean of 71% of the tectum removed, showed a deficit which was significantly different to normals, over the first 30 trials; the index was also unusually elevated during this period (Figs. 25 g and h). After the early trials, the level of both curves was approximately normal.

Summary of effects of tectal lesions on light avoidance learning and retention.

(a) Retention.

Although Sears' (1934) work suggested that, for simple paradigms such as classical conditioning the removal of the optic tectum did not affect retention, the work of Sanders (1940) and Botsch (1960) has led to the opinion that the tectum is involved in "higher learning". In view of the criticisms made of the interpretations of the post-operative deficits found by these workers, it seemed likely that experimental situations which did not require great visual acuity might allow discrimination between deficits due to lack of ability to discriminate

/the stimulus

the stimulus and surroundings, and lack of memory.

The average curves for both 5 and 15 second groups showed drops in level of avoidance after operation, but these were not comparable with the almost total deficits found by Sanders and Botsch. Furthermore, it seemed that there was a close correlation between the deficit and the ability to avoid, since comparison of average avoidance and shock index curves for both 15 and 5 second groups indicated that the former rose as the latter fell.

Consideration of individual fish, or of fish with similar results, confirmed this. Perhaps the most obvious example was that shown in Figs. 24 a, b and c. There was a high level of avoidance pre-operatively, but on the basis of avoidance alone, there would have seemed to be a total deficit. The activity records showed that there was a large conditioned response, and the shock index curve indicated that animals had considerable difficulty in escaping. Thus it was possible to attribute the deficit to motor failures, not to loss of retention. This interpretation held good in the other examples. Animals showing no post-operative avoidance deficits showed no rise in shock index, and their performance curves were like those of the control animals. Fish showing deficits in avoidance showed high shock indices, which latter decreased as the former increased. These last animals generally showed some startle reaction to the light, which was not seen in naive lesioned fish.

In view of these facts, and also of the results of the group of control fish shown in Fig.14 c (fish trained, then blinded, showed a retention of the avoidance, mediated via the diencephalon) it is

/probable

probable that the light stimulus could reach the brain, via receptos in the diencephalon, the pre-tectal and lateral geniculate nuclei of the optic tract, and via the remnants of the tectum. The results for simple conditioning support the conclusion that the light stimulus so received would be adequate to serve to activate the memory, so deficit is unlikely to be due to loss of ability to perceive brightness changes.

Results for pre-operatively trained fish on the simple conditioned reflex also suggested that in avoidance conditioning also, following Bitterman's (light/shock)/(response) analysis, the preliminary part of the avoidance conditioning, the light/shock association, should also be unimpaired. The activity shown by lesioned fish showing post-operative deficits supports this. It has been seen that these animals react as well to shock after as before operation, so it may be concluded that motor factors alone were involved. The shock index results support and confirm this.

(b) Learning.

Although the work cited above showed that the tectum was not the seat of retention, it seemed likely that on account of its visual, visuo-motor and general correlative functions outlined above, that it might well be responsible for the formation of more complex memories, even though these once formed, might be stored at a lower level. The results given here do not support that hypothesis; they suggest, as did those for simple conditioned responses, that learning can occur in the absence of the tectum, but that there may be some difficulty in implementing the response, on account of motor disturbances.

Once again, there was a close correlation between avoidance and

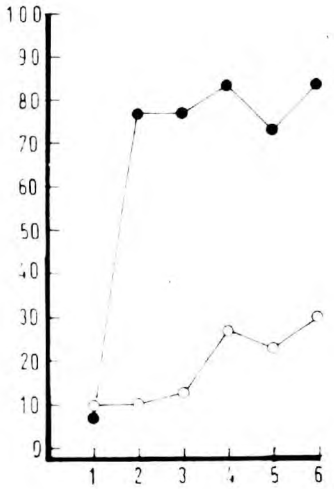


Fig. 26 a: Results for 5 animals trained to avoid light (CAS/UCAS interval 15 seconds), then operated with removal of optic tectum (79%). Re-trained after operation, and all attained $70\%^{+}$ correct avoidances for some days.

Open circles: 3 fish transferred to extinction (i.e. no reinforcement for response or lack of response).

Closed circles: 2 fish transferred to reversal (i.e. shocked in goal box if made avoidances).

Ordinate: percentage failures to avoid per day.
Abcissa: days' training (10 trials per day).

shock index curves. For example, the animals shown in Figs.25 c and d showed a poor level of performance over 80 trials, then a rise in avoidances. Their shock index curve showed the inverse, a high level of shocks (3^+) over the first 70 trials, dropping thereafter, somewhat erratically, but generally falling as the curve for avoidance rose. In contrast to this, animals shown in Figs.25 a and b showed a rapid learning, which correlated with a low shock index. The same phenomena were seen in both 15 and 5 second groups.

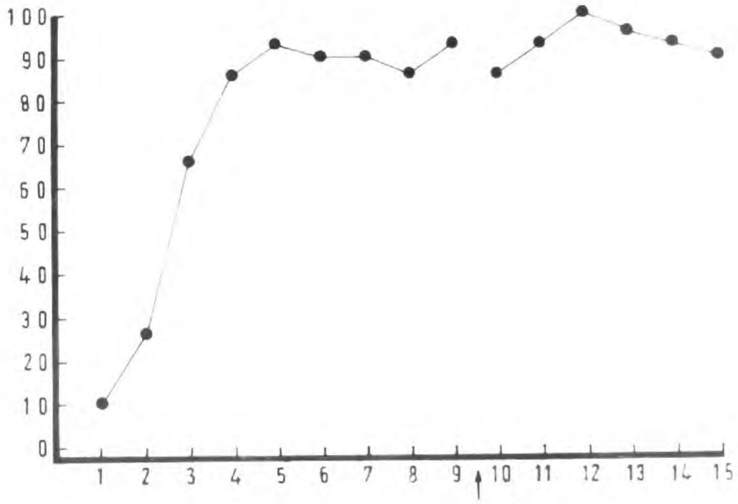
D. Reversal and extinction.

Five animals were trained on the 15 second light avoidance, then lesioned. When their performances had remained within the range 70-100% for some days, they were transferred, three to reversal, two to extinction training. The curves for these are shown in Fig.26 a.

The animals on extinction (open circles) showed a slower fall-off of the response than normals, but the two fish given reversal training (closed circles) showed a very rapid learning which was much faster than the control group (Fig.14b). The reversal animals would at first swim rapidly through the door as soon as the light came on, but after a time they showed a startle response, then using their pectoral fins, backed away from the door as rapidly as possible, and remained pushed against the far wall of the box for the rest of the trial. When the light was extinguished, they would swim forwards, and remain fairly quiet until the start of the next trial. 71.9% of the tectum had been removed in these fish.

Whilst the continued responses shown by these animals in extinction trials were to some account attributable to their increased excitability,

/which



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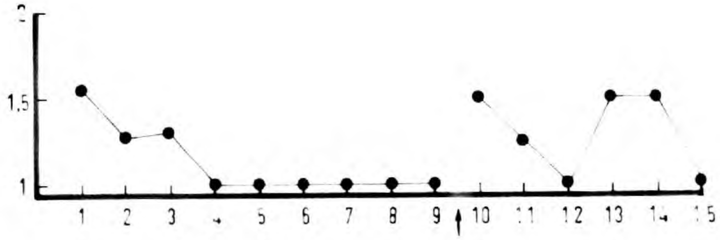
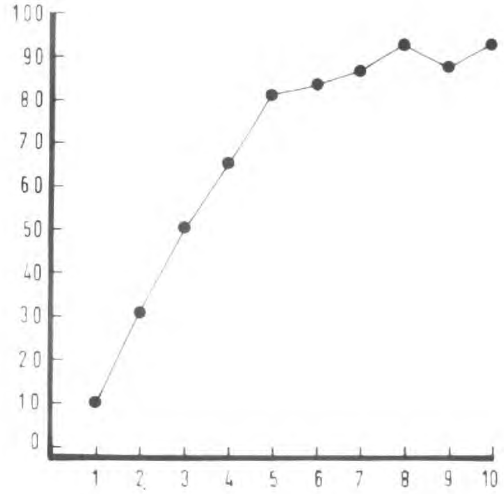


c

Fig. 26 b: Results for 3 animals trained to avoid sound (CAS/UCAS interval 5 seconds). Optic tectum removed (67%) at point indicated by arrow.
Ordinate: percentage avoidance per day.
Abcissa: days' training (10 trials per day).

Fig. 26 c: Shock index results for above group.
Ordinate: shock index per day (shocks/escapes).
Abcissa: days' training (10 trials per day).

d



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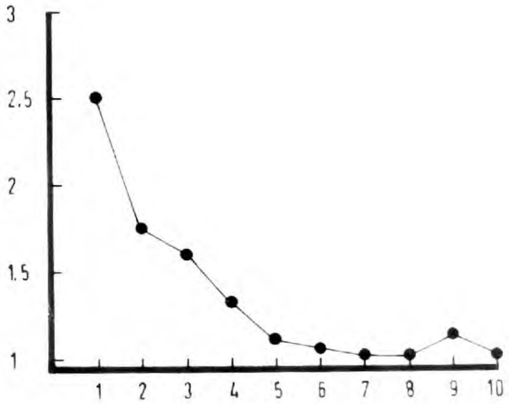


Fig. 26 d: Results for 5 animals with 78% of optic tectum removed, trained to avoid sound (CAS/UCAS interval 5 seconds).
Ordinate: percentage avoidance per day.
Abcissa: days' training (10 trials per day).

Fig. 26 e: Shock index results for above group.
Ordinate: shock index per day (shocks/escapes).
Abcissa: days' training (10 trials per day).

which has already been shown, it is difficult to explain the rapid reversal on this basis, since animals still maintained a high degree of activity, even when failing to avoid. There are no observations comparable to these as regards reversal, but Sears (1934) observed that in tectumless goldfish there was a slightly greater rate of extinction of a conditioned response. A crude hypothesis to explain these results may be that they are explicable on the basis of the lack of acuity caused by tectal lesions.

In the absence of ability to perceive more complex visual stimuli, animals may pay more attention to simple visual stimuli, since the visual "interruptions" of much of the environment are lacking. Thus switching "in" or "out" of the appropriate response may tend to become more "all or none", since the animals' ability to attend to the visual environment is so limited.

6. Sound avoidance.

A. Lesions after training.

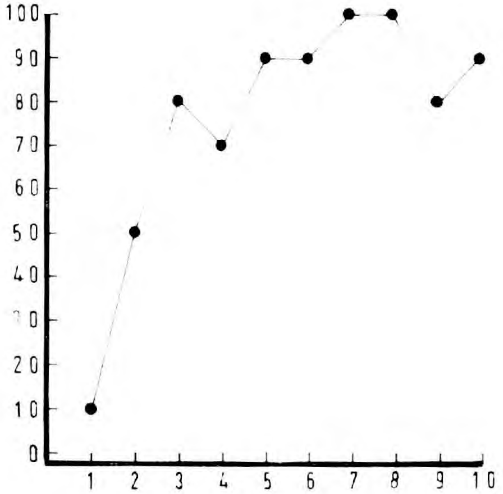
Fig. 26 b shows results for three animals trained before lesions, and given 50 trials after operation. The shock index is shown in Fig. 26 c. There was no deficit in these animals after operation, and the shock curves showed no change. 67.4% of the tectum was removed.

B. Lesions before training.

Figs. 26 d and e show the avoidance and shock index curves for five animals over 100 trials. The rate of acquisition was initially significantly lower than normals, but the percentage success soon reached a level comparable to theirs, and remained steady over 60 trials. The index was initially high and fell off over the same sort of period.

/78.3%

a



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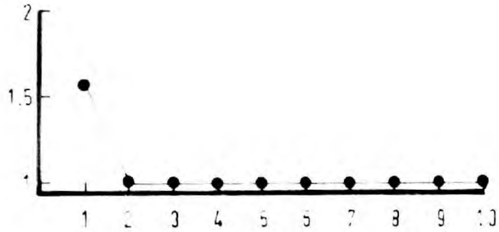
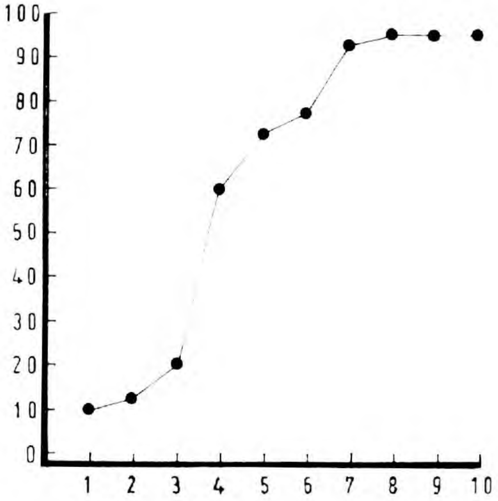


Fig. 27 a: Results for one animal with 84% of its optic tectum removed, trained to avoid sound (CAS/UCAS interval 5 seconds).
Ordinate: percentage avoidance per day.
Abcissa: days' training (10 trials per day).

Fig. 27 b: Shock index results for this animal.
Ordinate: shock index per day (shocks/escapes).
Abcissa: days' training (10 trials per day).



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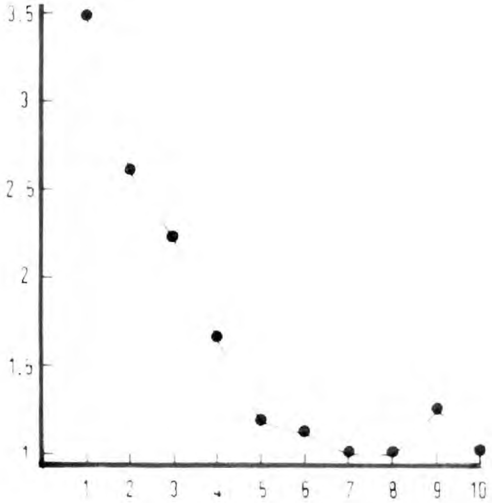
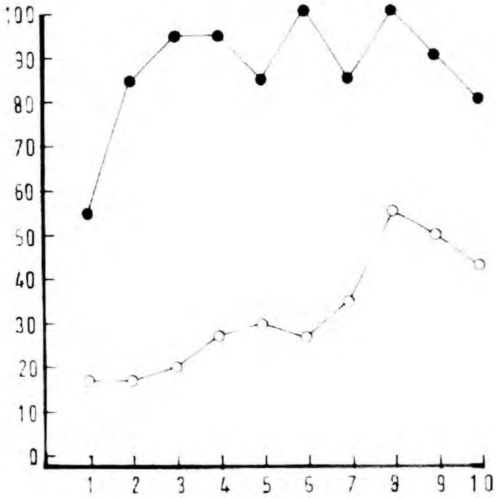


d

Fig. 27 c: Results for 4 animals with 77% of the optic tectum removed, trained to avoid sound (CAS/UCAS interval 5 seconds).
Ordinate: percentage avoidance per day.
Abcissa: days' training (10 trials per day).

Fig. 27 d: Shock index results for these animals.
Ordinate: shock index per day (shocks/escapes).
Abcissa: days' training (10 trials per day).

e



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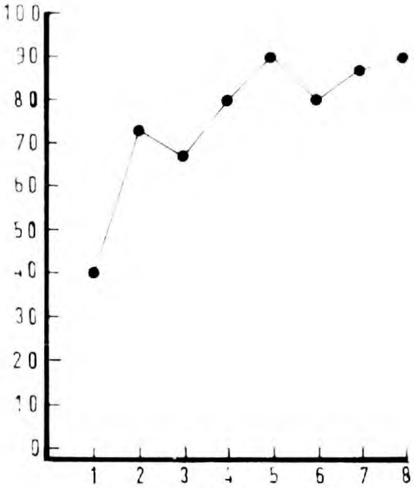


Fig. 27 e: Results for 5 animals trained to 70%[+] avoidance of sound after removal of 78% of optic tectum (CAS/UCAS).
Closed circles: Reversal (2 animals). (i.e. shocked if avoided).
Open circles: Extinction (3 animals). (i.e. no reinforcement at all).
Ordinate: percentage avoidance per day.
Abcissa: days' training (10 trials per day).

Fig. 27f: Results for 3 animals trained, then lesioned. (See Figs. 26b & c). Reversal (i.e. shocked if avoided).
Ordinate: percentage avoidance per day.
Abcissa: days' training (10 trials per day).

78.3% of the tectum had been removed.

C. More detailed examination of these results.

(i) Lesions after training.

There were no significant differences between the animals trained on this problem, either in avoidance or index.

(ii) Lesions before training.

