

6. Cuff-type methods depend on the detection of the turbulent flow of blood through the artery under the cuff which is produced when the cuff pressure is slightly lower than the arterial pressure. This turbulence produces an audible sound, the Korotkoff sound.
7. Exteroceptive feedback was used to facilitate the development of control of blood pressure. Augmented sensory feedback has been used extensively in studies of the control of heart rate. See (3).
8. These slides have been shown to be an effective operant reinforcer of the galvanic skin response. See G. E. Schwartz and H. J. Johnson, *J. Exp. Psychol.*, in press.
9. This was treated as a two-factor experiment with two levels of one factor (*up, down*) and repeated measures on the second factor (25 trials). For experimental treatments, $d.f. = 1/432$; for trials and the trial \times treatment interaction, $d.f. = 24/432$. See B. J. Winer, *Statistical Principles in Experimental Design* (McGraw-Hill, New York, 1962), p. 302.
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Learning in Fish with Transplanted Brain Tissue

Abstract. Material taken from fish embryos during gastrulation was implanted at prospective tectal sites in host embryos of the same age and species. When mature, the hosts were trained in a series of habit reversals. Two of six animals showed progressive improvement in reversal (a phenomenon not typically found in fish, but characteristic of higher animals), two showed unusually few errors, and two behaved normally. Differences in performance were correlated with differences in brain structure.

One way to study the role of the brain in learning is to study the learning of animals with less than the normal amount of brain tissue. The function of the missing tissue is inferred from differences in the learning of normal animals and animals whose brains are altered by ablation. The ablation method has been used to analyze differences in the learning of vertebrates of different classes (1). For example, adult rats, extensively decorticated at an early age, show fishlike behavior with respect to habit reversal. Now it is possible to

reverse the logic of the ablation experiment—to study the learning of animals with more than the normal amount of brain tissue. The first results of such a “supplementation” experiment suggest that transplantation of brain tissue may facilitate learning in simple vertebrates and even endow them with capabilities normally present only in more complex forms.

Although transplantation has not yet been accomplished in adult animals, Oppenheimer in work with *Fundulus* has shown that brain tissue can be sup-

plemented by grafting during early stages of development (2). In some cases, the grafting procedure produced suppression or rearrangement of primary brain structures; in others, there was duplication of structure. Oppenheimer described certain aspects of the behavior of her altered embryos, but killed them for histological study soon after they hatched (3). We report here some observations on the learning of supplemented animals which we reared to maturity.

Our work was done with *Tilapia macrocephala* (the African mouth-breeder), a species of fish more easily bred in the laboratory than *Fundulus* and about whose learning more is known (4). The surgical technique was a modification of the one developed by Oppenheimer. Embryos at stages 10 through 12 (5) were removed from the mouth of an adult male and rinsed repeatedly in sterile aquarium water to reduce bacteria. The specimens were placed in a dish of sterile water, and the operation was performed aseptically under high-power magnification of a dissecting microscope. Watchmaker's forceps sharpened to needlepoints were used to dechorionate host and donor embryos. Material from posterior regions of the donor embryo was removed with steel needles sharpened to knife-edges and implanted in the prospective tectal tissue of the host. Healing was so rapid that no mechanical device was necessary to keep the implant in place. The host then was transferred to a large covered petri dish containing a 1 percent solution of sulfadiazene in sterile aquarium water. After absorption of their yolk, the fry were fed, first Micrograin, and later trout chow. As they grew in size, the animals were transferred to progressively larger containers.

Although survival rates increased as the surgical technique was perfected, postoperative mortality was at best rather high. Many embryos starved because the grafting interfered with the normal development of mouthparts. In others, the grafts differentiated into non-nervous tissue (notochord, eye, or ear) which may have suppressed the development of brain structures necessary for survival. Tectal abnormalities may also have resulted in respiratory failure (6). We reared only ten experimental subjects to sexual maturity. Of the ten, two died from unknown causes while they were being trained to strike a target and take worms from an automatic feeder, and two failed to adjust satis-

