Lesions in the Medial Forebrain Bundle: Delayed Effects on Sensitivity to Electric Shock

Abstract. *Rats with bilateral lesions in the medial forebrain bundle demonstrate both an increased sensitivity to electric shock, as reflected by a lowered jump threshold, and a decrease in the concentration of serotonin in the brain. Both effects of the lesion are characterized by a delayed time of onset and gradual development, which approximates the time required for progressive nerve degeneration in the central nervous system. It is suggested that this behavioral effect of bilateral lesions in the medial forebrain bundle may be due to a central denervation supersensitivity.*

It is well known that changes in behavior produced by lesions in the central nervous system are not always permanent and that animals may, with time, demonstrate a gradual recovery. Such results have indicated that the effects of some lesions are not constant but may be a function of the time after surgery. In contrast, it has been generally assumed that there is an immediate onset of the behavioral effects of lesions as a consequence of the interruption of excitatory or inhibitory pathways in the central nervous system. Such a view has been supported by several reports of behavioral changes occurring within a few hours after lesions have been made. However, the assumption that the effects of a lesion occur immediately has never been thoroughly investigated, since in the majority of ablation studies the intervals between operation and testing have been approximately 7 days.

Research in this laboratory has revealed that certain subcortical lesions, including those placed in the medial forebrain bundle, can produce an increased sensitivity to electric shock. We now present evidence that this effect of lesions in the medial forebrain bundle does not occur immediately after destruction of tissue but requires time for its full development.

Experimentally naive male albino rats of the Holtzman Sprague-Dawley strain were housed two per cage and were given continuous access to food and water throughout the experiment. When the rats were 80 to 84 days old, bilateral electrolytic lesions were made in the medial forebrain bundle within the lateral hypothalamus in a manner previously described (*1*). The electrode was placed by means of a Krieg-Johnson stereotaxic apparatus at a point 2 mm posterior to the bregma, 1.5 mm lateral to the midline, and 9.5 mm below the surface of the skull. The lesion was produced by passage of a 2-ma current for 30 seconds. Rats not operated on and rats subjected to sham operations served as controls. In the sham operation the rats were treated in exactly the same manner as those which received lesions except that the electrode was never introduced into the brain.

All animals were then tested for their response to electric shock by the flinch-jump method of Evans (*2*), with the following modifications: the testing apparatus consisted of four boxes (25 by 38 by 30 cm) with grid floors and clear glass fronts; 10 rather than 14 series of shocks were employed; the shock intensities within each series were 0.1, 0.2, 0.3, 0.4, 0.5, 0.75, 1.00, 1.50, 2.00, and 2.50 ma and each shock was applied for 0.2 second. At each shock intensity the animal's behavior was rated as either: (i) no response, (ii) flinch (crouch or startle, rear paws never leave the grid), or (iii) jump (rear paws leave the grid or the rat runs vigorously, or both). In addition, the presence or absence of vocalization was recorded at each shock intensity. Flinch, jump, and vocalization thresholds were then arbitrarily defined as the lowest shock intensity at which the animal responded at least five out of ten times. As a special precaution, the identity of the rats was not known to the rater.

Separate groups of animals were tested 2, 4, and 10 days after surgery. Group 1, tested 2 days after being operated on, consisted of six rats with lesions, four unoperated, and eight sham-operated rats; group 2, tested on the 4th day after surgery, contained eight rats with lesions and eight sham-operated rats; group 3, tested 10 days after surgery, contained eight rats with lesions, four unoperated, and eight sham-operated rats.

Sixty days after surgery the brains of randomly selected rats were either prepared for histological examination of the locus and extent of the lesion or assayed for content of serotonin by the spectrophotofluorometric method of Bogdanski *et al.* (*3*), the quantities of reagents given by Udenfriend *et al.* (*4*) being used.

The sham operation had no significant effect on the flinch or jump threshold. When the data for the three groups were combined, the mean flinch threshold was 0.21 ma and the mean jump threshold was 0.82 ma for the eight controls, while for the 24 sham-operated controls these threshold values were 0.22 ma and 0.86 ma, respectively.

The effects of bilateral lesions in the medial forebrain bundle on jump threshold as a function of the time after surgery are shown in Fig. 1. Bilateral lesions had no significant effect on jump thresholds 2 days after surgery. On the 4th day, the animals with lesions demonstrated a significant decrease of 31 percent in their mean jump threshold as compared with sham-operated controls ($p < .05$). Thus the effects of bilateral lesions in the

Table 1. Effect of bilateral lesions in the premammillary nuclei (PM) and bilateral lesions in the medial forebrain bundle (MFB) on body weight and jump threshold at 11 days and on concentration of serotonin in the brain at 15 days after the lesions were made.