One animal learned in the normal fashion, as can be seen from Figs.27 a and b. It was found that 83.6% of its tectum had been removed. Four animals, however, showed significant deficits, and the shock index over the first thirty trials was high (Figs.27 c and d). 77% of the tectum of these animals had been removed.

D. Reversal and extinction.

Fig.27 e shows reversal and extinction for five animals lesioned before training, and transferred to these paradigms after attaining 70-100% avoidance. Two animals were reversed (closed circles), three extinguished (open circles). As in the case of the light-trained animals, the extinction animals performed worse than the controls, the reversal animals better. A mean of 78.3% of optic tectum was removed from these fish.

Fig.27 f shows results for three animals lesioned after training, and transferred to the problem after performing at a high level of avoidance for some days. The rate of reversal was very high, and the same behavioural phenomena were observed as noted for the light animals. 67.4% of the tectum was removed in these animals.

Summary of effects of tectal lesions on sound avoidance learning and retention.

/(a) Retention.

(a) Retention.

Since the optic tectum undoubtedly receives much of the visual input, it was of interest to see whether the effects of tectal removal were also seen if the stimulus for avoidance were of such a modality that it did not project to that area. Since Kappers, Huber and Crosby (1936) reviewed evidence and concluded that there was no evidence for auditory projection to the tectum, and Dijkgraaf (1949a) trained minnows to show a feeding response to a 1650 cps. note, which response was not abolished by tectal removal, sound seemed a worthwhile stimulus.

It can be seen from the results outlined above that the avoidance was not affected after operation. This supports earlier findings to the effect that unless motor difficulties were seen, there would be no retention deficit.

(b) Learning.

Results for these fish parallel those for the light trained fish - there were deficits in acquisition which correlated with inability to escape with any ease. The results are of further interest in correcting a suggestion brought to mind by the sound association retention results, that the motor deficit might be peculiar to the stimulus modality. The results for the control group of animals with eyes covered suggest that since the learning and shock index curves were normal (Figs.15 c and d) the deficit seen in tectal animals was due to purely motor defects, rather than to lack of visuo-motor co-ordination. These control animals had to learn the avoidance without such cues, and had little difficulty in making avoidances. Therefore, as in the case of the light-trained fish blinded after training (Fig.14 c) visual deficits due to

/tectal

Table 7a.

Activity of 2 fish before and after bilateral removal of the forebrain, → indicates the position of the operation.

DAY	ACTIVITY	DAY	ACTIVITY
1	724	15	36
2	576	16	26
3	152	17	6
4	194	18	6
5	706	19	0
6	398	20	4
7	676	21	0
8	582	22	0
9	412	23	0
10	370	24	0
11	436	25	0
12	290	26	0
13	944	27	0
14 →	258	28	0
Total	6718		78
%	100		0.86

Mean % of forebrain removed : 89.25%

tectal lesions would not seem to be the main cause of failure in these situations, though, of course, they would preclude the performance of any task requiring accurate vision.

C. Effects of bilateral removal of the forebrain.

1. General effects.

In one or two cases in the earliest removals, there was excessive damage to ventral blood vessels, and the loss of blood, combined with the pressure of the resultant clots over the surface of the brain, made it necessary to kill the animals. The practice of leaving a small amount of the medial basal area of the forebrain intact (see below) seemed to prevent this excessive bleeding, and survivals rose to nearly 100%. (The only deaths in operated fish occurred from the bleeding, early on, or later from fin rot. This disease may have attacked the fish because of the weakened condition due to operation; it was increasingly rare.)

Considering the size of the area removed, approximately 20% of the brain, excluding spinal cord, it was surprising how little the behaviour of lesioned animals differed from that of normals. On recovery from anaesthetic, lesioned fish would swim normally, and could not be differentiated from operated controls on the basis either of movement or orientation. When they were fed on the day after operation, they swam rapidly towards the pieces as they fell, and devoured them in the usual fashion. Forebrainless fish would show fairly normal "startle" reactions when the walls of their tank were banged, and would swim rapidly to avoid being caught in the net.

2. Activity changes.

Table 7a shows the activity of 2 animals, measured for 10 minutes each day for 14 days before and after total forebrain removal. The

/change

Table 8 a.

Activity produced by shocks in five forebrainless fish. (10 shocks per day; amount of activity expressed as a fraction of activity on the first day. Mean amount of forebrain removed : 86.9%).

DAY	ACTIVITY
1	1.0
2	3.3
3	4.0
4	5.5
5	6.4
6	3.4
7	8.4
8	5.3
9	6.4
10	4.1
11	7.5
12	7.4
13	5.2
14	4.5
15	3.9

Total activity (in number of pulses) : 7586

Equivalent figure for five normal fish:6960

Difference between individuals of each group - Mann Whitney U-test $T = 21$, $n = 10$, $P > 0.05$.

Table 8 b.

Conditioned reflex training in three forebrainless fish. (CS/UCS interval 5 sec., mean amount of forebrain removed 83.6%).

DAY	MEAN RESPONSE
1	0
2	7.3
3	0
4	5.0
5	10.3
6	0.7
7	33.3
8	27.3
9	16.67
10	54.0

Table 8 c.

Conditioned reflex training in five fish before and after removal of the forebrain. (CS/UCS interval 5 sec., mean amount of forebrain removed: 90%. Arrow indicates time of operation).

DAY	MEAN RESPONSE	DAY	MEAN RESPONSE
1	4.4	11	6.4
2	9.8	12	4.4
3	7.2	13	21.0
4	11.4	14	1.6
5	15.0	15	19.0
6	17.4	16	35.6
7	22.0	17	30.4
8	19.8	18	6.8
9	29.4	19	5.2
10	27.6	20	9.6
→			
	Mean 16.4		Mean 14.0

change in activity was remarkable, animals showed little sign of activity after the operation even if they had been quite mobile before. They would lie at the base of the box, and the only observable signs of life would be the slow movements of the gill covers, and occasional rather slow sweeps of the caudal fin. These contrasted with the rapid and highly excited movements of normal or operated control fish, even after some days' experience in the box. (Compare with results for normal fish, shown in Table 3a).

3. Reactivity.

Several stimuli, gentle banging of the side of the tank containing the box, a half second use of the buzzer, light flashes, had no effect on these fish; there was no increase in the activity as measured by the photocell, and no change in behaviour was seen by the observer; the animals remained still at the base of the training box.

Table 8 a shows results for 5 animals used to test the sensitivity to shock of these forebrainless fish. It can be seen that there was little difference in the overall intensity of response between operated and normal fish, and that, as in normals, the level of response rose rather than dropped over the 15 day test period. The contrast between the extremely quiet period before the shock and the period after it was much more marked than in normals. In no case were any abnormal reactions to shock developed.

4. Formation of simple CR's.

Table 8 b shows results for 3 naive forebrainless animals trained as outlined in the methods section.

Table 8 c gives the results for 5 animals trained on the problem,

/then

Table 8 d.

EXTENT OF LESIONS

GROUP LESIONS BEFORE TRAINING	MEAN % LESIONS	GROUP LESIONS AFTER TRAINING	MEAN % LESIONS
15 sec. light	86.75	15 sec. light	84.75
5 sec. light	94.50	5 sec. light	94.60
5 sec. sound	91.50	5 sec. sound	89.75
small light	94.80	small light	88.51
| vs —	91.05	| vs —	88.87

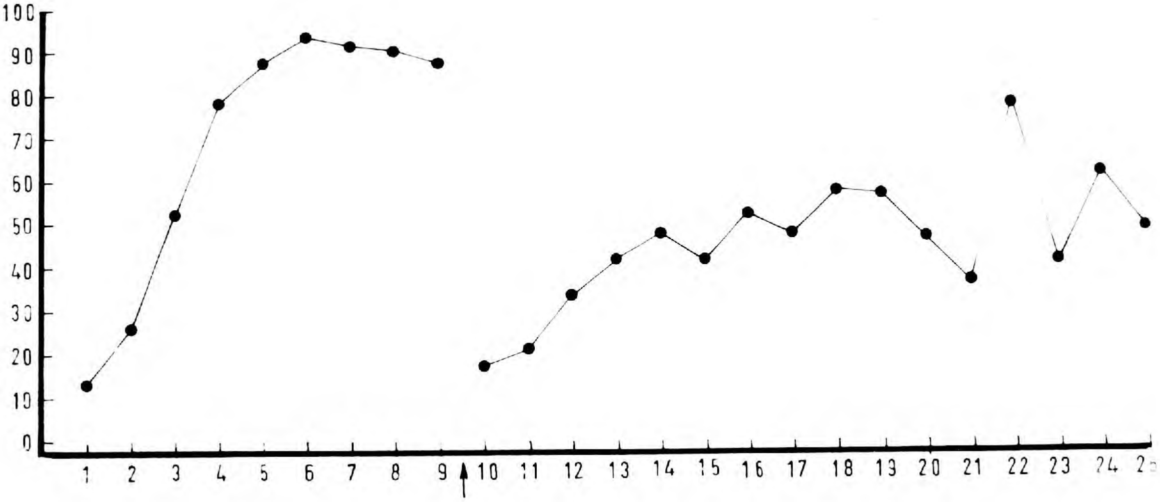


Fig. 28 a: Results for 8 animals, trained to avoid light (CAS/UCAS interval 15 seconds). Arrow indicates bilateral removal of forebrain (mean 85% removed).

Ordinate: percentage avoidance per day.
Abcissa: days' training (10 trials per day).

then lesioned. The mean of CR activity shown after operation was lower than that seen before the lesion. Two animals showed high CR on the first trial (unreinforced at the usual CS/UCS time) immediately after the recovery period, but showed no response at all ten trials later, after nine shocks to reinforce the learning. The control animals (see Table 4 a) showed the opposite phenomenon, more usual, of little response before the trials, and a fairly high one at the end of the session.

5. Overhead light avoidance.

A. Lesions after training. (Table 8 d gives details of extents of lesions to fish in the next sections).

(a) Light/shock interval of 15 seconds.

Eight animals were trained on this problem, and achieved a pre-operative level of avoidance of from 70-100%. Results are shown in Fig. 28 a. They were operated at the point indicated by the arrow. After the operation, all these fish showed a considerable deficit, some performing at 0%, others at 40-50%. The level of overall performance rose throughout the post-operative training, but individuals were very variable, performing quite well one day, and badly the next, whilst others remained unable to attain more than 30-50% correct trials per day. (Results for comparison of 50 post-operative trials for control and lesioned fish $T = 45$, $n = 15$, $P < 0.01$).

The post-operative behaviour of these animals was remarkable; previous to operation, the onset of the light had elicited a slight "startle" response, followed by a smooth and generally rapid avoidance. Operated animals, on the other hand, would generally sit still at the base of the box, even, in some cases, with their snouts in the doorway to the goal box, and would not show the slightest sign of disturbance

/when the

Table 9 a.

		Escape times before and after operations (sec).					Significance of difference
Light 15 sec.	Before	6.45	2.5	4.15	2.325	6.625	$P > 0.05$
	After	2.975	2.5	1.875	2.2	2.3	
	Before	2.225	1.45	2.325	5.85		
	After	14.25	1.325	2.2	5.625		
Light 5 sec.	Before	1.575	2.2	4.95	4.0	4.1	$P > 0.05$
	After	1.85	2.4	2.85	1.95	2.05	
Sound 5 sec.	Before	4.675	5.1	2.55	6.275		$P > 0.05$
	After	0.95	1.15	0.875	2.9		

Times taken for the last ten and first ten incorrect trials before and after operation. Differences expressed against results for normals.

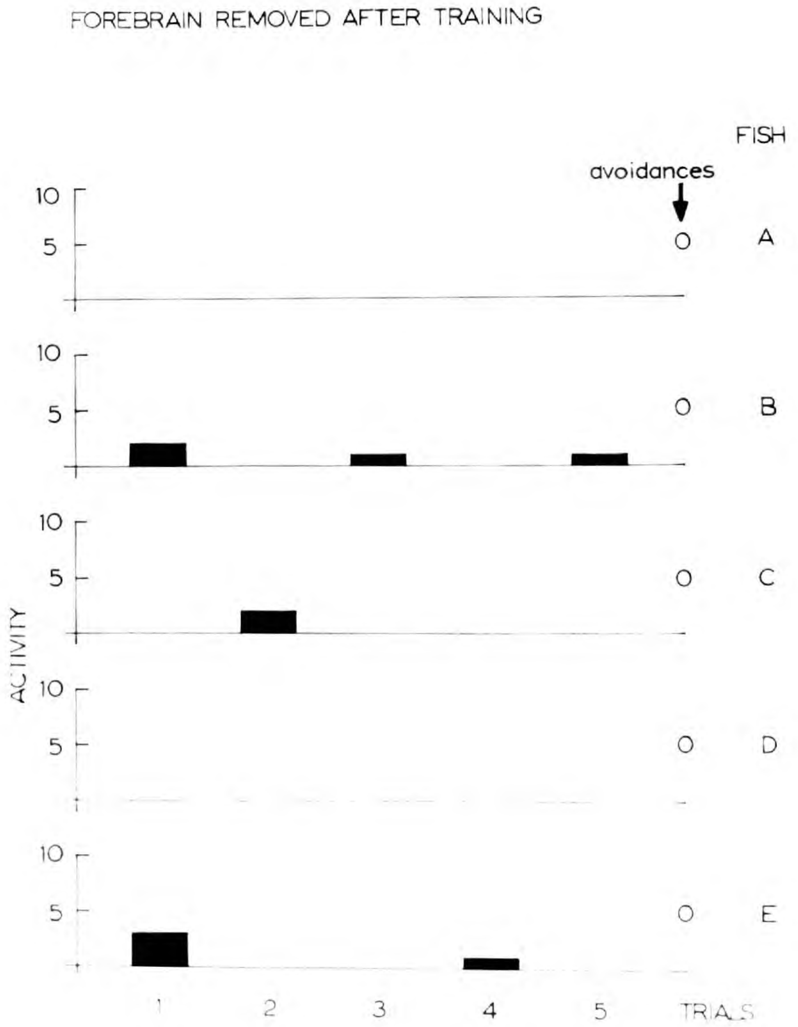


Fig. 29 a: Activity shown in the first 5 post-operative CAS/UCAS intervals (15 seconds) of 5 fish trained to 80% criterion before operation (removal of 80%+ of forebrain). Note that no avoidances were made.

Table 9 b.

Mean reaction times for first 10 trials of pre-operative training and the first 10 trials after operation.9 fish.

Before	After	%
19.75	15.98	80.80

Mean avoidance times.

Before	After	%
9.57	6.80	71.55

when the light came on. Avoidances were quite normal, the animals would rise rapidly from the base of the box, and swim quite rapidly through the door into the goal box. Escapes, too were quite normal, and Table 9a shows mean reaction times for escapes. These times were not significantly different to those of normals.

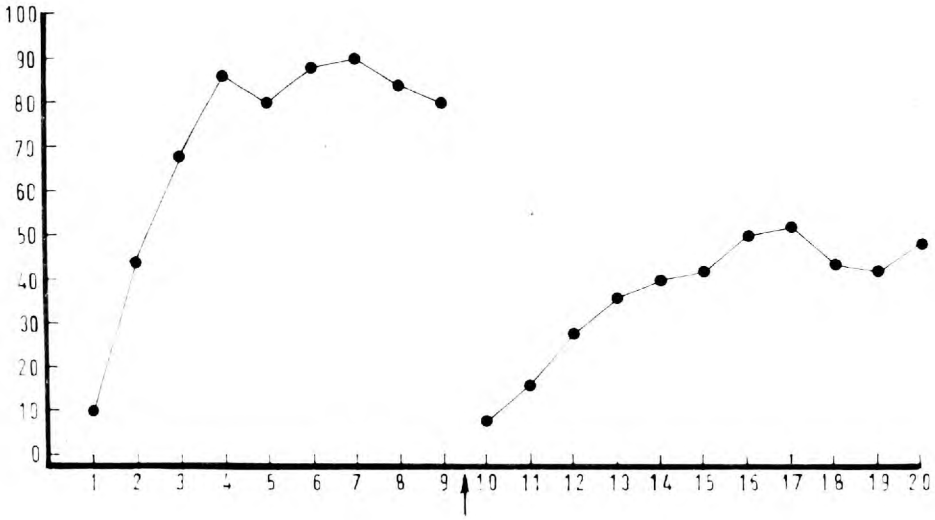
Fig.29 a shows activity readings for five of these animals, taken on the first five trials after operation. It can be seen that there were no "conditioned reflex" type of responses like those seen in the previous group of tectumless animals. Most animals regained some measure of activation due to the stimulus; some would show a very marked "startle" reaction after 20-50 trials, and in some cases would show high (60-90%) avoidances for short periods. It was equally common, however, for animals making this type of response to make short darts at the door, then withdraw, and fail to avoid. A number of animals hardly ever showed any alerting at the onset of the stimulus, and would remain quietly on the bottom of their box until the shock came, when they would escape rapidly. Every fish went through phases of behaving in this fashion, but most had days when they would make more directed movements, and would avoid more.