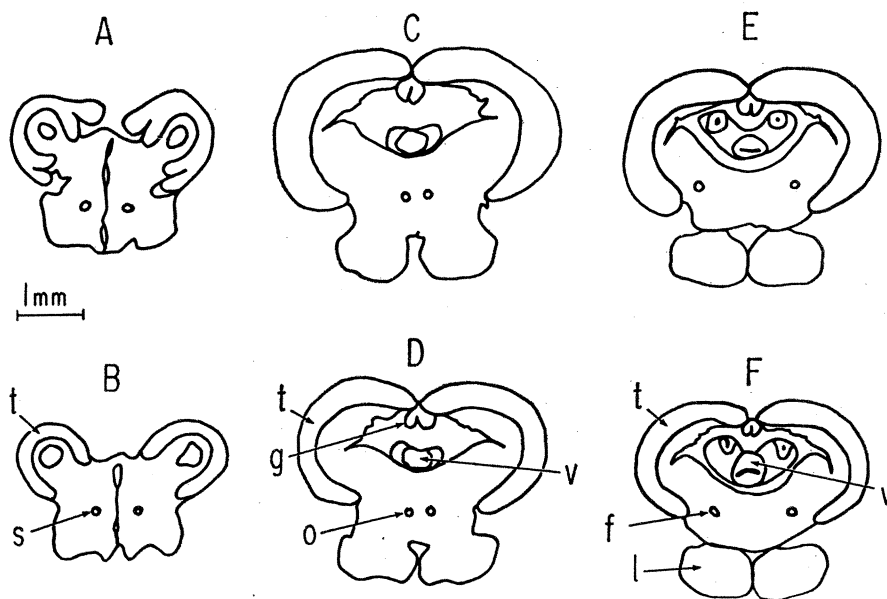


Fig. 1. Coronal sections through the mesencephalon showing (A) a supplementary tectal structure in animal No. 10; (C) tectal thickening in animal No. 4; (E) tectal thickening in animal No. 8; and (B, D, F) the normal brain of animal No. 1 at corresponding levels; (t) tectum opticum; (s) tractus striothalamicus and hypothalamicus; (g) torus longitudinalis; (o) tractus octavo-thalamicus; (v) valvula cerebelli; (f) fasciculus longitudinalis lateralis; and (l) inferior lobes.

factorily to the experimental situation. The six remaining animals were trained in a series of habit reversals.

Our choice of task was related to our choice of the prospective tectum as a grafting site. Higher vertebrates (reptiles, birds, and mammals) show progressive improvement in habit reversal, but fish typically do not (7). Since the tectum of the fish is regarded as homologous to the cortex of mammals (8), and since the results of ablation experiments suggest that the mammalian cortex plays an important role in progressive improvement (9), we thought that reversal learning in the fish might be enhanced by tectal supplementation.

Our fully automated training apparatus has been described (10). Each animal was carried in its individual living tank to a black Plexiglas enclosure. On each trial, two circular Plexiglas targets were illuminated with colored lights, and the response of the animal was to strike at one of them. Correct choice was rewarded with a live *Tubifex* worm; incorrect choice produced a period of darkness, after which the correct target alone was illuminated and the animal was rewarded for response to it. After learning to strike a target and to take worms from the feeder (7), three of the animals (Nos. 1, 4, and 6) were trained to discriminate between red and green targets, and choice of red (the less preferred color) was rewarded; the positions of the two colors were changed from trial to trial in balanced order. The other animals (Nos. 8, 9, and 10) were trained in a position discrimination; both targets were red, and choice of the left target was rewarded. There were 40 trials per day, and each animal was trained to the criterion of six or fewer errors per day, with no more than three errors in the last 20 trials.

