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INTRODUCTION

A STRIKING FEATURE in the neurology of teleostean fishes is that in at least some species the entire forebrain or the entire body of the cerebellum can be removed without causing any noticeable changes in behavior. Equilibrium, locomotion, feeding, and responses to gross stimuli appear to be unaffected by the operation. This phenomenon was well known to early experimentalists and led some investigators (e.g., Vulpian, 1886) to conclude that the teleost forebrain (apart from its olfactory function) played no role in behavior.

As a wider variety of techniques for the analysis of behavior were utilized, deficits in the behavior of forebrainless fish became apparent. In fact, the pendulum has swung in the opposite direction, and the forebrain is now said to be involved in a wide variety of behaviors, including orientation, schooling, reproduction, parental care, aggression, social facilitation, as well as the acquisition and performance of conditioned responses. These disparate functions ascribed to the forebrain led to the hypothesis of an underlying, unitary forebrain mechanism (Aronson, 1948, 1957, 1967). According to this hypothesis, the forebrain serves as a non-specific arousal mechanism that energizes behavioral processes organized in the midbrain and lower centers.

The cerebellum of mammals is concerned extensively with motor and proprioceptive functions, and because the histological structure of the cerebellum in all vertebrates is similar, some investigators have assumed that the functions must also be similar. This view of cerebellar function is obviously at variance with the findings of a number of investigators (Aronson, 1963), that the entire body of the cerebellum of fishes can be removed without causing obvious changes in locomotion and balance. On the other hand, Franz (1912) and Karamian (1956) have postulated that the cerebellum of teleosts has integrative and associative functions and is concerned with the establishment of conditioned responses.

The present series of experiments was designed to examine the role of the forebrain and cerebellum in the acquisition and performance of a conditioned avoidance response using light or sound as the conditioned stimulus. To accomplish this the following experiments were performed:

Experiment I: The effect of forebrain ablation on the acquisition of a conditioned avoidance response with light as the conditioned stimulus (operation before training).

Experiment II: The effect of forebrain ablation on the acquisition of a conditioned avoidance response with sound as the conditioned stimulus (operation before training).

Experiment III: The effect of forebrain ablation on the performance of a conditioned avoidance response with sound as the conditioned stimulus (operation after training).

Experiment IV: The effect of ablation of the corpus cerebelli on the acquisition of a conditioned avoidance response with light as the conditioned stimulus (operation before training).

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REVIEW OF LITERATURE

FUNCTIONS OF THE TELEOSTEAN FOREBRAIN

Anatomical Considerations

For many years the early neuroanatomists had categorized the teleost forebrain as an olfactory mechanism because of its close relationship with the olfactory mucosa and its apparent isolation from other sensory systems. Herrick (1922), while still emphasizing the olfactory connections, noted that parts of the cerebral hemispheres did not appear to receive secondary olfactory fibers. Much later, Aronson (1963) observed, particularly in those teleosts with highly specialized forebrains, that the greater part of the teleost pallium is devoid of secondary olfactory fibers. This has recently been confirmed by Nieuwenhuys (1967a) and is consistent with the conclusions of the many experimentalists who found that the forebrain has functions other than olfaction.

In the discussion that follows, the major divisions of the forebrain or telencephalon will be referred to as the "olfactory bulbs" and the "cerebral hemispheres." The latter are often termed "olfactory lobes" or "corpus striatum," especially in the older literature.

Electrophysiological Studies

Although ablation of part or all of the forebrain has been the most widely used method of studying forebrain function, bioelectric stimulation and recording have also been employed. Chauchard and Chauchard (1927) and Springer (1928) established that electrical stimulation of the cerebral hemispheres causes no motor responses. They showed that body movements of various kinds previously attributed to such stimulation were, in fact, due to spread of current to other parts of the brain.

Similarly, potentials evoked in the hemispheres by stimulating the eye of carp with light, as reported by Maliukina and Flerova (1960), were found to be artifacts by Voronin and Gusel'nikov (1958, cited by Kholodov, 1960) and Gusel'nikov, Onufrieva, and Supin (1964). The latter reported evoked potentials in the hemispheres only in response to stimuli arising from the olfactory tracts. Enger (1957), who occasionally observed evoked potentials in the cerebrum of codfish following photic stimulation, recognized that these might be artifacts. He did find an "arousal" reaction in the EEG recorded from the codfish cerebrum in response to light. Schadé (1959) and Schadé and Weiler (1959), however, were not able to find this response in goldfish.

Behavior Unaffected by Ablation of Forebrain

Extirpating the forebrain did not overtly change feeding, postural, or locomotory behavior of elasmobranchs (Steiner, 1886a, 1886b; Polimanti, 1913; Rizzolo, 1929) or of teleosts (Nolte, 1932; Hosch, 1936; Meader, 1939a; Aronson, 1948, 1957; Kamrin and Aronson, 1954; Hale, 1956a). Similarly, no changes in the acquisition or performance of conditioned responses were noted (Scharrer, 1928; Nolte, 1932; Hosch, 1936; Noble, 1936; Berwein, 1941). More recently, Karamian (1956), using a classical conditioning technique, and Kholodov (1960), using operant conditioning (bead-pulling), also found no qualitative or quantitative differences in performance between intact and forebrain-ablated goldfish and carp.

Role of the Forebrain in Behavior

As the techniques for behavioral investigation became more sophisticated an increasing body of work emerged, suggesting that the forebrain in various teleosts played an important role in behavior. In some instances the investigators concluded that this role was a direct one involving mediation of specific behavior patterns. Other investigators concluded that the forebrain is not involved in behavioral organization; rather, its function is one of arousal or facilitation.

Mediation of Specific Behavior Patterns

Since Striek (1924) determined that blinded, forebrainless minnows could no longer discriminate between odors but could still make taste discriminations, it has been assumed that the forebrain is responsible for the integration of olfactory information. Johnston (1911), Herrick (1922) and Papez (1929) considered the forebrain a center for correlation of taste and smell, and this hypothesis received support from Wrede (1932), who showed that blinded, forebrainless

minnows could not distinguish between tastes when the discrimination was normally made on the basis of taste in conjunction with olfaction. It should be emphasized that in these studies there were no control tests using olfactory bulb-ablated subjects. Thus the possibility exists that the integration may be taking place in the olfactory bulbs as well as in the remainder of the forebrain.

In recent electrophysiological studies, Hara, Ueda, and Gorbman (1965), and Ueda, Hara, and Gorbman (1967) obtained characteristic EEG responses from the olfactory bulbs when water from the home pond was infused in the nostrils of silver and chinook salmon. The response to home water is not evoked by strange water from other ponds. This discrimination between home and non-home spawning water persists after olfactory tract section (Gorbman, personal communication), indicating that at least some of the associations involving olfactory stimuli are mediated by the olfactory bulbs themselves.

Many investigators have reported changes in species-typical behavior patterns after ablating or placing lesions in the cerebral hemispheres, and a few have assigned a major role to the cerebrum in the coordination or integration of these patterns. Noble (1936, 1937, 1939) and Noble and Borne (1941) studied reproductive, parental, schooling, and fighting behavior in several cichlid, cyprinodont, and anabantid fishes. In *Hemichromis bimaculatus* and *Tilapia heudelotii macrocephala*, ablation of the forebrain (incorrectly called corpus striatum) resulted in such widespread and permanent deficits that the operated fish were unable to successfully mate or brood. Smaller lesions in the hemispheres resulted in deficits involving fewer components of these activities. Since different lesions produced the same deficit, and since different deficits were noted in the behavior of the same operated fishes during successive breeding periods, we find little evidence for localization of function in these studies. In species such as *Xiphophorus helleri*, where there is less need for synchronization in the mating patterns, nearly complete ablation of the forebrain interfered with, but did not prevent, normal mating. In all species studied by Noble, fighting patterns, which normally differ according to specific social situations, were markedly altered by forebrain ablation, but no reduction in vigor was noted. In cichlid fishes, schooling was permanently affected by forebrain removal. Noble concluded that the forebrain regulates social behavior by integrating the timing, balance, and form of the components of complex behavior patterns (Noble, 1936, 1939).

Similar conclusions were reached by Schönherr (1955) working with the three-spined stickleback, *Gasterosteus aculeatus*. He found that ablating the olfactory bulbs, unilateral ablation of the forebrain, or bilateral removal of just the rostral portion of the forebrain had no effect on reproductive behavior. Ablating the entire forebrain, however, resulted in such lack of integration and coordination of certain reproductive patterns of both males and females that normal courtship and mating did not occur. Schooling and aggressive behavior were similarly affected by forebrain ablation.

Segaar (1961, 1965) and Segaar and Nieuwenhuys (1963) have postulated very specific functions for localized areas of the cerebral hemispheres based on their detailed and extensive studies with *Gasterosteus aculeatus*. These deal with the relative changes in the aggressive (A), sexual (S), and parental (P) components of behavior of intact and forebrain-lesioned males during the two-week breeding cycle. During different stages of this cycle, the motivational levels of A, S, and P were found to vary in specific ways in the intact male. After operation the relative balance of these three tendencies was disturbed. Removal of rostral or lateral parts of the forebrain led to a change of balance in favor of parental care. Mediocaudal lesions led to increased aggressive behavior, continuous sexual behavior, and decreased parental care. More discrete lesions resulted in more discrete disturbances, leading Segaar to conclude that specific inhibitory and excitatory centers concerned with parental fanning exist in the dorsomedial nucleus of the cerebral hemispheres. In summarizing his findings, Segaar (1965) stated that the cerebrum is probably concerned both with integration of activities, as in nest-building, and with facilitation of activities, as in courtship and aggression.

FACILITATION OR AROUSAL FUNCTION OF THE FOREBRAIN

In most of the experiments reported in the literature, ablation of parts or even of all of the forebrain did not result in the loss of any partic-

lar behavior. Rather it resulted in changes in the frequency of occurrence of certain behavioral components or in a loss of efficiency, or synchrony. This led some investigators to conclude that the forebrain is not involved in the organization of specific behavior patterns. Rather it was postulated to have an arousal or facilitatory function.

Janzen (1933) was probably the first to attempt to conceptualize this aspect of forebrain function, which he construed as "initiative." He noted the following changes in goldfish after ablation of the forebrain: (1) opercular and eye movements became more uniform after operation; (2) operated subjects would follow the stripes in an optokinetic apparatus more consistently and for longer periods of time; (3) whereas intact fish would readily negotiate between the bars of closely spaced lattice work that divided the tank, forebrainless fish would not; (4) when given a choice of swimming from a main tank to a light or a dark chamber, intact fish exhibited definite individual preferences, while forebrainless fish tended to swim toward the same level of illumination from which they had started; (5) operates were "less cautious" in new situations; (6) whereas both intact and operated fish could learn to discriminate colors (using food as reinforcement), the latter took somewhat longer and "were not as certain in making their selection . . ."

Similar results were reported by other investigators. Changes in degree of locomotion were found in *Conger* (Polimanti, 1913), in minnows, *Phoxinus leavis*, and in gudgeons, *Gobio fluviatilis* (Hosch, 1936). Janzen's observation of the forebrainless fish's "lack of caution" was confirmed by Hosch (1936) who found that operated fish came out of their hiding places more readily and ate more quickly than intact fish in unfamiliar surroundings. Hosch also reported more uniform eye and opercular movements and similar optic rotary nystagmic behavior in his subjects.

Various investigators report changes in social behavior after forebrain ablation. Kumakura (1928) and Hosch (1936) found temporary deficits in schooling behavior in goldfish and minnows. Berwein (1941) reported that although forebrainless minnows schooled again several days after the operation, they accepted a strange fish into the school more readily than did intact fish. More permanent deficits in schooling were found in the jewel fish, *Hemichromis bimaculatus* (Noble, 1936), and in the percoid fishes, *Box salpae* and *Smaris alcedo* (Wiebalck, 1937).

A number of investigators (e.g., Shlaifer, 1939) have shown that the oxygen consumption of a school is likely to be less than the total consumption when each fish is measured in isolation. This is known as the "group effect." Koshtoiants, Maliukina, and Aleksandriuk (1960) showed that after forebrain extirpation in *Smaris smaris*, *Phoxinus phoxinus*, *Mullus barbatus*, *Gobius niger* and *Carassius carassius*, the "group effect" disappears.

As reported earlier, the forebrain has been implicated in the mediation of aggressive and reproductive behavior (e.g., Noble, 1936, 1937; Noble and Borne, 1941; Schönherr, 1955; Segaar, 1961, 1965). Aronson (1948, 1957, 1967) and Kamrin and Aronson (1954) have suggested that their results and possibly those of other investigators can be understood better by viewing the forebrain as a non-specific arousal mechanism rather than as a center for the organization of the behavior patterns. Aronson reported that although the frequency of performance of components of species-typical patterns of reproductive behavior declined after extirpation of the forebrain, the patterns themselves were not disrupted. He also pointed out that few, if any, behavior patterns are lost after forebrain ablation but they are performed less frequently or less efficiently (Aronson, 1963, 1967).

Other investigators have found this hypothesis useful. For example, Hale (1956a) noted a marked decrease in aggressive behavior in the sunfish, *Lepomis cyanellus*, after forebrain ablation. Although the fighting itself was normal in all respects, higher intensity stimuli were required to elicit fighting. He concluded that the behavior is organized at lower brain levels and that the forebrain facilitates the behavior.

Recent investigations have disclosed serious decrements in learning following forebrain removal, and some of these findings are interpreted in terms of the arousal hypothesis. Hale (1956b) found that grouped and isolated forebrainless sunfish responded much more slowly in a Welty-type maze than did corresponding intact fish. In addition about 50 per cent of the operates showed no evidence of learning in contrast to about 17 per cent of the controls.

Warren (1961) tested paradise fish, *Macropodus opercularis*, in a series of *umweg* (detour) problems and in a T maze involving reversal learning. He reported that extensive forebrain damage impairs *umweg* and reversal learning without producing gross sensory or motor deficits. He suggests that his findings are compatible with Aronson's (1957) hypothesis that the forebrain exerts a general non-specific facilitating effect on lower centers in the teleost brain. Savage (1968) has recently reported similar deficits in avoidance learning in forebrainless goldfish, which he also attributes to a decline in arousal or attention in these subjects.

Aronson and his co-workers have also investigated the role of the forebrain in learning. Aronson and Herberman (1960) readily trained West African mouthbreeders, *Tilapia heudelotii macrocephala*, to strike a small paddle in order to obtain food. Response latencies were soon reduced to a few seconds, and performance was very consistent. After ablation of the forebrain, response latencies rose perceptibly and performance became extremely variable. Following several months of testing, improvement was noted in some of the subjects.

Using a similar paradigm, Kaplan and Aronson, in a preliminary study (unpublished), trained *Tilapia* to discriminate between a vertically-striped and a horizontally-striped paddle. Before operation, the subjects responded during each trial with latencies generally less than three seconds. After training, at least 80 per cent of the responses were correct in every session. Ablation of the olfactory bulbs had no effect on the number and latency of responses, and only a slight, transitory effect on the percentage of correct responses. Ablation of the forebrain, however, had a drastic effect on all measures. The number of responses dropped precipitously and response latencies soared. The percentage of correct responses also dropped, but was extremely variable from session to session. Again, improvement was noted in some subjects with continued testing, but in others, performance continued to decline.

Aronson and Kaplan (1963) and Kaplan and Aronson (1967), using an avoidance conditioning paradigm, trained *Tilapia* to swim through a hole in a partition in the test tank (shuttle box) to avoid electric shock. Light was used as the conditioned stimulus (CS). With training, intact fish learned to respond with considerable regularity, and achieved at least 80 per cent avoidances in almost all sessions. Ablation of the olfactory bulbs did not affect performance. After ablation of the entire forebrain, however, the number of avoidances was markedly reduced, response latencies were higher, and performance became extremely erratic. Qualitative observations revealed that the operated fish no longer maintained efficient intertrial habits, such as waiting near the hole for the onset of light. Responses to the CS were neither so immediate nor so well oriented as before, and the swimming speed of some subjects seemed considerably less. As in the "approach" experiment (Aronson and Herberman, 1960), there was evidence of improvement in some subjects with continued testing. When the forebrain was ablated prior to training in three subjects, only one fish learned to avoid with some regularity, after an extensive training period. The results of these experiments were interpreted as further evidence of a nonspecific arousal function in the forebrain. Ablation of the forebrain in goldfish (Hainsworth, Overmier, and Snowdon, 1967) resulted in similar deficits in avoidance conditioning, which were also attributed to deficiencies in the arousal mechanism.

Other kinds of conditioning techniques also revealed deficits in forebrain-ablated subjects. Ingle (1965), testing Janzen's generalization (1933) that forebrainless goldfish lack "initiative," found that forebrainless subjects tested between three to five days postoperatively were clearly inferior to controls in spontaneously escaping from a small enclosure into a large tank. However, lesioned fish tested ten days after operation did not differ from controls. He also reported that forebrainless fish were inferior to controls in learning a left-right alternation problem but, paradoxically, were superior in learning a simple positional habit. He suggested that this could result from a reduction in forebrainless fish of the normal tendency to vary turning direction following several repetitive choices. Malyukova (1964) caused "distortion" of a previously learned shape discrimination in goldfish by stimulating the cerebral hemispheres with a weak electric current or by coagulating parts of the hemispheres. She reported that the conditioned response was restored in from one to three weeks.

The gradual improvement or recovery of function reported in some of these experiments

suggests that a compensatory process takes place in which other areas of the brain, at least partially, make up for the missing functional contribution of the forebrain. Aronson (unpublished) suggests that the cerebellum may be responsible for this compensatory process.

FUNCTIONS OF THE TELEOST CEREBELLUM

General Considerations

Although the mammalian cerebellum has been the subject of a vast amount of research, and much is known of its gross morphology as well as its histological structure, little is known about the way in which it actually functions (Bell and Dow, 1967). Even less is known about the function of the fish cerebellum.

The histological structure of the cerebellum is remarkably similar throughout the phylogenetic series (Larsell, 1967). Thus the fish cerebellum, like the cerebellum of other vertebrates, is composed of molecular, Purkinje, and granular layers. In gross morphology, however, the teleost cerebellum exhibits a unique specialization, the valvula, which projects anteriorly from the body of the cerebellum (corpus cerebelli) and occupies most of the optic ventricle. It should be noted that there is tremendous variation in the external morphology of the cerebellum in different species, not only in size but also in the relative proportion of its parts (Karamian, 1956; Rüdeberg, 1961; Aronson, 1963; Larsell, 1967).

Posture, Equilibrium, Locomotion

Eccles (1965) suggested that the cerebellum originally developed in relation to the vestibular and lateral-line receptor organs of primitive vertebrates. This concept corresponds with the classical picture of the cerebellum, so succinctly labeled by Sherrington as the "head ganglion of the proprioceptive system" (Sherrington, 1947). It has long been known, however, that in fishes, all of the sensory systems (with the possible exception of olfaction) have representations in the cerebellum (Franz, 1912; Larsell, 1967). Recent neurophysiological investigations (reviewed by Fadiga and Pupilli, 1964) have revealed cutaneous, auditory, visual, and cerebral-cortical representations in the cerebellum of mammals as well. These findings have led to some consideration of a broader view of cerebellar function. As Karamian (1956) has stated, "The cerebellum is undoubtedly closely connected with proprioceptive systems and with the vestibular apparatus and regulates their function, but this is not its only role."

With respect to the role of the cerebellum in the maintenance of posture and equilibrium and the regulation of locomotion, we find ourselves confronted with a number of experiments whose results are largely contradictory. Some studies indicate that there are no disorders following extirpation of the cerebellum (Baudelot, 1864 cited by Ten Cate, 1935; Vulpian, 1866 cited by Healey, 1957; Steiner, 1888; Corso, 1895 cited by Healey, 1957; Loeb, 1900; Polimanti, 1912a, 1912b; Aronson, 1948; Dijkgraaf, 1949). On the other hand, other studies show that cerebellar ablation does produce disorders of equilibrium, locomotion, and muscle tonus (Reisinger, 1919, 1926; Tuge, 1934; Karamian, 1956). In reviews of this material, Ten Cate (1935), von Buddenbrock (1953), Karamian (1956), Healey (1957) and Aronson (1963) pointed out that these contradictory findings may be the result of: (1) the lack of histological verification of the lesions, and (2) the use of different species as experimental subjects. With respect to the former, the possibility exists that in some instances variable amounts of cerebellar tissue were left, or, on the other hand, that other parts of the brain were inadvertently damaged. In some experiments, investigators failed to mention whether ablation of the cerebellum included the valvula or whether ablations of the body of the cerebellum included removal of the eminentia granularis. With respect to the second point, Karamian (1956), for example, obtained different results with different species after performing similar cerebellar ablations.

The basic similarity in the histology and connections of the cerebellum of all vertebrates led Healey (1957) to suggest that some of the basic functions of the cerebellum in mammals may also be found in fishes. According to Clark, Chung, Shine and Clark (1960), it appears that the cerebellar contribution is one of maintaining the proper posture in space by receiving impulses from a variety of sensory sources and sending the information necessary for synergistic action to the muscles. Thus, the function of the vertebrate cerebellum is not to pattern muscular responses but to facilitate their execution through the utilization of all available sensory experiences (Herrick, 1948). This concept of cerebellar function has received some experimental sup-

port, but it must be emphasized that there is a large body of evidence suggesting that this is not its sole function.

Associative and Integrative Functions

Franz (1912) was the first to suggest that the teleost cerebellum had associative and integrative functions analogous to those of the cerebral cortex of mammals. His hypothesis was not borne out by experiments in which minnows, following partial cerebellar ablation or lesions, could still be trained to make a conditioned response with sound and color as the conditioned stimuli (Dijkgraaf, 1949) or still retained a previously acquired conditioned response to color (Nolte, 1932).

Karamian (1956) on the basis of his own and other experiments presented a concept of cerebellar function similar to Franz's. He proposed that in teleosts the cerebellum is responsible for the establishment of "temporary connections" (conditioned responses), particularly those involving vision and hearing. His experiments involved removing the entire cerebellum or only the body of the cerebellum in goldfish and carp before or after training. He used a classical-conditioning paradigm in which electric shock was paired with a light or bell. When the entire cerebellum was removed prior to training, Karamian was unable to condition his subjects. When the entire cerebellum was ablated after the the response had been acquired, all evidence of conditioning disappeared.

When only the body of the cerebellum was removed, the outcome was variable. Karamian gave the results of only two subjects. In one, the conditioned response could not be acquired with any degree of stability during the one month of testing. In the other, the response was acquired in this period of time.

Karamian embarked on these experiments because he had unexpectedly found deficits in vision and hearing following removal of the cerebellum. To further elucidate the cerebellum's role in visual processes, he studied the effects of photic stimulation on the EEG of carp. Distinct changes were found in the electrical activity of the cerebellum after such stimulation, whereas no corresponding changes were noted in the forebrain. He therefore suggested that in teleosts the cerebellum and the midbrain are the main areas concerned with the formation of temporary connections as well as with motor functions, which in higher vertebrates are taken over by the forebrain.

Other Russian investigators offer further evidence for this hypothesis. Gusel'nikov and Ivanova (1958) also reported changes in the electrical activity of the cerebellum of carp in response to visual or auditory stimuli. Photic stimuli evoked a diphasic response plus an acceleration and increase in amplitude of the slow waves, whereas the response to sound was characterized only by the latter. Bianki (1963) reported that the ability of carp to form stable conditioned responses, when the unconditioned stimulus (US) was shock and the CS was elevation of pressure inside the swim bladder, was impaired after extirpation of the entire cerebellum or of either the body or the valvula. The responses were not affected by extirpating the forebrain. This investigator also reported that loss of half of the cerebellum precludes "acoustic space analysis" and concluded that the cerebellum takes part in acoustical conditioned responses (Bianki and Demina, 1963). Malyukova (1964) reported a drop in "food excitability and motor activity" followed by a loss of "motor food conditioned reflexes" after unilateral coagulation of the valvula.

Evaluation of these experiments is difficult since we often have access only to English summaries, abstracts, or review papers; and even here, the translations may be inadequate and the use of specialized terminology unclear. However, the results suggest that at least in some species the cerebellum plays an important role in the acquisition and performance of conditioned responses. Additional evidence is supplied by Aronson and Herberman (1960), who found that after ablation of the body of the cerebellum, fish that had been trained to strike a paddle in order to obtain food continued to respond, but with longer latencies. Removal of both the cerebellum and forebrain resulted in even longer latencies. The authors suggest that just as a basic function of the forebrain in fishes is the facilitation of responses organized in other parts of the brain, the cerebellum may have a comparable energizing action.

EXPERIMENT I—THE EFFECT OF FOREBRAIN ABLATION ON THE ACQUISITION OF A CONDITIONED AVOIDANCE RESPONSE WITH LIGHT AS THE CONDITIONED STIMULUS

MATERIALS AND METHODS

Subjects and Maintenance

The subjects were nine male and three female West African mouthbreeders, *Tilapia heudelotii macrocephala*, according to the orthography and specific designation of Thys van den Audenaerde (1964). They were selected on the basis of weight (between 9 and 11 grams) and healthy appearance from stock tanks of the American Museum of Natural History where they had been randomly bred for more than three decades. The subjects were individually housed in 2½-gallon aquariums filled with conditioned water that was continuously filtered through Dacron wool. The fish were maintained on standard Museum diet (Gordon, 1950) and weighed at regular intervals to ascertain if they were gaining normally. Water temperature was maintained at 80° ± 5°F. at all times. Tests were conducted in a separate room in which the test-tank water was similarly maintained at approximately 80°F.

