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A DEMONSTRATION OF A FALL IN BRAIN SEROTONIN FOLLOWING CENTRAL NERVOUS SYSTEM LESIONS IN THE RAT*

ALFRED HELLER, JOHN A. HARVEY and ROBERT Y. MOORE†

Departments of Pharmacology, Psychology, and Anatomy, The University of Chicago, Illinois, U.S.A.

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Abstract—Brain serotonin levels were determined 35 days after the placement of bilateral electrolytic lesions in the brains of male albino rats. Destruction of the medial forebrain bundle within the lateral hypothalamus produced a fall of 36 per cent in brain serotonin levels as compared with normal controls. Destruction of the septal region, dorsomedial midbrain tegmentum, and ventral midbrain tegmentum (areas contributing fibers to this region of the medial forebrain bundle) produced decreases of 12 - 15 per cent. Destruction of areas not related to this region of the medial forebrain bundle or destruction of other fiber tracts did not result in a significant fall in brain serotonin levels. These results are discussed in terms of a possible similarity between sectioning of peripheral and central nerve fibers, and it is suggested that the results may represent evidence for "serotonergic" fibers in the central nervous system. No correlation was found between the effects of a lesion on brain serotonin levels and its effects on the body weight, brain weight, or irritability of the animal.

THE NONUNIFORM distribution of serotonin in the central nervous system has been demonstrated in a number of studies. Using fluorometric techniques, Bogdanski *et al.*[1] and Kuntzman *et al.*[2] have noted the highest concentrations of serotonin in brain stem, hypothalamus, and limbic structures. Paasonen *et al.*,[3] using a bioassay procedure, have also demonstrated the presence of high levels of serotonin in parts of the limbic system. Many studies have previously shown that these areas of brain, generally containing high levels of serotonin, are important in autonomic function. The localization of serotonin to these central structures has indeed been given as part of the evidence for ascribing a neurohumoral role to this amine in the brain.[4] The relationship of the physiological and behavioral effects of ablation or stimulation of these autonomic structures to changes in brain serotonin content has, however, not been demonstrated.

As part of a study of the effect of lesions of the central nervous system on drug action and behavior,[5] we have attempted to determine whether central nervous system lesions have any effect on total brain serotonin levels.

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† Also in the Department of Medicine, Division of Neurology, The University of Chicago.

EXPERIMENTAL

One hundred and ninety-two male albino rats (Holtzman), 83 to 85 days old, were divided into 12 experimental groups. Bilateral electrolytic lesions were produced in the following structures: septal region, dorsomedial midbrain tegmentum, ventral midbrain tegmentum (at the level of the interpeduncular nucleus), lateral hypothalamus (medial forebrain bundle), medial hypothalamus, hippocampus, habenula, lateral pontine tegmentum, caudate nucleus, and cortical tissue overlying the septal region. A group of normal controls and a group of sham-operated animals were also included. A monopolar electrode consisting of a 24-gauge nichrome wire insulated with Beldenamel (Belden Wire Co., Chicago, Ill.) to a final diameter of 22 gauge was placed in the appropriate brain areas by means of a Kreig-Johnson stereotaxic apparatus. The lesions were produced by applying a direct current. All operations were performed under ether anesthesia and all animals received 25,000 units of procaine penicillin intramuscularly after the operation. In the sham operation the rat was anesthetized, the scalp incised and retracted, and the skull drilled, but the dura spared.

Immediately after operation all animals were housed one per cage, and on the fourth and seventh postoperative days were examined for possible effects of the lesion on irritability, body weight, or motor function. Seven days after operation all animals were housed two per cage until the time for serotonin assay on postoperative day 35. Animals were given food and water *ad libitum* throughout. Eight animals from each group were used for serotonin analysis with the exceptions noted in Table 1. The remaining eight animals from each group were prepared for histologic examination of the lesions. Brains were perfused with saline and formalin. The majority of the specimens were embedded in paraffin and a few were embedded in celloidin. Sections were taken at 0·2-mm intervals and stained with cresyl violet. For some specimens alternate sections were stained with cresyl violet and by the Woelcke method for myelinated fibers.