Table 9 b expresses the mean reaction times for these fish on the first day after operation as a percentage of the mean reaction times on the first day of training. It can be seen that there was a difference in the times, the post-operative results were uniformly lower than those for initial training. Also shown in this table are similar comparisons of the mean times to avoid on the correct trials for the same periods.

(b) Light/shock interval of 5 seconds.

/Five

a



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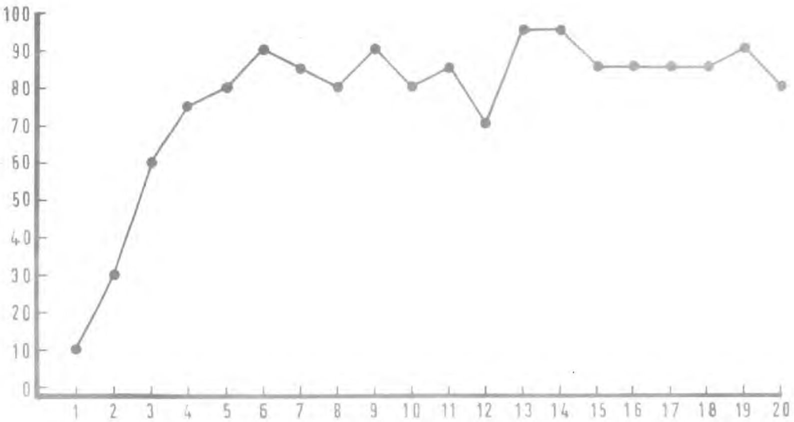
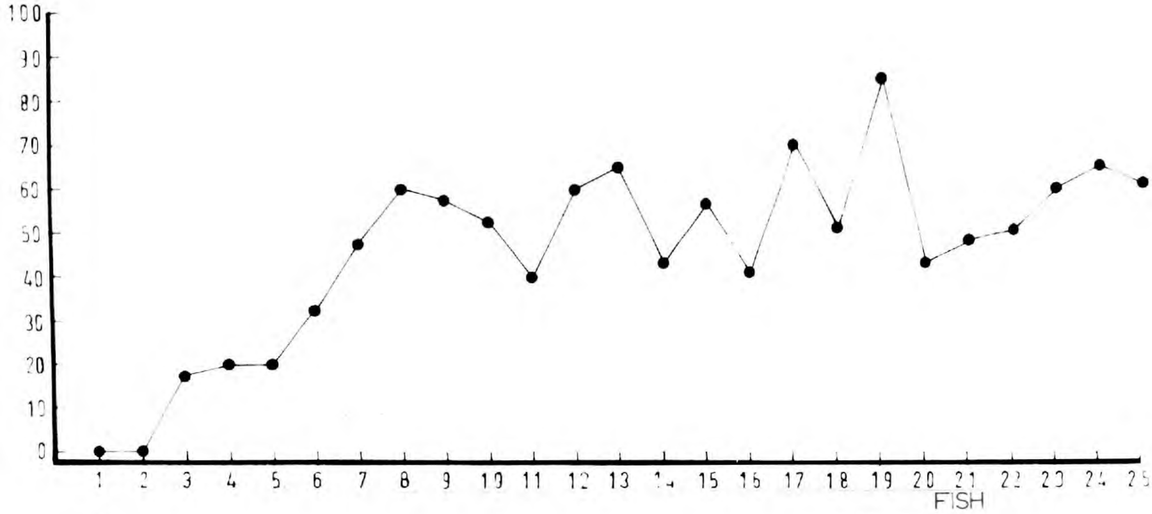


Fig. 30a: Results for 5 animals trained to avoid light (CAS/UCAS interval 5 seconds). Arrow indicates removal of 95% of forebrain.

Ordinate: percentage avoidance per day.
Abcissa: days' training (10 trials per day).

Fig. 30 b: Results for 2 animals with bilateral section of the olfactory tracts, trained to avoid light (CAS/UCAS interval 15 seconds).
Ordinate: percentage avoidance per day.
Abcissa: days' training (10 trials per day).



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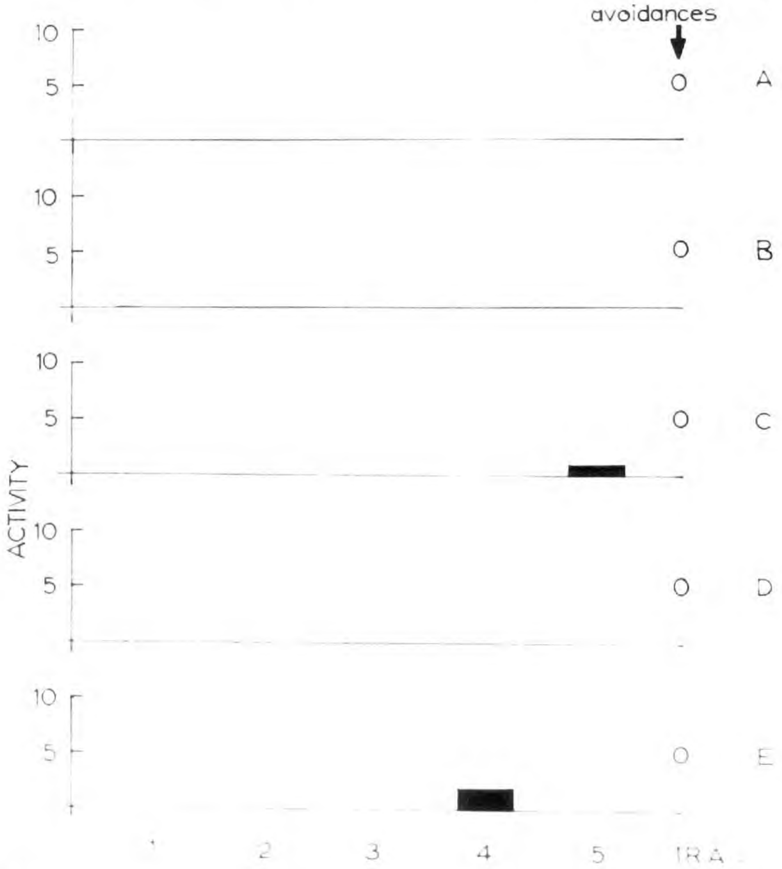


d

Fig. 30c: Results for 9 animals trained to avoid light (CAS/UCAS interval 15 seconds) after removal of 87% of the forebrain.
Ordinate: percentage avoidance per day.
Abcissa: days' training (10 trials per day).

Fig. 30d: Activity of 5 naive forebrainless fish during the first 5 CAS/UCAS intervals (15 seconds each) of light-avoidance training. Activity expressed as number of passes of the tail over the photocell.

Table 10 a.

Mean escape times over the first five days' training.

DAY	15 sec. light		5 sec. light		5 sec. sound	
	Normal	Operated	Normal	Operated	Normal	Operated
1	2.7	3.0	2.7	4.0	1.0	1.3
2	6.5	2.5	1.7	3.2	3.8	2.6
3	1.9	1.9	5.8	4.6	1.6	5.1
4	0.9	1.3	3.6	1.6	1.3	4.7
5	0.7	1.2	4.6	1.6	0.9	1.2

Five animals were trained on this problem, and the results are given in Fig. 30a. There was a large deficit following the operation, and the rate of re-acquisition of the performance was slow. The same phenomena were observed in these animals as were seen in those dealt with above, namely, the lack of alerting when the stimulus was presented, irregular performance from day to day, and gradually-developed "startles" to the light, not necessarily followed by avoidances. Table 9a gives the data for pre-and post-operative reaction times for escapes and avoidances.

B. Lesions before training.

(a) Controls.

Two animals were operated to cut both olfactory tracts, then trained. Their results are given in Fig. 30b. There was very little difference in these results from those for operated controls. Post-mortem and histological examination showed that the tracts had been severed.

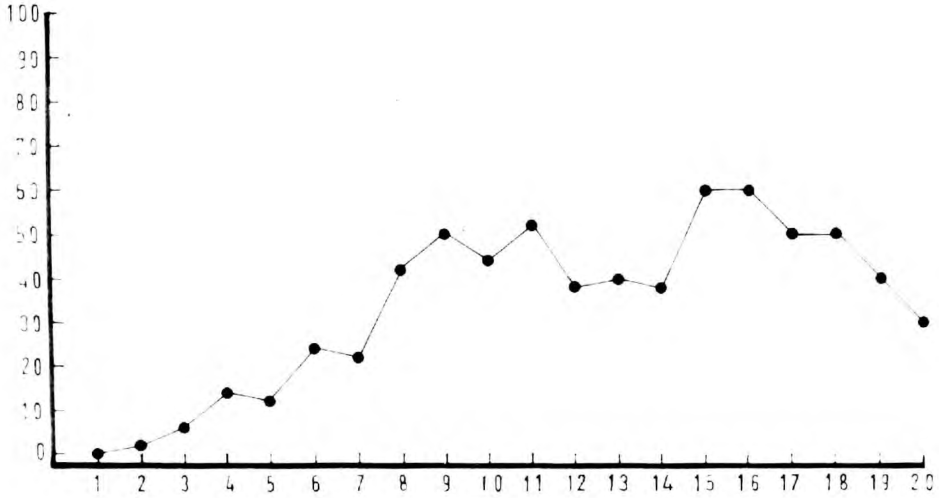
(b) Light/shock interval of 15 seconds.

Nine naive forebrainless animals were trained over 250 trials, and their results are given in Fig. 30c. The rate of learning was significantly inferior to that of normals ($P < 0.01$), and the level of percentage finally reached at the apparent asymptote was lower. Not one animal made an avoidance over the first twenty trials, compared with the average, for normal animals, of 17% for the first ten trials, and of 45% for the second ten. Fig. 30d shows the activity of five of these animals on their first five trials; there was little activity, and no avoidances were made.

Table 10a shows the mean reaction times for escapes, and compares

/them

a



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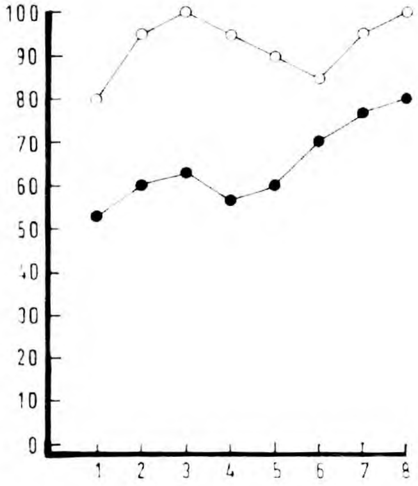


Fig. 31 a: Results for 5 animals with 95% of forebrain removed, trained to avoid light (CAS/UCAS interval 5 seconds).
Ordinate: percentage avoidances per day.
Abcissa: days' training (10 trials per day).

Fig. 31 b: Results for 5 animals shown in Fig. 30 c, transferred to
open circles: extinction (2) (no shocks for any contingency).
closed circles: reversal (3) (shocked if made avoidances).
Ordinate: percentage avoidance per day.
Abcissa: days' training (10 trials per day).

them with those for the control groups. It can be seen that the initial fall-off of the time was the same in both cases, but that there was no accompanying increase in avoidances in the lesioned animals. The initial lack of reaction to light gradually fell off, and in some cases there were quite violent reactions to the onset of the stimulus. Unlike normals, however, this arousal did not inevitably lead to the performance of avoidances. The rate of learning of these animals over the first 50 trials was significantly inferior to that of pre-operatively trained lesioned animals over the same period after operation ($T = 51$, $n = 16$, $P < 0.05$).

(c) Light/shock interval of 5 seconds.

The results for five naive lesioned fish are shown in Fig. 31 a. It can be seen that there was a similar deficit in the rate of learning to that seen above, no avoidances for the first few days, and a low asymptote. Table 10 a shows the mean reaction times.

The behavioural phenomena mentioned above were also noted in this group, and no special mention will be made of them. There was the same inferior rate of learning over the period described above, when compared with pre-operatively trained animals ($P < 0.05$).

C. Reversal and extinction.

Five animals were transferred from the 15 second group of post-operatively trained animals, and three were reversed, two extinguished. The latter extinguished very rapidly, the former took longer to achieve a high level of reversal. Results are shown in Fig. 31 b.

Summary of the effects of total forebrain lesions on light avoidance learning and retention.

/(a) Retention.

(a) Retention.

There was a large and long-lasting effect, on both 15 and 5 second situation retention, of forebrain lesions. In no case was there overlap between the control and lesioned groups - the latter all showed deficits ranging from 50% to 0% of the pre-operative level. The nature of the deficit was less obvious than that of the deficit seen in some animals after removal of the tectum. For example, mean escape times after operation were not different from those before, so that simple motor deficits, as seen in tectumless fish, could be ruled out. This was also supported by the almost total lack of reaction seen to the CAS, as measured by the activity recorder, and noted by the observer.

One possibility was that there might have been a simple slowing of the motor systems of the animals, so that response latencies rose. This was not supported by the escape time evidence given above, nor by comparison of post-operative avoidance results for the 15 and 5 second groups. If latency of avoidance had risen, it would have been expected that there would have been more difference in immediate post operative performance with greater performance of avoidances in the 15 sec. group, but both groups showed very similar results, with no significant differences ($T = 31$, $n = 14$, $P > 0.05$). It could of course be argued that times taken to avoid before operation should be considered, so that the time left of the CAS/UCAS interval could be taken into consideration. This would lead even more definitely to the conclusion that if motor slowing were the factor involved, it should be shown up by the use of short and long CAS/UCAS intervals. For example, using the data of Behrend and Bitterman (1962), whose results agree with those obtained for

/normal

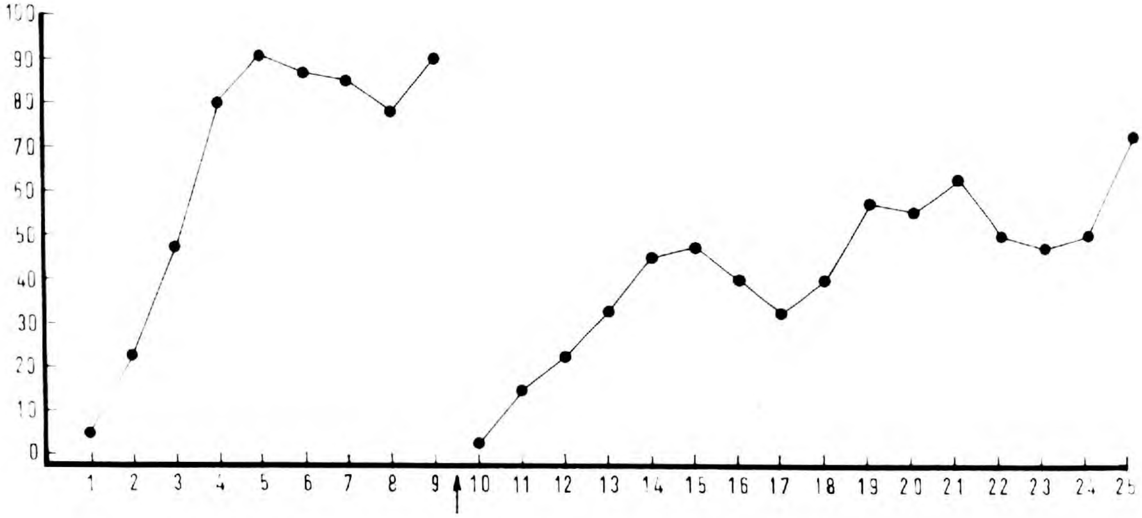
normal fish, the mean avoidance latency for a 5 sec. CAS/UCAS interval was c. 3 sec. after 90 trials, leaving about 2 seconds' extra avoidance time. On the other hand, the latency for the 15 second group, extrapolated from this data, and in agreement with figures given above, was c. 6.5 sec., leaving 8.5 seconds' extra avoidance time. Thus, if, as has been seen, avoidances could occur in the 5 sec. group, if motor slowing were operative, there should have been a much greater response in the 15 sec. group. A final fact in consideration of this is that if this factor were involved in the reduction of performance, it is difficult to explain the re-acquisition of avoidances. In view of the evidence above, it would seem more economical to postulate some mechanism less concerned with actual movement than with the control of initiation of movement.