Apparatus

The test apparatus consisted of a 35 by 20 by 25 cm. aquarium, divided by a translucent Plexiglas partition into two equal compartments. A black-rimmed hole, 4.2 cm. in diameter and situated 5.5 cm. from the bottom of the partition, provided the only access from one compartment to the other. Each compartment was equipped with its own set of electrodes. A 16 by 19 cm. stainless steel electrode plate was placed against each rear wall and a stainless steel coarse grid electrode that permitted observation into the test tank was placed against each front wall. The electrodes were connected to a 60-cycle sine wave shock stimulator that provided a continuous range of A.C. voltages. A 0.1-second digital timer, controlled by the stimulator switch, indicated the response latency.

The back and side walls of the test tank were painted light blue except for a 5-cm. clear, circular area centered on each side wall. Light from 40-watt lamps housed outside each side wall could be directed via a toggle switch, into one or the other compartment; thus each compartment could be illuminated and provided with shock independently.

Preliminary and Surgical Procedures; Grouping

All subjects had a preliminary training period of four to six sessions under actual test conditions, except that the light and shock were omitted. During this period the fish were trained to swim from one compartment to the other. A small fish net was used to guide them through the hole. When the fish had acquired relative proficiency in crossing, they were paired according to median latencies and speed in acquiring the response. One member of each of five pairs was assigned to the experimental group. The remaining member of each pair was designated either as a sham (four subjects) or as an olfactory bulb control (one subject). Both members of the sixth pair were designated as olfactory bulb controls. The experimenter knew which individuals had been paired but not the group to which each had been assigned.

Surgical procedures for all subjects were similar to those described by Kaplan and Aronson (1967) except that a 2.5 per cent solution of urethane was used as the anesthetic. In sham operates the entire surgical procedure was performed but the brain was left intact.

Training and Testing Procedures

The subjects were allowed several days to recuperate from the effects of the operation before their training was initiated. They were given five minutes to become habituated to the test tank prior to each session. All test observations were made from behind a curtain in which a small, rectangular window had been cut. To start a trial, the experimenter illuminated the compartment in which the fish was situated. This constituted the conditioned stimulus (CS). Intermittent shock (unconditioned stimulus—US), was automatically administered 2½ seconds later. Each shock pulse lasted 1.5 seconds with a 1.0-second interval between pulses. The voltage level ranged from three to five volts and was individually adjusted to produce moderate activity in each subject. A trial was manually

terminated either after a subject had completed a response or 30 seconds following the onset of the CS. Each trial lasted a maximum of 30 seconds. Three types of responses were possible: avoidance, escape, and "no crossing." A subject could "avoid" being shocked by swimming through the hole to the opposite compartment in response to the onset of light, during the 2.5-second CS-US interval. Thereafter, it could "escape" from the shock by swimming through during the remainder of the trial. If a fish neither avoided nor escaped, the response was termed "no crossing" and the latency scored as 30 seconds.

Subjects were given 15 trials a day (one session), five days a week. If, however, a subject made five consecutive "no crossings" during the first 10 trials or three consecutive "no crossings" after trial 10, the session was terminated to avoid injuring the fish by overshocking. The intertrial interval was designated as approximately 30 seconds. This was later changed to randomly determined intertrial intervals varying from 25 to 45 seconds.

Initially, the fish were guided through the hole with a small fish net during certain trials as prescribed by the following standard for net guiding:

1. Initial Sessions
   Trial 1: No guiding.
   Trials 2–15: Fish may be guided after each trial in which there is a "no crossing." After two or more unguided escapes are made during one session, the standard for subsequent sessions is followed.
2. Subsequent Sessions
   Trials 1 and 2: No guiding.
   Trials 3–15: If both trials 1 and 2 are "no crossings" trial 3 is guided. Subsequent trials are guided after three consecutive "no crossings."
3. Timing for All Guided Sessions
   If fish is nowhere near the hole, guiding is initiated 10 seconds after onset of the trial. If the subject is near the hole, guiding is initiated after 20 seconds.

Guiding was discontinued and regular testing began as soon as the fish made several unguided escapes from each compartment during a session. Net guiding was not resumed unless the subject showed signs of extinguishing the escape response. In such instances, the subject was guided until the escape response was reinstated, and data from the additional guided sessions were not included in the group figures. The following are definitions of the criteria used in the analysis of the data in this and in subsequent experiments:

1. Net guiding terminated: as soon as several unguided escapes from each compartment were made.
2. First consistent avoidances: calculated as the first of five consecutive sessions during which at least one avoidance response was made.
3. Avoidances stabilized: calculated as the first of five consecutive sessions during each of which a minimum of eight avoidances were made.
4. "No crossings" eliminated: calculated as the first of 10 consecutive sessions in each of which the subject avoided or escaped during every trial.

When the avoidance response had been established, sham trials were given occasionally to ascertain that the subjects were not responding to irrelevant cues. During a sham trial, the CS and the US were turned off, but the timers remained in operation. Thus the sounds that accompany the on-off switch and the timers could be heard without the conditioned or unconditioned stimuli operating.

Histological Procedures

After testing was completed, the brains of olfactory bulb and forebrain operates were removed, fixed in 10 per cent formalin, embedded in paraffin and sectioned at 10 μ. The sections were stained with gallocyanin (Einarson in Humason, 1962), and the extent of the lesions was determined by comparing the sections with similarly cut and stained sections of an intact brain. The brains of sham operates were examined under a dissecting microscope to determine whether any damage had been inflicted by the operation.

Statistical Procedures

The Mann-Whitney U test (Siegel, 1956) was used to determine levels of significance. As no significant differences between sham-operated and olfactory bulb-ablated subjects were found, they were grouped together in computation of p values.

RESULTS

Initial Training

The four subjects with sham operations and the three subjects with olfactory bulb ablations required significantly fewer training sessions

with net guiding than did the five subjects with forebrain ablations ($p=0.024\leftarrow$).[1] See table 1. The one sham-operated subject requiring 18 net-guided sessions (GN) proved to be anomalous in most other respects when compared with the remaining controls.

During the course of the experiment, four of the five forebrain-ablated subjects required additional net guiding when they appeared to be extinguishing the escape response. Between three and 20 net-guided sessions were given before the response was re-established. Of the seven control subjects, only GN required additional guiding and only during one session.

Acquisition and Performance of Conditioned Response

ESCAPE RESPONSE

There were no significant differences in the number of sessions before the first unguided escapes were made, the mean value being 7.5 sessions for the shams, 5.7 sessions for the olfactory bulb-ablated subjects, and 7.6 sessions for the forebrain-ablated subjects.

Following acquisition of the escape response, a marked difference between the performance of the experimental and the control groups emerged. All the subjects started with a high percentage of escapes followed by a subsequent decrease (fig.1). After approximately the twentieth session, there was an increase in the percentage of escapes made by the forebrain-ablated subjects, whereas the controls continued to decrease. In the case of the latter, this decrease in escapes was accompanied by a steady and sharply rising increase in the percentage of avoidance responses (fig.2). The initial decrease in escapes made by the forebrain-ablated subjects, on the other hand, was accompanied by an increase in "no crossings" and reflected the deterioration of the escape response after the termination of net guiding. After the additional net guiding that four of the five forebrain operates required, the percentage of escapes increased and the number of "no crossings" decreased.

[1] Throughout the paper we use the following symbols to designate one- or two-tailed tests of significance:
$\leftrightarrow$ = two-tailed test
$\leftarrow$ = one-tailed test, $u_1 < u_2$
$\rightarrow$ = one tailed test, $u_1 > u_2$
u_1 = pre-operative or control group
u_2 = post-operative or experimental group

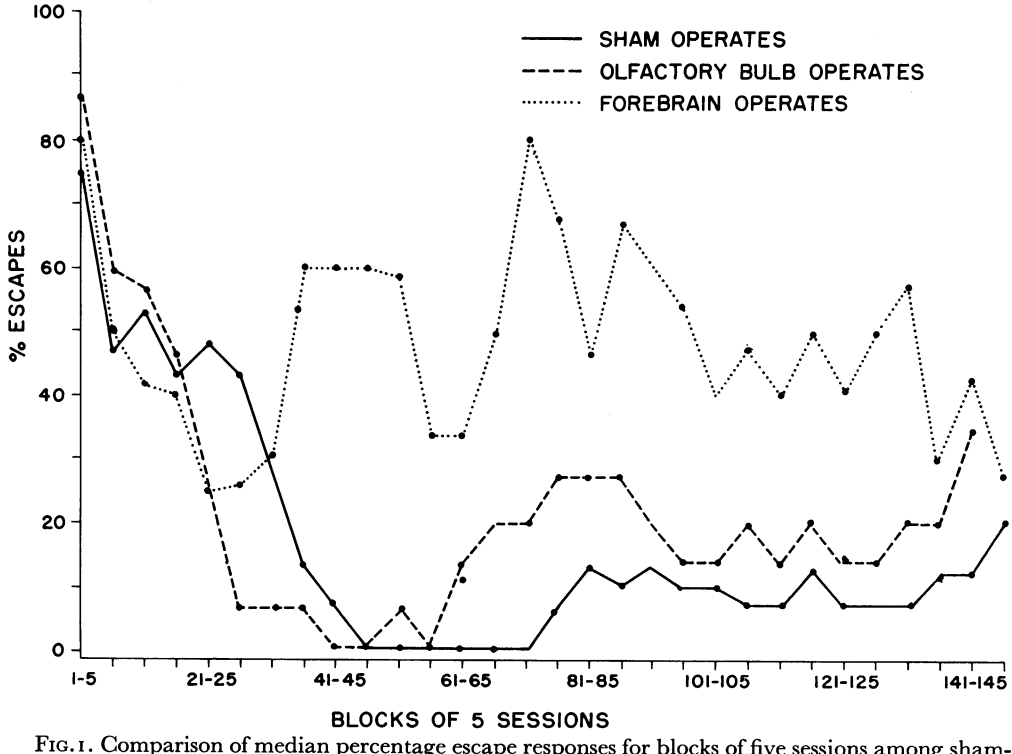

FIG. 1. Comparison of median percentage escape responses for blocks of five sessions among sham-operated, olfactory bulb-ablated, and forebrain-ablated subjects.

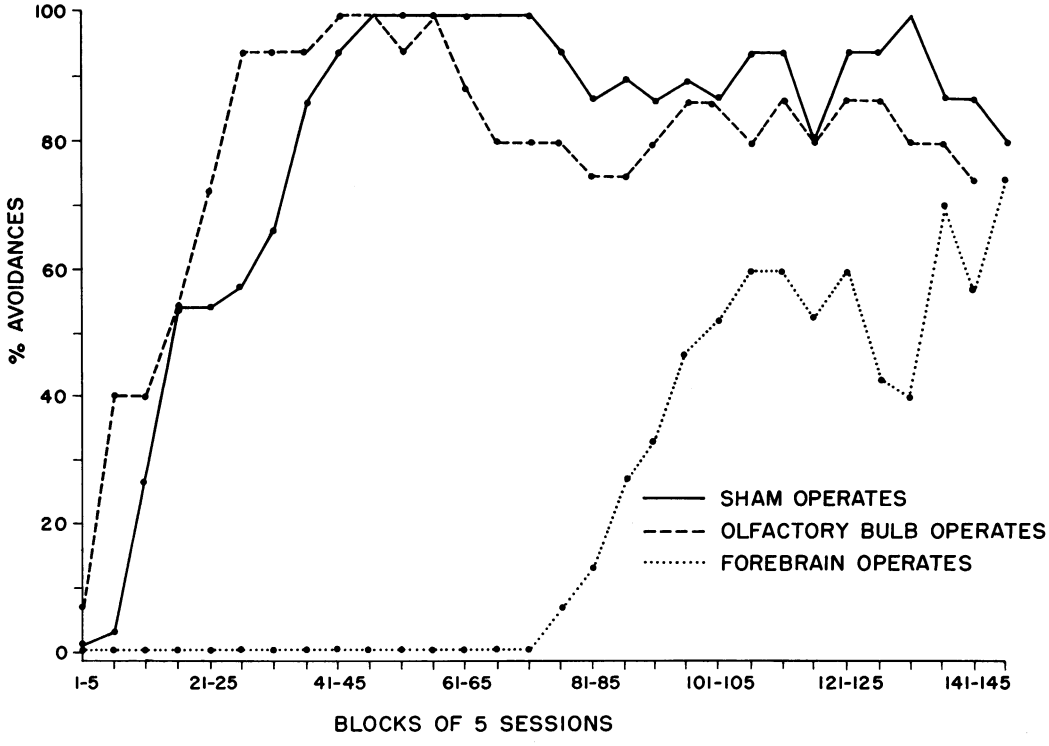

FIG.2. Comparison of median percentage of avoidance responses for blocks of five sessions among sham-operated, olfactory bulb-ablated, and forebrain-ablated subjects.

TABLE 1
ACQUISITION OF CONDITIONED RESPONSE BASED ON NUMBER OF SESSIONS TO CRITERIA[a]

	Net Guiding Terminated	First Avoidances	Avoidances Stabilized	"No Crossing" Eliminated
Sham-operated				
GG	6	3	46	2
GK	7	12	20	6
GN	18	36	42	40
GO	8	5	7	7
Mean	9.7	14.0	28.7	13.7
Olfactory Bulb-ablated				
GF	2	2	7	3
SB	7	8	40	12
SI	5	8	26	15
Mean	4.7	6.0	24.3	10.0
Forebrain-ablated				
GH	51	165[b]	165[b]	165[b]
GJ	7	19	46	39
GP	13	93	106	117
GQ	13	56	128	67
GR	13	107	115	113
Mean	19.4	88.0	112.0	100.2

[a] For definition of criteria, see text p.153.
[b] Criterion not reached; last session used in computation of mean.

AVOIDANCE RESPONSE

Even more striking differences were found in the acquisition of the avoidance response. Forebrainless subjects required more than six times as many sessions as the controls to perform their first consistent avoidances (table 1). This difference was highly significant ($p=0.001\leftarrow$). Actually, only four forebrain operates reached this criterion. The fifth subject (GH), although it began to make sporadic avoidances as it approached the end of the testing period, did not avoid during five consecutive sessions by its final (165th) session.

Once the controls began avoiding, their performance steadily improved. A high level of avoidance responses was soon achieved and consistently maintained (fig.2). Figure 3 represents the median response latency for each session of a representative sham-operated subject, GK. Note the low latencies, often well below the 2.5-second CS-US interval, and the generally small range of response. The olfactory

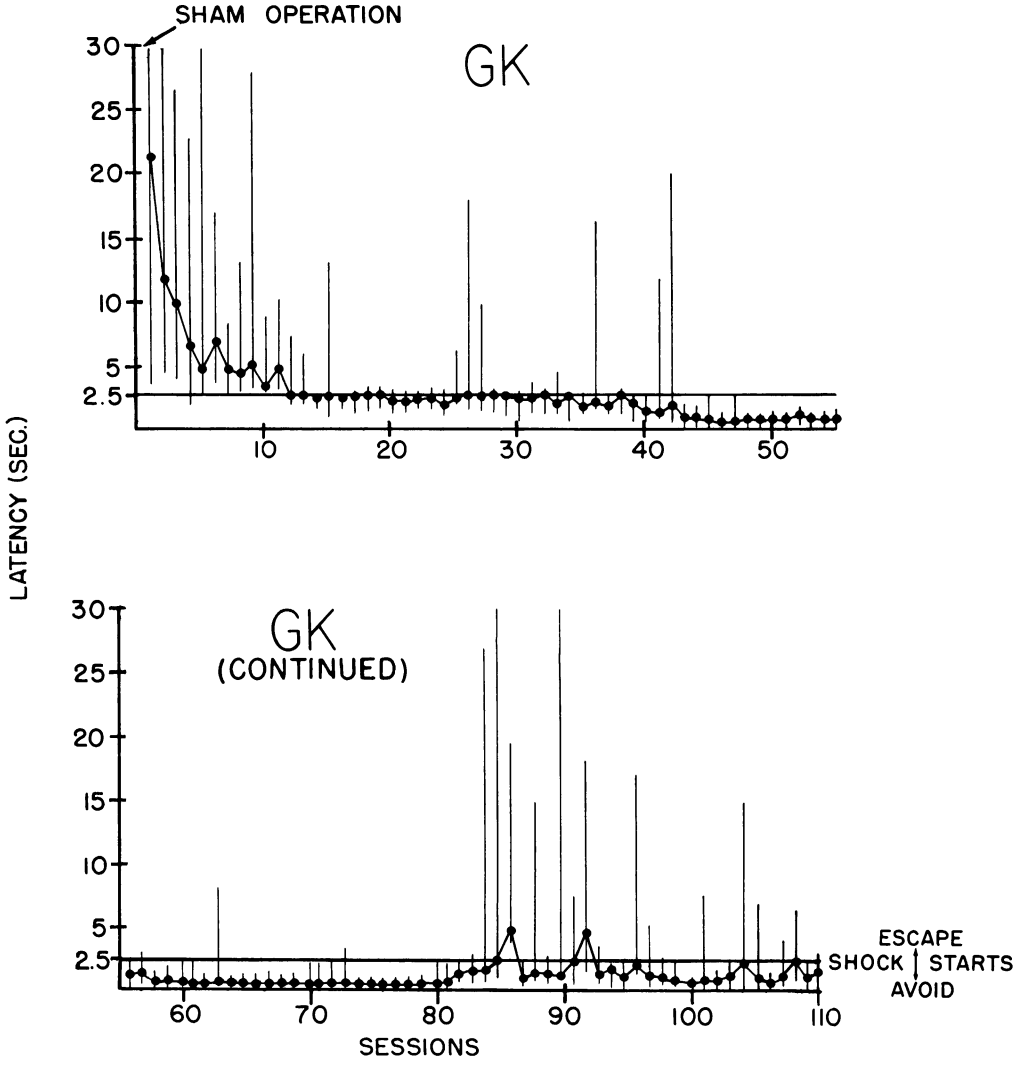

FIG.3. Median response latency in seconds for each session of a representative sham-operated subject, GK. In this and subsequent response latency graphs, the horizontal line at 2.5 seconds indicates the CS-US interval (shock starts). Vertical lines represent the range of response for each session.

bulb-ablated subject, GF (fig.4), presents a similar picture. Note, in contrast, the extreme variability of the representative forebrainless subject, GR (fig. 5). Latencies were high and the range of response extremely wide, indicating lack of consistency even within one session.

The avoidance response was considered stabilized when a minimum of eight avoidances were performed in each of five consecutive sessions. Forebrainless subjects required about four times as many sessions as did the controls to achieve this, and here, too, only four subjects reached criterion (table 1). This difference was highly significant ($p=0.001\leftarrow$). Even after achieving criterion, performance of the forebrain ablates continued to be more variable than that of the controls and the level reached was never as high (fig.5).

"NO CROSSINGS"

Another major difference between the control and experimental subjects was in the elimination of "no crossings." Almost as soon as the sham and olfactory bulb-ablated fish had learned the conditioned response, they either escaped or avoided during every trial. Forebrainless subjects, again, took much longer to do this. "No crossings" were considered eliminated when a subject responded in 10 or more consecutive sessions either by escaping or avoiding during every trial. Forebrainless subjects required almost 10 times as many sessions to eliminate "no crossings" with one subject failing to reach criterion after 165 sessions (table 1). Again, this difference was significant ($p=0.001\leftarrow$).

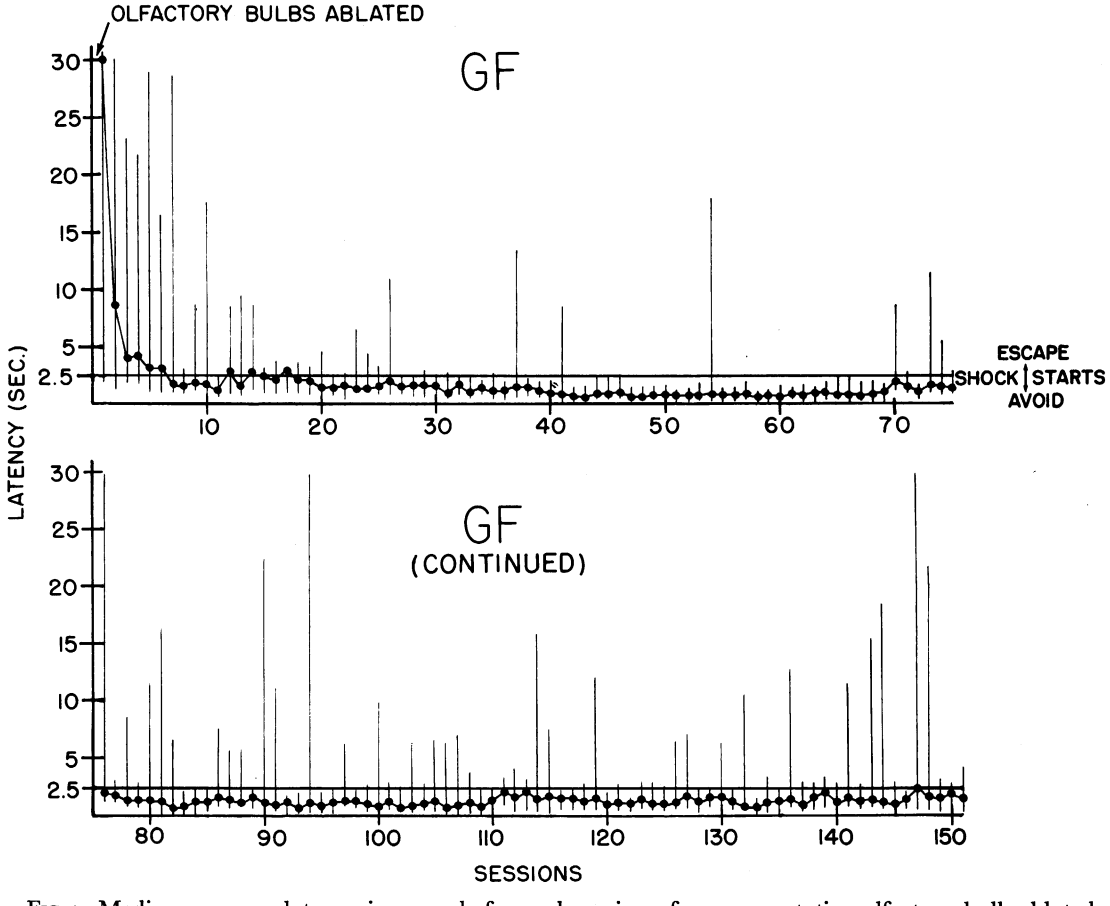

FIG.4. Median response latency in seconds for each session of a representative olfactory bulb-ablated subject, GF. (For explanation see fig. 3.)

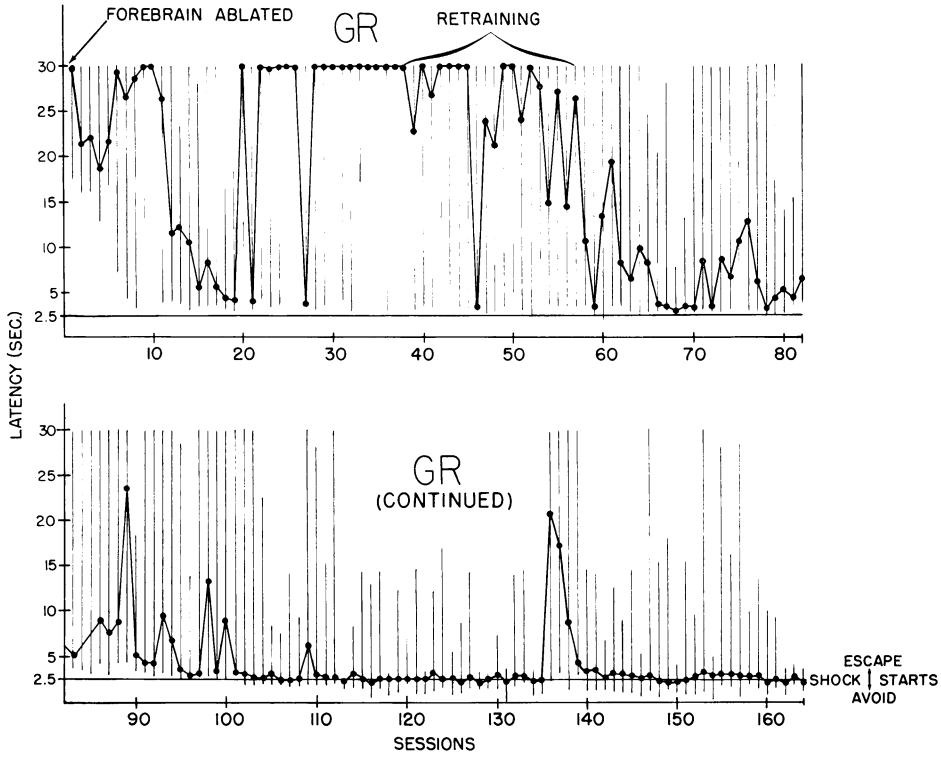

FIG.5. Median response latency in seconds for each session of a representative forebrain-ablated subject, GR. (For explanation see fig. 3.)

LATENCIES

The differences between the forebrain-ablated subjects and the controls are illustrated further in figure 6 in which we see two representative sessions of one forebrain-ablated and one sham-operated subject. The session on the left is relatively early, whereas that on the right is much later in the experiment. Each point represents the latency in seconds of one trial of the 15-trial sessions. Within each session the latency of the control subject is quite low and consistent, whereas in the forebrainless fish, even after it has learned to avoid, latencies are higher and performance more variable.