For the serotonin brain assays, rats were decapitated, the brain removed from the skull, blotted, weighed, and then homogenized in 2 volumes of 0·1 N HCl. After homogenization the entire brain sample was transferred to a 60-cc stoppered centrifuge bottle and analyzed for serotonin by the spectrophotofluorometric method of Bogdanski *et al.*[6], using the quantities of reagents given by Udenfriend *et al.*[7] The final acidified extract was activated at 295 mμ and the fluorescence read at 545 mμ in an Aminco Bowman spectrophotofluorometer equipped with an Osram lamp. Activation and fluorescence spectra were determined on known serotonin creatinine sulfate (Nutritional Biochemicals Corp.) and on the final acid extract of at least one rat brain from each experimental group. The activation and fluorescence maxima for the brain extracts and authentic serotonin were identical. In the studies reported here, no borate wash for 5-hydroxytryptophan was used, since in earlier experiments we found that the results were unaffected by the inclusion or omission of this wash. The brain serotonin levels are expressed as micrograms of serotonin per gram wet weight of tissue.

RESULTS

Anatomic localization of the lesions

Examination of the histologic material revealed that all the lesions were quite similar in histological appearance. In each lesion a central area of cavitation was

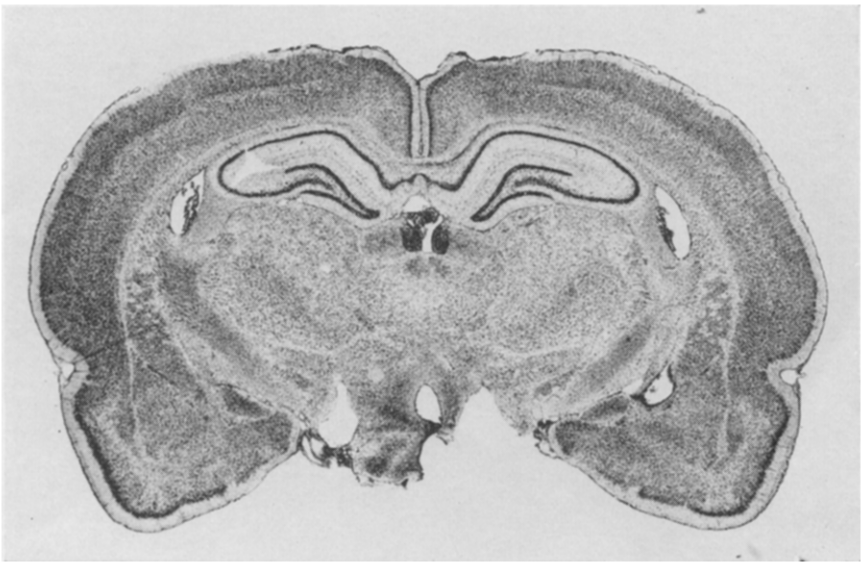

FIG. 1. Lateral hypothalamic lesion. Cresyl violet, 7·7×.

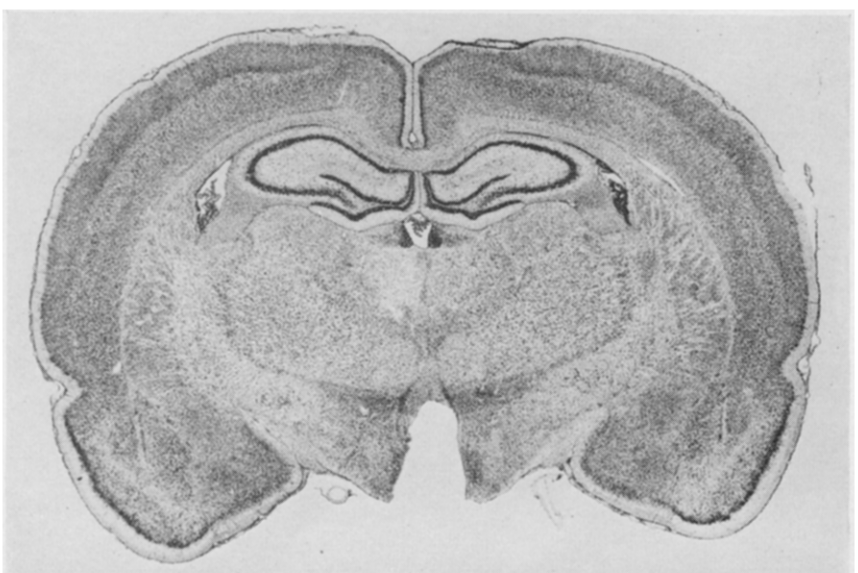

FIG. 2. Medial hypothalamic lesion. Cresyl violet, 7·7×.