Although fuller discussion of the problem will be made later, it is worth noticing that fish trained on the simple conditioned reflex training, then lesioned, did not show deficits, and that this concurs with the results of a number of workers, such as Bernstein (1962) and Karamyan (1956). These facts would argue that since no deficits were found, there must be some specific effect(s) of the lesion, not simple motor ones, or even simple ones on the switching "on" or "off" of the motor centres.

(b) Learning.

Although the rate of post-operative re-learning seen in the previous groups suggested that naive forebrainless fish would also show poor learning, it was necessary to test this. In addition, although there seemed to be a total deficit after lesion, it was important to

/assess



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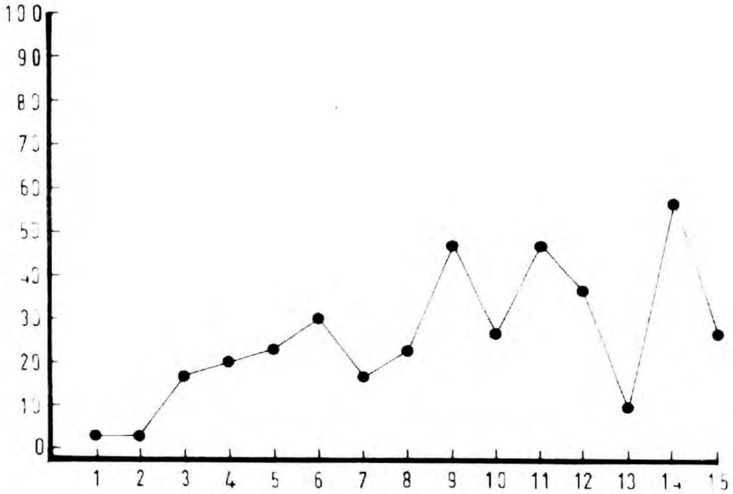


Fig. 32 a: Results for 4 animals trained to avoid sound (CAS/UCAS interval of 5 seconds). Arrow indicates removal of 90% of forebrain.
Ordinate: percentage avoidance per day.
Abcissa: days' training (10 trials per day).

Fig. 32 b: Results for 3 animals trained to avoid sound (CAS/UCAS interval 5 seconds) after removal of 92% of the forebrain.
Ordinate: percentage avoidance per day.
Abcissa: days' training (10 trials per day).

assess the reality of this - it might be that poor post-operative learning was making some retention. The results obtained confirmed this. Though the rate of re-learning was slow, it was in both cases (15 and 5 second) significantly faster than in naive lesioned fish. This argued that there was some retention which survived the lesion, and which facilitated re-learning.

Whilst it has been seen that forebrainless fish were unreactive, there has been proof that their ability to escape was unimpaired by the lesion, and that their reactions to shock were as great as those of normals, showing no sign of lessening with continuation of such punishment. Thus it may be assumed that insofar as reward (shock) contributed towards promoting activity, and the appropriate activity, these fish were similar to normals. That the effect of forebrain removal might be merely to reduce sensitivity to shock which was normally mediated via the olfactory tracts was disproved by the results for two animals trained after bilateral section of the olfactory tracts. These results would suggest that neither effectiveness of shock, nor ability to respond in appropriate fashions, were impaired, so that it remained to test the effects of either varying the modality of the stimulus, or varying the intensity of one modality. Both these approaches were investigated.

6. Sound avoidance.

A. Lesions after training.

Four animals were trained to a criterion of 80-100%, and were operated. The post-operative performance was very similar to that of the light avoidance groups, and is shown in Fig.32 a. Initially, there

/were

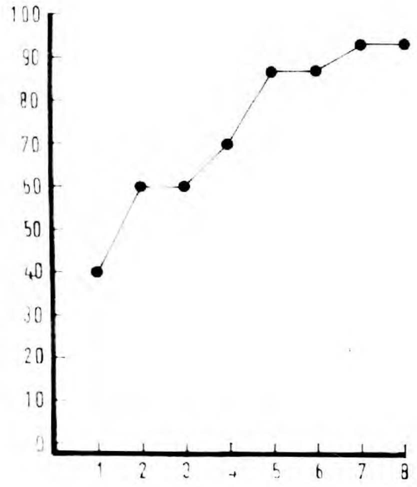


Fig. 32c: Results for the animals shown in Fig. 32b, transferred to reversal training (i.e. shocked for avoiding).
Ordinate: percentage avoidance per day.
Abscissa: days' training (10 trials per day).

were no signs of activity when the buzzer came on, but as training proceeded, animals tended to be activated more consistently than those in light avoidance groups. It seemed as if the vibrations, especially those at the onset of the noise, were stimulating the animals mechanically, and were for this reason more effective than light. This increased arousal did not, as in previous cases, cause an automatic avoidance, and fish would swim to and fro in the start box in the light/shock interval without making an avoidance. Table 9 a shows mean reaction times on incorrect trials both before and after the operation, and it can be seen that there was no increase after operation in the time taken to escape.

B. Lesions before training.

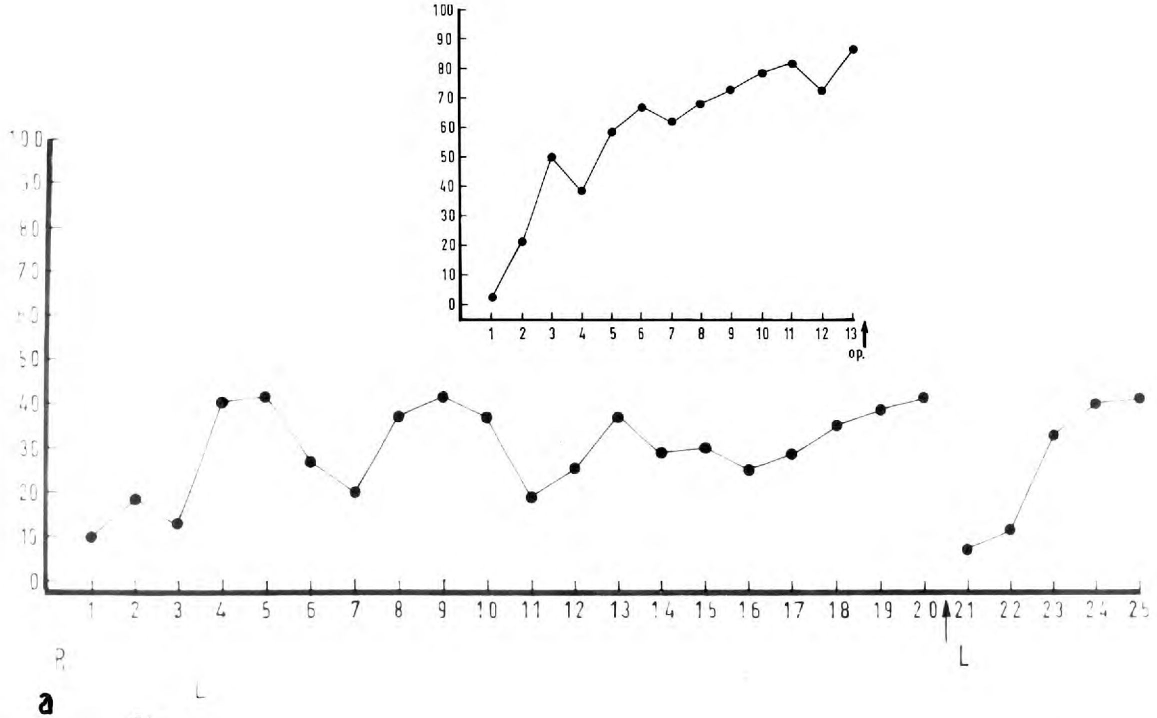
Fig. 32 b shows curves for three animals trained on the sound avoidance after being lesioned. The curve obtained was significantly different to that obtained for normals, and the final level of performance was lower. The variability of response in the lesioned animals after some days' training is better shown here than in other cases, on account of the smaller number of subjects. One animal tended to alternate, one day remaining inert, the next day achieving up to 70% correct avoidances. The others were more consistent, generally achieving 20-50% avoidances. Table 10 a gives details of the reaction times for these animals.

C. Reversal.

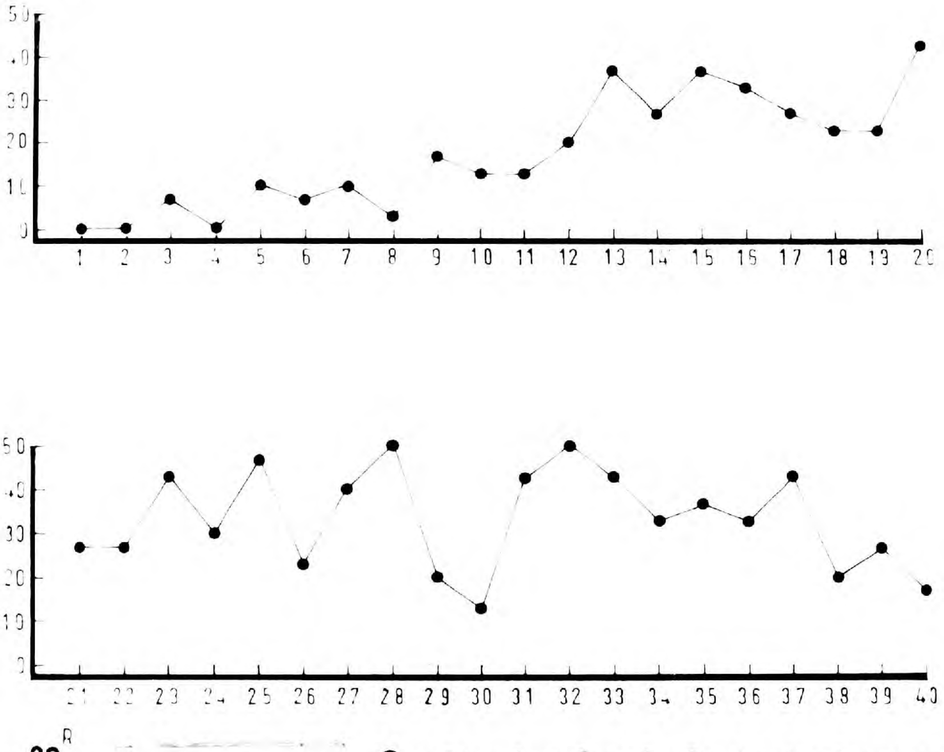
Fig. 32 c shows results for the three animals just mentioned when transferred to a reversal problem.

Summary of the effects of forebrain lesions on sound avoidance learning and retention.

/(a) Retention.



a



b

Fig. 33 a: Results for 6 animals trained preoperatively to 78% avoidances over the last 50 of 130 trials, 89% of forebrain removed. Small light avoidance (CAS/UCAS interval 5 seconds). Arrow indicates transfer of training from RHS to LHS.
Ordinate: percentage avoidance per day.
Abcissa: days' training (10 trials per day).

Fig. 33 b: Results for 3 animals trained to avoid a small light (CAS/UCAS interval 5 seconds) after removal of 91% of forebrain.
Ordinate: percentage avoidance per day.
Abcissa: days' training (10 trials per day).

(a) Retention.

The results obtained paralleled those obtained for the light-avoidance groups. Previously-trained animals showed a total deficit after operation, and naive lesioned fish were slow to learn. There was no change in average escape times after operation, and no sign of immediate post-operative "startle" responses to the stimulus.

(b) Learning.

Again, the rate of learning of the naive lesioned fish was inferior to that of the previously trained lesioned fish, over about 50 trials, the two curves then became more similar in average. These results confirmed those for the light groups, and suggested that since the effect was not stimulus modality-specific, there was some more subtle central cause.

7. Small light avoidance.

A. Lesions after training.

A group of 6 animals was trained over 130 trials, then lesioned, and their results are shown in Fig. 33a. These animals had achieved a mean of 78% over the last 50 trials pre-operatively. It can be seen that there was a considerable deficit, and that levels of avoidance rarely reached 50%. In addition to this, there was a deficit in the inter-ocular transfer trials. Animals showed little response to the light in early trials, and only slowly developed reactions to it; these reactions vanished almost completely when the animals were trained from the LHS. Escapes were again good, and pre-and post-operative escape times were 7.86 and 7.7 seconds respectively. The post-operative deficit was significantly different ($T = 21$, $n = 15$, $P < 0.01$) from the controls'

/post-operative

post-operative scores, measured for the first ten trials.

B. Lesions before training.

Fig. 33 b shows results for three animals trained after bilateral forebrain removal. Comparison of these results with those for normals will show that they were inferior, and comparison with the results for animals trained then lesioned, as above, shows that the rate of learning after operation was inferior in the present group (for first 50 post-operative trials $T = 6$, $n = 9$, $P < 0.05$). Changing the side seemed to make little difference to the level of avoidance, the 20% drop seen being well within the limits of variation of the group. The animals were unaffected by the stimulus for some 20 trials, and the number of trials where an alerting was seen did not rise very fast.

Summary of small light avoidance results.

These results confirm those preceding, and also show one or two points worthy of note. Firstly, although the pre-operatively trained fish showed a deficit after operation, their rate of re-learning was comparable with that seen in the other groups, though the asymptote was lower. Although three of the naive lesioned fish died because of water contamination, the results for those remaining do suggest that the slower rate of learning seen in these fish was due to the lessened intensity of the light stimulus. Another interesting fact was that whilst there was good interocular transfer of the light avoidance capability in the animals trained only post-operatively, there was a large drop in avoidances in the animals trained pre-operatively, when trained on the untrained side.

These results suggested a number of further experiments. Firstly,

/since

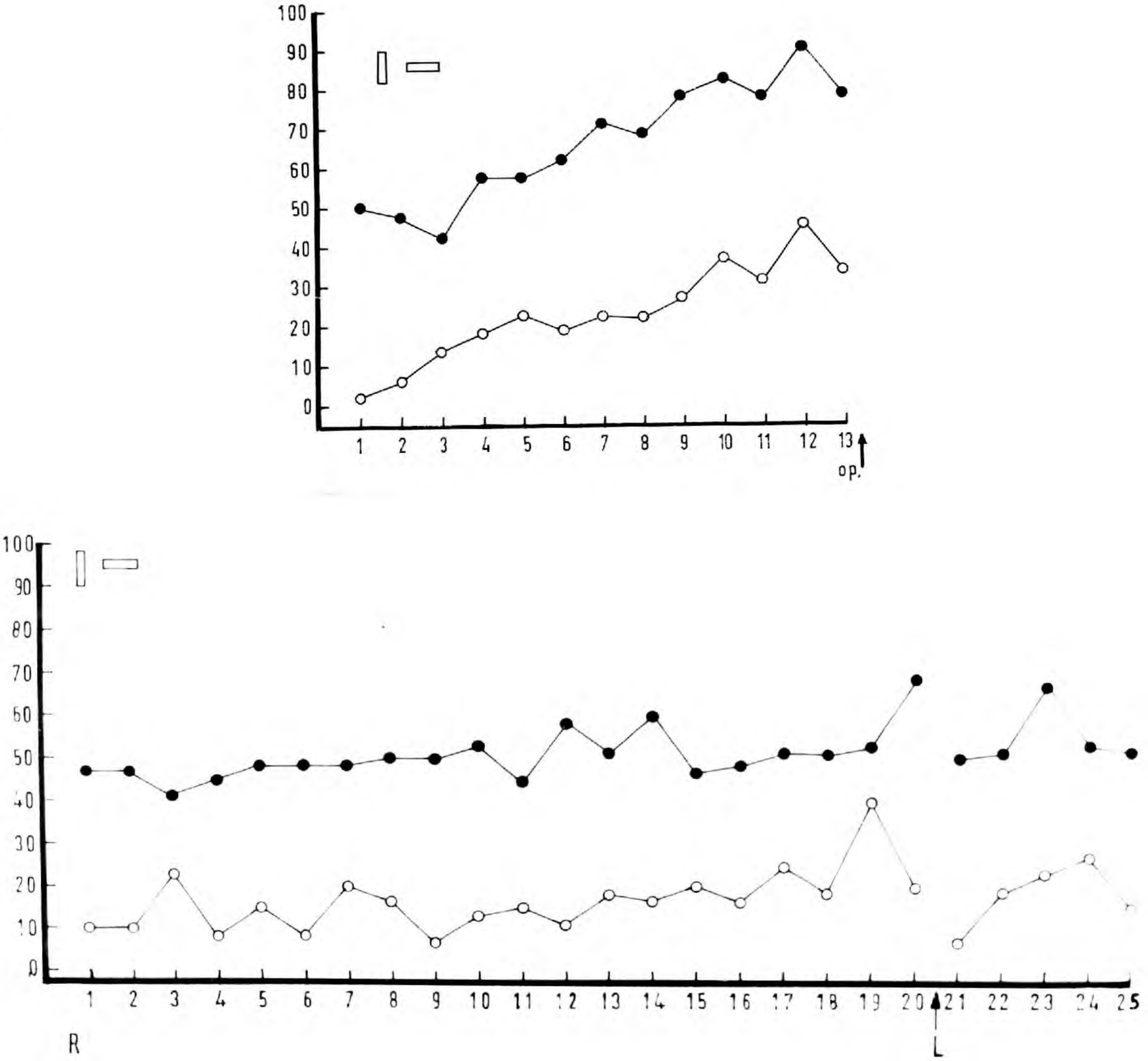


Fig. 33 c: Results for 6 animals trained with | + vs — - over 130 trials, attaining 80% correct responses over the last 50 trials. 89% of forebrain removed. Arrow indicates transfer of training from RHS to LHS. (CAS/UCAS interval 5 seconds). Closed circles: discrimination per day (correct + or - trials). Open circles: avoidances (irrespective of correctness).