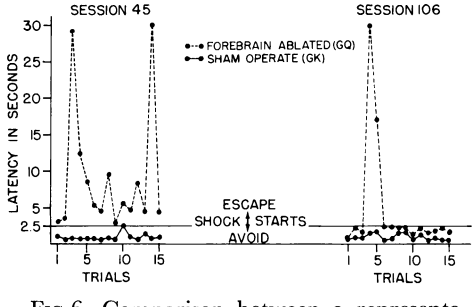

FIG.6. Comparison between a representative sham-operated subject (GK) and a representative forebrain-ablated subject (GQ) of latencies for each trial of one session early in the experiment and one session near the end of the experiment.

OTHER ASPECTS OF PERFORMANCE

Several other behavioral parameters were measured. First, the number of responses to the CS, defined as any overt body movement following the onset of light, were tabulated. These responses included swimming in any direction, head turning, and avoidance. The controls began responding to the light very early in the experiment, and by sessions 41 to 45 were making a response during almost every trial (fig. 7). From this point on variability was slight, the median generally ranging from 80 to 100 per cent for each block of five sessions. The percentage of responses made by the forebrain operates, on the other hand, remained at a much lower level. They did not reach a median of 100 per cent until sessions 81 to 85 (i.e., in twice as many

sessions as were required by the controls) and variability of this measure remained high until sessions 91 to 95.

At the beginning of the experiment many of the controls spent some time nosing the partition, but they did so less frequently and virtually eliminated this behavior after sessions 26 to 30 (fig.8). Forebrain-ablated subjects nosed the partition more frequently and continued until sessions 106 to 110.

Differences were noted also in the intertrial behavior of the controls and the experimental animals. Many of the sham-operated and olfactory bulb-ablated controls learned to wait at the hole. This resulted in avoidances achieved with low latencies and with the least expenditure of energy. Forebrain operates began to wait at the hole much later in the experiment, and they never reached as high a level as did the controls. The median percentage of trials in which sham, olfactory bulb-ablated, and forebrain-ablated subjects waited at the hole is illustrated in figure 9. Toward the end of the experiment, controls did not wait at the hole as frequently, and the three groups remained at approximately the same level.

The number of times subjects crossed from one compartment to the other during the intertrial interval (crossing over) was also recorded. This response was highly variable within the controls, as well as within the experimental subjects. In general, the rate of crossing over was higher in the controls except at the end of the experiment.

Neither in this nor in subsequent experiments did subjects respond by crossing over during a sham trial, indicating that they were indeed responding to the CS, and not to extraneous cues.

Histological Examination

Sham-operated Subjects: It was determined by gross examination under a microscope that no brain damage had been inflicted by the sham operations.

Olfactory Bulb-ablated Subjects (Fig.22):

GF: The olfactory bulbs were ablated except for the dorsal caudal end of the anterior olfactory nucleus.

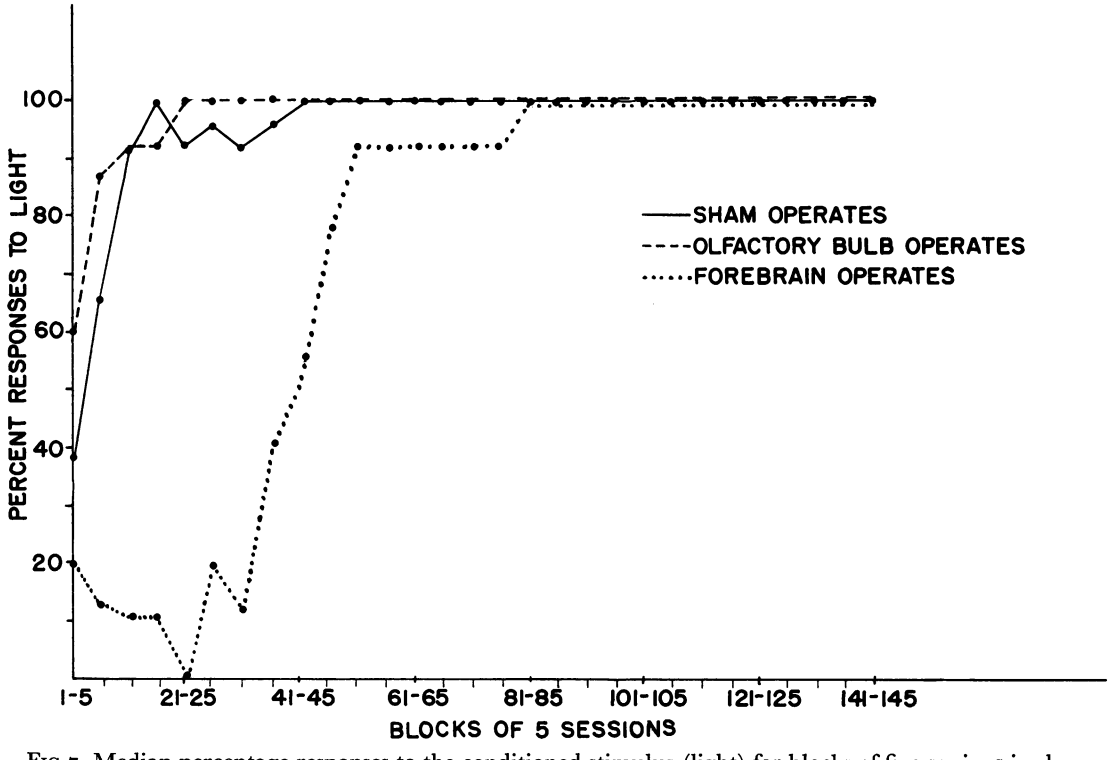

Fig.7. Median percentage responses to the conditioned stimulus (light) for blocks of five sessions in sham-operated, olfactory bulb-ablated, and forebrain-ablated subjects.

SI: Both olfactory bulbs were completely ablated. In neither case was there any forebrain damage.

SB: The olfactory bulbs were completely ablated and there was slight damage to the ventral edge of the area dorsalis medialis of the forebrain. The rest of the forebrain was intact.

FOREBRAIN-ABLATED SUBJECTS: These operations were quite similar, with some variations in the amount of forebrain tissue left and in the degree of invasion of the preoptic area.

GQ (FIG.22): Both lobes of the forebrain were ablated except for the ventral edge of the nucleus precommissuralis inferior. The preoptic area, including the nucleus entopeduncularis and nucleus preopticus magnocellularis, was intact.

GP (FIG.22): Both lobes of the forebrain were ablated except for the ventral caudal edge of the nucleus lateralis on the left side. The anterior dorsal edge of the preoptic area was invaded with more damage seen on the right side. Here the nucleus entopeduncularis was partially ablated, whereas on the left, it was intact. The nucleus preopticus magnocellularis, as well as the rest of the preoptic area, remained intact.

GJ (FIG.23): Both lobes of the forebrain were completely ablated. The anterior portions of the preoptic area were also ablated. Farther back, only the dorsal part of the preoptic area was ablated with about one-half of the nucleus entopeduncularis remaining on the left side. Still more caudally it was intact. The nucleus preopticus magnocellularis was also intact.

GR (FIG.22): This lesion was almost exactly like that of GJ except that slightly more of the dorsal portion of the preoptic area was invaded so that more of the nucleus entopeduncularis was ablated.

GH (FIG.22): The operation was similar to the last two described except that more of the preoptic area was ablated including most of the nucleus entopeduncularis. The nucleus preopticus magnocellularis remained intact.

No correlation between the amount of brain tissue ablated and avoidance performance of the forebrain-ablated subjects was found. However, the subject that performed most poorly (GH) was also the subject with the most extensive invasion of the preoptic area.

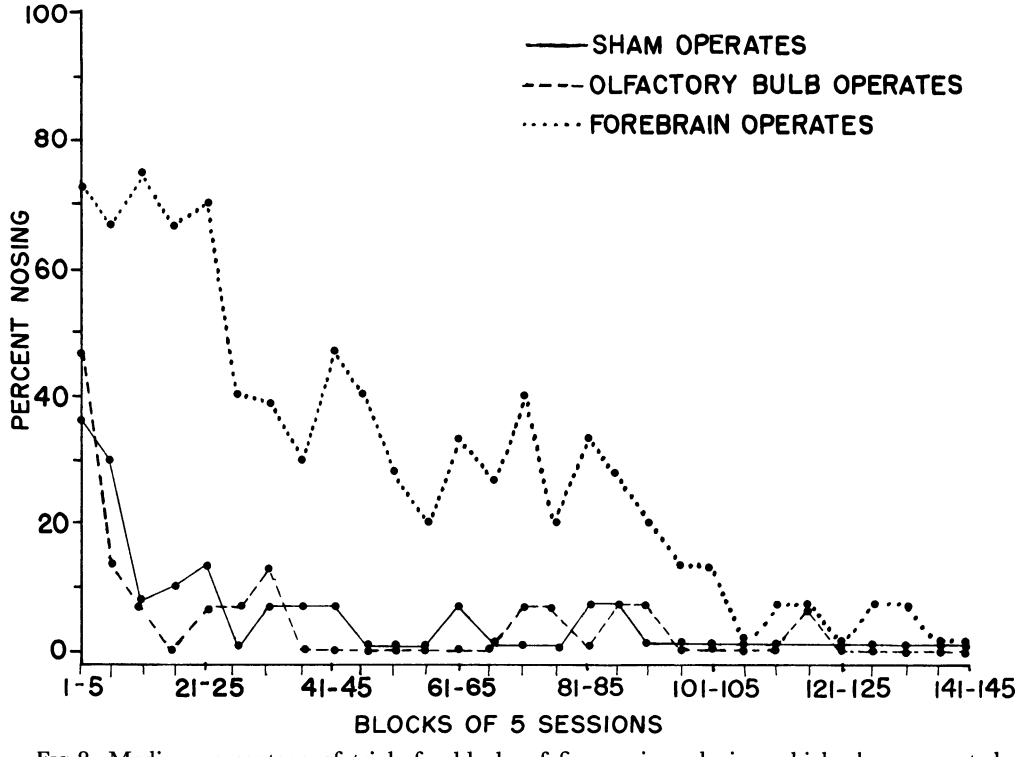

FIG.8. Median percentage of trials for blocks of five sessions during which sham-operated, olfactory bulb-ablated, and forebrain-ablated subjects nosed the partition before making a response.

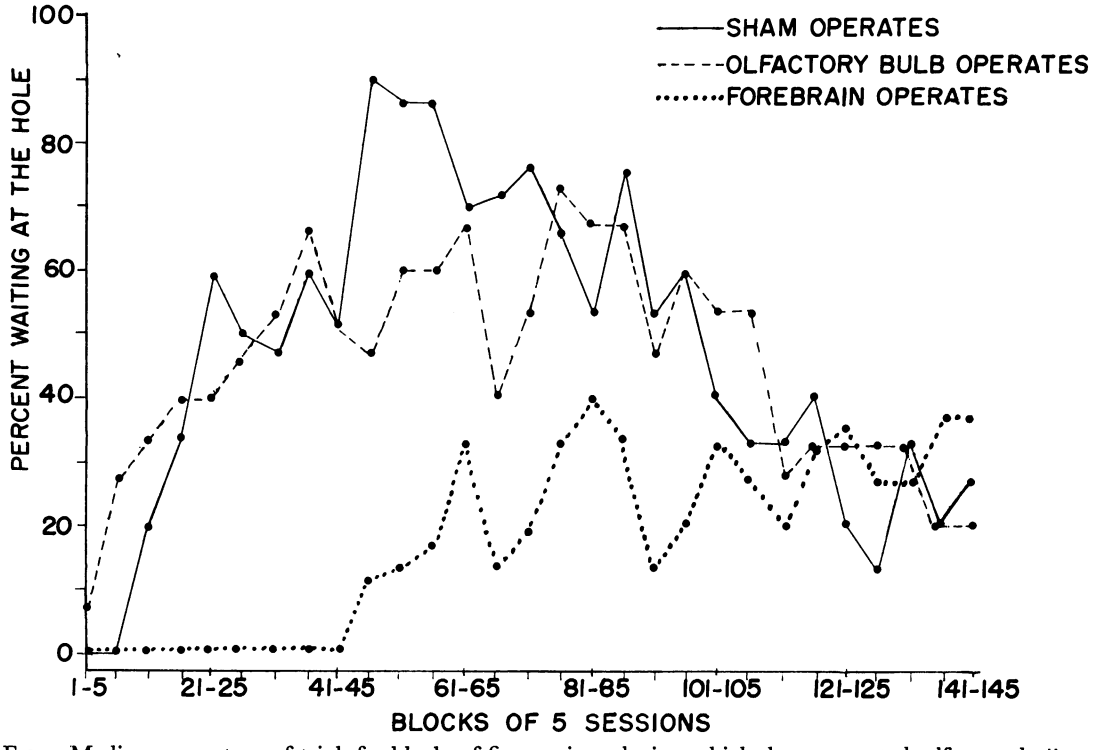

FIG. 9. Median percentage of trials for blocks of five sessions during which sham-operated, olfactory bulb-ablated, and forebrain-ablated subjects waited at the hole just before onset of a trial.

EXPERIMENT II — THE EFFECT OF FOREBRAIN ABLATION ON THE ACQUISITION OF A CONDITIONED AVOIDANCE RESPONSE WITH SOUND AS THE CONDITIONED STIMULUS

MATERIALS AND METHODS

Subjects and Maintenance

The subjects were 14 male *Tilapia heudelotii macrocephala* that weighed between 14.0 and 16.8 grams at the time they were selected from the stock tanks. Housing, maintenance, and testing conditions were similar to those described in Experiment I.

Apparatus

The test apparatus consisted of a 58 by 29¾ by 37 cm. (20-gallon capacity) aquarium into which a 32 by 18 by 22 cm. Plexiglas shuttlebox was placed. The floor of the shuttlebox was raised 14 cm. from the floor of the aquarium, and its supports rested on a mat of rubberized hair, which was 3 cm. high. The partition and the electrodes were similar to those in Experiment I. The shock pulses, each lasting 500 msec. were generated at the rate of one pulse every 2 seconds by an electronic square wave stimulator.[1] These pulses were monitored on a cathode ray oscilloscope, which measured the voltage across the electrodes.[2] Shock level was adjusted for each subject, as in the first experiment, and ranged from 6 to 8 volts. The shock was delivered independently to either compartment of the shuttlebox by means of a toggle switch. The conditioned stimulus (CS), a 500 Hz pure tone, was generated by an audiogenerator connected to a radio amplifier, and was monitored on an A.C. voltmeter. It was delivered via a 25-watt driver unit on which a 2-inch rubber bulb was sealed with Silastic caulk to the open end. The driver unit was centrally placed under the floor of the shuttlebox. The intensity of the CS was maintained at approximately 31 dB. re one microbar at the hole, diminishing to 21 dB. at the end wall of the tank. The noise level with the timers on measured approximately 10 dB.

[1] The stimulator used in Experiment I was abandoned since on some occasions the sine wave shock appeared to injure the fish.

[2] The output indicated on the stimulator could not be used to monitor the shock level because of the high and varying conductance of the conditioned aquarium water in the test tank.

A 0.1-second digital timer, controlled by the stimulus switch, indicated response latencies. The randomly determined intertrial intervals were timed on a manually operated timer. A trial was initiated by the experimenter who, by pressing a switch, turned on the audiogenerator and two automatic timers. One timer turned on the shock 5 seconds after the onset of the CS, and the other shut off the apparatus at the end of 30 seconds. If an avoidance or escape response was executed during the trial, the apparatus was turned off manually.

Preliminary Training, Grouping, Surgical, and Histological Procedures

All subjects underwent one preliminary net-guided session under actual test conditions, but without sound and shock. They were then randomly assigned to the experimental or control groups and underwent either forebrain ablation or sham operations. These operations were performed without revealing the identity of the animals to the experimenter. Subjects were allowed to recuperate fully from the effects of the operation (one to two weeks) before net guiding was initiated. One experimental and one control animal died early in the experiment, leaving a total of 12 subjects.

Net guiding and testing procedures were similar to those described in Experiment I. Histological procedures were also similar, except that a modified method of preparing the stain was used (Berube, Powers, Kerkay, and Clark, 1966).

Statistical Procedures: The Mann-Whitney U test (Siegel, 1956) was used to determine levels of significance.

RESULTS

Initial Training

Forebrain operates required approximately three times as many net-guided sessions as did the sham-operated controls (table 2). This difference was significant at the 0.01 level←. Only one forebrain-ablated subject, A14, required additional net guiding after sessions 22 and 27

TABLE 2
ACQUISITION OF CONDITIONED RESPONSE BASED ON NUMBER OF SESSIONS TO CRITERIA[a]

	Net-Guiding Terminated	First Avoidances	Avoidances Stabilized	"No Crossing" Eliminated
Sham-operated				
A1	7	7	25	62
A4	13	17	38	31
A6	13	10	119	52
A12	6	6	28	46
A15	7	8	11	20
A16	6	12	17	18
Mean	8.7	10.0	39.7	38.2
Forebrain-ablated				
A3	11	10	71	25
A5	12	13	52	126[b]
A7	33	35	95	60
A11	35	46	—[c]	—[c]
A14	54	107	128	126
A17	19	28	31	139[b]
Mean	27.3	39.8	75.4	95.2

[a] For definition of criteria see text p.153.
[b] Criterion not reached; last session used in computation of mean.
[c] Fish died before criterion reached.

when it appeared to be extinguishing the escape response.

ACQUISITION AND PERFORMANCE OF THE CONDITIONED RESPONSE

Both groups began making unguided escapes at almost the same time, the mean number of sessions being 3.0 for the controls and 3.3 for the experimental animals. Both also rapidly increased the number of escapes during the first five sessions reaching a level of about 55 per cent (fig.10). As in Experiment I, however, the controls showed a subsequent decrease in the percentage of escapes (fig.10) concurrent with an increase in avoidances (fig.11). The forebrainless subjects, on the other hand, after an initial drop when net guiding was discontinued, maintained a fairly constant level of escapes.

More striking differences were seen in the acquisition of the avoidance response. In comparison to the controls, the forebrainless subjects required almost four times as many sessions before they began making consistent avoidances ($p=0.01\leftarrow$), and almost twice the number of sessions ($p=0.04\leftarrow$) before they stabilized their avoidance performance (table 2). The sham operate (A6) that required 119 sessions to stabilize its performance was quite anomalous in this respect.

OTHER MEASURES OF PERFORMANCE

The elimination of "no crossings" was also achieved relatively early by the shams (table 2), who did so within 18 to 62 sessions. Only three of the five surviving forebrainless subjects reached criterion before the end of the experiment ($p=0.04\leftarrow$). That the sham-operated subjects acquired the conditioned response and achieved a stable level of performance much earlier than did the forebrain operates is further illustrated in figures 12 and 13. The response latencies for each trial of a representative session relatively early in the experiment and toward the end of the experiment are compared in a sham and a forebrain-ablated subject in figure 12. During session 37, the sham had already acquired a stable conditioned response, whereas the forebrain operate was still responding with a great deal of variability, even to the US (shock). By session 100, the sham maintained its consistent level of performance, but the forebrain operate, although it had acquired the conditioned response, was still highly variable. In figure 13, the

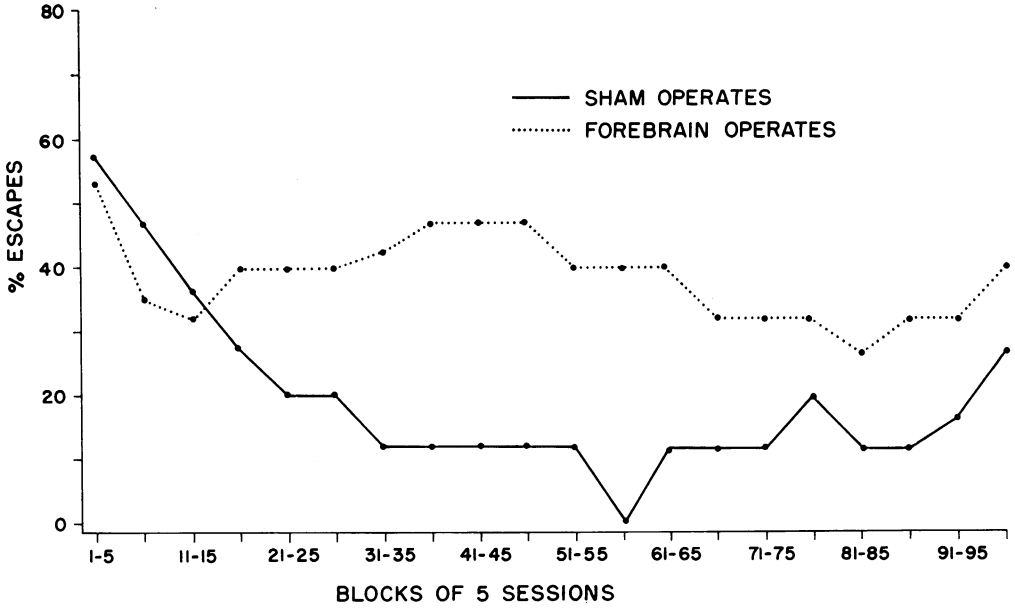

FIG. 10. Comparison of median percentage of escape responses for blocks of five sessions between sham-operated and forebrain-ablated subjects

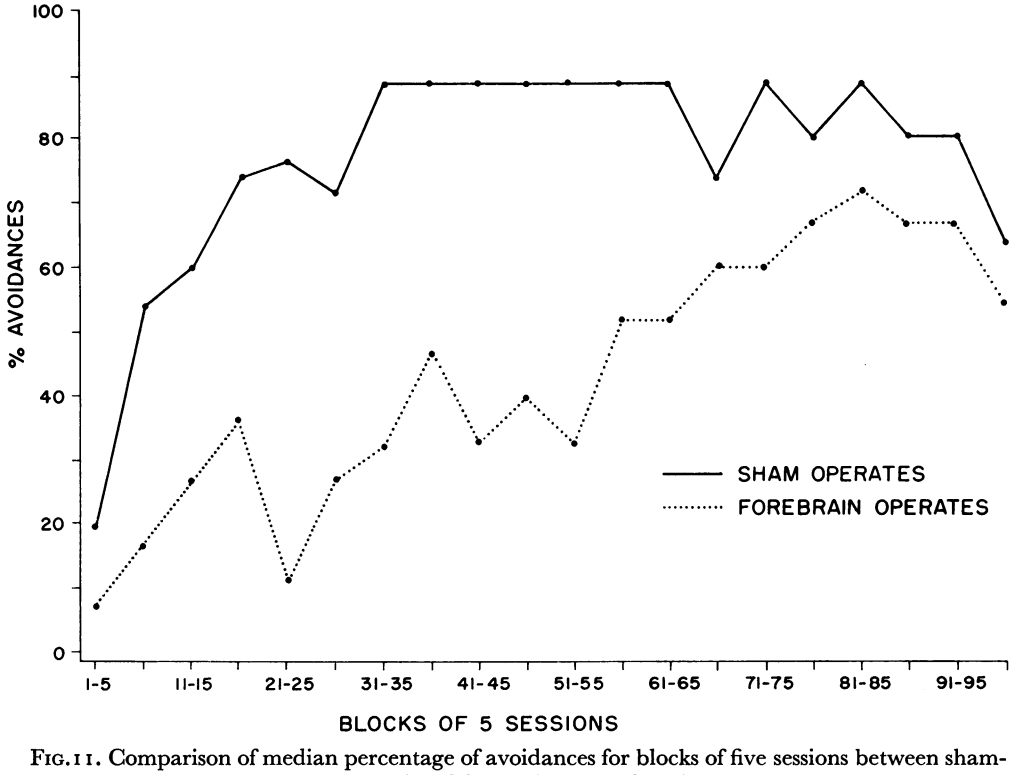

FIG. 11. Comparison of median percentage of avoidances for blocks of five sessions between sham-operated and forebrain-ablated subjects.

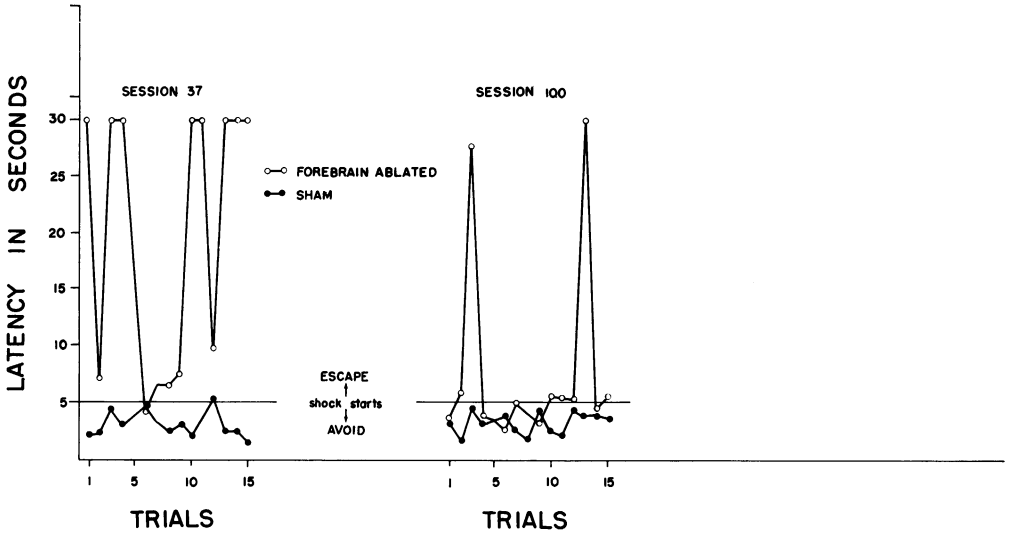

FIG. 12. Comparison between a representative sham-operated subject (A16) and a representative forebrain-ablated subject (A17) of latencies for each trial of one session early in the experiment and one session near the end of the experiment.