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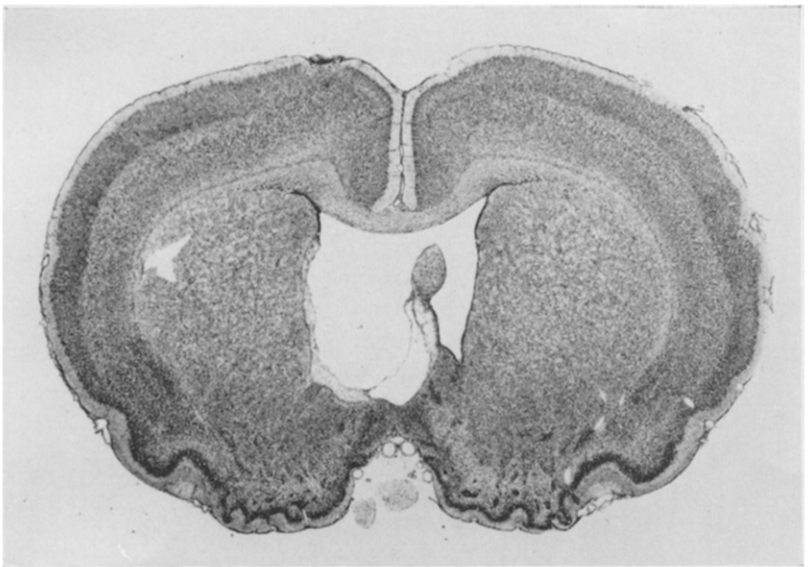

FIG. 3. Septal lesion. Cresyl violet, 7·7

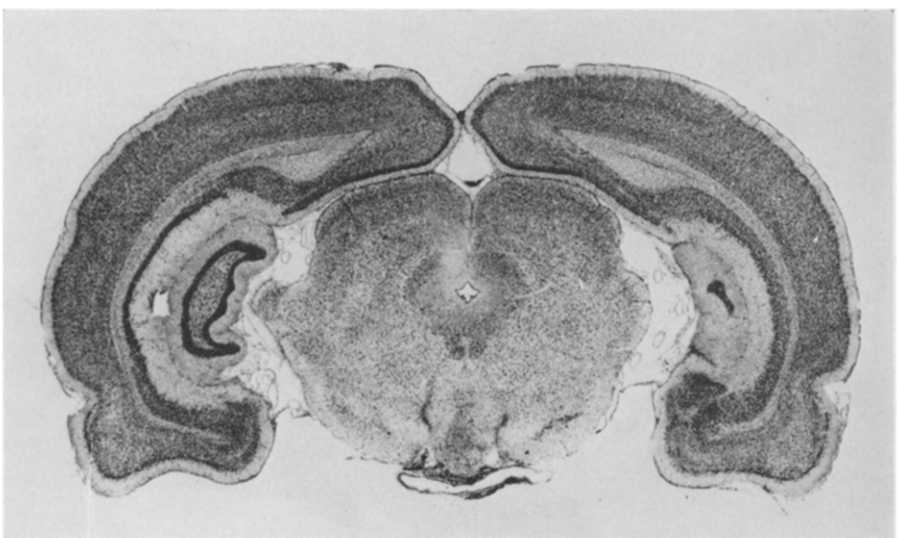

FIG. 4. Ventral midbrain tegmentum lesion. Cresyl violet, 7·7 . The small bilateral lesion can be seen just above the ventral border of the section near the midline.