As each animal reached the criterion of learning in the original problem (R_0), the correct and incorrect stimuli were reversed—the choice of green now was rewarded for the first three animals, and choice of right for the others. After reaching criterion in this first reversal (R_1), each animal was reversed again, and so on (R_2, R_3, \dots, R_n). Training was terminated when the animals contracted a disease which also affected unoperated mouthbreeders living in other parts of the laboratory. Five of the animals died, and the remaining subject (No. 4) was killed with a 3 percent urethane solution. All specimens were fixed in Bouin's solution. Then their brains were removed, embedded in Paraplast, sec-

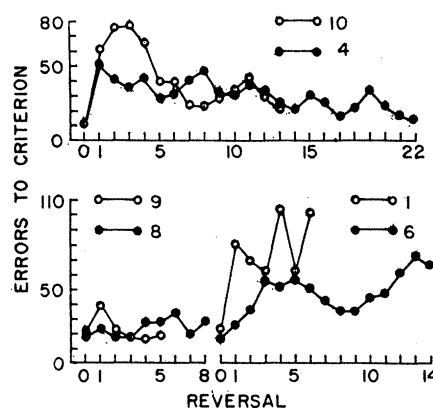


Fig. 2. The performance of the six animals plotted in terms of errors per reversal. Reversal 0 is the original problem. The curves for animals Nos. 4 and 10 (above) show progressive improvement. The curves for animals Nos. 8 and 9 (lower left) show error scores which are unusually low throughout. The curves for animals Nos. 1 and 6 (lower right) are characteristic of normal mouthbreeders.

tioned at 10μ , and stained by Davenport's modification of the Bodian silver-impregnation technique (11). The brains of 24 control animals of the same age and parentage were prepared for study in the same way.

Of the six experimental animals, two had brains which were indistinguishable from those of the control animals, and four showed marked tectal abnormalities. The normal brains were those of Nos. 1 and 6. Three coronal sections through the mesencephalon of No. 1 are sketched in Fig. 1. Section B was taken about 0.5 mm from the cephalic end of the tectum, section D from the middle region, and section F about 1 mm from the caudal end. Although abnormalities in brain structure can be produced in fish embryos by mechanical injury (2) and by antibiotics (12) the fact that these two brains were normal suggests that the changes in structure found in the other four were not due to such secondary aspects of the operative procedure. As Oppenheimer noted, grafted tissue fails occasionally to effect a junction with the nervous system of the host, and may be sloughed off or absorbed into the host tissue (2); that perhaps is what happened here. Whatever the reason for our failure to alter the tecta of these animals, they serve the function of sham-operated controls.

Three kinds of tectal abnormality found in the brains of the other four animals are illustrated in Fig. 1. The brain of No. 10 showed a supplementary layered structure, about 1 mm in longitudinal extent, overlying the anterior third of the tectum. The new struc-

ture may be seen clearly in section A, which was taken at the same (relatively anterior) level as the normal section B. The brain of No. 4 showed a substantial increase in tectal thickness, which may be seen in section C, taken at the same (medial) level as the normal section D. The thickening was evident in all regions of the tectum of this animal although other brain structures were of normal size, and it could be traced to an increase in the width of the tectal layers rather than to an increase in the number of layers. The brains of Nos. 8 and 9, both very much alike, also showed tectal thickening, which was confined principally to the posterior third of the tectum. The thickening may be seen in section E, which was taken from the brain of No. 8 at the same (relatively caudal) level as the normal section F. Nothing remotely like these tectal abnormalities was found in the control brains. The supplementary tectum of No. 10 was, of course, unique, and the enlargements in the brains of the other three animals were evident to the naked eye. A simple ranking test (with the two normal brains assigned median ranks) shows the sample of six experimental brains to be different from the control sample at the 1 percent level of confidence. The locus of abnormality in the experimental brains corresponded to the locus of implant.

Individual learning curves plotted in terms of errors to criterion over reversals (Fig. 2) show that all of the animals (whose training was begun at different times) reached criterion in the original problem and at least five subsequent reversals. The curves also show three kinds of performance. Those for animals Nos. 1 and 6 illustrate the inefficient reversal performance typically found in normal mouthbreeders (7). Those for animals Nos. 4 and 10 show significant progressive improvement ($P < .05$ in each case by Fisher's exact test), and examination of the fine grain of the performance (analysis of errors over blocks of trials) shows it to be indistinguishable from that found in rats (13). The curves for animals Nos. 8 and 9 show another kind of performance which is unusual in fish—not progressive improvement, but error scores which are very low throughout. In experiments with large numbers of normal fish, an occasional animal does give some indication of improvement over reversals. Behrend and Bitterman (7) reported "two or three" in a sample of 75. If we take 4 percent as the population value, the probability of two or

more such animals in a sample of six is only about 3 percent. Animals with low error scores like those of animals Nos. 8 and 9 are at least as rare, and the probability of two such animals in a sample of six is therefore at least as low. To find four such deviant animals in a sample of six is even less likely.