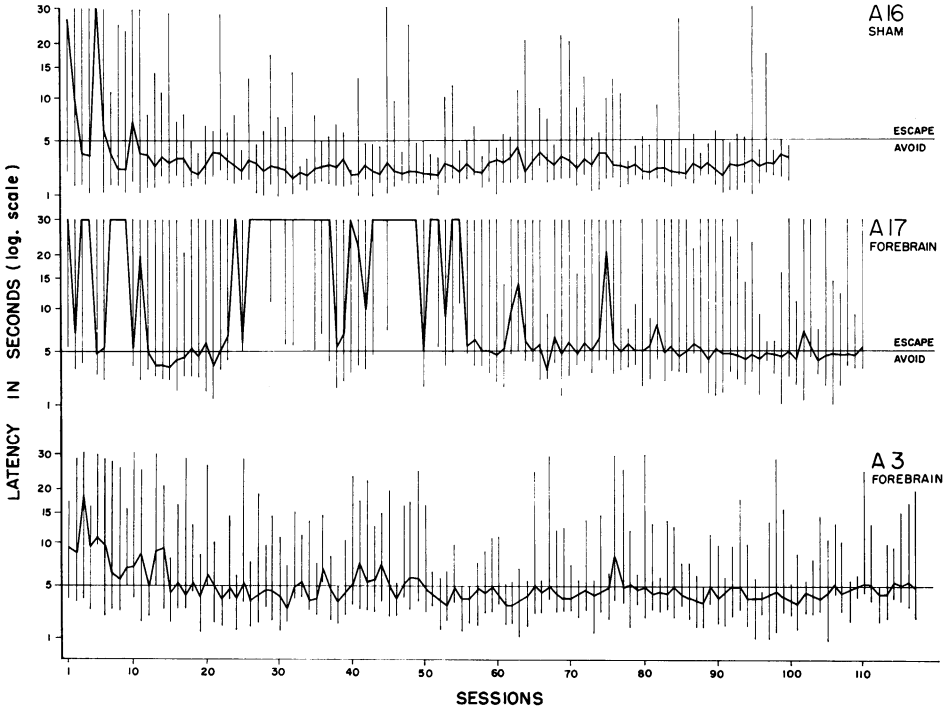

FIG. 13. Median response latencies for each session of a representative sham-operated subject (A16), a representative forebrain-ablated subject (A17), and the forebrain-ablated subject that performed best (A3). Horizontal line at 5 seconds indicates the CS-US interval. Vertical lines represent the range of response for each session.

median latency and range of response for each session in a typical sham and two forebrain operates are compared.

Subject A17 is a representative forebrain operate. Its early performance was extremely variable both within and between sessions. Note in particular the number of sessions in which more than 50 per cent of the responses were "no crossings" (median latency of 30.0 seconds) and the wide range in latencies for escape and avoidance. After approximately 55 sessions, the escape response was well established, and still later the avoidance response was stabilized, but the degree of variability remained high. Note, however, that variability decreased somewhat after a long period of testing. Forebrain-ablated subject A3 achieved the highest level of performance in the experimental group. In some instances its performance was better than that of the poorest control. Even so, its behavior was more variable than that of the typical sham, and median latencies were higher, tending to hover around the 5.0-second line.

During the initial tests both sham and forebrain operates were responding to the CS at approximately the same level (fig.14) but after session 10, the increase in response to the CS was higher and more consistent in the shams. At approximately the fifty-fifth session, the responses to the sound were similar in both groups, with the median percentage for the shams still being slightly higher during most sessions.

The mean number of intertrial crossings was higher for the shams than for the forebrain operates between session one and 75. After the seventy-fifth session they were almost the same. This response, however, was variable within both subjects and groups. There appeared to be a relationship between the acquisition of the avoidance response and crossing over. In general the onset of crossing over preceded by a few sessions the onset of avoiding, whereas stabilization of the avoidance response was often accompanied by a high level of crossing over. After considerable testing, however, crossing over became more variable and decreased in some animals.

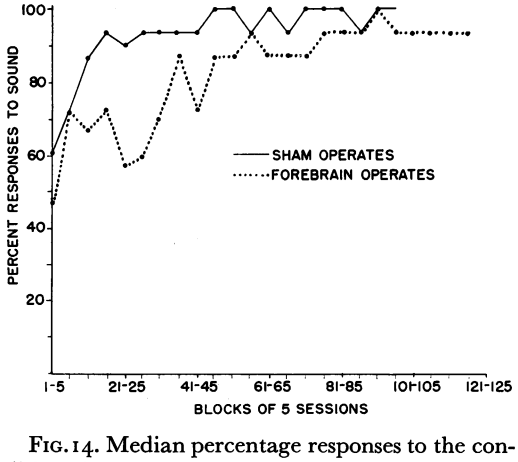

FIG. 14. Median percentage responses to the conditioned stimulus (sound) for blocks of five sessions in sham-operated and forebrain-ablated subjects.

In Experiments II and III, the fish did not wait at the hole as often as they did in Experiment I. Rather, they tended to wait at the end walls of the tank, facing away from the partition. This difference may have been due to the fact that the walls of the test aquarium were unpainted. The fish may have been attracted more by visual stimuli outside the tank than by the partition, which was not transparent. Nosing the partition occurred infrequently in this experiment. Tabulation of these items was therefore discontinued.

Histological Examination

Sham-operated Subjects: Gross examination of the sham-operated subjects showed no damage to the brain.

Forebrain-ablated Subjects (Fig.23): In four of the subjects, A3, A5, A7, and A17, the entire preoptic area was intact. In A14 the preoptic area was intact except for the anterior dorsal edge which, with the anterior end of the entopeduncular nucleus, was ablated. The amount of forebrain tissue that had been ablated was more variable. In A14, both lobes of the forebrain were entirely ablated. In A5, both lobes of the forebrain were removed except for the anterior ventral half of the nucleus lateralis and a small piece of disorganized tissue in the area of the nucleus precommissuralis inferior. The operation of subject A7 resembled that of A5 except that much of the nucleus precommissuralis inferior had been ablated along with the nucleus lateralis. Farther caudally the remaining portion of the nucleus precommissuralis inferior was disorganized.

Both lobes of the forebrain were largely ablated in A3 and A17. However, the nucleus precommissuralis intermedia was mostly intact, and the ventral edge of the nucleus precommissuralis superior was also intact.

As in Experiment I, no correlation was found between the extent of the lesion and avoidance performance. However, the subject with the greatest amount of brain tissue ablated (A14) was, again, the subject that performed most poorly.

EXPERIMENT III — THE EFFECT OF FOREBRAIN ABLATION ON THE PERFORMANCE OF A CONDITIONED AVOIDANCE RESPONSE WITH SOUND AS THE CONDITIONED STIMULUS

MATERIALS AND METHODS

Subjects, Maintenance, and Apparatus

The subjects were 10 male and one female *Tilapia heudelotii macrocephala* that weighed between 9 and 14 grams when selected from the stock tanks. Housing, maintenance, and testing conditions were similar to those described in Experiment I, and the test apparatus and general procedures were the same as those in Experiment II.

Experimental Design and Procedures

In this study, all the subjects were trained to make the conditioned avoidance response before surgery. When they were performing at a consistent level, they were subjected to a series of operations as shown in table 3.

The first six subjects were given sham operations and retesting began the next day for a varying number of sessions to ascertain the effects of the operation. When it was confirmed that sham operation had no effect on the performance of the conditioned avoidance response the remaining subjects were spared this procedure. All subjects then underwent olfactory bulb ablation and following that were retested. The number of sessions given to each subject after these operations are shown on the individual graphs (figs.15–18). Once the effects of olfactory bulb ablation had been determined, the remainder of the forebrain was ablated and subjects were retested. Operative procedures were the same as in Experiment I, and histological procedures were the same as in Experiment II.

TABLE 3
Subjects and Operations

Subject	Sham Operation	Olfactory bulbs Ablated	Forebrain Ablated
AB	X	X	X
AC	XX[a]	X	X
AI	X	X	X
AK	X	X	X
AP	X	X	X
AR	X	X	X
AL		X	X
AS		X	X
AT		X	X
AW		X	X
AZ		X	X

[a] AC had two sham operations.

Statistical Methods: The Wilcoxon matched-pairs signed-ranks test (Siegel, 1956) was used to determine levels of significance where necessary.

RESULTS

Performance before Operation

The subjects were given a variable number of pre-operative sessions (from 19 to 85) before surgical procedures were begun. This number was determined by the speed with which the subjects acquired the avoidance response and achieved a stable level of performance. Because of these differences and the fact that each subject acted as its own control, the data are presented, for the most part, as individual rather than as group scores, although central tendencies are noted.

By sessions 21 to 25, all the subjects were avoiding in at least eight out of 15 trials per session. They all continued to improve, but reached different levels of proficiency by the end of the pre-operative testing period (table 4). As in previous experiments, "no crossing" virtually disappeared (table 5). Response latencies were generally low and, for the most part, stable as indicated by the small range (figs.15–18).

Effects of Sham Operation

Of the six subjects that underwent sham operations, five continued performing at the pre-operative level or showed improvement (figs.15; 16, AB; 17, AP; 18, AI). The sixth subject initially avoided somewhat less but soon regained its former level (fig.16, AK; table 4).

Other aspects of performance, such as responses to the sound (table 6), crossing over (table 7), waiting at the hole (table 8), and waiting at the back wall (table 9), were essentially unaffected by the sham operation.

Effects of Olfactory Bulb Ablation

Following olfactory bulb ablation, performance remained approximately at pre-operative levels in regard to median and range of latency (figs. 15–18). There was, however, a significant decrease ($p = 0.01 \leftrightarrow$) in the percentage of avoidances achieved during the first five sessions after operation (table 4). By the last five sessions after olfactory bulb ablation, eight of the subjects increased their per cent of avoidances, and the differences before and after operation were no longer significant ($p > 0.05 \leftrightarrow$).

There were no significant differences before and after olfactory bulb ablation with respect to "no crossing" (table 5), responses to the CS (table 6), intertrial crossings (table 7), waiting at the hole (table 8), and waiting at the back wall (table 9). The p value for each of these items was $> 0.05 \leftrightarrow$.

In summary, sham operation appeared to have had no effect on performance of the conditioned responses, whereas olfactory bulb ablation had a more or less transitory effect.

Effects of Forebrain Ablation

Immediate Effects

In contrast to the effects of sham and olfactory bulb operation, ablation of the forebrain resulted in clear-cut and drastic changes in performance of the conditioned response, even after discarding the first five post-operative tests to allow for the effects of diaschisis. The percentage of avoidance (table 4) decreased sharply as the percentage of escapes (table 10) and of "no crossings" (table 5) increased correspondingly. Performance became variable with an increase in range of latencies within sessions and from day to day (figs. 15–18). During the five sessions just prior to forebrain ablation, the subjects responded to the sound in 93.1 per cent

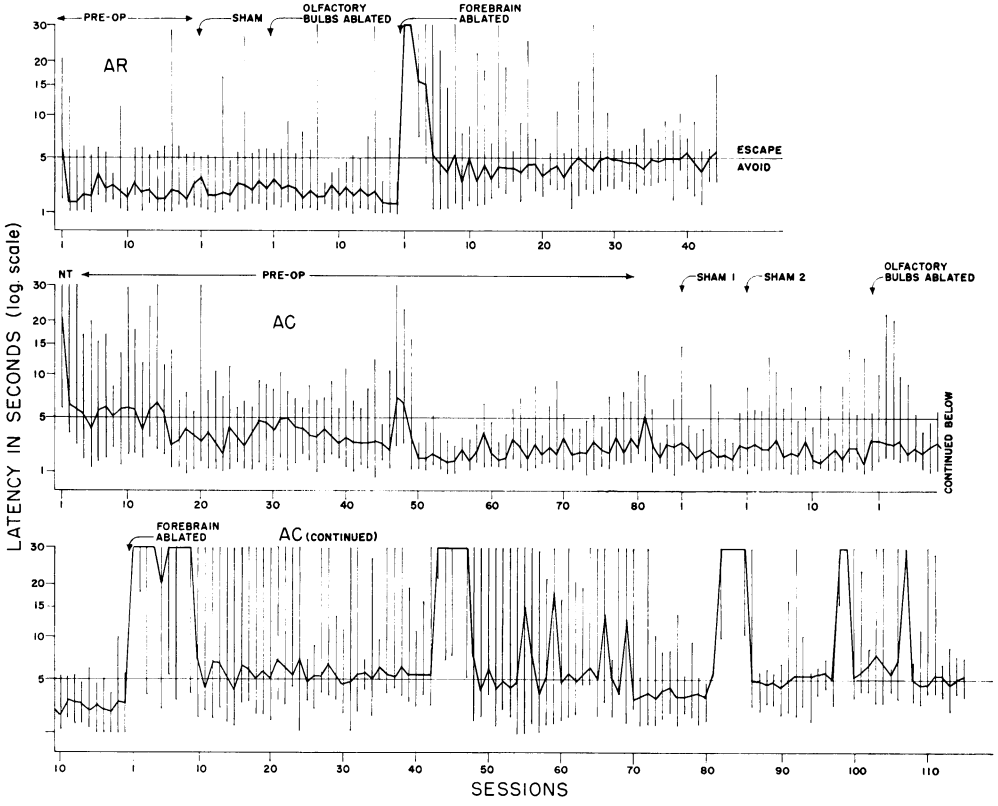

Fig. 15. Median response latency in seconds for each session of subjects AR and AC. In all response latency graphs, the horizontal line at 5 seconds indicates the CS-US interval. Vertical lines represent the range of response.

of the trials. Their responses dropped to 27.5 per cent during sessions six to 10 after forebrain ablation (see table 6 for individual figures).

As stated, subjects avoided or escaped during almost every trial pre-operatively, after sham operation, and after olfactory bulb ablation. Following the removal of the forebrain, nine of the subjects showed a tremendous increase in "no crossing" (table 5). Two subjects (AP and AR) changed little in this respect.

Response latencies generally increased and became more variable after forebrain ablation (figs. 15–18). Escape latencies increased in nine subjects but stayed approximately the same in AP, the tenth subject (table 11). The eleventh subject, AI, made no avoidances and so few escapes during the first 20 sessions after forebrain ablation that mean latencies for these sessions were not computed.

The difference between the mean of the last 10 escape latencies before ablation of the forebrain and the mean of the first 10 escape latencies after ablation of the forebrain (eliminating sessions one to five) was found to be significant at the 0.005 level ←.

The intertrial behavior of the subjects also changed drastically. All waited at the hole much less frequently (table 8). Eight subjects waited at the back wall more frequently; one subject, AC, waited there less frequently and two others, AL and AT, waited there approximately the same number of times (table 9). The mean number of intertrial crosses meanwhile declined in all subjects (table 7).

LONG-TERM EFFECTS

The long-term effects of forebrain ablation were variable, both in terms of the individual subjects and the parameter under consideration. Regarding performance as a whole, all subjects

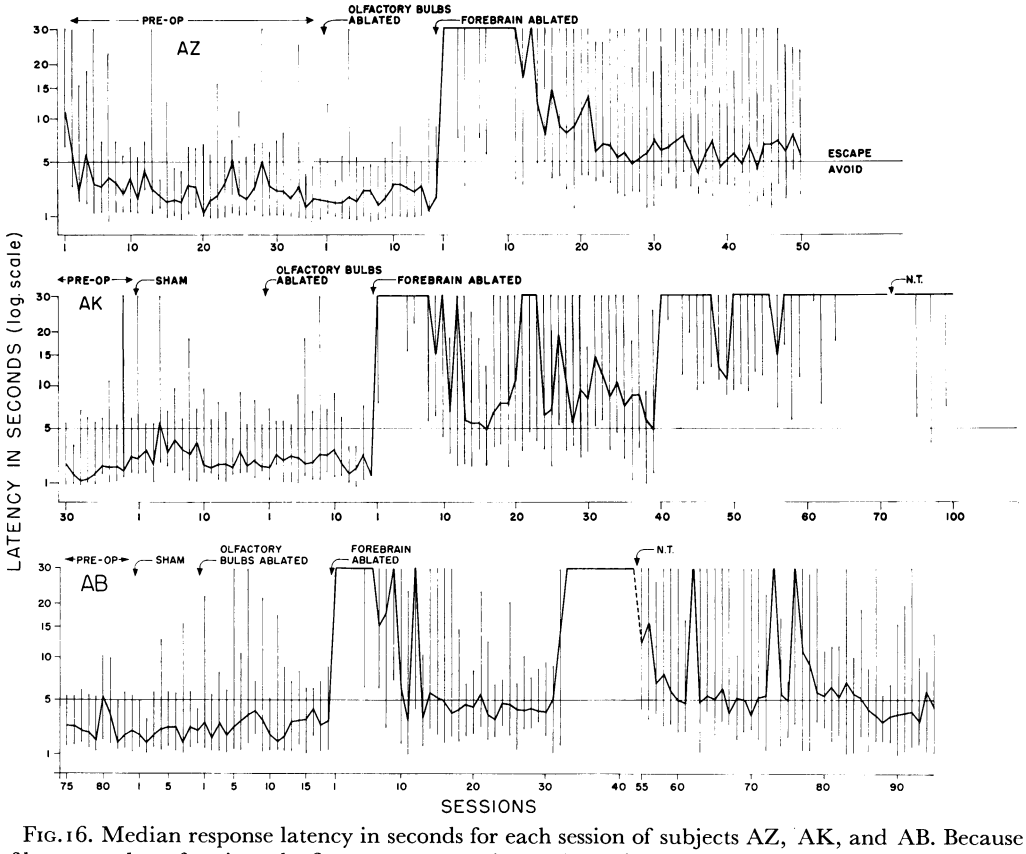

FIG. 16. Median response latency in seconds for each session of subjects AZ, AK, and AB. Because of large number of sessions the first 29 pre-operative sessions of AK and the first 74 of AB are omitted. (For further explanations see fig. 15.)

Abbreviation: N.T., net guiding.

TABLE 4

PERCENTAGE OF AVOIDANCE RESPONSES FOR BLOCKS OF FIVE SESSIONS

Subject	Pre-operative	Sham Operation		Olfactory Bulbs Ablated		Forebrain Ablated						
	Last Five Sessions	Sessions		Sessions		Sessions						
		First Five	Last Five	First Five	Last Five	6–10	26–30	46–50	66–70	86–90	106–110	126–130
AB	76.0 (80–84)[a]	92.0	93.3[b]	84.0	73.3	11.1	73.3	1.3	62.7	76.0	52.0	17.3
AC	81.3 (81–85)	93.3	89.3[c]	80.0	92.0	19.6	38.7	30.8	54.7	65.3	55.4	—
AI	70.7 (52–56)	70.7	85.3	74.7	69.3	0	8.3	4.8	2.7	9.3	—	—
AK	82.7 (35–39)	72.0	88.0	85.3	94.7	1.8	16.7	0	0	0	—	—
AP	97.3 (15–19)	96.0	94.7	90.6	96.0	53.3	61.3	29.3	16.0	—	—	—
AR	86.7 (15–19)	96.0	81.3	84.0	94.1	56.0	66.7	61.3[d]	—	—	—	—
AL	64.0 (77–81)	—	—	61.3	77.3	0	16.1	4.2	13.3	—	—	—
AS	76.0 (26–30)	—	—	70.6	92.0	6.4	65.3	72.0[e]	—	—	—	—
AT	85.3 (41–45)	—	—	80.0	96.0	8.8	45.3	62.7	—	—	—	—
AW	74.7 (29–33)	—	—	53.3	85.3	20.0	57.3	25.3	—	—	—	—
AZ	96.0 (32–36)	—	—	90.7	90.7	0	37.3	32.0	—	—	—	—

[a] Session numbers.
[b] Last four sessions.
[c] Second sham operation.
[d] Based on sessions 41–45.
[e] Based on sessions 44–48.

TABLE 5

PERCENTAGE OF "NO CROSSING" FOR BLOCKS OF FIVE SESSIONS

Subject	Pre-operative	Sham Operation		Olfactory Bulbs Ablated		Forebrain Ablated						
	Last Five Sessions	Sessions		Sessions		Sessions						
		First Five	Last Five	First Five	Last Five	6–10	26–30	46–50	66–70	86–90	106–110	126–130
AB	0	0	0[a]	1.3	0	49.2	0	23.1	8.0	2.7	4.0	9.3
AC	0	0	0[b]	0	0	58.9	2.7	36.9	5.3	1.3	12.3	—
AI	0	0	0	0	0	91.4	54.2	44.4	14.7	10.7	—	—
AK	1.3	2.7	0	0	0	57.1	18.0	41.3	92.9	33.3	—	—
AP	0	0	0	0	0	1.3	1.3	0	4.0	—	—	—
AR	0	0	0	0	1.3	1.3	1.3	0[c]	—	—	—	—
AL	0	—	—	0	1.3	63.5	33.9	19.7	17.3	—	—	—
AS	1.3	—	—	1.3	1.3	70.2	1.3	0[d]	—	—	—	—
AT	0	—	—	0	0	82.4	8.0	2.7	—	—	—	—
AW	2.7	—	—	2.7	0	16.0	1.3	0	—	—	—	—
AZ	0	—	—	1.3	0	92.6	5.3	2.7	—	—	—	—

[a] Last four sessions.
[b] Second sham operation.
[c] Based on session 41–45.
[d] Based on sessions 44–48.

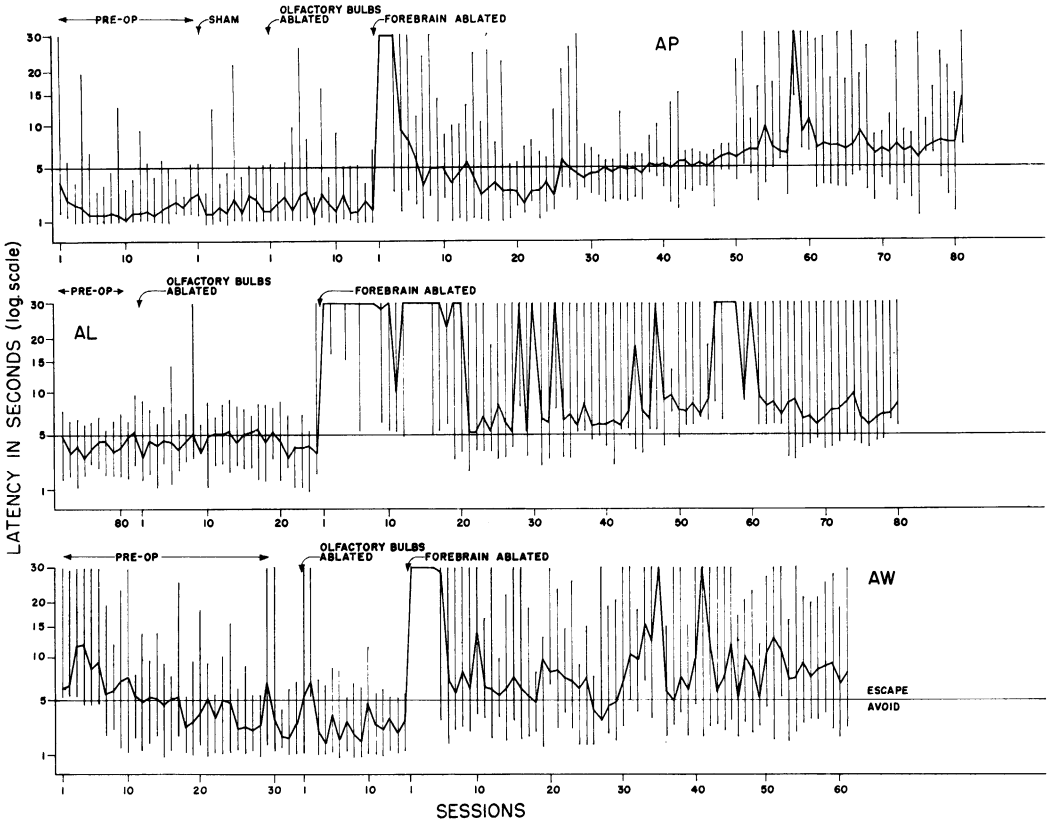

FIG. 17. Median response latency in seconds for each session of subjects AP, AL, and AW. Because of the large number of sessions, the first 71 pre-operative sessions of AL are omitted. (For further explanations see fig. 15.)