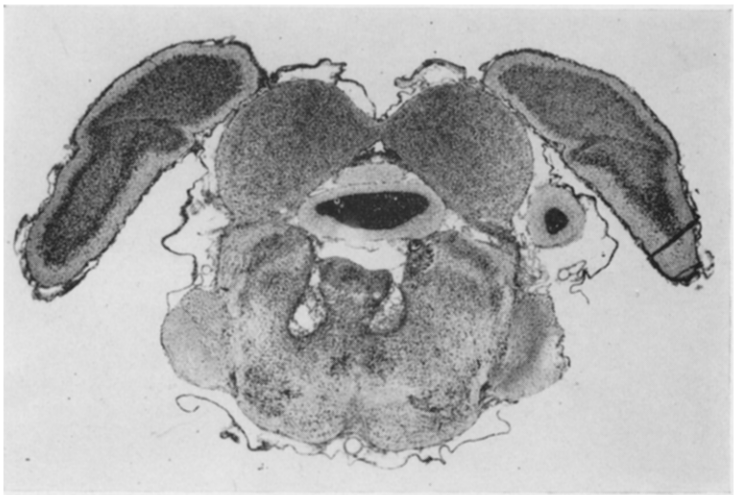

FIG. 5. Dorsomedial midbrain tegmentum lesion. Cresyl violet, 7·7×.

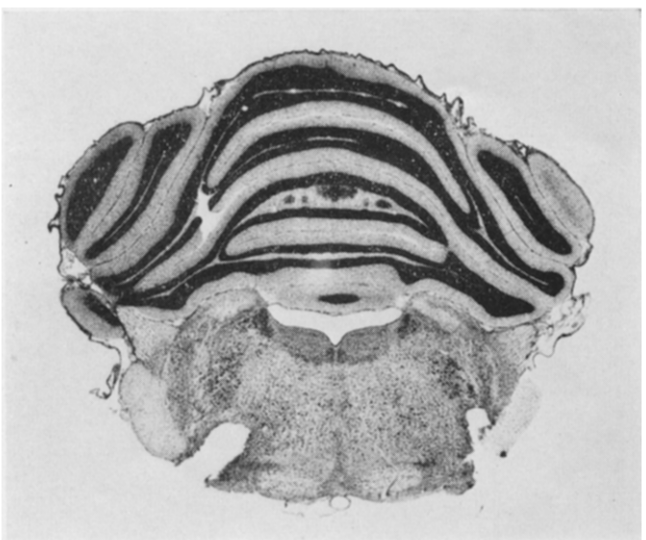

FIG. 6. Lateral pontine tegmentum lesion. Cresyl violet, 7·7×.

surrounded by a shell of reactive gliosis. The diameter of this glial shell varied somewhat from lesion to lesion. Outside the shell of gliosis the neural tissue appeared to be normal. An electrode tract entered each lesion dorsally. Infrequently, incidental damage, such as hemorrhagic infarction, was associated with the passage of the electrode through the brain substance. Since such incidental damage was infrequent, it will not be discussed further. A description of the specific anatomic locus of each type of lesion follows. The descriptions emphasize the nuclear areas and tracts that were most consistently included in each type of lesion. Incidental variations in the location and extent of the lesions are also mentioned. Photomicrographs of sections through typical examples of several of the lesions are shown in Figs. 1–6.

Lateral hypothalamus (*Fig. 1*). In this group, bilateral lesions were placed in the lateral hypothalamic area at the level of the ventromedial nucleus of the hypothalamus. The size of the lesions was variable, but in each brain there was some damage bilaterally to the lateral hypothalamic region. Fig. 1 shows a moderately large lesion on one side and a small one on the other. In each case at least some of the fibers of the medial forebrain bundle were transected at this level. Many brains indicated complete or nearly complete bilateral transection of this fiber system. Larger lesions tended to invade surrounding areas in one or more directions. Lesions extending medially from the lateral hypothalamus invaded the medial nuclear area and the ventromedial nucleus in particular. Those extending laterally invaded the subthalamic nucleus and the medial border of the cerebral peduncle. Lesions extending dorsally involved the zona incerta and fibers of the medial lemniscus. Occasional lesions damaged fibers of the fornix or mammillothalamic tract, but the constant area of neural destruction in these brains was the lateral hypothalamus, including fibers of the medial forebrain bundle dispersed through it.