Ordinate: performance (%) per day.
Abcissa: days' training (10 trials per day).

since in all avoidance situations there had been a deficit following forebrain removal, it was of interest to examine a slightly different paradigm, that of a "go/no go" situation. Here, fish would be required to make different responses to different shapes. The situation used has already been outlined. If the deficit seen above had been only in the "go" mechanism, and the animals were still capable of making correct responses, there should be failures only in "go" responses, so that the overall level of avoidance should be, by analogy with the situations above, about 20%, or half that of the normals. If only the "go" responses were affected, that is, if some relatively simple motor switching were affected, the level of discrimination should be about 70%, that is, "no go" (no errors) 50% + "go" 20% correct.

The second interesting possibility was that of using unilateral lesions to determine the effects of these on performance with each eye separately, and hence to assess laterality of forebrain function.

8. I vs - training.

A. Lesions after training.

Fig. 33 c shows results for six animals trained over 130 trials, attaining a mean of 80.6% correct responses over the last 50 trials, then lesioned. Over the first 70 post-operative trials, the group performed at just below chance level, whilst the percentage avoidances did not increase in the normal fashion. Some animals made very few avoidances over the 250 trials of the experiment; others were more active, but their levels of discrimination did not rise very much, though they might show some quite high scores on certain days. The post-operative deficit was significantly different ($T = 21$, $n = 21$, $P < 0.01$) from the post-operative performance of controls, as assessed over the first ten

/trials.

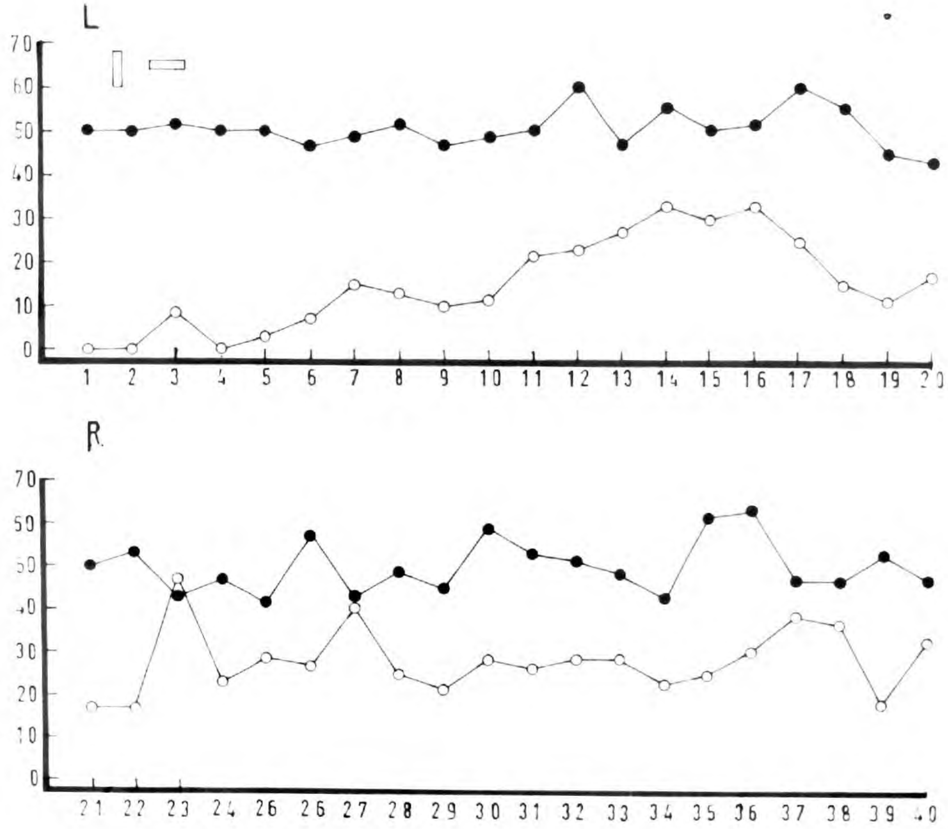


Fig.34 a: Results for 6 animals trained | + vs — - after removal of 91% of forebrain. (CAS/UCAS interval 5 seconds). L , R :side of training.
Closed circles: discrimination per day (+ or - correct).
Open circles: avoidance (irrespective of correctness).

Ordinate: percentage performance per day.
Abcissa: days' training (10 trials per day).

trials. This applied both to discrimination and avoidance results.

After 200 trials on the RHS, these animals were trained on the LHS. The low level of the percentage discrimination (near chance level, 50%) made it impossible to determine whether or not there had been inter-ocular transfer, but there was a drop in the percentage avoidance, so it was assumed that there was a deficit in this. The level of avoidance soon reached the pre-transfer level, and the level of discrimination did not change significantly, over fifty trials.

B. Lesions before training.

Six naive forebrainless animals were trained on this problem, and given 200 trials on each side. The results are shown in Fig. 34a. The percentage avoidance curve rose very slowly, and the discrimination curve remained around chance level, since animals tended to avoid both shapes if they did reach a fairly high level of avoidance. Some animals rarely avoided, others would reach quite high levels of discrimination for one day, then drop below chance level the next day. The level of avoidance over the first 50 trials was inferior to that of the previous group post-operatively ($T = 21$, $n = 12$, $P < 0.01$) but the level of discrimination was not different ($T = 34$, $n = 12$, $P > 0.05$).

In view of the variability and nearness to chance level of the discrimination curve, it was impossible to say whether inter-ocular transfer occurred, but the avoidance curve did not drop as much as that for the previous group, so it was suggestive of a measure of transfer.

Summary of results for 1 vs - .

These results show the previously-noticed effect on avoidances of forebrain lesions. Contrary to the prediction made on the hypothesis

/of correct

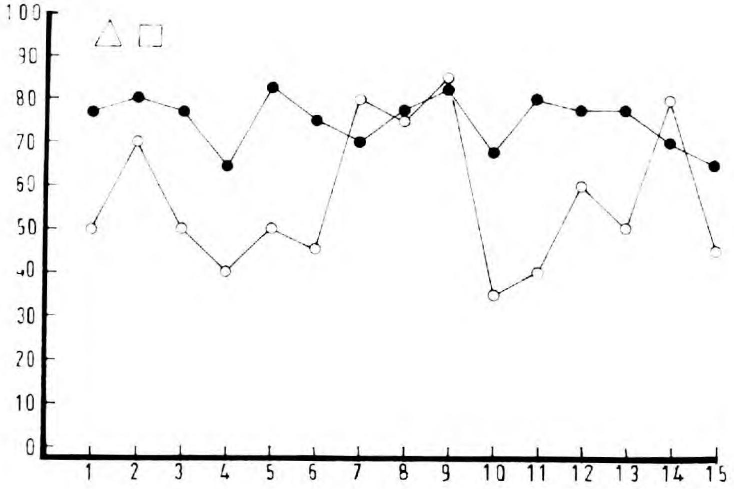


Fig. 34b: Post operative results for the animals whose pre-operative training is shown in Fig. 21 d.
Closed circles: 4 animals given control operation.
Open circles: 2 animals with 78 and 86.5% of the forebrain removed.

Ordinate: percentage correct responses per day.
Abcissa: days' training (10 trials per day).

of correct discrimination and deficit affecting only "go" shape responses, discrimination was at chance level. Many errors were made to the non-aversive shape, and though there was individual variation, on average the animals failed to show much discrimination between the shapes. Thus it was necessary to postulate that the lesion had affected some important part of the memory system, rather than just affecting speed of responses. This will be amplified below.

The same phenomenon was noticed with regard to interocular transfer as had been mentioned for the small light avoidance animals. The previously-trained lesioned fish showed a drop in avoidances on changing the eye being trained, whilst this was not seen in the naive forebrainless animals. Whilst this effect was not prolonged, it did agree with that noted above, and suggested that unilateral interference with the system might yield useful data.

9. △ vs ▯ training. Fig.34b.

Whilst it had been seen from the results just outlined that the re-development of the avoidance ability did not necessarily mean the re-acquisition of discriminative capability, as expressed by percentage correct results, it was of interest to assess the effects of ablation on discrimination as opposed to avoidance. Therefore the forebrain was removed in two of the animals previously trained and four given the control operation. After operation the lesioned fish could still approach the boxes, but the accuracy of response was lower; that of the controls was not. These results were very preliminary, and there is need for more work of this sort. Nevertheless, since there was no pre-operative variability of the kind seen in the lesioned fish after operation, it seemed that the change was a significant one.

/10. Split brains.

Table 11 a.

Details of performance of split animals.

Operation

Small light		Last trials before operation	Trained side after operation	Untrained side after operation
A		8, 7, 7	7, 8	0, 1, 4, 7, 8
	Total	69.15%	75%	54%
B		8, 10, 7	8, 7	0, 0, 5, 7, 9
	Total	65.75%	75%	64%
C		9, 8, 9	6, 7, 5	0, 3, 6, 8, 8
	Total	63.25%	60%	71.25%
| vs. —				
A	Disc	9, 8, 9	9, 8, 9	5 3 3 4 8 8 9
	Total	71.25%	86.7%	69.2%
	Av	4, 5, 4	4, 3, 4	0 2 6 5 7 5 4
	Total	24.1%	36.7%	43.8%
B	Disc		7, 8, 8, 9, 9	5 5 6 7 8 8
	Total		66.5%	70.75%
	Av		2, 3, 3, 4, 4	0 0 1 2 3 3
	Total		15.65%	22.5%
C	Disc		7, 7, 7, 9, 7	6 5 7 7 7 6
	Total		74%	73%
	Av		2, 2, 2, 4, 2	1 0 2 2 2 1
	Total		24%	29.3%
D	Disc		7, 8, 9, 8, 9	6 6 6 8 9
	Total		63.75%	74.5%
	Av		2, 3, 4, 3, 4	1 1 1 3 4
	Total		13.75%	29.4%

Disc = Discrimination
Av = Avoidance

All scores are per day.

Totals are percentages for <u>all</u> trials, not merely those shown.

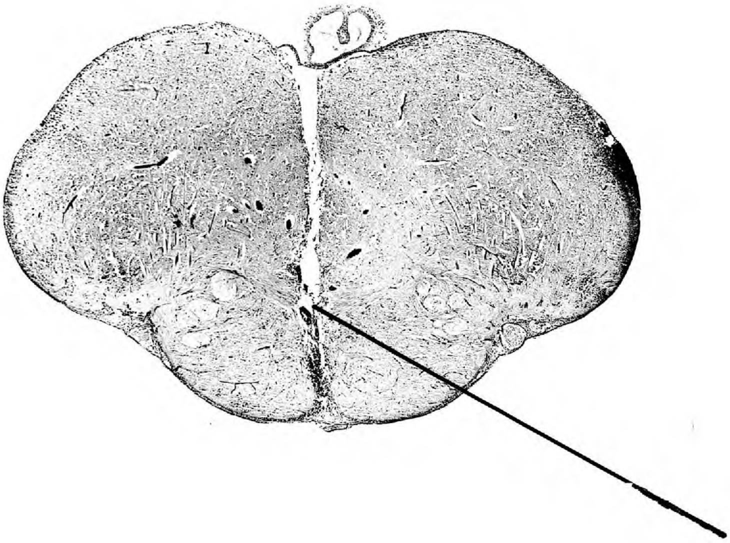


Fig. 35 a: Brain of fish I — A, showing split anterior commissure (arrowed).

10. Split brains.

As mentioned above, the operation of splitting the anterior commissure was not often successful; there was degeneration of a number of brains, and these were rejected. Table 11 a shows results for a number of animals which histological examination showed to have been split without other damage. Such a brain, that of animal |— A, is shown in Fig. 35 a.

Learning of discrimination was quite good in both normal and split animals. After lesion, the trained side showed good retention of the problem. When animals were trained on the untrained side, there was generally no response at all, not even the "startle" reaction seen in normal animals which had difficulty on their first transfer trials. The fall in level of performance was significant below the 0.05 level, and the fall in the level of avoidance was significant at the 0.01 level, when these fish were compared with normals. Comparisons were made between the rates of re-learning on the untrained side, and the rates of learning of the transfer group of control fish shown in Fig. 20a. There were no significant differences ($P > 0.05$).

These results supported the tentative hypothesis that there might be some involvement of the forebrain in transfer of information laid down unilaterally. If the presence of the forebrain did no more than facilitate motor reactions, cutting the commissure should have had no effect on the ability of the fish to show interocular transfer of response capability. On the other hand, if there were some effect on learning, which was lateralised, and which information was normally transferred via the commissure, the effect seen would be explicable.

Table 12 a.

Forebrain removed unilaterally. Performance on both sides.

	Damaged (i.e. contralateral eye trained) %	Undamaged %
Small light		
A	13.95	44
B	10	35.3
C	15	46
| vs —		
A Disc.	48	74.5
Av.	32.5	38.5
B Disc.	48	76.3
Av.	28	36.7
C Disc.	52.5	71
Av.	6	23
D Disc.	51.5	73.5
Av.	3.5	28

Table13 a.

Lesions made electrolytically - effect on | vs — learning.

% Performance

		Damaged		Undamaged		LA	CA	LFB
		Disc.	Av.	Disc.	Av.			
GROUP 1	FISH A	50	52	74	31	5	2½	4
	B	45	47	75	32	2½	4½	0
	C	54	58	73	26	1½	4½	0
	D	50	12	81	35	5	4½	0
	E	45	14	75	28	4½	4½	0
GROUP 2	FISH A	70	33	92	46	5	0	3½
	B	64	28	83	30	5	2½	0
	C	49.5	0.5	61	13	5	0	4
	D	57	26	77	31	5	4	0
	E	54	5	79	31	5	4	0

Damage is marked off in terms of severity of lesion in the region of the anterior commissure, where lesions were made.

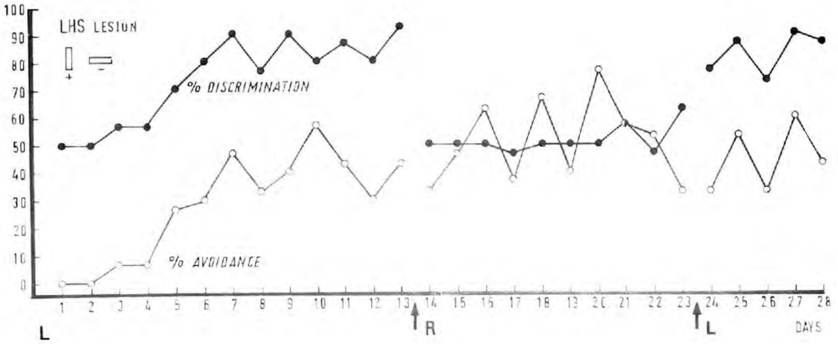
(0 = no damage; 5 = totally destroyed).

LA = Lateral area

CA = Central area

LFB = Lateral forebrain bundle

a



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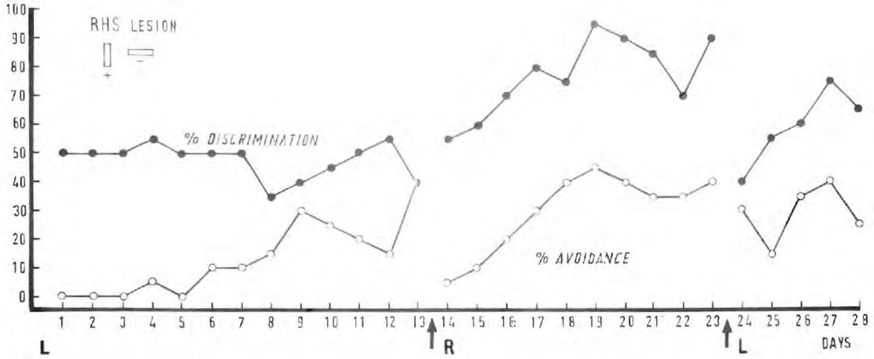


Fig. 36 a: Results for 3 animals, with LHS lateral and central areas damaged. Arrows indicate change of side of training.
Closed circles: discrimination (correct + or –).
Open circles: avoidance (irrespective of correctness).