TABLE 6

PERCENTAGE OF RESPONSES TO CONDITIONED STIMULUS (SOUND) FOR BLOCKS OF FIVE SESSIONS

Subject	Pre-operative Last Five Sessions	Sham Operation Sessions First Five	Sham Operation Sessions Last Five	Olfactory Bulbs Ablated Sessions First Five	Olfactory Bulbs Ablated Sessions Last Five	Forebrain Ablated Sessions 6–10	Forebrain Ablated Sessions 26–30	Forebrain Ablated Sessions 46–50	Forebrain Ablated Sessions 66–70
AB	89.3	98.7	95.0[a]	96.0	86.7	25.4	90.7	19.2	80.0
AC	94.7	94.7	94.7[b]	88.0	97.3	50.0	69.3	49.2	74.7
AI	93.3	92.0	94.7	90.7	77.3	14.3	22.9	15.9	24.0
AK	92.0	82.7	94.7	97.3	100	8.9	26.4	1.6	0
AP	97.3	96.0	96.0	93.3	100	66.7	84.0	86.7	33.3
AR	88.0	97.3	84.0	89.3	98.7	73.3	94.6	93.3[c]	—
AL	76.0	—	—	72.0	88.0	0	57.1	46.5	64.0
AS	82.7	—	—	76.0	94.7	6.4	86.7	94.7[d]	—
AT	94.7	—	—	88.0	98.7	17.6	82.7	88.0	—
AW	88.0	—	—	73.3	89.3	36.0	74.7	49.3	—
AZ	96.0	—	—	98.7	93.3	3.7	60.0	53.3	—

[a] Last four sessions.
[b] Second sham operation.
[c] Based on sessions 41–45.
[d] Based on sessions 44–48.

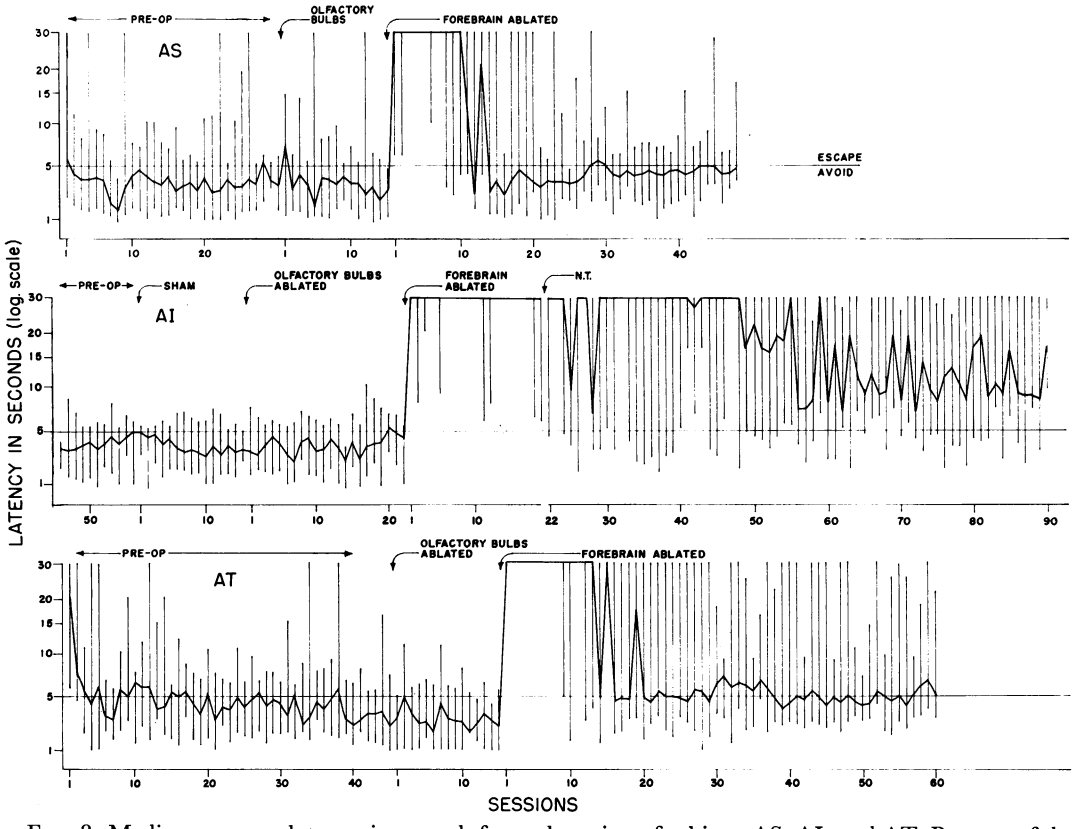

FIG. 18. Median response latency in seconds for each session of subjects AS, AI, and AT. Because of the large number of sessions, the first 45 pre-operative sessions of AI are omitted. (For further explanations see fig. 15.)

Abbreviation: N.T., net guiding.

TABLE 7

MEAN NUMBER OF INTERTRIAL CROSSES PER SESSION FOR BLOCKS OF FIVE SESSIONS

Subject	Pre-operative Last Five Sessions	Sham Operation Sessions First Five	Sham Operation Sessions Last Five	Olfactory Bulbs Ablated Sessions First Five	Olfactory Bulbs Ablated Sessions Last Five	Forebrain Ablated Sessions 6–10	Forebrain Ablated Sessions 26–30	Forebrain Ablated Sessions 46–50	Forebrain Ablated Sessions 66–70
AB	22.6	15.6	20.7[a]	18.2	15.2	2.6	14.8	8.4	17.4
AC	29.4	33.6	29.4[b]	23.6	26.6	5.6	26.6	10.4	10.6
AI	15.2	17.0	20.8	18.2	22.8	0.2	3.8	2.0	0.2
AK	17.4	13.8	13.0	11.2	14.2	1.4	7.8	1.0	15.2
AP	26.0	20.2	19.6	21.8	17.2	6.8	6.0	10.4	—
AR	13.0	19.4	14.6	15.2	22.2	15.8	18.0	16.2[c]	10.0
AL	16.6	—	—	20.2	12.0	0.2	5.6	9.4	—
AS	19.8	—	—	19.8	15.6	0.6	12.2	13.0[d]	—
AT	14.2	—	—	19.0	28.4	2.6	21.4	25.2	—
AW	14.4	—	—	18.8	15.8	5.6	21.8	23.8	—
AZ	30.2	—	—	31.6	25.8	0	21.0	19.4	—

[a] Last four sessions.
[b] Second sham operation.
[c] Based on sessions 41–45.
[d] Based on sessions 44–48.

TABLE 8

PERCENTAGE OF WAITING AT HOLE (BEFORE TRIAL) FOR BLOCKS OF FIVE SESSIONS

Subject	Pre-operative Last Five Sessions	Sham Operation Sessions		Olfactory Bulbs Ablated Sessions		Forebrain Ablated Sessions			
		First Five	Last Five	First Five	Last Five	6–10	26–30	46–50	66–70
AB	76.0	78.7	86.7[a]	82.6	69.3	6.3	1.3	6.4	5.3
AC	48.0	34.7	66.7[b]	64.0	44.0	16.1	2.7	9.2	46.7
AI	85.3	92.0	64.0	46.7	34.7	2.9	8.3	4.8	2.7
AK	84.0	72.0	64.0	50.7	61.3	7.1	2.8	0	0
AP	61.3	64.0	54.7	37.3	60.0	9.3	1.3	1.3	0
AR	68.0	57.3	65.3	44.0	64.0	17.3	1.3	0[c]	—
AL	56.0	—	—	20.0	21.3	17.3	0	0	2.7
AS	41.3	—	—	22.7	25.3	2.1	0	0[d]	—
AT	9.3	—	—	10.7	8.0	2.9	2.7	0	—
AW	85.3	—	—	62.7	61.3	10.7	0	0	—
AZ	33.3	—	—	50.7	45.3	11.1	1.3	2.7	—

[a] Last four sessions.
[b] Second sham operation.
[c] Based on sessions 41–45.
[d] Based on sessions 44–48.

TABLE 9

PERCENTAGE OF WAITING AT BACK WALL (BEFORE TRIAL) FOR BLOCKS OF FIVE SESSIONS

Subject	Pre-operative Last Five Sessions	Sham Operation Sessions		Olfactory Bulbs Ablated Sessions		Forebrain Ablated Sessions			
		First Five	Last Five	First Five	Last Five	6–10	26–30	46–50	66–70
AB	17.3	13.3	1.7[a]	10.6	12.0	65.1	52.0	83.3	74.7
AC	28.0	30.7	20.0[b]	29.3	48.0	33.9	62.7	50.8	40.0
AI	9.3	2.7	12.0	4.0	32.0	80.0	79.2	82.5	68.0
AK	8.0	9.3	17.3	26.7	22.7	75.0	73.6	84.1	82.1
AP	36.0	33.3	42.7	50.7	32.0	81.3	97.3	93.3	85.3
AR	24.0	34.7	14.7	22.7	35.0	65.3	93.3	81.3[c]	—
AL	26.7	—	—	62.7	56.0	55.8	69.6	43.7	28.0
AS	41.3	—	—	70.7	61.3	80.9	93.3	96.0[d]	—
AT	72.0	—	—	57.3	82.7	82.4	85.3	77.3	—
AW	14.7	—	—	29.3	38.7	72.0	88.0	90.7	—
AZ	54.7	—	—	40.0	45.3	77.8	66.7	64.0	—

[a] Last four sessions.
[b] Second sham operation.
[c] Based on sessions 41–45.
[d] Based on sessions 44–48.

TABLE 10

PERCENTAGE OF ESCAPE RESPONSES FOR BLOCKS OF FIVE SESSIONS

Subject	Pre-operative	Sham Operation		Olfactory Bulbs Ablated		Forebrain Ablated						
	Last Five Sessions	Sessions		Sessions		Sessions						
		First Five	Last Five	First Five	Last Five	6–10	26–30	46–50	66–70	86–90	106–110	126–130
AB	24.0	8.0	6.7[a]	14.7	26.7	39.7	26.6	11.5	29.3	21.3	44.0	73.3
AC	18.7	6.7	10.7[b]	20.0	8.0	21.4	58.7	32.3	40.0	33.3	32.3	—
AI	29.3	29.3	14.7	25.3	30.7	8.6	37.5	50.8	82.7	80.0	—	—
AK	16.0	25.3	12.0	14.7	5.3	41.1	65.3	58.7	7.1	23.2	—	—
AP	2.7	4.0	5.3	9.3	4.0	45.3	37.3	70.7	80.0	—	—	—
AR	13.3	4.0	18.7	16.0	4.6	42.7	32.0	38.7[c]	—	—	—	—
AL	36.0	—	—	38.7	21.3	36.5	50.0	76.1	69.3	—	—	—
AS	22.7	—	—	28.0	6.7	23.4	33.3	28.0[d]	—	—	—	—
AT	14.7	—	—	20.0	4.0	17.4	46.7	34.7	—	—	—	—
AW	22.6	—	—	44.0	14.7	64.0	41.3	74.7	—	—	—	—
AZ	4.0	—	—	8.0	9.3	7.4	57.3	65.3	—	—	—	—

[a] Last four sessions.
[b] Second sham operation.
[c] Based on sessions 41–45.
[d] Based on sessions 44–48.

TABLE 11

MEAN ESCAPE LATENCIES (IN SECONDS)

Subject	Pre-operative Last 10[a] Escapes	Olfactory Bulbs Ablated First 10[b] Escapes	Olfactory Bulbs Ablated Last 10[a] Escapes	Forebrain Ablated Sessions 6–10[c]	16–20	26–30	36–40	46–50	56–60	66–70	76–80
AB	6.1	10.6	6.4	12.8	10.2	5.9	—[d]	—[d]	11.7	7.0	11.7
AC	6.4	10.9	6.1	11.5	7.1	6.1	8.7	7.3	12.2	13.6	6.9
AI	5.6	5.8	6.2	—[d]	—[d]	8.9	12.8	15.9	14.1	15.8	17.3
AK	6.3	6.1	6.3	13.7	8.0	11.2	12.1	16.7	11.9	—	—
AP	6.7	8.6	7.9	7.6	8.8	7.2	5.6	5.7	8.3	8.3	8.2
AR	8.9	5.8	6.1	8.6	7.9	6.3	5.7	—	—	—	—
AL	6.1	6.7	5.8	13.8	9.7	7.6	6.3	6.9	8.3	8.1	8.0
AS	5.5	8.3	5.8	17.1	6.8	7.7	6.1	7.1	—	—	—
AT	7.6	7.2	6.2	10.8	7.0	9.0	8.5	10.0	8.3	—	—
AW	5.9	6.9	5.5	11.5	13.5	8.9	8.2	9.4	8.6	—	—
AZ	8.0	6.3	6.7	9.1	9.3	8.6	10.2	9.2	—	—	—
Grand Mean	6.6	7.6	6.3	11.7	8.8	7.9	8.4	9.8	10.4	10.6	10.4

[a] Means based on last 10 escapes before operation.
[b] Means based on first 10 escapes after operation
[c] Means based on first 10 escapes within each block of five sessions.
[d] Too few escapes performed to compute mean.

that were tested long enough showed a great deal of variability within the general trend of their performance, although trial-to-trial and session-to-session variability eventually decreased in most animals. Note, for example, in AC (fig.15), that, after forebrain ablation, periods in which performance was relatively good are interspersed with several sessions in which performance was very poor. This degree of variability was not seen before the forebrain was ablated. Some subjects, AC (fig.15), AZ (fig.16), AS and AT (fig.18), improved as testing continued; some, AK (fig.16), AP (fig. 17), deteriorated; others, AR (fig. 15), AB (fig.16), AL and AW (fig.17), AI (fig.18), showed no definite trend. They were either extremely variable or remained at the same level.

The percentage of avoidance responses increased in all subjects after the initial sharp drop, but in some subjects, this measure varied greatly from one block of sessions to another (table 4). Although seven subjects occasionally attained a level of avoidance responding that was within the range of their control performance, in no instance did they attain as high a level as they had previously reached (table 12). It should be stressed that even when the percentage of avoidance responses was comparable with that achieved by the animal before forebrain ablation, the avoidance latencies were generally higher. Median latencies before forebrain ablation were often well below the 5-second CS-US interval, whereas after forebrain ablation, they were quite close to or well above it (figs.15–18).

TABLE 12
HIGHEST PERCENTAGE OF AVOIDANCES ATTAINED FOR BLOCKS OF FIVE SESSIONS

Subject	Before Forebrain Ablation	After Forebrain Ablation
AB	93.3	76.0
AC	98.7	88.0
AI	85.3	12.3
AK	93.3	26.7
AL	73.3	30.7
AP	98.3	85.3
AR	96.0	84.0
AS	92.0	86.7
AT	96.0	62.7
AW	85.3	57.3
AZ	94.7	46.7

Escape latencies in most instances tended to decrease with continued testing (table 11). However, the mean of the 10 escape latencies in the final block of tests was still significantly higher than the mean of the last 10 escape latencies before forebrain ablation ($p=0.005\leftarrow$). In all cases, individual variability remained high compared with pre-operative performance. In 10 subjects, "no crossings" tended to decrease as testing continued (table 5). Again there was some variability but less than in the avoidance responses. In all subjects, responses to the sound at first increased over the level reached immediately after forebrain ablation, but there was a great deal of variability between blocks of sessions (table 6). In two subjects there was a subsequent decrease over the initial level.

In eight subjects, waiting at the hole decreased with continued testing (table 8). Waiting at the back wall either increased or was extremely variable (table 9). Intertrial crossings increased somewhat in all subjects but varied considerably (table 7). In most cases, however, crossing-over remained at a lower level than had occurred before forebrain ablation.

Pre-operatively, all subjects reached criteria (see p.153) for performing consistent avoidances, stabilizing avoidances, and eliminating "no crossing" (table 13). After removal of the forebrain, all eleven subjects reached criterion for consistent avoidances, but only six reached criterion for stabilizing avoidances, and only five eliminated "no crossings." In all three measures the majority of subjects took considerably longer to achieve the above criteria.

HISTOLOGICAL EXAMINATION

In all instances where sham operations had been performed, the brains were examined under a dissecting microscope to ascertain that no brain damage had been sustained before the olfactory bulbs were ablated. Before forebrain ablation, the brains were similarly examined to make certain that the olfactory bulbs had indeed been removed. In each instance the operation was considered successful, as there was no visible brain damage to areas caudal to the olfactory bulbs.

On examination of the stained sections, it was determined that the entire forebrain was ablated in eight subjects (AC, AI, AK, AP, AR, AS, AW, AZ). In AB (fig.23) both lobes were ablated except for the ventral edge of the nucleus

TABLE 13

NUMBER OF SESSIONS TO FIRST AVOIDANCES, STABILIZATION OF AVOIDANCE RESPONSES AND ELIMINATION OF "NO CROSSINGS" BEFORE AND AFTER FOREBRAIN ABLATION

Subject	First Avoidances		Avoidances Stabilized		"No Crossings" Eliminated	
	Before	After	Before	After	Before	After
AB	6	10	13	16	13	18
AC	2	9	16	70	4	115[a]
AI	7	33	17	90[a]	17	90[a]
AK	6	10	9	100[a]	25	100[a]
AL	7	19	20	80[a]	15	80[a]
AP	3	3	3	7	4	17
AR	2	4	4	9	3	15
AS	1	8	3	14	11	29
AT	5	16	24	38	16	60[a]
AW	9	5	29	61[a]	12	16
AZ	7	14	10	50[a]	19	50[a]

[a] Criterion not reached. (See text p.153 for definition of criteria.) Number indicates last session.

lateralis. In AT (fig.23) the nucleus lateralis was mostly intact with the rest of the forebrain ablated. Parts of several nuclei were spared in AL (fig.24). These included the posterior parts of the nucleus lateralis and the nucleus precommissuralis inferior, as well as part of the nucleus precommissuralis anterior. The rest of the forebrain had been ablated.

There was more variability with respect to the amount of preoptic area that was invaded. In five subjects, AB (fig.23), AL, AR, AI, and AZ (fig.24) the preoptic area was completely intact. In two subjects, AC (fig.23) and AP (fig.24), the anterior portion of the preoptic area, including the nucleus parvocellularis and the nucleus entopeduncularis was ablated. More caudally, the ventral portion of the preoptic area was spared and from the level of the nucleus preopticus magnocellularis the preoptic area was intact. The anterior end of the preoptic area was ablated in AW (fig.24) as was the nucleus entopeduncularis on the right side. The nucleus preopticus magnocellularis was intact. The operation on AS (fig.24) was very similar to that of AW with somewhat more invasion of the preoptic area. The entire entopeduncular nucleus on the right side and part of it on the left side had been ablated. In AI (fig.24) part of the preoptic area, including the nucleus entopeduncularis was ablated. In AK (fig.24) slightly more of the preoptic area was ablated, but in other respects, the operation was similar to that of AI.

As in Experiments I and II, we find that only in the subjects with the most extensive lesions, AI and AK (fig.24), does there seem to be a correlation with the degree of deficit resulting from forebrain ablation. These two subjects suffered the greatest deficits after the forebrain was ablated. It should be noted, however, that another subject, AL (fig.24), with a less extensive lesion performed about as poorly as these two.

EXPERIMENT IV — THE EFFECT OF ABLATION OF THE CORPUS CEREBELLI ON THE ACQUISITION OF A CONDITIONED AVOIDANCE RESPONSE WITH LIGHT AS THE CONDITIONED STIMULUS

MATERIALS AND METHODS

EXPERIMENT IVa

THIS EXPERIMENT may be considered a pilot study during which apparatus and procedures were tested and improved. Five male and three female *Tilapia heudelotii macrocephala* were the subjects, light was the CS, and the US was shock. Equipment and procedures were similar to those used in Experiment I except that only three to four sessions per week were administered and the stimulator used was a square wave stimulator similar to the one employed in Experiments II and III. In addition, the CS-US interval, which was 2.5 seconds during most of the experiment, was increased to 10 seconds during the last 11 sessions to see if subjects who were not avoiding could do so if they had more time.

EXPERIMENT IVb

This experiment was a repetition of Experiment IV–a with the following differences: (1) 14 males were used as subjects; (2) the CS-US interval was 5 seconds throughout most of the experiment, but was increased to 10 seconds during the last 10 sessions for the cerebellar ablates that survived to the end of the experiment; (3) five sessions per week were administered; (4) subjects with cerebellar ablations were tested for a longer period of time.

PROCEDURES: Pre-operative procedures in both experiments were similar to those used in Experiment I. Control subjects underwent sham operations, whereas the body of the cerebellum (corpus cerebelli) was ablated in the experimentals. Surgical and histological techniques were similar to those described earlier. The Mann-Whitney U test (Siegel, 1956) was used to determine levels of significance.

RESULTS

EQUILIBRIUM, POSTURE, LOCOMOTION

Following ablation of the cerebellum, some fish exhibited minor disturbances of equilibrium mainly in the form of rocking from side to side. However, this disappeared within 24 hours, and subsequently the cerebellum-ablated subjects could not be distinguished from the sham-operated subjects on the basis of posture, locomotion, or feeding behavior.

ACQUISTION AND PERFORMANCE OF THE CONDITIONED RESPONSE

All subjects learned to escape with the aid of a net, the cerebellum-ablated subjects requiring two to three times as many net-guided sessions as did the controls (table 14). Once the controls had learned to escape, "no crossings" were almost completely eliminated, and the avoidance response was subsequently acquired (table 15). This was reflected in median latencies, which generally remained below the CS-US interval (table 16).

The cerebellum-ablated subjects presented a different picture. The escape response was acquired more slowly, and even after it was acquired, "no crossing" remained at a high level (table 15). The conditioned avoidance response was not acquired with any degree of stability by any of these subjects, for while six fish reached criterion for making their first consistent avoidances (at least one avoidance response during each of five consecutive sessions) none of them reached criterion for stabilization of the avoidance response (at least eight avoidance responses during each of five consecutive sessions). In fact, the three subjects in Experiment IVa that reached criterion for first

TABLE 14
MEAN NUMBER OF NET-GUIDED SESSIONS BEFORE REGULAR TESTS WERE BEGUN[a]

	Experiment IVa	Experiment IVb
Sham-operated	5.7 (1–9)[b]	6.1 (3–11)
Cerebellum-ablated	17.5 (6–22)	10.6 (4–20)

[a] See p. 153 for criterion for terminating net guiding.
[b] Range.

TABLE 15

PERCENTAGE OF AVOIDANCES (A), ESCAPES (E), AND "NO CROSSING" (NC) FOR BLOCKS OF TEN SESSIONS IN EXPERIMENT IVb

Sessions	Sham-operated				Cerebellum-ablated			
	A	E	NC	No. of Subjects	A	E	NC	No. of Subjects
1–10	14.5	33.8[a]	9.9	7	1.7	11.1[a]	22.3[b]	7
11–20	38.0	53.0[a]	9.0	7	2.0	37.2	55.5	7
21–30	71.3	25.8[a]	3.0	7	1.4	61.9	36.5	7
31–40	77.8	21.2	1.0	7	4.1	66.1	29.8	7
41–50	82.5	16.7	0.9	7	4.1	63.2	32.7	7
51–60	96.1	3.9	0	6	4.6	66.5	28.9	6
61–70	96.5	3.4	0	6	2.0	59.8	38.2	5
71–80	—	—	—	—	0.2	49.1	50.7	5
81–90	—	—	—	—	0.3	16.4	83.3	5
91–100	—	—	—	—	0.3	21.2	78.5	5
101–110	—	—	—	—	0.5	27.7	71.8	5
111–120	—	—	—	—	1.1	37.7	61.2	5

[a] Net-guided escapes not included.
[b] The percentage of "no-crossing" in sessions 1–10 is unexpectedly low because some trials were net guided.

consistent avoidances did so only after the CS-US interval was increased to 10 seconds. Only one sham operate failed to meet these criteria before it died (after 57 sessions). This difference in avoidance behavior was the most striking difference between the control and the experimental groups (table 17). Four cerebellum-ablated subjects in Experiment IVb never avoided at all. Two avoided only intermittently (usually one avoidance every three to four sessions), whereas the fish with the highest level of avoidances did so only one to four times per session during the last 20 sessions. In Experiment IVa, the four cerebellum-ablated subjects made totals of only 1, 3, 8, and 10 avoidances during the entire experiment.

TABLE 16

MEDIAN LATENCY (IN SECONDS) FOR EVERY 10 SESSIONS IN EXPERIMENT IVb[a]

Subject[b]	Sessions 1–10	11–20	21–30	31–40	41–50	51–60	61–70
Sham-operated							
NB	14.2	6.5	2.0	2.3	2.2	1.9	1.8
NC	3.0	2.5	2.2	2.5	2.6	2.8	2.3
ND	11.2	8.1	4.8	2.0	1.6	2.0	2.1
NH	18.3	7.2	1.9	1.9	2.1	1.9	2.1
NI	21.0	9.4	2.1	1.7	1.5	1.3	1.0
NK	11.0	4.9	4.3	2.0	1.5	1.8	1.9
Mean	13.1	6.4	2.9	2.1	1.9	2.0	1.9
Cerebellum-ablated							
NE	23.8	30.0	24.8	30.0	30.0	30.0	30.0
NJ	27.2	30.0	30.0	19.7	30.0	22.2	30.0
NL	22.0	30.0	22.7	19.6	18.2	14.9	18.7
NS	19.7	30.0	30.0	30.0	30.0	30.0	30.0
SL	21.4	19.1	9.1	8.0	8.5	11.0	9.5
Mean	19.0	23.1	19.4	17.9	19.4	18.0	19.7

[a] Net-guided sessions omitted.
[b] Subjects that died during the experiment not included.

TABLE 17

NUMBER OF SUBJECTS IN EXPERIMENTS IVa AND IVb AND MEAN NUMBER OF SESSIONS TO REACH CRITERIA[a]

	First Avoidances	Avoidances Stabilized	"No-Crossing" Eliminated
Sham-operated (11)[b]			
No. of Subjects	10	10	10
No. of Sessions	14.4 (1–34)[c]	23.1 (3–51)	16.9 (1–44)
Cerebellum-ablated (11)			
No. of Subjects	6	0	2
No. of Sessions	44.3 (3–63)	—	51 (39–63)

[a] See text p. 153 for definition of criteria.
[b] Total number of subjects.
[c] Range.