Medial hypothalamus (*Fig. 2*). These lesions mainly involved the ventromedial nucleus and the periventricular nucleus, including its arcuate portion. Many extended anteriorly into the anterior hypothalamic area and the diffuse supraoptic nucleus. Occasional lesions extended into the dorsomedial nucleus or into the lateral hypothalamic region. Very occasionally the mammillary nuclei were damaged.

Septal region (*Fig. 3*). These lesions consistently ablated the greatest part of the septal area. Nuclei invariably involved were the medial and lateral septal nuclei, the nucleus septohippocampalis, and the dorsal part of the nucleus of the diagonal band of Broca. The lesions frequently damaged the dorsal part of the nucleus accumbens and extended posteriorly to damage the bed nucleus of the stria terminalis and the posterior septal nucleus. The precommissural fornix and Zuckerkandl's olfactory bundle were invariably destroyed. Some lesions involved the postcommissural fornix, the ventral hippocampal commissure, the anterior commissure, the stria terminalis (never bilaterally), the corpus callosum, or the caudate nucleus. No lesion extended ventrally into the preoptic region or the medial forebrain bundle. The area destroyed by this lesion has been shown by Nauta[8] to provide a large number of fibers entering the medial forebrain bundle to distribute throughout the lateral hypothalamus.

Hippocampus. The lesions were placed bilaterally in the midportion of the hippocampus, usually transecting it at this level. They ablated only a relatively small portion of the total volume of this structure but were consistent in size with other lesions in this study. Some of the lesions extended medially into the lateral thalamus, and a few invaded overlying cortex laterally. Some precommissural fornix fibers from the region of this lesion are distributed to the lateral preoptic region but do not extend caudally beyond this. It is well known that postcommissural fibers distribute to the medial hypothalamus and particularly to the mammillary bodies.[8]

Ventral midbrain tegmentum (*Fig. 4*). The very small lesions in this group of brains were placed laterally and posteriorly in the region of the interpeduncular nucleus. Damage to this nucleus was usually slight, but adjacent fiber pathways related to the medial forebrain bundle[9, 10] were consistently damaged. The lesions occasionally extended laterally into the cerebral peduncle and substantia nigra or dorsally into the medial lemniscus.

Habenula. The lesions in these brains were placed in the midline and ablated the greatest part of the habenular complex. In all cases they extended posteriorly and ventrally to completely transect the fasciculus retroflexus. In so doing they destroyed parts of the dorsomedial and parafascicular thalamic nuclei and adjacent nuclei of the pretectal region. Most of the lesions ablated the posterior commissure.

Dorsomedial midbrain tegmentum (*Fig. 5*). Two areas of destruction were present dorsomedially in the caudal midbrain tegmentum involving the nucleus reticularis pontis oralis and the nucleus centralis superior. These nuclei were never completely ablated. The deep tegmental nucleus of Gudden was involved in all cases, but this was sometimes only unilateral. Some lesions extended dorsally into periventricular gray or ventrally into the nucleus tegmenti reticularis pontis. The lesions in this group destroyed fibers known to distribute into the medial forebrain bundle system of the lateral hypothalamus[10, 11] as well as into ascending dorsal pathways of the reticular formation and descending fibers related to the extrapyramidal motor system.

Lateral pontine tegmentum (*Fig. 6*). These lesions destroyed large parts of the superior olivary complex, the lateral lemniscus and its ventral nucleus, the trapezoid body, and the spinothalamic tract. In all there was some involvement of the nucleus reticularis pontis caudalis laterally and in many this was quite extensive. These lesions also often involved the brachium pontis and the main motor and sensory nuclei of the trigeminal nerve.

Caudate nucleus. The lesions were placed dorsally and laterally in the caudate nucleus and were confined to it except for some extension dorsally into fibers of the corpus callosum. The lesions transected fibers of the internal capsule as they run through the substance of the nucleus.

Cortex. These lesions ablated isocortex of the dorsomedial aspect of the hemisphere, dorsal and somewhat rostral to the septal region. They usually encroached upon subcortical white matter but not upon any other subcortical structures.