Ordinate: percentage performance per day.
Abcissa: days' training (10 trials per day).

Fig. 36 b: Results for 2 animals, with RHS lateral and central lesions. Captions as for 36 a.

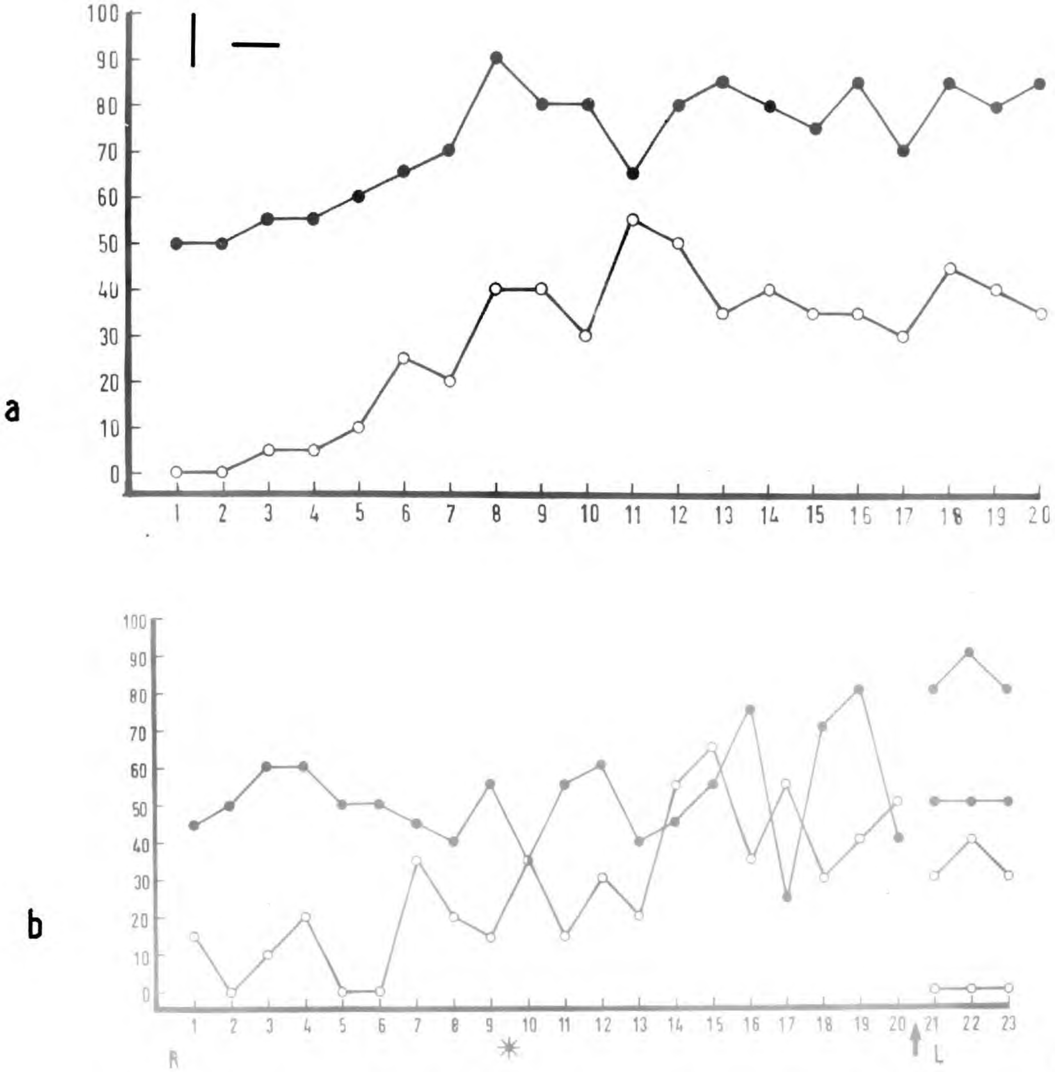
Since the rates of learning of the untrained side of these fish were comparable with those of the problem transfer animals, where the stimulus association was being learned de novo, it would seem that there was some fundamental involvement of the forebrain in learning. It remained to assess the results of unilateral lesions, to see if they would fit into this framework, and if there was any localisation of function in the telencephalon, as suggested by the work of Segaar and Nieuwenhvys (1963).

11. Partial unilateral lesions (All animals unsplit).

Unilateral removal of the forebrain reduced avoidances, but performance was better on the side contralateral to the intact hemisphere. The results for some of these animals are summarised in Table 12 a.

The most interesting results were obtained using the electrolytic lesion technique described above. Two groups of five animals were lesioned, but not split. The extents of the lesions are roughly assessed in Table 13 a. This also sets out the performances of these animals. It can be seen that there was a significant ($P < 0.01$) difference in performance between intact and lesioned sides. The level of avoidance was not always low, but the level of discrimination was on average not very much above chance level. The variability of some of these animals can be gauged from consideration of the learning curves. Fig. 36 a shows mean results for three animals (A, B & C) of group one. The lesion was to the LHS of the brain, and training to the LHS eye gave good learning. On transfer training, whilst there was good avoidance, there was a tendency to avoid shapes indiscriminately, and the level of discrimination was low. Fig. 36 b shows results for two other

/animals



Figs. 37 a and b: Results for 2 animals with LHS lateral and central area lesions. Star indicates additional surgery to one fish. Arrow and letters indicate change of side of training.

Closed circles: discrimination (correct + or - responses).

Open circles: avoidances (irrespective of correctness).

Ordinate: percentage of trials.

Abcissa: days' training (10 trials per day).

animals (D & E) in group 1. Learning with the damaged side was poor, and there was a deficit in avoidances on transfer. The intact side learned well, and it seemed that there was some transfer, since the damaged side showed better performance when trained again. Figs.37 a and b show results for two animals (A & B) of group 2. The undamaged side learned rapidly, and there was a consistent level of performance over 120 trials. When the damaged side was trained, the level of avoidance dropped, then rose after about 100 trials, whilst the level of performance increased, but slowly and erratically, with some very high values being achieved after much training. The star on the baseline indicates when fish A damaged itself, and lost its skull cover; this was replaced, and in doing so it was observed that the brain appeared quite normal. When the same brain was examined histologically, it was found that the "intact" half of the brain had been badly damaged presumably when the skull cap was replaced. This was borne out in subsequent training, since fish A (the lower curves) showed no avoidance when trained to this side, fish B (the upper curves) showed its previous level of avoidance.

Although some of these lesions were small it was not possible to make any definite statements as to the functions of specific areas. For example, although the area immediately above the lateral forebrain bundle was damaged in fish A of group 1, and fishes A and C of group 2, there was no correlation of lesion with impairment of avoidance or of accuracy. Both impairments occurred, but it was not possible to attribute one or the other to specific damage to one brain area. It can be briefly stated that unilateral lesions to the median dorsal

/segment

segment of the brain had no effect on learning of either the small light or horizontal/vertical problems. The lateral and central areas seem to be closely related functionally, so that no separate functions are yet attributable. Further work, with more discrete lesions, may throw light on the problem.

DISCUSSION AND CONCLUSIONS

1. Introduction.

The introduction to this thesis will, it is hoped, have conveyed the impression that whilst in many ways the data relating to discrimination by fish in a variety of sensory modalities are large, the corresponding data relating to brain function are surprisingly small. There has been a tendency for workers to spend much time and energy on fish projects, then to turn to other fields for research. The value of some of these individual contributions such as those of Janzen, Nolte, Hale and Warren is considerable, but it is probable that if even one worker had confined himself to this field, and applied himself, there would not have been the rather simple concepts, and the lack of controls, which have prevailed to the present time.

Thus many reviews quote Botsch's (1960) work on the optic tectum as having shown that this area is the memory centre of fish, when, as has been shown above, there is reasonable doubt that the animals had accurate enough form vision to allow them to display the presence or absence of retention. A similar example is that of the relation of the cerebellum to learning and retention. Aronson (Kaplan and Aronson (1963 & 1965) and Aronson (1967)) has shown that removal of the cerebellum before and after training on avoidance problems causes large deficits. This is an interesting observation, but the suggestion that some part of the memory system has been affected is not realistic when the results of other workers using fish with cerebellar lesions are taken into account (see for example, Tuge 1934a). The obvious control would be to test the ability of these lesioned fish to orientate and to learn and retain new motor patterns, before confusing the issue with the introduction of more elaborate paradigms. Nieuwenhuys (personal communication) is at present using this approach, and it will be interesting to compare his results with those of Aronson. However, Karamyan (1956) has shown that fish are unable to develop conditioned responses in the absence of the cerebellum, and this would provide reason for caution in proposing too simple an explanation.

Ingle (unpublished) has shown that conditioned heart rate responses can occur in fish with cerebellar lesions, and more recently workers in Karamyan's laboratory have found that both learning and retention of light/shock associations with heart response are possible despite cerebellar ablation. They have also found that normal CR's, the increase of skeletal motor activity, are unaffected by the lesion, though this work is in early stages (Karamyan, communication to Ingle). The lack of obvious controls seen in some of these experiments would hardly be tolerated if the authors were dealing with higher vertebrates, and such lack reduces the value of these interesting observations.

It is hoped that it will be possible here to present a crude explanation of the functioning of two parts of the fish memory system, and to try to relate as much as possible of the data to this.

2. Functions of the optic tectum in learning and retention.

The difficulty of investigating the optic tectum as a memory centre has been outlined above. The simplest way round these difficulties is to use training methods which as far as possible avoid using modalities affected by tectal lesion. Although he did not make the point, and did not consider tectal involvement in other than memory functions, Sears (1934) was correct in using simple conditioned reflex training in the first study of the relation of the area to learning and retention. The stimulus, the onset of a large overhead light, was so diffuse that even with quite extensive lesions, there would still be some tectum left to receive the signal, and as has been seen, there are other areas capable of carrying visual information from the eye to the lower centres of the brain. In addition to this, there exists a separate receptor, the pineal complex, which allows detection of the stimulus, even in the unlikely instance of the separate nuclei of the optic tract not serving to relay visual information. As regards output, Sears was measuring increases in activity, and orientation reflexes, not the performance of some complex response, so that even if there were interference with the ability to make more complex actions, the response would still be visible.

In view of these unwitting avoidances of the problems associated with tectal removal, it is significant that Sears did not find deficits when he removed the tectum, either before or after training. His results have been confirmed here for light/shock association, a paradigm which he avoided on account of the generalisation effect, but there is good agreement as to the lack of effect of the lesion on both learning and retention.

The reason for investigating the more elaborate forms of behaviour was that it was hoped to make assessment of the function of this region in the formation and retention of more complex responses. For reasons given above, there would be little point in trying to train lesioned fish on more complex visual problems. From Sears' work, relating to visual stimuli, and from Dijkgraaf's (1949a) work on the effects of tectal removal on the retention of auditory responses, it seemed likely

/that

that use of these stimuli would not introduce receptor deficits such as would prevent detection of signals. The control groups of fish with blinders showed that for both avoidance tasks, there was ability to make the response. Thus any deficit found must be due to causes other than blindness effects. One possibility, unlikely in view of the results reported here for the effect of tectal removal on conditioned reflexes, was that removal of the tectum might reduce the sensitivity to shock of these animals. Results for animals shocked over a fortnight showed that this was not an important factor, these animals showed as much reaction following reinforcement as did normals.

In view of the controls outlined above, any deficits found could be related to one of two causes; the damaging of a memory centre, or the disruption of motor control areas. Consideration of the reaction times of lesioned animals should allow discrimination between these alternatives. Mention has already been made of the high degree of correlation between the percentage of avoidances each day, and the shock index, or shocks/escapes for that day. In no case where there was a low, or normal shock index, did an animal fail to learn as rapidly as normals, or to show as good retention. Where the index was high when the animal was learning, the rate of learning, as expressed by the percentage avoidance scores, was low. Where animals were trained, then lesioned, and it was found that there was a high index, there was generally observed a conditioned reflex response, indicating that there was retention of the stimulus/reinforcement association, even if the paths allowing implementation of the response were affected by the lesion. The possibility of there being such motor aberrances was noted in the introduction.

/It was

It was not possible to correlate motor failures with the amount of tectum removed; indeed, in most cases, it seemed that it was the animals with the smaller lesions which showed the greatest effects. This may have been because of the presence of some 30%+ of the tectum remaining intact; it may be supposed that in the animals with more complete lesions there would be little competition between the centres controlling movement, such as the cerebellum and thalamic motor nuclei, and the remnants of the tectum. In those fish with some tectum remaining, there may have been some interference with these areas from the remaining tectum, causing difficulty in co-ordination. This may explain the increased activity seen in most animals.

It was frequently noticed that tectumless fish would not react to food dropped into the tank until it was on the bottom, when presumably the smell reached them. It has been noted that Cronly-Dillon et al. (1966) made similar observations on fish with optic tract severed in one brachium only, so that optic input was still going to one half of the tectum, and the geniculate and other nuclei were receiving their input. If Sanders (1940) and Botsh (1960) had controlled for this visual deficit, they might have come to conclusions rather different to those published. These facts emphasise the difficulty of investigating the function of the tectum in more complex behaviour; that behaviour almost inevitably contains a considerable visual component, and the scotoma produced by tectal lesion would affect it.

The optic tectum is not necessary for the learning or retention of simple conditioned reflexes - the work of Sears (1934) and Dijkgraaf (1949a) and results given above show this. Neither is the tectum

/necessary

necessary for the learning or retention of more complex responses to similar conditioned stimuli. Deficits in avoidance in tectumless fish as compared with normal fish, were only seen when there were obvious signs of inability to respond. This inability was not due to blindness, but to some lack of ability to respond caused by the lesion. It would thus seem unlikely that the conditioned reflex and avoidance associations were organised in the tectum. With regard to more complex visual stimuli, the situation is complicated by the blindness caused by removal of the analysis area. Thus Sanders' (1940)and Botsch's (1960) work is inconclusive as to the presence or absence of memory in the tectum. It is reasonable to suppose that the functions of analysis and addressing are connected. Thus a shape is analysed, and this very process serves to address it to the appropriate response systems. The tectum may serve this dual function for visual stimuli. In some theories of retention, the coded result of analysis is seen as being addressed to the appropriate response by a further complex addressing system. Although von Foerster (1965) has pointed out the difficulty of consulting a catalogue sequentially, to achieve this addressing, it is possible that an hierarchical system might serve. There is considerable evidence that such a system operates at the level of analysis (see for example Hubel and Wiesel (1963)), and there is therefore no a priori reason why such should not exist for addressing, so that coded stimuli of different modalities could be linked. The experiment outlined below may allow some statements to be made as to the probability of these hypotheses.

3. Suggestions for further work on the optic tectum.

The way round this impasse may be the technique at present being used in an attempt to gain a less ambiguous estimate of tectal function

/in retention

in retention. This is to train fish, then remove the tectum and test, and test again when the tectum has regenerated, during which period there has been no further training. It now seems unlikely to be one of the functions of the tectum to "read in" or to store the motor information necessary for the performance of tasks, as once seemed possible. It has already been shown that responses can be learned, stored and read out in the absence of the tectum.

There are, three main hypotheses as to the function of the tectum in visual learning, and the experiment to be outlined should allow discrimination between them. The first is that the whole association may be tectally organised; that analysis, storage of the analysed product, and switching to the appropriate response may be located in the area. This is somewhat unlikely on the basis of experiments described above, but there might be separate organisations for simple and more complex visual memories. A second quite reasonable possibility is that the tectum analyses the stimuli it receives, and stores the results of analysis on the basis of their importance to the animal, and only passes on to lower centres analysed and coded versions of stimuli, which make "sense" to the memory only if presented in this form. The third possibility is that analysis occurs in the tectum - this function of analysis would seem to be undoubted - and that this coded message is transmitted to the lower areas of the brain, to the "store", perhaps, using Sutherland's (1964) model, where it can be used by the memory, or to the memory direct. (It is of interest that one of the possibilities outlined here offers the chance of being able to see whether there is an easily-separable "store" as opposed to memory function in the fish brain).