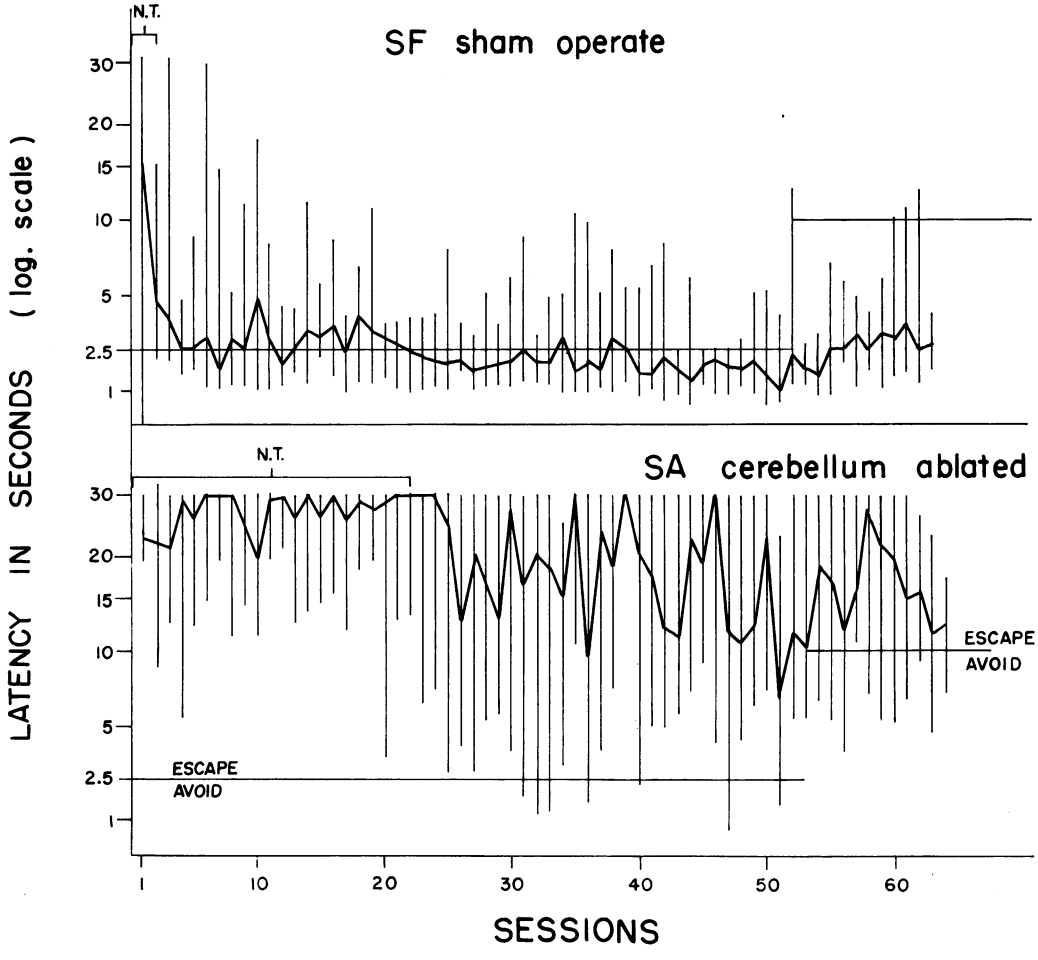

FIG. 19. Median response latency in seconds for each session of representative sham-operated subject (SF) and a representative cerebellum-ablated subject (SA) in Experiment IVa. CS-US interval increased to 10 seconds for last 11 sessions. (For further explanations see fig. 3.)

Abbreviation: N.T., net-guided sessions.

All sham operates eliminated "no crossing," (for criterion see p. 153) whereas only two cerebellum-ablated subjects accomplished this (table 17). "No crossing" continued at a high level in the other cerebellar operates throughout the experiment (table 15).

The clearest picture of the differences in performance between the experimental and the control subjects can be obtained by examination of representative graphs of median latencies (figs. 19–21). The performance of the shams was similar to that of controls in previous experiments in terms of speed, level of avoidance responding, and consistency of response. The cerebellum-

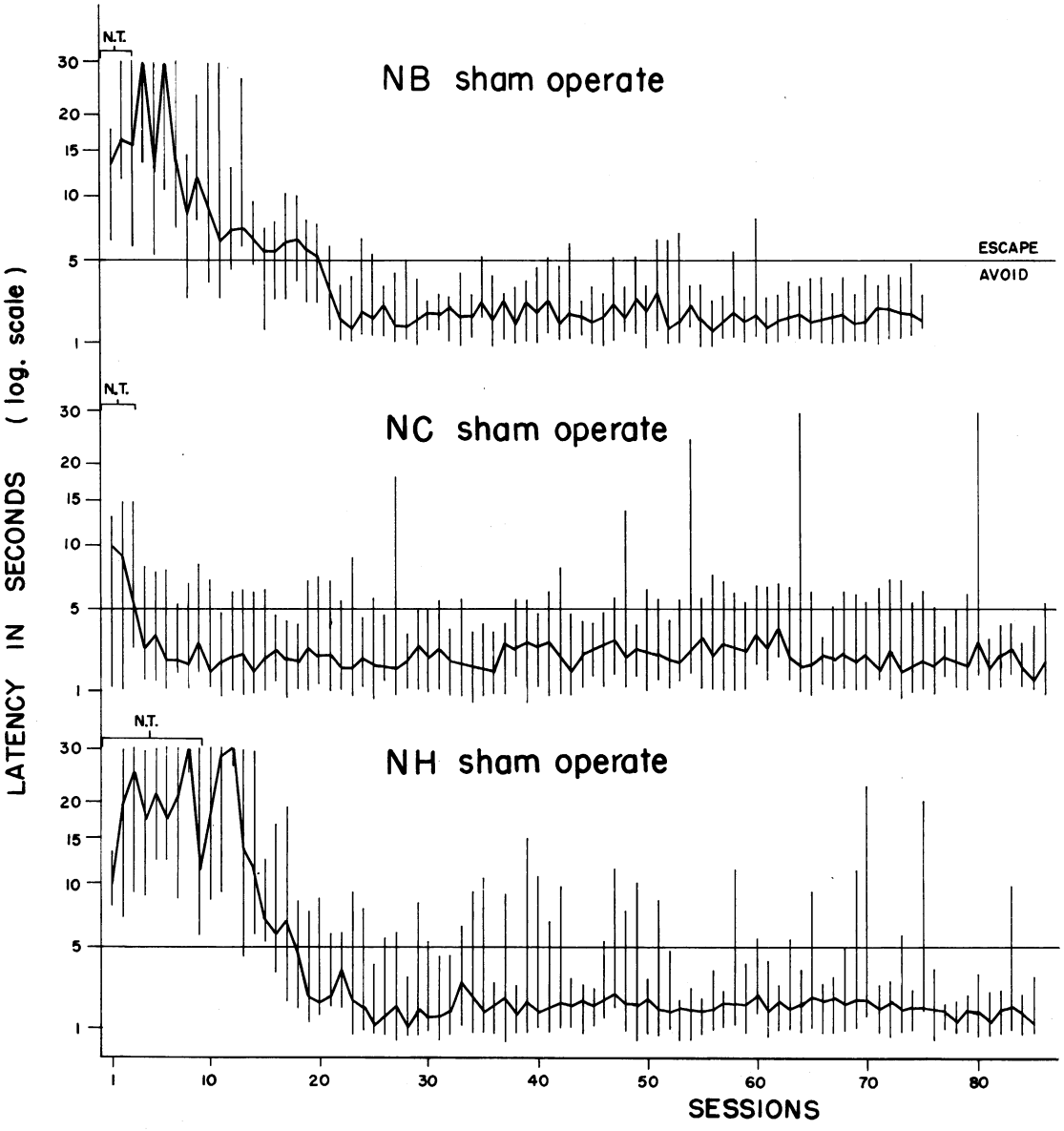

FIG. 20. Median response latency in seconds for each session of three representative sham-operated subjects in Experiment IVb. (For further explanations see fig. 13.)

Abbreviation: N.T., net-guided sessions.

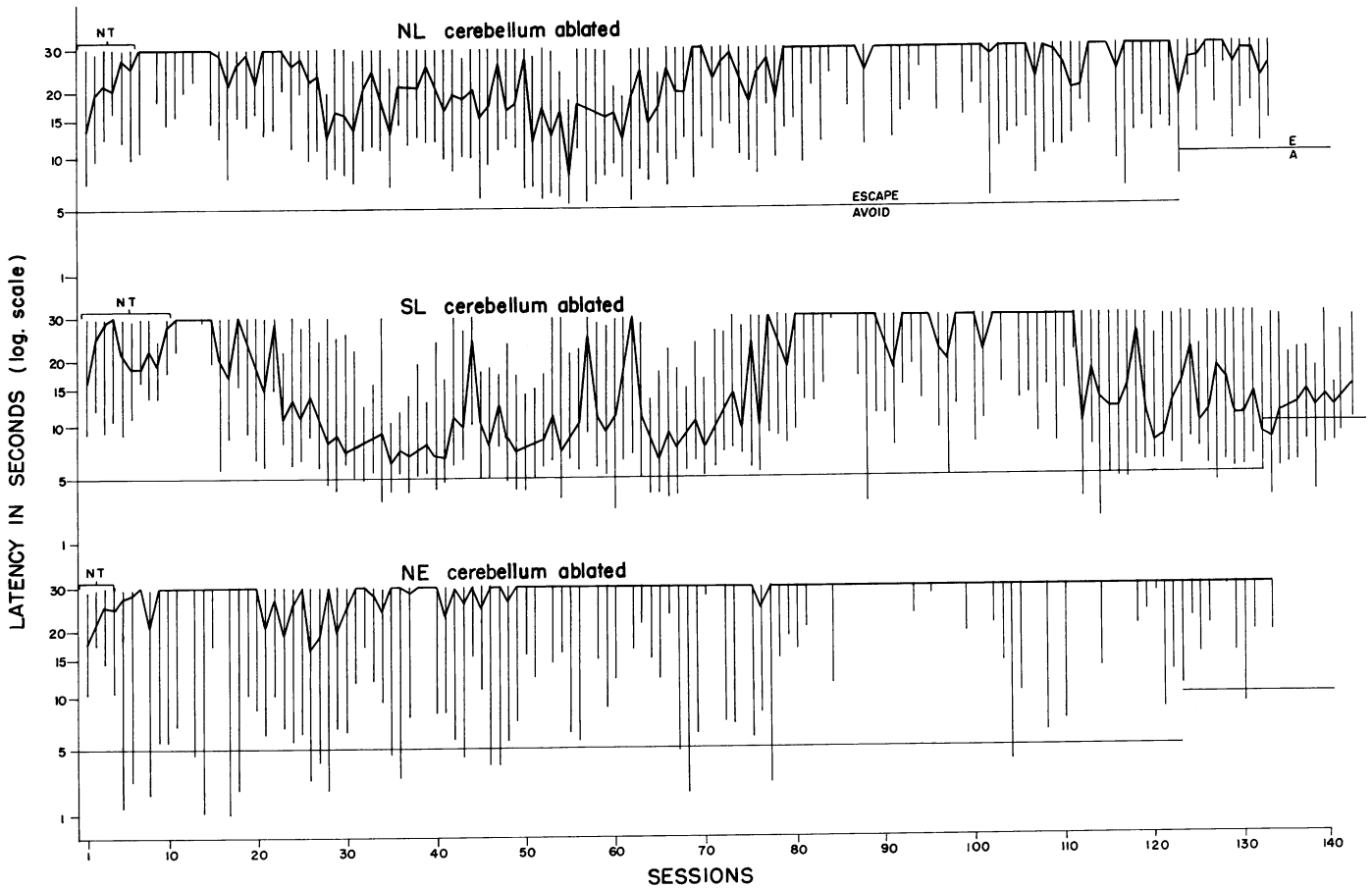

FIG. 21. Median response latency in seconds for each session of three representative cerebellum-ablated subjects in Experiment IVb. CS-US interval increased to 10 seconds for last 10 sessions. (For further explanations see fig. 13.)

Abbreviations: A, avoid; E, escape; N.T., net-guided sessions.

ablated subjects, on the other hand, were much slower, rarely avoided, and were extremely variable.

Increase in CS-US Interval

When the CS-US interval was increased from 2.5 to 10 seconds in Experiment IVa, median latencies of all sham operates increased (SF, fig.19). Three of the shams avoided significantly more, with SK (the sham with the poorest performance) showing the greatest increase (table 18). Three of the four cerebellar ablates also avoided significantly more, but the level of the best operate was still far below that of the controls. The CS-US interval was also increased to 10 seconds for the last 10 sessions of the surviving cerebellar ablates in Experiment IVb. The three survivors that had never avoided (see, for example, fig.21, NL) did not do so under these conditions. Another subject, NE (fig. 21), showed no improvement. The remaining subject, SL (fig.21), did show a significant increase in the percentage of avoidances; however, its performance remained considerably below that of the controls (table 18).

TABLE 18

Percentage of Avoidances before and after Increase of CS-US Interval to 10 Seconds

Subjects	CS-US Interval 2.5 seconds[a]	5 seconds[a]	10 seconds[b]
Sham-operated (Exp. IVa)			
SC	84.7	—	85.3
SF	91.3	—	98.0[c]
SK	8.0	—	37.3[c]
GS	70.0	—	90.0[c]
Mean	63.5	—	77.7
Cerebellum-ablated (Exp. IVa)			
SA	1.4	—	21.1[c]
SG	1.5	—	0
GV	3.6	—	21.5[c]
GW	0	—	4.6[c]
Mean	1.6	—	11.8
Cerebellum-ablated (Exp. IVb)			
NE	—	0	1.0
NJ	—	0	0
NL	—	0	0
NS	—	0	0
SL	—	1.3	28.0[c]
Mean	—	0.3	5.8

[a] Last 10 sessions before increase.
[b] First 10 sessions after increase.
[c] Significant at 0.01 level→.

Qualitative Observations

As a group, the cerebellar operates waited at the hole much less frequently than did the sham operates (table 19). The latter responded to the CS during 50 per cent of the trials in the first 10 sessions, increasing steadily until they responded during almost all of the trials (table 19). Their cerebellum-ablated counterparts began responding at a lower level, reached a high point of 79.6 per cent in the middle of the experiment, and then declined.

A more marked difference was found in the percentage of trials during which the partition was nosed (table 19). Shams nosed the partition during 79 per cent of the trials of the first 10 sessions. This dropped steadily until they nosed in less than 4 per cent of the trials by the time testing was discontinued. Cerebellum-ablated subjects, on the other hand, maintained about the same level of nosing (between 86.7 and 98.3 per cent) throughout the experiment.

Histological Examination

Sham-operated Subjects: Gross examination of the brains indicated that no damage had been inflicted by the sham operations.

cerebellum-ablated subjects

General Results: The body of the cerebellum was almost completely ablated in all subjects, with variation only in the amount of tissue remaining in the deeper parts. In no instance was the valvula injured. Except for one fish, (SG, fig.25), in Experiment IVa, in which a very slight nick to the medial edge of the left tectum was noted, there was no damage to any other parts of the brain. The following description of lesions, therefore, pertains solely to the body of the cerebellum.

Individual Subjects GV, Fig.25: The molecular layer was completely ablated except for a small portion of the anterior ventral portion. The anterior portion of the granular layer was mostly intact, as was the Purkinje layer. Posteriorly, the granular layer was almost completely obliterated and few Purkinje cells were seen. The eminentia granularis appeared to be intact. A small part of the stratum granulare ventralis and the stratum fibrosum remained. The cerebellar crests were intact.

TABLE 19

PERCENTAGE OF TRIALS FOR BLOCKS OF 10 SESSIONS DURING EXPERIMENT IVb

Sessions	Waited at Hole before Trial		Responded to Light[a]		Nosed Partition	
	Sham-operated	Cerebellum-ablated	Sham-operated	Cerebellum-ablated	Sham-operated	Cerebellum-ablated
1–10	3.7	0.8	50.0	33.1	79.0	89.7
11–20	5.4	0.8	78.0	53.3	73.0	88.5
21–30	22.8	0.8	90.0	64.8	39.0	86.7
31–40	27.3	0.1	94.0	68.0	25.1	94.0
41–50	24.8	0.3	97.0	75.0	16.4	95.8
51–60	20.2	0.1	99.7	79.6	3.5	98.3
61–70	25.4	0.3	99.5	73.2	2.2	97.1
71–80	18.3	0.9	99.6	68.3	3.9	96.2
81–90	—	1.3	—	54.5	—	88.7
91–100	—	0.7	—	53.4	—	87.9
101–110	—	0.4	—	56.6	—	92.0
111–120	—	0.4	—	55.5	—	95.6

[a] Onset of CS.

SA, FIG.25: The anterior end of the granular layer was largely intact as was a small portion of the anterior edge of the molecular layer. The Purkinje layer was mostly ablated. Caudally, the granular layer on the left side was completely ablated, whereas the ventral edge remained on the right. The molecular and Purkinje layers were completely ablated on the left side but only partially on the right. The stratum granulare ventralis was completely ablated, but a portion of the stratum fibrosum remained. The cerebellar crests were untouched.

GW, FIG.25: A small portion of the anterior ventral edge of the molecular layer remained. Also, the anterior part of the granular layer remained, but more caudally both layers were completely ablated. The Purkinje layer was completely ablated as was the eminentia granularis and the stratum granulare ventralis. All but the anterior end of the stratum fibrosum was ablated. The cerebellar crests were intact.

SG, FIG.25: This operation was similar to that of GW. It appeared, however, that all of the molecular layer was ablated and that in the stratum granulare ventralis, a small portion of the anterior edge was left.

NE, FIG.25: This subject had the greatest amount of tissue remaining in Experiment IVb. Of the molecular layer, a small amount of the most anterior ventral lateral parts remained, and a small portion of the anterior ventral portion of the granular layer was found. A small portion of the Purkinje layer also remained. The eminentia granularis, stratum granulare ventralis, stratum fibrosum and cerebellar crests were intact.

NS, FIG.25: A small portion of the anterior ventral edge of the molecular and granular layers remained. A small portion of the Purkinje layer was seen. About one-third of the stratum fibrosum was left anteriorly. The eminentia granularis, the stratum granulare ventralis and the cerebellar crests were completely intact.

NL, FIG.26: This operation resembled that of NS except that the stratum fibrosum was completely ablated and slightly more of the Purkinje layer remained.

NJ, FIG.26: The anterior ventral portions of the granular layer and the lateral portion of the molecular layer on the right side remained. A small portion of the Purkinje layer (particularly on the right side) also remained. The eminentia granularis on the right side was intact but was largely ablated on the left. The stratum granulare ventralis was also invaded, especially on the left. A small portion of the stratum fibrosum remained. The cerebellar crests were intact.

SL, FIG.26: A small portion of the anterior ventral portion of the molecular and granular layers were seen. The Purkinje layer was completely obliterated. On the right side the eminentia granularis was ablated, but on the left it was almost intact except for the dorsal edge. The stratum fibrosum was largely ablated

except for the anterior ventral edge. As far as could be seen, a small portion of the ventral edge of the stratum granulare ventralis on the left side remained. Unfortunately, because some brain sections were lost, this could not be verified. The cerebellar crests were intact.

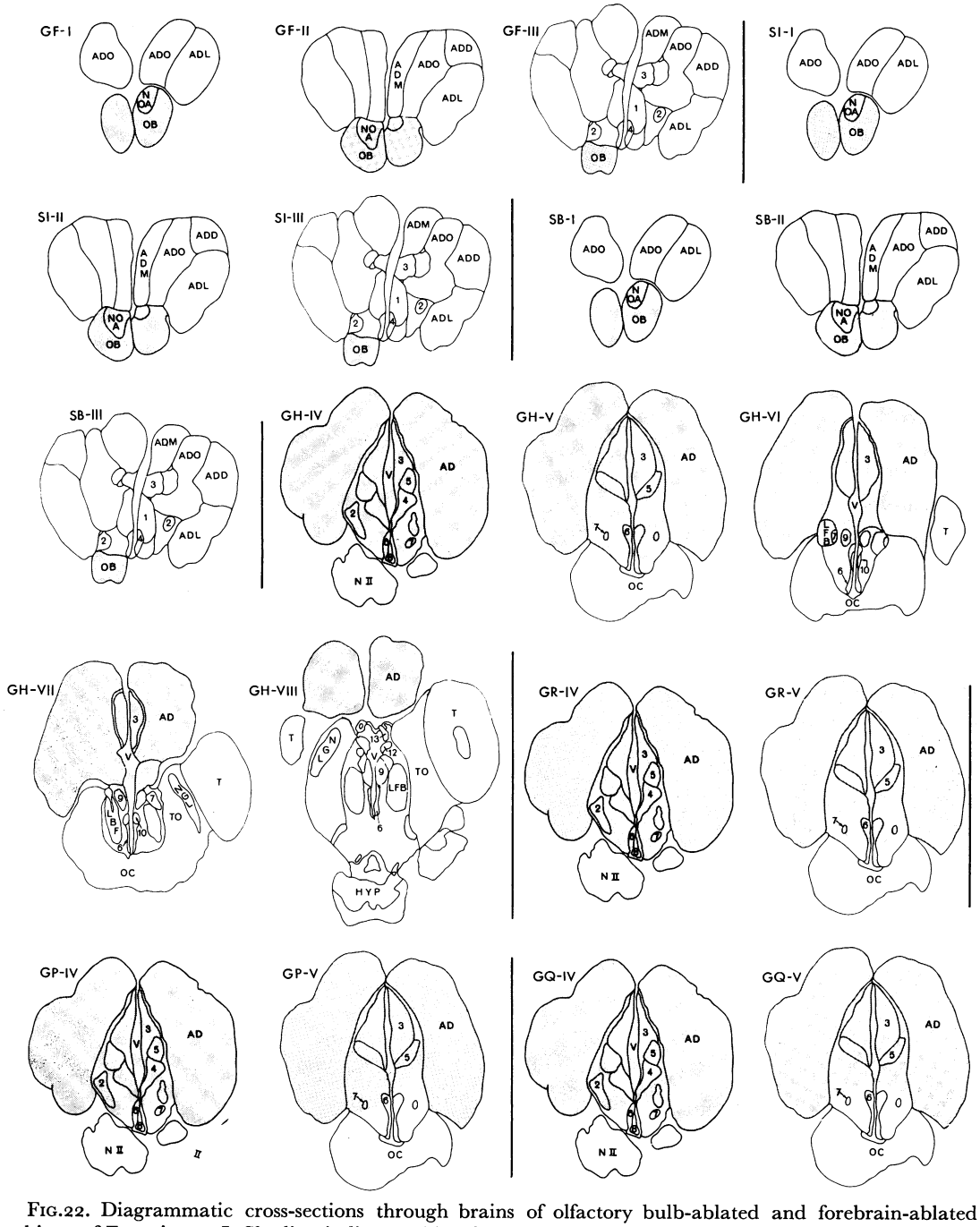

FIG. 22. Diagrammatic cross-sections through brains of olfactory bulb-ablated and forebrain-ablated subjects of Experiment I. Shading indicates ablated areas. Cross-hatched areas indicate disorganized forebrain tissue. Sections VII and VIII of subject GH represent the ablations at these levels for all forebrain-ablated fish in figures 22–24 except section VII of subject AC, figure 23. × 9.5 Key to abbreviations and section levels indicated by Roman numerals, p. 192.