Group	*n*	Mean body weight (g)	Mean jump threshold (ma)	Percentage of change from sham-operated	Mean brain concentration serotonin (μg/g)*	Percentage of change from sham-operated	Range for serotonin
Sham-operated	4	371	0.62		0.60		0.57–0.62
Bilateral PM	5	339†	.58	− 6	.58	− 3	.55– .61
Bilateral MFB	5	337†	.34†	−45	.46†	−23	.45– .48

* Serotonin values based on three animals from each group. † Mean value significantly different from the mean of sham-operated controls ($p < .05$). In each case there was never more than one overlapping value between the groups with lesions and sham operations.

medial forebrain bundle on jump threshold do not occur until some time between the 2nd and 4th days after the lesions are made; the decrease in jump threshold reaches its maximum of approximately 42 percent by 10 days after operation ($p < .01$). When groups 1 and 2 were retested 30 and 25 days after operation, respectively, the rats with lesions demonstrated essentially the same decrease in jump threshold as the rats with lesions in group 3.

In contrast to the effects of the lesions on jump threshold, there were no significant differences between the mean flinch or vocalization thresholds of the rats with lesions and the control rats. Further, the flinch and jump responses of the animals with lesions were not detectably different in strength from those of the controls, even at the highest shock intensities.

As originally reported by Morgane (*5*), bilateral lesions in the medial forebrain bundle produced a temporary aphagia and adipsia which lasted between 1 and 4 days after operation. As a result, the animals with lesions demonstrated significantly lower mean body weights as compared with sham-operated controls at 4 and 10 days after operation (−15 and −13 percent, respectively; $p < .05$ for both comparisons). However, there were no significant differences in body weight on the 2nd, 25th, or 30th day after operation. The effects of bilateral lesions in the medial forebrain bundle on jump threshold do not, therefore, appear to be related to the effects of the lesions on body weight. Further, within the range of body weights obtained in this study, there was no correlation between body weight and jump threshold of individual animals.

It has been demonstrated that bilateral lesions in the medial forebrain bundle cause the concentration of serotonin in the brain to be reduced by approximately 36 percent (*6*) and that this reduction in serotonin does not occur immediately but requires time to develop (*7*). Data obtained by Harvey *et al.* for the effects of bilateral lesions in the medial forebrain bundle on the concentration of serotonin in the brain at various intervals after surgery (*7*) are presented in Fig. 1. The time course for the effects of bilateral lesions in the medial forebrain bundle on concentrations of brain serotonin is similar to the time course for the effects of the lesions on jump threshold. This comparison appears to be valid, since the lesions we made were comparable with those made by Harvey *et al.* (*7*), both with respect to the anatomical locus of destruction and with respect to the changes which they produced in the concentration of serotonin in the brain. The lesions we made consistently transect the fibers of the medial forebrain bundle in the lateral hypothalamus at the level of the premammillary nuclei. The mean concentration of serotonin in whole brain was found to be 0.62 μg/g for four sham-operated rats (range 0.60 to 0.63) and 0.44 μg/g for four rats with bilateral lesions (range 0.41 to 0.47). Thus the bilateral lesions we made decreased the serotonin content by 29 percent compared with sham-operated controls.

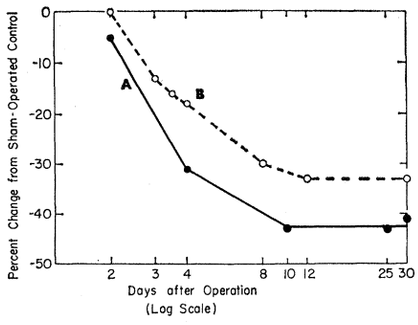

Fig. 1. *A*, Time course of the decrease in jump threshold in rats after bilateral lesions were made in the medial forebrain bundle. *B*, Time course of the decrease in the concentration of serotonin in the brains of rats after bilateral lesions were made in the medial forebrain bundle, as reported by Harvey *et al.* (*7*). For both curves the effects of the lesion are expressed as a percentage change from the mean values of sham-operated controls.

In Table 1, we summarize the data obtained from a second experiment in which the effects of bilateral lesions in the medial forebrain bundle were compared with the effects of lesions in the premammillary nuclei on body weight and jump threshold at 11 days and on concentration of serotonin in the brain at 15 days after operation. The placement of the electrode for the lesions in the medial forebrain bundle was the same as in the preceding experiment. For the bilateral lesions in the premammillary nuclei, the electrode was placed at a point 0.75 mm medial to its position for the lesions in the medial forebrain bundle. A 1-ma current delivered for 15 seconds was used in order to reduce lesion size and thus prevent overlap in the locus of destruction. At the termination of the experiment, two animals were selected from each group with lesions for histological determination of the locus and extent of destruction. The brains from the remaining three animals in each group, including three sham-operated control rats, were assayed for serotonin content at 15 days after operation (4 days after measurement of jump threshold).

The lesions in the medial forebrain bundle were centered in the lateral hypothalamus at the level of the premammillary nuclei and between the column of the fornix and cerebral peduncle, but they never extended into the latter two structures. Although these lesions were smaller in size than those in the preceding experiment, they produced an equivalent amount of damage to that portion of the lateral hypothalamus containing the fibers of the medial forebrain bundle. The lesions in the premammillary nuclei were of the same size as the lesions in the medial forebrain bundle and were centered in the medial hypothalamus between the ventricle and fornix. These lesions had as their major effect the destruction of the premammillary nuclei. In addition, the posterior portions of the dorsomedial and ventromedial nuclei were always ablated. In both brains the lesion involved the fornix laterally and a portion of the arcuate nucleus medially, but such destruction was never bilateral. There was no overlap in the areas destroyed by the two hypothalamic lesions.