Animals are being trained to discriminate between a triangle and a square, of equal area, presented simultaneously. Controls will be made for preference, and the training biased against the preferred shape (the square). Half the animals will then be lesioned, and half used as operated controls. Retention tests will be run immediately after the operation, and animals will then be left for about 4 months. (It is assumed, of course, that the animals without the optic tectum will show a postoperative deficit. The results of various workers, including Dijkgraaf (see Healey 1957) and Botsch (1960) suggest that such a deficit should occur). The hypotheses will allow the following predictions: in the first case, if the memory is entirely tectal, there should be a total deficit, and re-training after regeneration should take as long as the initial training; in the second, if analysis and storage are tectal, there should be a deficit, possibly not in the mode of response, but in the correct shape to which to respond. The correct coded message from the tectum should not initially be present in this model, and only when this is being transmitted to the memory should the animal be able to show signs of retention of the association; thus in this example, the retraining should be shorter, to a set criterion, than in the previous example, on account of the retention of the code/response association. If, as in the third hypothesis, the analysis alone is tectal, there should have been complete regeneration of the tectum, and consequent total re-integration of tectal function, so that there should be no more deficit in these animals than in the operated controls. One interesting possibility is that if the second of these alternatives should prove to be the case, there would be some evidence for some separation

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of the memory and the store. If the third case should prove true, there would be evidence for separation of the analysis and storage and/or memory functions in the brain.

This experiment would offer the rather unusual example in biology of being able to test an animal with lesion, and after repair of this. It is hoped that by the time this thesis is examined this experiment will have been terminated, and allow some more definite statements to be made as to the functions of the optic tectum in retention.

4. Functions of the forebrain in learning and retention.

Contrary to the expectations of the earlier workers, the forebrain seems to be a much more interesting area than the tectum when considered in relation to the "higher functions" of the brain. There were rather obvious deficits associated with the removal of the tectum, and once these were controlled for there were no signs that this area could be considered to be involved in the elaboration or storage of simple avoidance associations. Visual analysis might well be confined to this area, but there was no evidence that any of the more central mechanisms responsible for these associations lay there.

5. Comparison of results with those of other workers.

Results reported here are generally in agreement with those of other workers in the field. Forebrain removal reduced the activity of animals, as noted by Kumakura (1927) and Janzen (1933). The lesion did not prevent learning or retention of simple conditioned reflexes involving light/shock association. Baru (1955) and Karamyan (1956) both found that removal of the forebrain had little effect on learning or retention of light/shock and sound/shock associations, and on conditioned feeding res-

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ponses. Similarly Bernstein (1962) found that removal of the forebrain did not impair the formation of conditioned heart responses to colour stimuli.

The deficits following forebrain removal found for simple avoidance situations parallel those reported by two other groups of workers. Hainsworth, Overmier and Snowdon (1967) found comparable deficits both in learning and in retention in goldfish trained to avoid light with a 15 second avoidance interval. Their results agree with those outlined in the present work in showing that the lesion has no effect on escape ability, and that the post-operative escape times are no different to those noted pre-operatively. Aronson and Kaplan (1963 and 1965) working with Tilapia macrocephala, found that both learning and retention of light avoidance, reinforced by shock, were impaired after bilateral removal of the forebrain. Aronson (1967) has recently reported that retention of sound avoidance is also impaired by the lesion. In all situations, he reports that the deficit is associated with inability to avoid. Escape times rise rapidly after the lesion, and only fall slowly. This is certainly not the experience with goldfish. In contrast with the two sets of results obtained for goldfish, Aronson finds that Tilapia will attain a level of performance comparable with that found in normal animals. Results presented here, and those of Hainsworth et al. (1967), suggest that there is a long-lasting deficit. The number of post-operative trials given by Aronson (about 100) is similar to that of Hainsworth et al. (100) but less than that given in most of the present experiments.

Whilst there is agreement amongst the results of these workers as

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to the presence of deficits in learning and retention of simple avoidances following forebrain ablation, Ingle (Ph.D. thesis) has found no such effects in goldfish. It is difficult to see where lies the difference in method, but since he gives blocks of upwards of 30 trials per day, it is possible that the lack of effect is a product of this schedule. This cannot be the entire explanation of the differences between avoidance and conditioned reflex results, since whilst Bernstein (1962) and Ingle (unpublished) have used large numbers of trials per day, the Russian workers have used ten or less, as has the present author. Unfortunately, Ingle has never trained animals, then lesioned them; this might have shown up the effects of the damage.

The effects of the lesion on discriminatory responses, both in the "go/no go" situation, and in the simultaneous avoidance situation, agree with those reported by Aronson and Herberman (in preparation). These workers found that removal of the forebrain in <u>Tilapia</u> previously trained to press one paddle (vertical stripes) for food and not the other (horizontal stripes) had the effect of reducing to near chance the overall accuracy of response. These results agree with those obtained here, in that even if responses could occur, they were not necessarily the correct ones. There is some quantitative discrepancy between these results and those of earlier workers, but Janzen's (1933) results suggest some impairment, as do those of Berwein (1941) whilst Nolte's results (1933) need further confirmation. Thus generally there is quite good agreement between results reported here and those of other workers.

6. Lack of deficits due to simple interruptions of motor and sensory modalities.

/The deficits

The deficits caused by the removal of the forebrain were of a subtler and less obvious nature than those caused by removal of the tectum, and it might be supposed that this sort of deficit would be peculiar to memory areas. Thus, for example, it would not be expected that the ability to move and co-ordinate would be affected by interference with a centre responsible for switching in stimulus/response pathways. Evidence has been presented above that animals with total forebrain lesions were as capable as normal animals of performing the actions necessary in avoidance situations, and that their reactions to shock were as great as those of the intact fish. Furthermore, the intensity of this response did not decrease with considerable repetition of shock, so it was unlikely that these animals were showing long-lasting avoidance deficits because they had an increased threshold to shock. Lesioned fish fed normally, and would survive indefinitely; Ingle (personal communication) finds that fish survive less than one month after operation, but the author, in common with such observers as Aronson (1957) has found that unless fungus or other diseases occur, the animals can be kept for many months.

One suggestion (Ingle 1965b) as to the cause of forebrain removal effects was that there was a long recovery period after operation and that if fish were allowed sufficient time, they would perform normally. This was made on the basis of Ingle's work with the spontaneous escape paradigm outlined above. He found that fish tested three days after operation were inferior to normals, but that if fish were left ten days, they were not inferior. This is invalidated with regard to application to other paradigms, since Ingle (1965b) and Bernstein (1962) used recovery periods of less than one day for Y maze training and heart-rate conditioning respectively, and found no effects of the lesion. The effect

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should have been maximal at this point on Ingle's interpretation. Another point is that in the present work fish were trained for up to 40-50 days after lesion; if there had been a drop in lack of reactivity due to recovery from shock, there should have been a corresponding rise in performance - this was not observed. Finally, Hainsworth et al. (1967) left groups of fish for one day, and eight weeks before starting training, and found no difference in learning ability. Post-operative shock can therefore be dismissed as a factor in these results.

In the absence of any direct optic connection, it would seem unlikely that removal of the forebrain would affect vision, and the experimental evidence supports this view. For example, Hale (1956b) and Segaar and Nieuwenhuys (1963) found that fish with total and partial forebrain lesions reacted to stimuli, if not in the appropriate fashion. Similarly Janzen (1933) observed that lesioned fish showed increased optokinetic responses to a rotating vertically-striped drum. The work of the investigators using conditioned reflex techniques has shown that there are few effects on learning and retention of these tasks if the forebrain is removed, and this again supports the non-involvement of the area in vision. For example, Karamyan (1956) and Bernstein (1962), the former using a simple light stimulus, the latter using red and green, and different brightnesses of white, both found no change in performance after removal of the telencephalon. The evidence as regards lack of forebrain involvement in audition is scanty, but to try to prove such direct connection from the study of the anatomy of the brain would be even more difficult than trying to prove a similar point with regard to light. Besides this, it has already been seen that there was a similar deficit in animals trained to avoid light as in those trained to avoid sound. If there were any specific effect on light/sound reception alone, it would hardly be expected that there should be such similar effects on both learning and retention in these groups. Thus in the absence of any definite effects on locomotion, feeding, reactions to shock, vision or audition, it is reasonable to attribute the deficits observed to some central mechanism concerned perhaps with the acquiring, storing or reading out of associations.

7. Facilitation.

The early results obtained in the work reported here suggested that the forebrain might be functioning in some sort of "activator" capacity. Thus in the simple avoidance situations, its function might have been to amplify the commands fed out lower in the brain, so that the animal could react faster. Its effect might have been almost as unspecific as Aronson has suggested. In Aronson's view (1957) the forebrain functions as a non-specific facilitator of brain action organised at lower levels. This hypothesis has often been used to explain the results of various authors, but whilst it provides a relatively easy avoidance of explaining the effects of forebrain lesions, it is hoped that further discussion will outline its shortcomings.

Firstly the term "facilitation" is a loose one, when used with reference to behaviour. Whilst the term is meaningful and precise when used in physiology, there are no bases for its use in animal psychology. If in the absence of any definition of the precision needed, the term is taken to mean "speeding of activity at lower levels" as Aronson (1967) has suggested, then the lack of this general effect should be readily

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observable at all levels of animals' behaviour once the forebrain is removed. If there are only specific deficits, however, there is good reason to assume that some specificity of forebrain involvement in memory may be expected.

It is fairly obvious from results outlined in this thesis, and from the work of the reproductive ethologists, that there are some aspects of behaviour which are relatively little affected by forebrain lesion, whilst others are almost eradicated. Lesioned animals feed, swim, and show certain parts of their social behaviour quite normally, whilst if there are deficits these are in the type of response produced, not in the co-ordination of the response. For example, in Segaar and Nieuwenhuys' (1963) work, fish would produce well-integrated parental behaviour in quite the wrong context. Similar evidence comes from consideration of the behavioural data. The author's results for the effects of forebrain removal on avoidance, those of Hale (1956a) for maze learning, and those of Warren (1961) for reversal, all show a very marked difference between operated and control fish. On the other hand, the results of Karamyan (1956) and Bernstein (1962) for conditioned reflexes, of Janzen (1933) for simultaneous discrimination, and of Ingle (1965b) for position learning show that there are some situations in which operated fish may be much less retarded, and almost as accurate as normals.

Turning from the more elaborate forms of behaviour to the more reflex, much the same phenomena are seen. If there were some non-specific effect, it would be expected that there should be a slowing of such actions as respiratory movements, rate of swimming, rate of response to food and rate of eye movement. Both Janzen (1933) and Hosch (1936) showed that forebrainless fish showed not an overall slowing of respiratory and eye movement rates, but a decrease in the rate of change of these, even though the average might be much the same as in normal fish. Finally, if the effect of the forebrain were entirely efferent, as this hypothesis suggests, it is difficult to see why there should be large ascending tracts, and why, on presentation of novel stimuli, there should be a definite arousal reaction, paralleling that in the tectum.

8. Activation.

It is thus difficult to support the idea of the forebrain simply as a non-specific facilitator of lower centres. Let us turn to the idea of it as an activator, being responsible for amplifying motor orders transmitted from the midbrain. Certainly, such an hypothesis as this would fit the facts observed for the effect of forebrain lesions on the learning and retention of simple avoidance problems, and explain why lesioned fish should take much longer to run a maze after operation. This was the first idea which was suggested after obtaining the avoidance results. The conditioned reflex experiments were then performed, and it was found that there was rapid learning, and fair retention in lesioned fish. These results agreed with those of Karamyan (1956) and Bernstein (1962), and since the activation hypothesis would have predicted deficits in these cases, it seemed unlikely that such an explanation was sufficient. The later results obtained here, which showed that there was interference with discrimination, even if avoidances were occurring, both with bi- and unilateral lesions, supported some more complex explanation. On the ethological side, Segaar and Nieuwenhuys' (1963) results showed that there was some localisation of function, and that there might well be specificity in the function of

/the forebrain.

the forebrain. Nevertheless, whilst the idea of activation alone as the explanation of forebrain function might not seem tenable, the concept may have some use when considered together with another mechanism, to be discussed later.

It is worth examining this idea in some more detail in order that it may lead to the formulation of another hypothesis. The use of avoidance situations and situations where reaction times can be lengthy, such as mazes and approach situations, should show up the effect of activation. Thus it would be supposed that the memory would be intact in the lower parts of the brain, but that the speed of implementation of the response would be lowered. The supposition from this would be that if the response occurred, it should be the correct one, since the appropriate stimulus/response channels would be "trying" to use the alerting facilities of the forebrain. Thus, in the successive avoidance situation used by the present author, as avoidances increased, so, on the basis of this idea, should have overall percentage correct response. If activation occurred, the correct memory trace should gain output, and allow avoidance. There would also be the prediction that since there had been habituation to the non-aversive shape, and since it had been shown that forebrainless fish habituated much faster than normals, there should be no errors to this shape. Both these ideas were proved wrong. Even more definite proof of this separation of activation and discrimination comes from consideration of the results for unilaterally-lesioned fish: in many cases there was a good avoidance ability, but discrimination was poor. Aronson and Herberman (in preparation) have found a similar result using a bar-pressing response in <u>Tilapia</u>. The preliminary results for △ vs □ in BIII also involving approach ~~and~~

/~~food~~

~~food reward~~ support this.

The evidence for simultaneous discrimination situations is rather fragmentary, as has been outlined in the introduction. Janzen's (1933) results and the shape results of Nolte (1933) support the idea that even if more time were allowed for responses, the accuracy of these responses would be less than that of normals. The only other of the older workers to have examined this situation was Hosch (1936) who found no effect of forebrain removal on learning. The sole repetition of work of this sort, though involving operant responses (not too different from the approach responses used by these workers) is that of Aronson and Herberman mentioned above. They showed that discrimination between paddles with vertical and horizontal stripes was poorer and more variable after removal of the telencephalon. Berwein's (1941) results, on the effect of the lesion on the learning of a sequential "go/no go" situation involving heat perception also show that even if the paradigm is not one which places stress on temporal patterns of response, there are considerable effects of forebrain ablation.

Similar conclusions as to the need to suppose greater specificity of effect are seen in the work of the observers who have used maze situations. Zunini (1954) and Hale (1956a) both used simple mazes, involving relatively straight paths without "decision points". The activator idea supports the loss of speed here, but it does not support the deficits found by both Warren (1961) and Ingle (1965b) both using situations which required animals to make turning decisions; both workers found that their operated animals were inferior to controls in their ability to learn the situation, though it is unfortunate that they did not test the effect of forebrain removal on previously-acquired

/associations.

associations.

The data from ethology are harder to interpret. It has already been noted that fish with total or partial forebrain lesions can be aroused by the sight of socially or reproductively significant stimuli, but that their responses may not be those which would be expected in that situation. This type of aberrance, which is seen as well as lack of reaction, agrees with the results reported above. These results do not bear comparison with the training data in that the latter allowed some separation of temporal and choice factors, whereas in ethological situations, the temporal factors are extremely important. Nevertheless, these results are roughly comparable with those of the author for discriminatory avoidance; responses could be made after some time, but these were not necessarily the correct ones.

9. Function of the forebrain in reinforcement.

There is thus need to look for some more specific cause of these effects of removal of the forebrain. It is obviously closely connected with the processes of learning and retention in some way. Hale (1956a) went so far as to state that there was "loss of all previously-formed associations" after removal of the forebrain in animals which had learned to run his maze. Whether or not he intended to imply that the forebrain was functioning as a store of memory, as the statement suggests, this is one possibility that can be discounted with ease. For example, to quote the author's work on simple avoidance situations, it has been shown that both for light and sound/shock associations, there was retention in preoperatively trained animals after removal of the entire forebrain. In addition to this, it has been seen that conditioned reflex training is less affected by removal of the forebrain either before or after training, and that animals seem to be able to learn shape discriminations, showing less deficit in simultaneous than in successive problems.

Despite this, it is obvious that whatever mechanism is involved, it must lie in close functional relation to the learning centres of the animals, and whatever the hypothesis as to the functioning of the forebrain, it must achieve the rather difficult task of explaining deficits in learning as well as in retention. The first suggestion would be that neither the formation nor the retention of stimulus/reward association (i.e. the strengthening of an analyser) is interfered with by bilateral removal of the forebrain. Karamyan's (1956) and Bernstein's (1962) results for conditioned reflex training agree with those of the present author in showing that animals are capable of forming and retaining associations in the absence of the forebrain. Similarly, results described above for simple avoidance suggest that there was some retention of association after removal of the forebrain, and the results of Nolte (1933) support this. Thus it seems unlikely that the stimulus/reward pathways were interrupted. This leads to the consideration that the pathways linking this association with response were interrupted. Such a conclusion is more tenable, but there is then need to explain why certain types of response were affected whilst others were not; it is unlikely that there is a motor output via the forebrain, and the anatomical evidence supports this point of view.