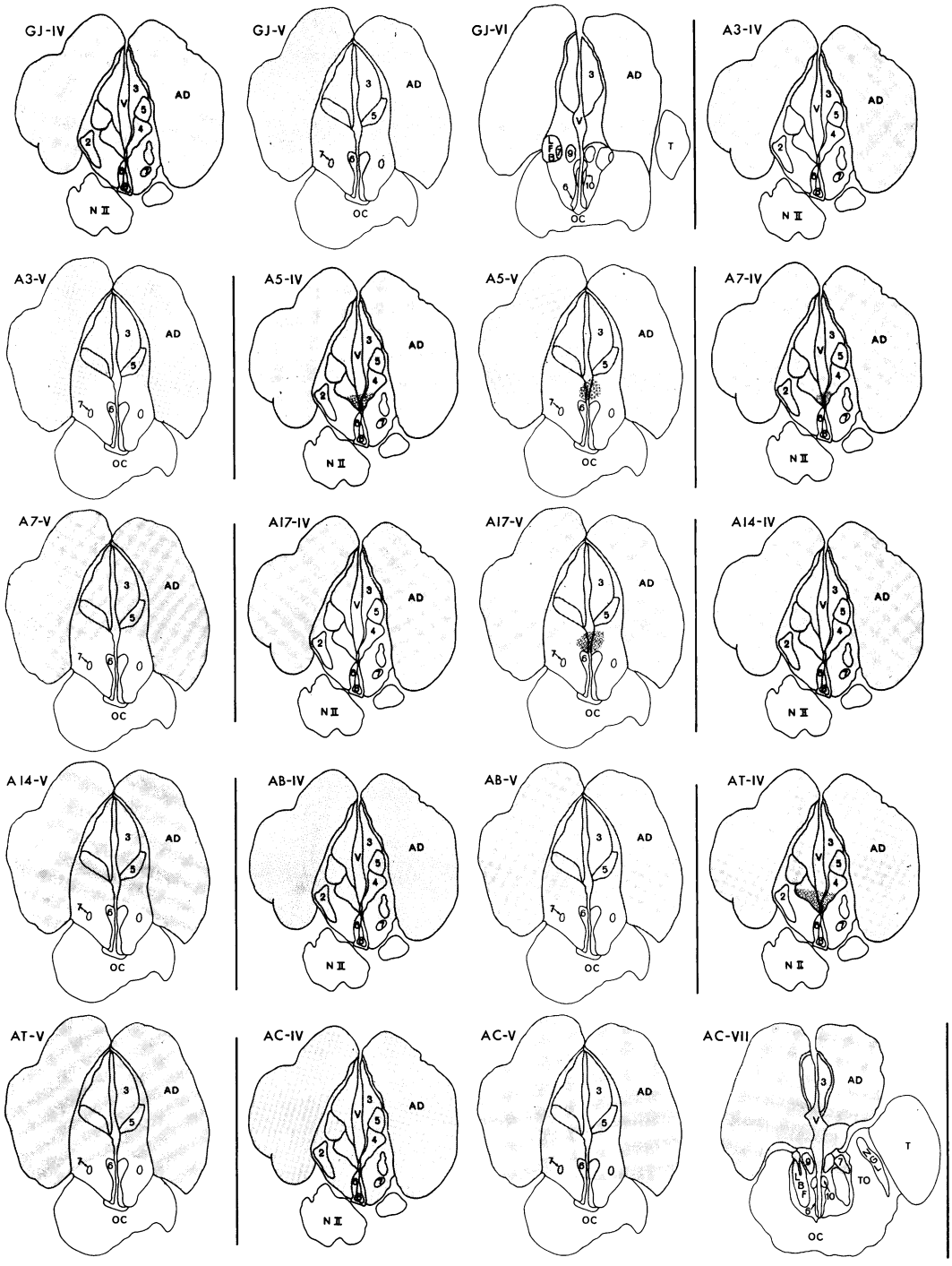

FIG.23. Diagrammatic cross sections through brains of forebrain-ablated subjects of Experiments I, II, and III. × 9.5 (For details see fig.22.)

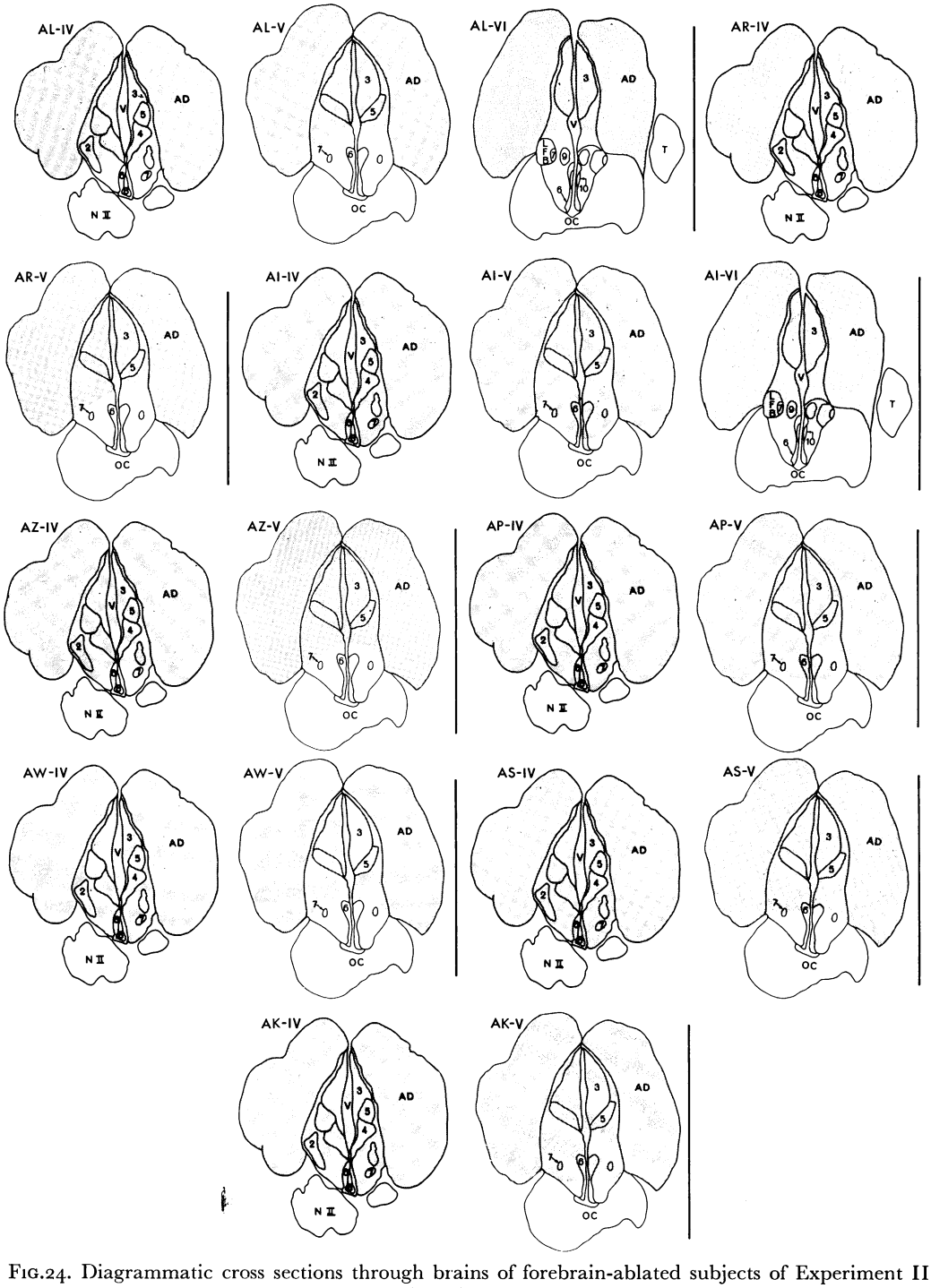

FIG.24. Diagrammatic cross sections through brains of forebrain-ablated subjects of Experiment III. × 9.5 (For details see fig.22.)

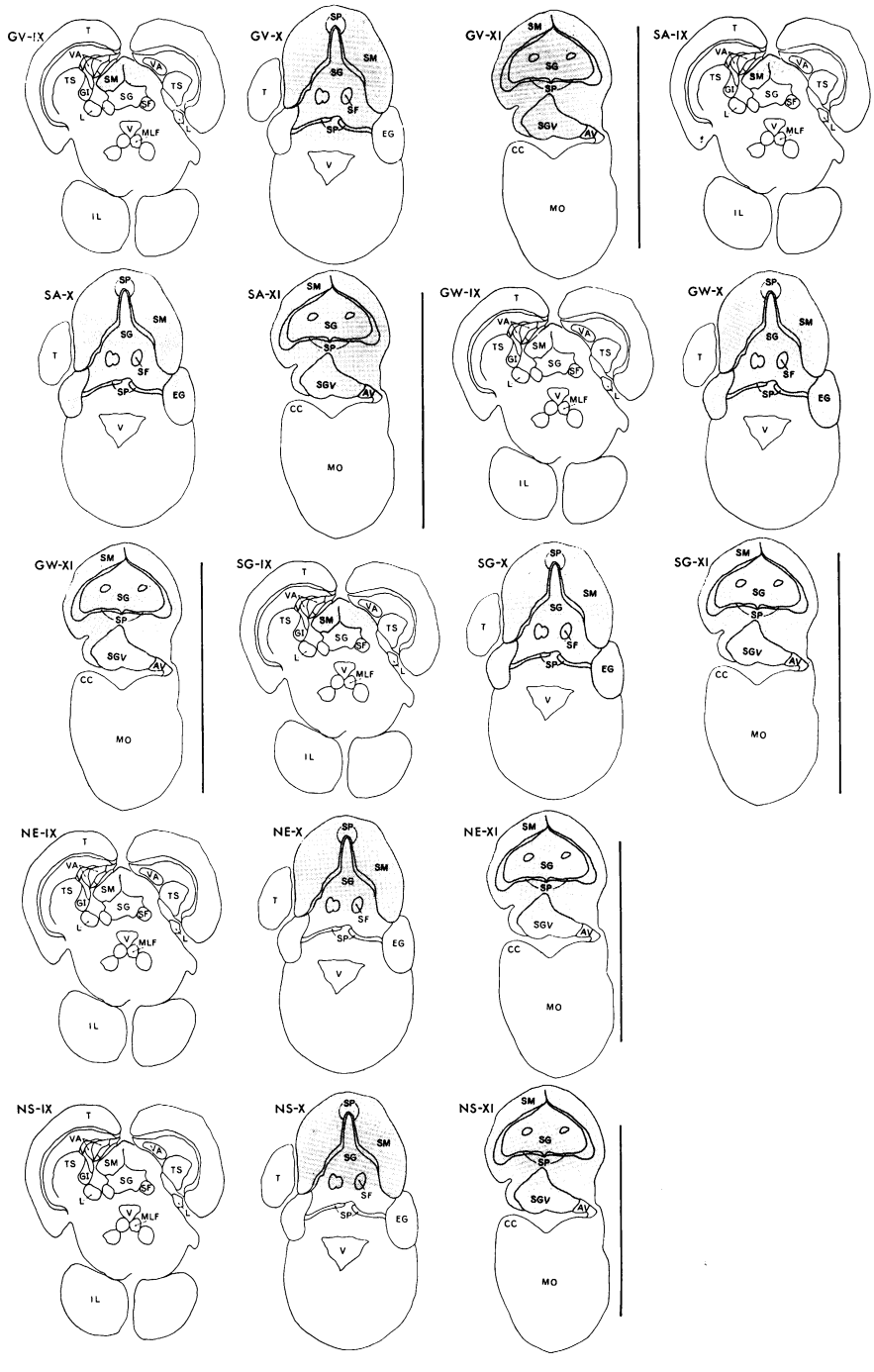

FIG. 25. Diagrammatic cross sections through brains of cerebellum-ablated subjects in Experiments IVa and IVb. × 7.9. (For details see fig. 22.)

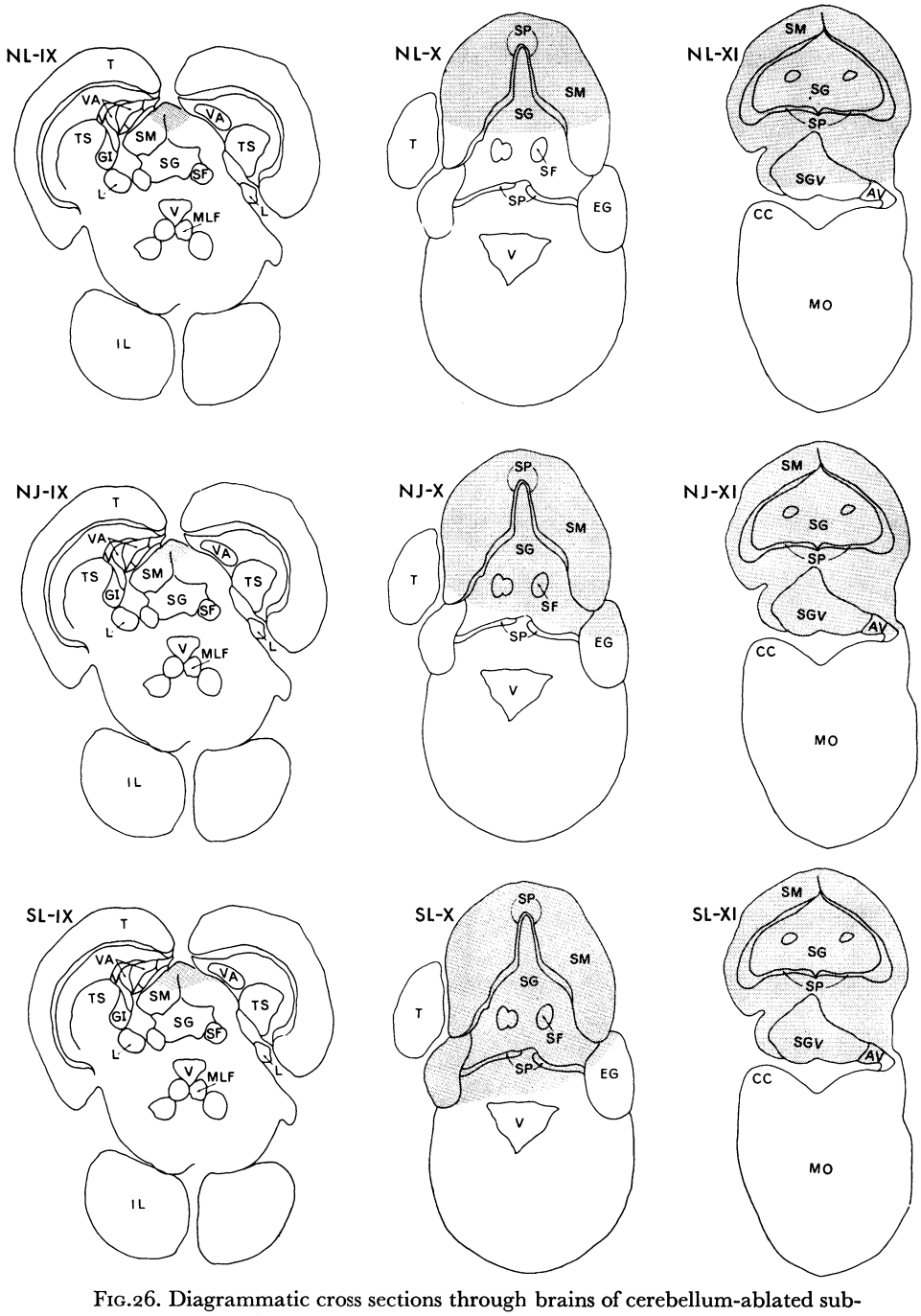

FIG.26. Diagrammatic cross sections through brains of cerebellum-ablated subjects in Experiment IVb. × 12.4. (For details see fig.22.)

HISTOLOGICAL CROSS-SECTIONS OF OPERATED BRAINS

Levels of Sections

I. Cross-section through the forebrain at the anterior edge of the olfactory bulbs.
II. Cross-section through the forebrain at the middle of the olfactory bulbs.
III. Cross-section through the forebrain at the posterior edge of the olfactory bulbs.
IV. Cross-section through the forebrain at the anterior end of the preoptic area.
V. Cross-section through the forebrain at the level of the nucleus precommissuralis superior.
VI. Cross-section through the forebrain at the level of the nucleus preopticus magnocellularis (anterior end).
VII. Cross-section through the forebrain at the level of the nucleus preopticus magnocellularis (posterior end).
VIII. Cross-section through the posterior end of the forebrain and the anterior end of the diencephalon.
IX. Cross-section through the tectum, caudal end of the valvula, and anterior end of the body of the cerebellum.
X. Cross-section through the body of the cerebellum at the level of the eminentia granularis.
XI. Cross-section through the posterior end of the body of the cerebellum at the level of the stratum granulare ventralis.

ABBREVIATIONS

AD, Area dorsalis
ADD, Area dorsalis pars dorsolateralis
ADL, Area dorsalis lateralis
ADM, Area dorsalis medialis
ADO, Area dorsalis olfactosomatica
AV, Auricle
CC, Cerebellar crests
EG, Eminentia granularis
GI, Ganglion isthmi
HYP, Hypophysis
IL, Inferior lobe of hypothalamus
L, Nucleus lateralis valvula
LFB, Lateral forebrain bundle
MLF, Medial longitudinal fasciculus
MO, Medulla oblongata
NII, Optic nerve
NGL, Nucleus geniculatus lateralis
NOA, Nucleus olfactorius anterior
OB, Olfactory bulb
OC, Optic chiasma
SF, Stratum fibrosum
SG, Stratum granulare
SGV, Stratum granulare ventralis
SM, Stratum moleculare
SP, Stratum Purkinje
T, Tectum
TO, Tractus opticus
TS, Torus semicircularis
V, Ventricle
VA, Valvula
1, Nucleus precommissuralis anterior
2, Nucleus lateralis
3, Nucleus precommissuralis superior
4, Nucleus precommissuralis inferior
5, Nucleus precommissuralis intermedia
6, Nucleus preopticus parvocellularis
7, Nucleus entopeduncularis
8, Recessus preopticus
9, Medial forebrain bundle
10, Nucleus preopticus magnocellularis
11, Nucleus subhabenularis
12, Nucleus habenularis
13, Epiphysis

DISCUSSION

THESE STUDIES HAVE DEMONSTRATED THAT: 1) Deficits in performance of a conditioned avoidance response result from ablation of the forebrain. These deficits occur whether the conditioned stimulus (CS) is visual or auditory. 2) Deficits are also found in the acquisition of a conditioned avoidance response when training is initiated after forebrain ablation. 3) The deficits found in both types of experiment (ablation before or after training) are similar and do not seem to be due to any specific sensory or motor defect. 4) Compensation takes place after many months of testing as most forebrainless subjects eventually acquire the avoidance response, performing more efficiently and less erratically than in the early post-operative tests. 5) Ablation of the body of the cerebellum results in more severe deficits with respect to acquisition of a conditioned avoidance response than does ablation of the forebrain.

FOREBRAIN STUDIES

The results of our experiments provide additional evidence that the teleost forebrain (in some species at least) plays an important role in the acquisition and performance of conditioned avoidance responses. Two major differences were found between forebrain-ablated subjects and controls. The first was that the forebrain-ablated subjects took longer to acquire the avoidance response. The second was that after acquiring the avoidance response, forebrain ablates performed less efficiently and less consistently. The additional efficiency and speed of reaction that the forebrain provides has obvious adaptive value.

Ablation of the olfactory bulbs resulted in deficits in performance, which, in contrast to the effects of forebrain ablation, were slight and transitory. As these deficits were so transient, they may have been due to operative trauma, but the possibility exists that the olfactory bulbs may contribute in a small way to the facilitatory process.

FOREBRAIN FUNCTION AND AROUSAL: MOTOR PROCESSES

How well does the arousal hypothesis (Aronson, 1948, 1963, 1967; Aronson and Kaplan, 1968) explain the results obtained with respect to motor, sensory, and associative processes? Most studies have agreed that the forebrain plays no direct role in the maintenance of posture, equilibrium, or locomotion. Changes in degree of locomotion and in reactivity have, however, been reported (e.g., Ferrier, 1879, cited by Ten Cate, 1935; Polimanti, 1913; Hosch, 1936). Our observations confirm that whereas no postural or swimming abnormalities resulted from forebrain ablation, locomotion may have been affected indirectly. For example, some forebrainless subjects exhibited very slow swimming speeds in the test situation, although at other times normal speeds were observed.

The lower rate of crossing over, the delayed response to the CS, and the longer latencies for avoidance and escape shown by the forebrainless fish may all be manifestations of the effect of deficits in the arousal system on motor processes.

FOREBRAIN FUNCTION AND AROUSAL: SENSORY PROCESSES

There is no positive evidence in these experiments that sensory processes, aside from olfaction, were directly affected by forebrain ablation. Forebrainless subjects were as quick and as accurate in seizing small pellets of food dropped into their home tanks as were the controls. They were often observed swimming toward the near side of the tank and nosing the wall when a food pellet was dropped into an adjoining tank. In addition, they were quite agile in avoiding being caught in a net, and they responded in the same way as did the controls to a loud tap on the tank or a disturbance of the water. Nevertheless, some of the results could also be interpreted as effects on sensory processes. For example, there was a noticeable difference initially between the responses of the controls and the forebrain-ablated subjects to the CS, particularly when the CS was a light; controls began responding and reached a high level of response much sooner than did the forebrainless fish. Forebrain ablates were much more variable, responding to the CS as often as the controls during some sessions and then becoming less responsive. Another difference was that the responses of the controls were usually immediate and those of the forebrainless fishes in many cases were delayed.

It should be noted that even in the experiments in which sound was the CS, the response itself was visually oriented so that the deficits seen after forebrain ablation may have resulted from some interference with visual processes. Several authors have postulated that the forebrain may have some special relation to the visual system. Aronson (1948) was particularly impressed with the fact that the components of courtship and sex behavior that declined most after forebrain ablation were those that were visually oriented. Bernstein reported that forebrain ablation does not affect brightness discrimination in goldfish, but does result in loss of hue discrimination (1961a, 1961b). This conclusion was later modified (Bernstein, 1962) to state that the operation resulted in "a selective suppression of the color visual system." However, since function was completely restored four hours after surgery, the possibility that the initial effect was due to trauma cannot be ruled out.

Kholodov (1959 cited by Kholodov, 1960) reported that defensive conditioned reflexes with a sound or magnetic field as the CS were not affected by ablation of the forebrain. However, when light was the CS, the operation affected the stability of the conditioned reflex.

The relationship between the forebrain and the visual system has also been investigated electrophysiologically. Changes in the electrical activity of the forebrain following photic stimulation have been reported in codfish (Enger, 1957), in goldfish, and carp (Schadé and Weiler, 1959; Voronin and Gusel'nikov, 1959; Malikuina and Flerova, 1960). Some investigators, however, offer convincing evidence that the evoked potentials recorded from the forebrain in response to light were actually artifacts (Voronin and Gusel'nikov, 1963; Gusel'nikov, Onufrieva and Supin, 1964).

Using a different technique, Timkina (1965) reported that electrical responses to photic stimuli recorded from the surface of the optic tectum of carp were modified when the forebrain was strychninized, or when the ipsilateral olfactory tract was stimulated electrically. Response latencies became longer, and the amplitude of the response became smaller. Responses in the forebrain to electrical stimulation of the ipsilateral olfactory tract were modified when strychnine was applied to the tectum, or when the eyes of the fishes were stimulated by light. In a related study, Zagorul'ko (1965) found that strychnine spikes evoked from the forebrain of carp were suppressed by rhythmical photic stimulation. Removal of the forebrain resulted in increased amplitude and duration of photically evoked responses from the tectum, whereas both chemical (strychnine) and electrical (square wave) stimulation of the forebrain inhibited evoked responses to light. Zagorul'ko offered this evidence in support of the hypothesis that the teleost forebrain exerts a regulatory influence on the activity of midbrain visual centers.

An additional piece of evidence is offered by Aronson and Kaplan (1968). In an unpublished study, Aronson noted a correlation between large eyes, visually dominant behavior, elaborate visual mechanisms in the midbrain and diencephalon, and elaboration of the pars dorsolateralis of the dorsal (pallial) area of the forebrain. Although the evidence is as yet inconclusive, there are indications that the forebrain has a facilitatory role (facilitation including both excitation and inhibition) with respect to both motor and sensory processes which are mediated in lower brain centers.

Forebrain Function and Arousal: Associative Processes

The associative processes are more difficult to deal with because we have, as yet, no direct way of measuring them as distinct from motor or sensory processes. If, however, we examine the various associations that are involved in a conditioned avoidance response, we may be able to get some indications of the underlying mechanisms. We can separate these into: (1) associations between the unconditioned stimulus, US (shock), and the unconditioned response, UR (escape), although strictly speaking, an escape is not "unconditioned" as the fish must learn to make this response (This is in contrast to experiments that use a classical conditioning technique, in which the subject is not trained to make a specific response); (2) associations between the US and the conditioned stimulus CS (light or sound); (3) associations between the CS and the conditioned response, CR (avoidance); and (4) other associations.

1. Associations between the US and the UR involve integration of the sensory input and the sensory and motor aspects of performing the escape response. Both forebrain-ablated and control subjects began escaping at about the

same time, and the percentage of escapes actually increased in trained subjects after the forebrain was ablated. This indicates that the forebrain is not necessary for the association between the US and the UR to be established and that this association is not lost after forebrain ablation. This does not mean, however, that the escape response was altogether unaffected by forebrain ablation. In fact, the escape response was not performed as effectively by forebrain-ablated subjects. Responses were often delayed, slow, or poorly oriented. Subjects might first swim in the wrong direction, or nose the partition or walls of the tank. This was reflected in the longer escape latencies found in most forebrainless subjects. In contrast, Hainsworth *et. al.* (1967) reported no differences in escape latencies in forebrainless and sham-operated goldfish. The possible explanations for these as well as other contradictory results will be discussed later.

2. The association between the US and the CS involves sensory-sensory integration. Again, the evidence at hand indicates that these associations could still be made and were retained after forebrain ablation, but they were less stable. We must mention again that forebrainless fish do make responses to the CS that indicate an association between light or sound and shock. These responses include swimming that is correctly oriented toward the opposite compartment, turning or excited swimming in some other direction, and circling movements. Naïve fish tested with light alone were never seen to respond in this way. They tended to remain quiet or to swim to a corner and quiver their fins. The fact that some investigators have reported no effect of forebrain ablation on the acquisition or performance of a classical conditioned response (e.g., Karamian, 1956; Bernstein, 1961a) can be interpreted as further evidence that the association between US and CS has not been erased. However, both in our studies and in that of Hainsworth *et al.* (1967) performance of the CR was severely affected by forebrain ablation, whereas performance of the UR (escape) was affected much less or not at all. This suggests that associations between the CS and the US, although not obliterated, may have been disturbed.