The best evidence that our grafting procedure affected both the brains and the behavior of our experimental animals may be found in the correlation between the brain structure and the behavior. The two animals with normal brains (Nos. 1 and 6) behaved normally. The probability of this correspondence alone is only about 2 percent. Furthermore, the two animals with unusually low error scores (Nos. 8 and 9) showed tectal thickening which was restricted to the caudal region, whereas the two animals with progressive improvement (Nos. 4 and 10) showed changes in the anterior tectum (No. 10 a new structure; and No. 1, a general thickening that included the anterior region). Our results thus suggest that supplementation experiments have an important role to play in the analysis of brain function.

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They were directed medially approximately 4 mm inward from the lateral surface and about 1 mm below the dorsal surface of the superior temporal gyrus. Nine electrodes were implanted similarly, but on one side only, in the auditory cortex of a second monkey (M-10). The surgical procedure and method of implanting electrodes have been described (2). Intracranial stimulation through pairs of electrodes was provided by an isolated constant current generator, with a continuously variable 60-hz output of from 1 to 1000 μ amp (root mean square).

Bipolar and monopolar evoked potentials elicited by pure tones were recorded from these electrodes. For monopolar recording the indifferent electrode consisted of five screws distributed around the skull. Potentials were recorded with standard neurophysiological equipment, including Grass P511 preamplifiers and a Tektronix oscilloscope. Frequency response of the system was limited by low and high half-amplitude filters set at 3 hz and 2 khz. Signals from the preamplifiers were also fed to the analog-to-digital converter of a Digital Equipment Corporation (PDP-8) computer for on-line analysis. The computer was programmed, first to sample (at 4 khz) a 100-msec episode of cortical activity starting from the onset of the tone, and then to determine the latencies of the first positive-going peak and the first negative-going peak, and the peak-to-peak amplitude of the signal (3). This procedure was followed for each evoked potential. Statistics based upon these individual measurements were then calculated.

The behavioral procedures have been described (2, 4). Training and testing were conducted in a sound-deadened, electrically shielded, double-walled experimental chamber (Industrial Acoustics). During experimental sessions each subject was restrained in a standard primate chair. Each subject's head was further restrained so that Permoflux (PDR-600) earphones could be placed directly over the external auditory meatus. The sound generation and calibration equipment and procedures have been described (5). The tone was electrically switched with a rise and fall time of 5 msec. Intensities of all tones were measured at the opening of the external auditory meatus with a calibrated probe tube and Bruel and Kjaer condenser microphones. All sound intensities given in this report are in decibels relative to 0.0002 dyne/cm².

Evoked Potentials and Auditory Reaction Time in Monkeys

Abstract. Monkeys with bipolar stimulating and recording electrodes in primary auditory cortex were trained to release a key to the onset of a pure tone. Substitution of direct cortical stimulation for the pure tone resulted in a reduction of 15 milliseconds in the latency of the behavioral response. This changed latency agreed with the latency of the primary evoked potential recorded from the animals. Systematic related changes in the amplitude of the central response and in the latency of the behavioral response followed changes in the intensity and frequency of the acoustic stimulus.

Monkeys can be trained to respond rapidly (within 200 msec) to the onset of acoustic stimulation. Replacing the acoustic stimulus with direct electrical stimulation through electrodes in the primary auditory cortex results in a more rapidly executed behavioral response. This shift in latency agrees with the initial latency of the acoustically evoked primary potential recorded from the same electrodes. Moreover, concurrent behavioral and electrophysiological measurements indicate that

characteristics of the evoked potential and behavioral response vary systematically with changes in the frequency and intensity of the acoustic stimulation. The findings suggest an effective approach to the study of relations between cortical function and behavior.

Bipolar platinum-iridium electrodes were implanted bilaterally in the primary auditory cortex (I) of a monkey (M-9) (*Macaca irus*). The electrodes were placed when sufficient bone was removed to reveal the fissural patterns.