10. Addressing and activation.

One possibility, which seems to be the most likely, is that the forebrain is in some way yet to be specified, concerned with the addressing of reinforcement values to specific stimulus/response associations. This is likely on several considerations. Firstly, the results of the

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ethological workers suggest that forebrain lesions do not inevitably cause overall depression of activity, and that there may be inappropriate but well-developed responses to stimuli. Secondly, the results reported here for discrimination, and those of Aronson and Herberman, quoted above show that responses can occur without their being correct. There is also the evidence from this thesis, and from other workers, that forebrainless fish are more unreactive than normals, and less disturbed by novel stimuli. Finally, there is the physiological evidence both for recording and for stimulation. Enger (1957) and to a lesser extent Schadé and Weiler (1959) showed that arousal reactions to novel stimuli, or to changes in environment were seen in the optic tectum, but that these were paralleled by reactions in the telencephalon. The Russian workers (Voronin and Gusel'nikov (1959) and Maliukina and Flerova (1960)) quoted by Aronson (1963) also found such a telencephalic arousal. Boyd and Gardner (1962) showed that stimulation of the forebrain in free-swimming fish had the effect of causing avoidance of stimulation. (It would be extremely interesting if there were found to be two possible values of such stimulation, located at different parts of the forebrain; this would suggest that both negative and positive reinforcement were being dealt with by this area). These results would suggest that perhaps there might be two activities of the forebrain, a simple "activating" one, seen in responses to novel and significant stimuli, and which would serve to maintain attention, and a more complex one of ensuring correct responses.

The actual stimulus/reward association may take place elsewhere than in the forebrain, and be to some extent separable from the association/response system. On a purely psychological basis, this last idea is not different from that of Behrend and Bitterman (1962) already quoted, as regards learning of avoidances, and would be in accord with Sutherland's (1964) suggestion that analysis and attachment of appropriate responses may be different processes. Thus the relevant analyser system would be strengthened, without the attachment of any definite response. Behrend and Bitterman (1964) showed that if goldfish were given conditioned reflex training, with light/shock association, then transferred to an avoidance situation with the same light/shock interval, there was rapid acquisition of avoidance capacity. This would suggest analyser/response attachment of the type postulated by Mackintosh et al. (1966).

11. A model.

It is possible to envisage two interlinked functions to be present in the forebrain. Firstly an activator one, set in motion by the appearance of significant stimuli or novel situations, and secondly, an address-holding mechanism, whereby analyser and response channels are kept linked during the performance of tasks, and whose integrity is necessary for correct read-out of appropriate responses. Whilst the need to postulate the latter will, it is hoped, seem reasonable on the basis of evidence, it is perhaps necessary to explain why the former should be included, since an earlier section rejected it as the entire explanation of forebrain function.

(The use of the terminology "present in the forebrain" is not meant to imply that it is considered that there must be such circuits present, but that the overall effect of removal of this area is to remove the functions described, whether this be by actual removal of units mediating such effects, or by interference with the circuitry of the entire brain

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to such an extent that these changes are caused at a lower level. As Gregory (1963) has pointed out, removal of areas of brain is a gross procedure, rather analagous to that of removing valves from a radio set, only at a much more complex level. To conclude that since removal of a valve produces a whistle from the loudspeaker, that valve must serve as a "whistle suppressor" is a truism only slightly grosser than the explanation given here of brain function).

As was shown above, arousal occurs in the forebrain even if the stimulus concerned is completely unconditioned; if this function were wholly identifiable with that of addressing, there should be no responses to other than trained or innately-significant stimuli. The data given here for habituation would support this; it has been seen that normal fish will take several hours to habituate to new conditions; they would never have experienced the training box before. Habituation in forebrainless fish was so fast as to be almost instantaneous. Finally, comparison of animals with total and partial forebrain lesions, in the avoidance situations used, shows that though discrimination was impaired in both groups, there was a difference in the ability to make avoidances. There was some slight suggestion of difference in localisation of these functions, but further work is needed to clarify this. Nevertheless, the fact that activation of the animals could occur without there being correct discrimination would provide some evidence for the idea of separation.

As regards the addressing function, the point may briefly be made that it is supposed that since there is some evidence for separate formation of stimulus/reinforcement and stimulus/reinforcement/response associations, there must also be some separate addressing of the

/reinforcement

reinforcement values associated with these. (It should be pointed out here that no assumption is being made as to the laterality of memory, either in the forebrain or in the lower areas; this will be dealt with separately in a later section).

12. Explanation of some of the results on the basis of the model.

Habituation would be rapid in forebrainless fish on account of their lack of arousal, due partly to removal of the activator, and partly to the reduction of the effects of the analysers on the motor areas. In the instance of conditioned reflexes, no response the animals could make would avoid the UCS, so that the addressing system (and hence the forebrain) would not be used, since no response would be preferentially reinforced. Perhaps the analyser system has an unspecific activating effect on the motor centres, and this may be strengthened without the involvement of the forebrain. (In avoidance learning, this would serve to alert the motor centres until the correct response was read out). Removal of the forebrain should affect avoidance learning by preventing the strengthening analyser from being linked to the appropriate response. There should be some retention after removal of the forebrain following training, since the relevant analyser would have been strengthened. Since one channel ("go!") was always in use, there might be more ease in learning in lesioned fish than in the case of discrimination situations, which would require the use of two outputs. The addressing failure would be more obvious here. In the example of simultaneous discrimination, comparison of the "activation" produced by the two analyser systems might allow the animals to make more accurate responses, even if one shape only were addressed to a "go" response in the intact animal.

/13. Laterality

13. Laterality of engram.

The term "engram" is used here to include both the midbrain and forebrain acquired and stored components of the behavioural system under discussion. It seems that the entire memory system is to a large extent duplicated on each side of the body, and that transfer of the relevant information is accomplished via the commissures. Evidence for this lateralisation has been presented in the introduction, on the psychological side from the work of Schulte (1957) and Ingle (1967), and on the neurological side from the work of Mark (1967), and the present author. The correlation of these with the model would be that visual stimulus/reinforcement (analyser) information was transmitted via the tectal or some midbrain commissure, and that the analysis/response information was transmitted via the anterior commissure of the forebrain, together with activation relating to the shapes. The fact that the relearning on the untrained side in fish with forebrain split after training was comparable to the learning of animals trained previously on the motor aspects of the situation would suggest that there was no laying-down of forebrain information on the ipsilateral side, and that transfer occurred by the naive side drawing on the information of the trained side. A similar interpretation is suggested by results reported here for the lateralisation of effect of unilateral lesions.

Ingle (personal communication), and Regestein (1967) have shown that unilateral hypothalamic lesions in goldfish have the effect of producing avoidance deficits on the side contralateral to the lesion, so that there is some evidence for the lateralisation of the lower memories, which was suggested by Mark's results.

14. Evolution of the functions of the forebrain.

In view of the conclusions outlined above, as to the function of

/the telencephalon

the telencephalon, an evolutionary explanation of the development of forebrain function could be drawn, and it was surprising to find how well this agreed with Sherrington's (1906) hypothesis as to the reasons for the development in higher vertebrates of the cerebral cortex from the rhinencephalon. The olfactory sense was probably one of the first senses to be well-developed in early vertebrates, since it provided a large amount of specific information about the environment. Thus it might be assumed that though some light detection system would be present, there would be a tendency in early vertebrates towards being macrosmatic. The esturine theory of vertebrate origins would place more emphasis on the distance sense of smell than on that of vision not only for the reasons of lesser complexity, but also because the water in such areas would be so turbid that the latter sense would not be of much value.

It may be assumed that the primitive forebrain might serve three roles in the capture of prey. Firstly, the analysis and classification of odours detected by the olfactory epithelium. Secondly, an activation of the lower areas of the brain to set in motion the appropriate motor activities, via the already-formed associations in the midbrain. Thirdly, the maintenance of this connection, so that during the pursuit, the animal would keep after its quarry, and when it had received its reinforcement by eating this, the appropriate strengthening of the "attack" channels would occur. When the more complex sense organs of the visual system evolved, it is assumed that it was evolutionarily more economical to use this system, and to generalise between sensory modalities, as has been seen in the introduction can be done, than to develop an identical system for each sensory modality. Thus the fore-

/brain

brain may have come to serve as a motor addressing system and an activator for all the sensory systems of the animals.

Thus even though there has been the divergence mentioned in the introduction, of actinopterygean and higher vertebrate lines, there seems to have been a surprising retention of similarity of function.

15. Possibilities for further work on the telencephalon.

As will have been seen from the introduction, there have been rather conflicting reports as to the effect or lack of effect of forebrain lesions on the learning and retention of simultaneous visual discriminations; the latest work, that of Aronson and Herberman (unpublished) and the author, show that there may be deficits, but there is need to repeat some of the older work. Aronson used the operant technique already described, and the author an approach/avoidance situation, which may not be comparable with the approach situation of the others. Also, the early workers, with the exception of Nolte (1933) failed to give any indication of the size of their lesions, and since Nolte was most definite in stressing lack of effect, it is of importance to duplicate this work, bearing in mind the prediction made from the model that there should be impairment of discriminatory responses. It will also be worth comparing the results from this experiment with those from a similar one using successive rather than simultaneous discrimination where the model predicts that there should be much more impairment, amounting almost to total deficit on average. (Since there would be no cues from simultaneous comparison of the shapes, and feed-out of differential activation from the analysis centres, and since the response would be the same in each case, the animals should "go" or "no go" to the same extent for each shape.)

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A second experiment would be to assess the effect of delayed rewards. If the forebrain does hold open the channels linking analysis and response, as supposed, then there should be far greater impairment of operated than normal animals when confronted with such a situation, since the area responsible for the maintenance of such connections over long periods would have been removed. A further, and related possibility, is that since the forebrain seems to serve as a "holding" system, there may be less effect of lesions if trials are close together than if they are spaced. This may be the explanation of the difference between results reported here and those of Aronson and Hainsworth et al., and those of Ingle. (The former, using about 10 trials per day find avoidance deficits following forebrain removal, the latter, using 30-50 trials per day, does not). It would be interesting to test the effect on learning of massed versus spaced trials, with similar inter-trial intervals. The model would predict greater learning in the former than in the latter group in lesioned fish, though the reverse might well be true in normals, on account of over-training.

A third prediction would be that if the strengthening of the analysis system is separable from the analysis/response system, as is being assumed, then within the limits of loss of discrimination ability caused by forebrain removal on the initial discrimination, there should be no extra difficulty, as compared with normals, in performing generalisations to various shapes. It is assumed that the ability to generalise lies in the analyser part of the system, so that since responses are the same, the differential activation produced by the midbrain will still allow comparison, if that system can itself manage the generalis-

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ation. Since that system will be intact in both normal and lesioned animals, this ability will be the same in both groups, and thus the effect of presenting modifications of shapes should be as predicted.

Another possibility is to test the effect of applying low-voltage stimulation to the forebrain during learning. If this were below the aversive threshold,there might be expected to be some "scrambling" of the analysis/response paths, so that retention could not occur. This could be tested, without stimulation, at a later time. Such an experiment might also allow the static and dynamic effects of the forebrain to be assessed. If the animal was stimulated during learning, and not in the retention test, and showed deficit, it might be assumed that the formation of associations had been affected. If an animal was allowed to learn, then stimulated during retention, and showed no deficit, it might be assumed that there were laid down the appropriate paths, which did not need the mediation of the forebrain for their operation. (In view of the results outlined above this possibility would seem unlikely). The other possibility would be that there would be a deficit; this would suggest that there was a dynamic effect of the forebrain, and that it did not serve merely to establish paths, but to maintain them.

Further work will be done on the effects on discriminatory avoidance of unilateral forebrain lesions. It may be possible to discriminate between forebrain areas mediating alerting and avoidance, and those which allow the correct response to be made. In the results outlined above for the preliminary studies, it has been seen that certain lesions, in the central and lateral areas, sometimes have had the effect of interfering with discrimination whilst allowing avoidances to occur. At one point it seemed possible that the commands were transmitted via the lateral forebrain bundles, and that the correct addressing was the responsibility of the central area; lesions to the latter area would allow activation, but the wrong connections were being made. As will have been seen from consideration of the lesion extents, this does not seem to hold in all cases, but it is hoped that with more animals, and finer lesions, some localisation of function may be found.

One last possibility, which seems to have been ignored by all workers in the field, is that there may be hormonal influences on behaviour, resulting from interference with the pituitary of the lesioned animal. Brief mention was made in the introduction of the anatomical connection of the forebrain nucleus preopticus pars magnocellularis with the pars nervosa of the pituitary, and of Kandel's (1964) findings of the effects on the nervous activity of its axons of stimulation of the olfactory organs. Although there was put forward evidence which suggested specific neural mechanisms, for example, the effects of the lesion on one type of behaviour but not on another, even when the same degree of stress was involved (e.g. conditioned reflex versus avoidance conditioning) there is need for definite proof that hormonal influences are not playing a major part in producing the deficits observed. It is difficult to devise a relatively simple experiment which would give proof of this, since the effects of hormones such as thyroxin are so general that it would be expected that almost all the animal's activities would be affected, and it would not be possible to suppose some specific action on the nervous system. Nevertheless, it may be worth trying the effect of some of the hormones most possibly involved in "activation", such as thyroxin and the catecholamines, to see whether or not they have any effect both on activation ability and, more important, on accuracy of discriminative responses. If there were some depletion of hormone reserves responsible for the effects of forebrain removal seen above, there should be greater deficits in learning in fish trained long after lesion (when the pituitary reserves would be exhausted) than immediately after lesion. Hainsworth et al. (1967) showed that for survivals of 8 weeks and 1 day this was not so. This gives some reason to suppose lack of hormonal cause of forebrain effects.

16. Conclusion.

As regards investigation of the "memory" proper, the stimulus/reinforcement association centre, even if "centre" is the correct concept, there would seem at present few hopeful signs. From evidence presented here, it is suggested that this is not organised for the paradigms considered in either forebrain or optic tectum, and that for the moment, until there is proof to the contrary, it is reasonable to eliminate the cerebellum. It is possible, in view of the confluence of tracts from many regions in the thalamus and the known reinforcement importance of the hypothalamus (in higher vertebrates) that it is here that an association centre may lie. For example, there is in the dorsal hypothalamus a nucleus known variously as the nucleus rotundus, glomerulosus, or hypothalamicus anterior. This is a bilateral body, with numbers of large cells surrounded by quite dense nueropil; it has connections with the tectum, anterior cerebellum, from the medial and lateral forebrain bundles, and the lower hypothalamus. Although its connections are only expressed here in terms of tracts connecting areas, since in the absence of any reliable degeneration staining technique in fish it is not possible to be specific about direction in this region of the brain, the connections at least suggest that there is correlative

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action of some importance proceeding in this area.

There have as yet been few attempts to investigate the regions of thalamus and hypothalamus; the work of Ingle (unpublished) and Regestein (1967) suggests that there are deficits in learning after suction-ablation lesions, but unfortunately these workers have never used the more informative technique of training, then lesioning. Perhaps it would be more surprising if lesions in these regions failed to show deficits (Savage (1967)) since if it is supposed that the association centres lie in these regions, there must be widespread and unspecific damage to so many parts of these that their continued functioning must be well-nigh impossible unless the lesions are of an accuracy and fineness as yet unused in fish, and in any case the area is one of such general importance as to cause hasty death of lesioned fish. Whilst the use of the electrolytic lesion technique elaborated by the author provides the possibility of making small lesions, there is as yet no method for accurate placings, so such lesions would seem too inaccurate for the approach to prove fruitful. If the hypothesis be true of analysers serving also as addressing systems, then the search for the association centres is even more fruitless, since destruction of the analyser will destroy the addressor.

It is on account of the difficulties outlined here, both of method, and even more of interpretation, that it may be more useful to confine research to more peripheral systems, such as the forebrain, in the hope that some insight may be gained into the working of these with respect to the association centre(s). There is also need for some work on the functions in learning and retention of responses of the cerebellum. Lesion methods are suited to trying to examine the outlying systems of

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the memory, but the central area(s) would seem so complex that this crude technique is not adequate. Perhaps if recording electrodes could be directed there, it might be possible to gain some idea of the changes involved, but for the moment, the "simple" systems of the outlying parts of the brain are likely to provide more information as to the higher integrative action of the nervous system.

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