3. The association of the CS with the CR (avoidance) involves higher level integration, as it is built upon the previous associations between the US and the UR and between the US and the CS. In addition, the time factor is more important in performance of the CR. In our experiments, subjects had only 2.5, 5, or 10 seconds in which to avoid, but they had the remainder of the 30-second trial (20 to 27.5 seconds) in which to escape. Our studies have shown that forebrain-ablated fish can learn to avoid, but take much longer to do so, first, because the establishment of the associations between the US and the CS and between the CS and the CR take longer, and, second, because even after these associations are established, they are not as stable as those of the controls. Once control fish have learned to escape and avoid, improvement in performance proceeds in a fairly linear fashion and is remarkably consistent. Forebrain-ablated fish are, on the other hand, extremely variable, with periods of improvement interspersed with periods of deterioration.

In fish trained prior to operation, avoidances show a steep decline immediately after the operation. Hainsworth *et al.* (1967) interpreted this as a complete loss of retention of the avoidance response, which is then relearned. They pointed out that it took the subjects whose forebrains had been ablated after training just as long to reach a criterion of 70 per cent avoidances as subjects whose forebrains had been ablated before training. In our previous investigations, however, it was reported that during the first sessions after forebrain ablation, some subjects performed better than they did in subsequent tests (Aronson and Herberman, 1960; Kaplan and Aronson, 1967). The fish still made some conditioned approach responses and avoidances or escapes. Deterioration of performance then set in and performance reached a low level—often with no responses at all. Following this, improvement, at times sudden and dramatic, was noted in some animals. This is not the classical picture of retention loss followed by relearning. Most of the evidence related to the function of the forebrain, either in performance of species-typical behavior or in the acquisition and performance of conditioned responses, indicates that the forebrain is not directly responsible for the organization of the behavior or for retention. The deficits that result from extirpation of the forebrain suggest, rather, that the forebrain is concerned with facilitating or modulating these underlying processes. It seems

more likely that it is the processes concerned with retrieval, attention, or coordination of the ancillary behavior that result in efficient, stable performances that are disrupted by ablation of the forebrain. We have, consequently, preferred to interpret our results as decrements in performance rather than as a complete loss of retention.

When a trained animal does not perform after some experimental manipulation, it is exceedingly difficult to interpret the results in terms of underlying mechanisms. Has retention of the associations been abolished or is there interference with retrieval processes? Is the animal unable to perform or is his motivation to do so lessened? Neither our experiments nor that of Hainsworth *et al.* (1967) provide final answers to these questions.

4. As there are apparently no changes in classical conditioning after forebrain ablation (Karamian, 1956; Bernstein, 1961a), it seems probable that the decline in arousal is mainly in the more complex associative (integrative) functions of the brain. During the learning of a conditioned avoidance response, many levels of conditioning also take place, e.g., autonomic conditioning, as well as conditioning to incidental stimuli that occurs prior to onset of the US. In addition, second or third order conditioning may take place, i.e., neutral stimuli may become associated with the CS. Incidental responses may also be inadvertently reinforced. The control subjects seemed able to utilize these accompanying conditioning processes effectively. For the most part, they learned to inhibit inefficient responses and to pay attention only to relevant cues. Increased levels of avoidance responding, for example, were accompanied by inhibition of nosing the barrier and an increase in waiting at the hole. In short, the control subjects appeared to have formed a "gestalt" of the situation. The forebrainless subjects began to respond in a similar manner much later, when the compensatory processes began to function. Even after nine months of testing, however, few of these animals reached the level of efficiency and stability of the controls.

It is apparent that the forebrain is not essential for avoidance learning, as forebrainless subjects do eventually learn. It has been found, however, that the forebrain is necessary for learning a conditioned avoidance response quickly and for performing that response efficiently and consistently. In examining all these aspects of the CR, we have found no evidence of a specific deficit in forebrainless fish, but rather decrements in behavior that could be attributed to interference with sensory, motor, and associative processes. As in the case of the deficits found in species-typical behavior (see p. 147) these results can well be explained by Aronson's arousal hypothesis, namely, that the forebrain acts as a non-specific energizer or activator of lower centers that are responsible for mediating the behavior.

Arousal Defined

What exactly do we mean by the term arousal? This concept has been used to describe a variety of phenomena which appear to have a common basis. Herrick (1948) viewed arousal as a diffuse, non-specific function that prepared the organism to respond to more specific olfactory, visual, or other stimuli. In this sense, arousal is akin to attention, which is a more focused, selective kind of arousal. Electrophysiologically, an aroused brain is characterized by desynchronized, low voltage, fast activity in fish (Enger, 1957) as well as in mammals (Moruzzi and Magoun, 1949). This EEG activation has been correlated with reduced reaction time (Lansing, Schwartz, and Lindsley, 1959), and thus from the point of view of behavior, arousal may be defined operationally in terms of reduced reaction time.

In our conditioning experiments, reduced escape and avoidance latencies have been considered as one of the signs of arousal, and we have attempted to describe the characteristics that lead to such low latencies in the aroused animal. During the intertrial interval, the control subjects seemed to be aroused in the way that Herrick (1948) defined arousal; that is, they were prepared to respond to the onset of the CS by swimming to the other compartment. Their waiting at the hole and their immediate responses to the CS are evidence for this. Similarly, ducking the head in and out of the hole and intertrial crosses may also be considered as "anticipatory" responses. Again, this kind of arousal is distinctly related to attention. In contrast, the delayed responses of the forebrain-ablated subjects to the CS, their waiting at other places in the test tank, and their slow swimming indicate that they were not so well prepared. Furthermore, the responses of the

forebrainless subjects were often circuitous or poorly oriented. In many cases, the first response to the CS was a turning movement, even when the animals had been facing in the correct direction before the CS was presented. They also spent time nosing the walls of the tank or the partition before entering the hole. Interestingly, as the responses of the forebrain ablates improved, they too acquired some of the more efficient ways of responding.

Comparisons with Other Vertebrates

Because of the atypical embryological development of the teleost forebrain, it is difficult to homologize its various specialized areas with those of other vertebrates. Some neuroanatomists (e.g., Nieuwenhuys 1967b), although stressing the fact that the teleostean forebrain consists of both pallial and subpallial structures, suggested that more specific homologies cannot be made. Others, however (see Droogleever Fortuyn, 1961), considered the major portions of the teleost forebrain as homologous to those rhinencephalic structures in mammals (hippocampus, pyriform cortex, amygdala, and septum) that form the core of the limbic system. This system has also been characterized as a non-specific modulator of behavior patterns organized in other parts of the brain (Gloor, 1960). In the analysis of the changes in behavior that follow ablation of the forebrain, we can see some similarities with the kinds of changes produced by destruction of the hippocampus and amygdala in mammals. Douglas and Pribram (1966) and Douglas (1967) have suggested a model whereby the hippocampus functions as an inhibitory gating mechanism which reduces or eliminates irrelevant stimuli or stimuli associated with non-reinforcement. The amygdala, on the other hand, acts as a register, increasing attention to stimuli as a function of reinforcment. It is possible that analogous counterparts of these two processes underlie the arousal function of the forebrain.

Investigations of the causes of mental deficiency in human beings have recently implicated the arousal level as a possible major factor (Clausen, 1966; Rosvold, 1967). Clausen suggested that, "slight impairment of the ascending reticular activating system would make a less efficient arousal mechanism resulting in generally impaired performance on all tasks." Obviously, the anatomical and physiological mechanisms underlying the teleost and the mammalian arousal systems are very different. It is significant, however, that in two such widely separated groups, functioning arousal systems, which may involve some homologous structures, have been implicated as of prime importance to learning and performance.

Deterioration, Variability, and Compensation

The initial deterioration of performance following forebrain ablation was not the only observed change in behavior. The variability of performance and the subsequent improvement were also quite striking. The behavioral deterioration may be related to the deterioration of some physiological process resulting from lack of stimulation or reinforcement from the forebrain. One possibility is that reverberating circuits in lower centers run down when no longer primed by the forebrain. In this connection it should be noted that the major tracts of the forebrain form a descending system running caudally to the diencephalon and midbrain. Another possibility is that the deterioration is related to the depletion of some biogenic substance normally supplied by the forebrain. For example, acetylcholine has been implicated in normal waking or in the "arousal" mechanism of the cerebral cortex (Celesia and Jasper, 1966). It has also been suggested that norepinephrine produces a non-specific state of arousal (Schildkraut and Kety, 1967).

We hypothesize that the improvement in performance displayed by most forebrainless subjects after several months of testing is due to the building up of some compensatory mechanism. This may involve the development of new connections or the expanded function of some other part of the brain, possibly the cerebellum. It is probable that deterioration and compensation do not proceed at the same rates and that both the session-to-session variability and the general post-operative trend are results of these two opposing processes.

Possible Explanations for Contradictory Findings

In view of the impressive differences between forebrain-ablated and control fish disclosed by our studies, as well as by those of other investigators, it seems puzzling that some investigators reported no changes in learning

behavior after forebrain ablation (see p. 146). There are several possible explanations for this.

(1) Variations in experimental design, apparatus and techniques. It is possible that the experimental design and testing procedures used may have minimized or masked the deficiencies. Some experiments were not carried on long enough to show variability in performance. Several of the negative studies used a classical conditioning paradigm (most of the Russian studies were of this type), and it is quite possible that classical conditioning, which is far less complex than avoidance conditioning, may not be affected by forebrain ablation.

(2) Species differences. The forebrains of various teleosts, although retaining certain basic features, may show complex histological differentiation or dramatic enlargement of certain pallial areas (Meader, 1939b). This suggests that extrapolation from one group of teleosts to another should be made with caution. Most of the negative studies were performed on ostariophysine species, which have a less-differentiated forebrain than that of *Tilapia*.

(3) Incomplete removal of the forebrain. Few of the negative studies included histological examination of the brains, and some did not even offer descriptions of the lesions based on gross examination. In addition to the difficulties of surgery one must consider the possibility of regeneration (Segaar, 1965).

CEREBELLAR STUDIES

General Discussion

Reference has already been made to the compensatory process that appears to take place in the brain after forebrain ablation, and to the suggestion (Aronson, unpublished) that this process might occur in the cerebellum. It was immediately apparent from our studies that ablation of the cerebellum resulted in a far greater deficit than did forebrain ablation. Some cerebellar ablates never learned to avoid at all. Others avoided only intermittently, and even the best cerebellar ablate did not reach criterion for stabilization of the avoidance response; that is, this fish could not avoid even 50 per cent of the time. The cerebellar ablates that learned to avoid intermittently did show some improvement after the increase in CS-US interval to 10 seconds. As these fish did not swim more slowly than did the control subjects, it is suggested that the additional time was needed for integration of the incoming information and for the organization of the response. No compensatory process similar to that noted in forebrain-ablated subjects was evident in the cerebellar albates. Some subjects showed no avoidance learning even after 130 sessions over a period of seven months.

Qualitative Observations

The fact that, at best, the experimental subjects responded to the CS in only 80 per cent of the trials could indicate deficits either in sensory or in associative processes. Indeed, Karamian (1956) reported decreased responsiveness to visual stimuli after ablation of the body of the cerebellum, but this was based on rather crude and unquantified tests, such as response to the onset of a light and the approach of a hand or a net in a well-lighted aquarium.

Because most cerebellum-ablated subjects did not learn either to avoid or to wait at the hole, it would appear that they simply had not learned to associate the CS with the US, or had learned to a low and unstable level. That they persisted in nosing the partition indicates that they also had not learned to inhibit irrelevant responses. It should be mentioned that many subjects nosed other parts of the tank as well, particularly the wall they happened to be facing at the onset of either the CS or the US. As the shams were learning the conditioned avoidance response, their responses to the onset of the CS were more and more oriented toward the partition, which they then nosed. After the avoidance response had been acquired, nosing tended to drop off. Although most of the cerebellum-ablated subjects did not learn to avoid, many of them did learn to orient correctly in response to the CS, at least some of the time, swimming toward and nosing the partition.

Hypotheses Concerning Cerebellar Function

What, then, is the function of the cerebellum, particularly with regard to the conditioned avoidance response? In 1905 the Russian physiologist V. M. Bekhterev (quoted by Karamian, 1956) asserted that, "this astonishing variety and contradiction of views and conclusions of various authors only shows that despite the enormous amount of work, we have advanced little in the study of the functions of the cerebellum . . ." What progress has been

made in the past 60 years can be assessed by the statement of Bell and Dow (1967) regarding cerebellar function, "No one really knows exactly what it does or how it does it."

Not surprisingly, most of the studies of cerebellar function have been performed on mammals. Karamian (1956), one of the few investigators to make a systematic study of the cerebellum and the forebrain throughout the phylogenetic series, had suggested that the fish cerebellum, "participates in the formation of temporary connections" when vision and hearing are involved. Karamian's experiments concerned with conditioning in teleosts were performed on goldfish and carp, the extirpations involving either the entire cerebellum or only the cerebellar body. In the former case, severe disturbances of motor activity, as well as decreased responsiveness to visual and auditory stimuli resulted. His subjects did not survive for more than 26 days. During that time, conditioned responses could not be established. When only the body of the cerebellum was removed, the locomotory disturbances were not as severe. Conditioned responses could be established in some subjects, if the CS were a tone, but this required a longer period of training than was needed for intact subjects. When the CS was a visual stimulus, stable conditioned responses could not be established. Karamian suggested that this difference was due to the uneven distribution of visual and auditory projections in the cerebellum, the visual elements being associated more with the cerebellar body, the auditory elements associated more with the valvula.

In our study only the body of the cerebellum was removed and light was used as the CS. In agreement with Karamian, it was found that stable conditioned responses could not be established. In contrast to Karamian's finding, however, we found little or no evidence of disturbances of balance or locomotion. In some subjects transitory rocking was noted after the operation, but this usually disappeared in an hour or two. It is possible that this discrepancy can be attributed to species differences, because Karamian himself obtained greater or lesser disturbances depending on the species he used.

COMPARISON OF FOREBRAIN AND CEREBELLAR STUDIES

It may be of value, at this point, to compare results obtained in our experiments after ablation of the forebrain and after albation of the cerebellum.

Avoidance: Forebrainless subjects eventually learned to avoid even though they took longer than the controls and did not reach as high or as stable a level of performance. Some cerebellum-ablated subjects never made any avoidance responses at all. Some made intermittent responses, but rarely accomplished more than three to four avoidances during a 15-trial session. The distributions of the performances of the controls, and forebrain- and cerebellar-operates, may be likened to that of three normally distributed curves arranged serially with tails overlapping (fig. 27). In summary, forebrain-ablated fish are still capable of acquiring a conditioned avoidance response and of performing it, eventually, with some degree of stability, whereas cerebellum-ablated subjects are, at best, capable of acquiring the avoidance response only to a very low and unstable level.

Escape: Forebrain operates learned to escape as quickly as the controls did although performance, at first, was more variable and latencies were higher. Cerebellar operates took longer to acquire the escape response and escaped less frequently than their sham and forebrainless counterparts, and escape latencies were higher. Thus the escape response was affected more by ablation of the cerebellum than by ablation of the forebrain.

"No Crossing." Forebrainless subjects eventually learned to avoid or escape during each trial so that in time "no crossing" was almost eliminated. Cerebellum-deprived subjects never learned to do this, consequently during each session there were some trials in which neither an avoidance nor an escape was made.

Compensation: Whereas forebrainless subjects improved in all aspects of the conditioned response, the cerebellar ablates gave no indication of progressive improvement. This suggests that with respect to acquisition of a conditioned avoidance response no similar compensatory process takes place after ablation of the cerebellum as after ablation of the forebrain.

Conclusions: The differences outlined above indicate that the role of the forebrain in learning processes is ancillary and to a large extent can eventually be compensated for. The role of the cerebellum, on the other hand, appears to be essential for establishment of stable conditioned responses. The reasons why forebrain ablates

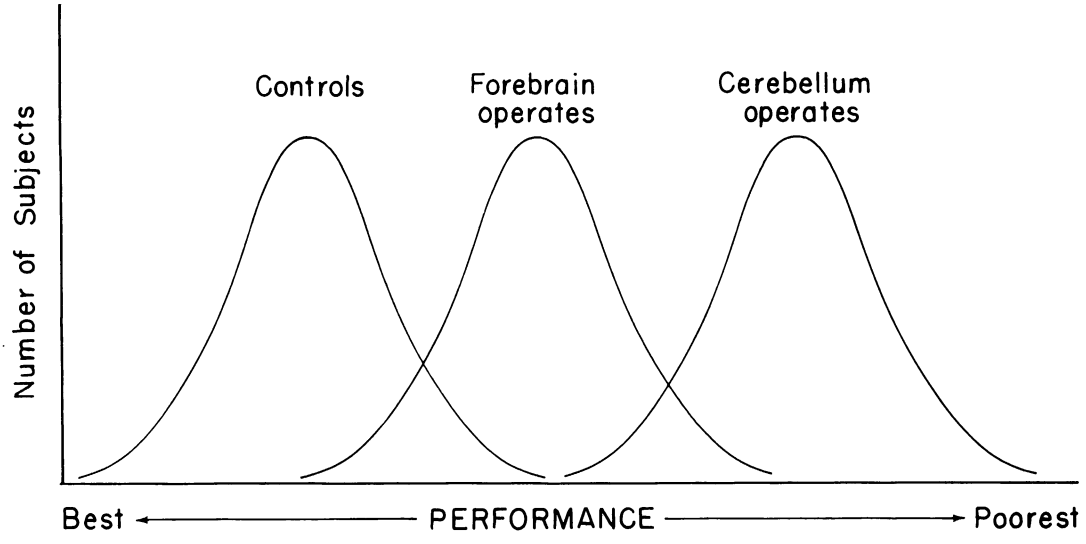

FIG. 27. Hypothetical distribution of over-all performance of control, forebrain-ablated, and cerebellum-ablated subjects.

and cerebellar ablates did not avoid appear to substantiate this. Forebrain-ablated fish gave evidence of having learned to associate the CS with the appropriate responses necessary to avoid the shock. When they did not succeed in avoiding, it was often because their responses were slow, late, or poorly oriented. Thus they appeared to be less well prepared for the onset of a trial and less efficient in performing the response than were the controls. Fish with cerebellar ablations did not seem to have acquired all the necessary associations. They responded less often to the CS and these responses were in many cases not directed toward the opposite compartment. Although they never swam slowly (in fact they sometimes appeared to be hyperexcitable) they circled, dove toward the bottom of the tank, nosed, and responded in other inappropriate ways to the onset of the CS or the US.

It thus appears that the body of the cerebellum plays a major role in the acquisition of a conditioned avoidance response. After ablation of the cerebellar body, only temporary and unstable conditioning can be established, indicating that some other area of the brain, possibly the midbrain, must also participate in this process. It appears, however, that neither this area nor any other area can compensate for the loss of the cerebellum, at least not as observed by us during the seven months of our experiment.

The forebrain, on the other hand, although not directly responsible for the establishment or retention of a conditioned avoidance response, nevertheless indirectly affects many of the sensory, motor, and associative processes by means of its non-specific facilitatory or arousal function.

SUMMARY

THE FUNCTION of the forebrain and the cerebellum in learning in the teleost fish *Tilapia heudelotii macrocephala* was studied. An avoidance conditioning paradigm with light or sound as the CS and shock as the US was used. The design of the four experiments performed was: Experiment I—operations performed prior to training; forebrain ablated in experimental subjects; sham operations performed or olfactory bulbs ablated in controls; CS was light. Experiment II —operations performed prior to training; forebrain ablated in experimental subjects; sham operations performed on controls; CS was sound. Experiment III—operations performed after training; subjects first underwent sham operation and then olfactory bulb ablation, or only the latter; following a period of retesting, both lobes of the forebrain were ablated; CS was sound. Experiment IV, a and b—operations performed prior to training; body of the cerebellum ablated in experimental subjects; sham operations performed on controls; CS was light.

All subjects were trained to avoid or escape from intermittent shock by swimming through a hole in a partition that divided the test tank into two equal compartments. Intact, sham, and olfactory bulb-ablated controls quickly learned to avoid the shock, and performance was stabilized soon after, with a high percentage of avoidance responses and consistently low latencies. These were achieved by the efficient intertrial behavior of the subjects, as well as by their speed and accuracy. When the forebrain was ablated prior to training, subjects took much longer to acquire the avoidance response. Even after long periods of testing, performance remained unstable, with variable latencies and fewer avoidance responses than those of the controls. When the operations were performed after training, it was found that sham operation had no effect on performance, whereas ablation of the olfactory bulbs had only a slight, transitory effect on the performance of some subjects. Ablation of the forebrain, however, resulted in marked and long-lasting deficits. Immediately after operation, avoidances dropped to a low level with a concurrent increase in the number of trials in which subjects neither avoided nor escaped. With continued testing, the performance of some subjects of Experiments I to III improved, but none reached the consistently high levels of avoidance responses and low latencies characteristic of the performance of the controls. On the other hand the performance of a few animals deteriorated in an erratic manner.

Ablation of the body of the cerebellum had, at most, only a very transitory effect on equilibrium. It did, however, result in more severe deficits in the acquisition of a conditioned avoidance response than did ablation of the forebrain. Some cerebellum-ablated subjects never avoided at all, but those that did, performed at a very low level. Even escape performance was below that of a typical forebrain operate with higher latencies and fewer responses. Unlike the forebrain operates, cerebellum-ablated subjects showed no improvement with continued testing.

Results are interpreted as being consistent with our hypothesis that the forebrain acts as a facilitator of behavior which is organized in other brain centers. The subsequent improvement noted in the performance of many forebrain-ablated subjects is regarded as an indication that a compensatory process is taking place in some other part of the brain. The body of the cerebellum appears to play a more essential role in the acquisition of a conditioned avoidance response and may, in fact, be directly concerned with learning processes.

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ADDENDUM

SINCE THE COMPLETION of the manuscript, several recent papers on the function of the teleost forebrain have come to our attention. Savage (1968) studied avoidance behavior of goldfish in a conventional shuttlebox using massed versus spaced trials. Massed trials facilitated learning by the forebrainless fish, but interfered with learning in intact fish. He concluded that in forebrain-deprived fish there is more rapid decay of memory than in normal subjects. In a second experiment Savage (1968) used a delayed compound stimulus prior to the CS and found deficits in attention to the relevant stimulus, a finding which conforms with our arousal hypothesis (Aronson and Kaplan, 1968). In a further study Savage and Swingland (1969) tested forebrainless fish in a simple operant situation and obtained additional evidence for deficits in arousal.

Karamian, Malyukova and Sergeef (1966) studied food acquisition, defensive reflexes and special forms of orienting reflexes in species of marine and freshwater fish and concluded that the telencephalon participates to various degrees in the performance of complex forms of conditioned reflexes. This conclusion would be anticipated from our non-specific activation hypothesis. In a study of the optomotor response Sherman (1969) found that forebrainless *Tilapia mossambica* circumscribed fewer revolutions per minute, reversed less frequently when the drum was reversed, and showed poorer swimming movements than did the control subjects. These results are interpreted as deficits in arousal.

Fiedler (1968) and Demski (1969) stimulated various areas of the telencephalon electrically in free-swimming wrasses and sunfish, and evoked specific patterns of behavior such as nest building, quivering, thrusting, chewing, and yawning. Fiedler accounted for his results by noting that descending fibers from the forebrain impinge directly on motor centers of the midbrain. Since the same behavioral responses were elicited by stimulation of various areas of the forebrain, Fiedler described this phenomenon as "... diese scheinbar diffusen Reizeffekte im Vorderhirn. . ." This interpretation lends further support to the hypothesis of non-specific forebrain function.

In the experiments by Dewsbury and Bernstein (1969) a shuttlebox was also used, but one in which the task was simplified by the use of a minimal barrier. In this situation differences in behavior between intact and forebrainless goldfish were fewer, a result that we had previously predicted from our arousal hypothesis (Aronson and Kaplan, 1968, p. 118). Similarly, Overmier and Curnow (1969) did not find decrements in behavior in a simple classical conditioning situation. The authors of these two papers do not accept the concept of arousal as a major function of the forebrain. Dewsbury and Bernstein refer to the forebrain as a "highly specific information processor," whereas Overmier and Curnow relate the deficits in avoidance learning after forebrain removal to the "instrumental component" of this behavior.

In consideration of these opposing points of view the following items are relevant:

1. These authors base their conclusion on experiments involving one type of behavior, namely avoidance conditioning, whereas we have shown that forebrain deprivation affects almost every type of behavior studied, and that any theory of forebrain function must account for a large array of behavioral changes. A hypothesis based on "non-specific function" seems to fit this situation best.

2. These authors take a rather narrow view of arousal as equivalent to activity, a view very different from ours. We view arousal as a non-specific, non-directive effect on a variety of neural processes, sensory, integrative, and motor. These effects may be excitatory or inhibitory. Although in most experiments, forebrain deprivation appears to result in a decrease in arousal, yielding sluggish, less-reactive subjects, forebrainless fish may also become overreactive and even violent (Hosch, 1936; Sherman, 1969). Our conclusions in the present experiment were supported by qualitative observations which showed that a variety of behavioral changes occur after forebrain extirpation.

3. The neuroanatomical and neurophysiological evidence indicates that the connections of the forebrain of teleosts constitute primarily a descending system impinging on the diencephalon, midbrain, and medulla. An ascending system from the dorsal thalamus, which carries specific sensory information to the forebrain in

higher vertebrates, is lacking in teleosts. Ascending fiber tracts are limited and come mostly from the hypothalamus. It is very doubtful that these fibers carry the kind of specific information that the hypotheses of these investigators require. From the total evidence presently available, it is more likely that the forebrain exerts a generalized regulatory action on lower centers where specificity of function is evident.