Both hypothalamic lesions produced equally significant decreases in body weight as compared with sham-operated controls. However, only the bilateral lesion of the medial forebrain bundle was effective in producing significant decreases in both the jump threshold and in the serotonin concentration of the brain.

Our results suggest that the lesions in the medial forebrain bundle may be inducing some progressive changes in the remaining portions of the brain which require time for their full development. These changes can be measured both chemically, as decreases in brain concentration of serotonin, and behaviorally as increases in the sensitivity of an animal to electric shock. As previously pointed out (*7*), the time course for the fall in brain serotonin following lesions in the me-

dial forebrain bundle is in reasonable agreement with the experimental findings available regarding the time course of degeneration in central nervous system fibers. It is possible, therefore, that both the chemical and behavioral changes are related in some fashion to the effects of progressive nerve degeneration in areas of the brain lying outside the primary locus of the lesion.

It is not clear whether the close correspondence between these changes in brain serotonin and jump threshold reflects some functional role for serotonin in determining the sensitivity of an animal to electric shock. It is interesting, however, that lesions placed in the septal area of the rat have also been reported to produce significant decreases in serotonin concentration of the brain (*6*) and an increased sensitivity to electric shock (*9*).

It is well established that a denervated or decentralized smooth muscle (for example, iris or nictitating membrane) is more sensitive to the effects of medullary catecholamines released by painful or stressful stimuli (*8*). Further, the development of such a supersensitivity in a smooth muscle has been demonstrated to follow a time course which approximates the time required for the progressive degeneration of the severed nerve. The development of an increased sensitivity to painful stimuli following lesions in the medial forebrain bundle is strikingly analogous to those effects of peripheral nerve section. If we use the terminology of Cannon and Rosenbleuth (*8*) a lesion in the medial forebrain bundle may prove to be a "central denervation" and the resultant increased sensitivity to electric shock may represent a "central denervation supersensitivity."

Regardless of the interpretation of these findings it is clear that the effects of some central nervous system lesions on behavior cannot be interpreted categorically but must be defined with respect to the time after surgery. Further investigations of the progressive anatomical or neurochemical changes induced by a lesion in the remaining portions of the brain may help to clarify the nature of such time-dependent phenomena.

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Interhemispheric Reversal of Mirror-Image Oblique Lines after Monocular Training in Pigeons

Abstract. *Pigeons, with one eye open, were reinforced for pecking at a 45° oblique line (/). When the opposite, untrained, eye alone was open, pigeons responded maximally to the mirror-image (135° \) of the training stimulus (45° /). This unexpected interocular reversal of mirror-image stimuli has not been reported for any other species.*

In the visual system of the pigeon, the primary afferent input from each eye projects to the contralateral optic tectum. Both anatomical (*1*) and physiological (*2*) data indicate that there is complete crossing of the optic nerve fibers at the optic chiasma. However, visual information presented to only one eye must reach the ipsilateral half of the brain because pigeons trained monocularly on color, brightness, and certain form discriminations can perform the same task using the untrained eye (*2–4*).

The functional capacity of the interhemispheric integrating systems has been examined in a variety of species. Many investigators have studied the extent of transfer of a monocularly trained discrimination as it is affected by difficulty (*5*); degree of transformation of the test stimulus (*6*); or as a function of the visual dimension (that is, color, form, brightness) primarily involved (*7*). Since patterns which are mirror-image pairs provide the same amount of visual information, they are a convenient vehicle for studying interhemispheric transfer as a function of alterations in the shape and number of elements of the discriminative stimuli. In this report, two experiments are described, each concerned with the interhemispheric transfer of mirror-image oblique lines which can be distinguished only by their angular orientation. Pigeons are able to discriminate oblique lines, although this discrimination has proved more difficult for octopuses, fish, and children (*8*) to learn than a discrimination of horizontal versus vertical lines.

In the first experiment, generalization along a continuum of angular orientation was examined in pigeons after they were given monocular training on a single angle. Once an organism has been reinforced for responding to a particular stimulus (S+), it also responds to other stimuli which share properties in common with S+. Response rate decreases as a function of the distance of these stimuli from S+ along a particular continuum and thereby indicates which stimuli an organism regards as "similar" to a given stimulus (S+) associated with reinforcement. Several investigators (*9*) have found that pigeons will generalize along a continuum of angular orientation after binocular training, but there have been no studies of interhemispheric transfer of generalization following monocular training.

Five experimentally naive, male Carneau pigeons were trained to peck a translucent key, 25 mm in diameter, mounted on a vertical panel. A 45° line, 3 mm in width and 25 mm in length, was projected on the key from a miniature display unit. The box was dark except for the discriminative stimulus during training and testing sessions. All the pigeons wore brass goggles (*2*) which permitted restriction of visual stimulation to one eye during training sessions. The pigeons were maintained at 75 to 80 percent of their "free feeding" weight.

The pigeons were trained in a stan-