Effect of central nervous system lesions on brain serotonin levels

Table 1 presents the mean brain serotonin level in micrograms per gram for each experimental group and the percentage change from the normal value of 0·59 μg/g. Lesions in the septal region, dorsomedial midbrain tegmentum, and ventral midbrain tegmentum produced a significant fall in brain serotonin level ranging from 12–15 per cent, whereas the lateral hypothalamic lesion (medial forebrain bundle) produced a decrease of 36 per cent as compared with normal controls. Although the decrease seen with septal and midbrain tegmentum lesions is small, this finding has been replicated on two separate occasions for the septal lesions and on one separate occasion for the dorsomedial midbrain tegmentum lesion. The small variation from normal values seen with lateral pontine tegmental, hippocampal, medial hypothalamic, and habenular lesions (± 7 per cent) were not significant. Cortical, caudate, and sham-operated controls were essentially identical with normal animals.

TABLE 1. EFFECT OF CENTRAL NERVOUS SYSTEM LESIONS IN THE MALE ALBINO RAT ON BRAIN SEROTONIN LEVELS

Experimental group	N	Mean brain serotonin level (μg/g)	Change from normal (%)	P*
Normal control	8	0·59		
Sham-operated	7	0·61	+ 3	
Lateral hypothalamus (medial forebrain bundle)	8	0·38	− 36	>0·005
Ventral midbrain tegmentum	8	0·50	− 15	>0·01
Dorsomedial midbrain tegmentum	8	0·51	− 14	>0·01
Septal region	8	0·52	− 12	>0·01
Medial hypothalamus	8	0·55	− 7	
Habenula	8	0·55	− 7	
Hippocampus	8	0·56	− 5	
Cortex	8	0·58	− 2	
Caudate	8	0·59		
Lateral pontine tegmentum	5	0·63	+ 7	

* *P* values were obtained from a 2 × 2 contingency table[16] using the μg/g value obtained from each animal. A *P* value of 0·01 indicates only one overlap between the experimental and control group. A *P* value of 0·005 indicates no overlap.

Gross behavioral effects of the lesions

As previously reported by Brady and Nauta,[12] animals with septal lesions demonstrated an immediate postoperative irritability which disappeared with handling and was no longer detectable 12 days after operation. Lesions in the lateral hypothalamus, medial hypothalamus, and hippocampus also produced a transient irritability which was less severe than that displayed by septal rats. There was no measurable increase in irritability caused by the other lesions reported in this study.

A quite unexpected and dramatic effect on emotionality was seen in animals with lesions in dorsomedial midbrain tegmentum. These animals were easily handled and displayed no irritability to such manipulation. However, when placed two per cage at 7 days after operation, seven of the eight pairs of rats displayed severe attack behavior toward each other, resulting in multiple lacerations of the body. This behavior continued until postoperative day 35, in varying degrees of severity. A more complete

description of this behavior will appear elsewhere.[13] There appeared not to be any relationship between this behavior and brain serotonin levels, however, since two of the animals, although not displaying the fighting syndrome, also showed a significant fall in brain serotonin. Animals with septal, hippocampal, medial hypothalamic, and lateral hypothalamic lesions did not demonstrate irritability at the time of serotonin assay nor was there any relationship between their immediate postoperative irritability and brain serotonin level.

Although there did not appear to be any relationship between irritability and brain serotonin levels, a separate control experiment was carried out to investigate further any possible effect of septal irritability on brain serotonin levels. Eight septal and eight sham-operated control rats were placed one per cage immediately after operation and left undisturbed until the time for serotonin assay 35 days later. Another group of eight septal and eight sham-operated control rats was placed one per cage and handled once a day from the time of operation until the time for serotonin assay, 35 days later. These handled septal rats demonstrated the usual transient postoperative irritability but were tame and easily handled at the time of assay. The nonhandled septal and sham-operated control rats were first handled at the time of assay, and the septal rats responded in the manner usually seen immediately after operation. They squealed, urinated, defecated, and attacked the gloved hand vigorously during the entire minute they were being held. There was, however, no difference between the brain serotonin levels of the two sham-operated groups or between the two septal groups, but both septal groups had significantly lower brain serotonin levels (— 15 per cent) than either of the sham-operated controls. It thus appears that serotonin levels in brain may be altered by central nervous system lesions but this alteration does not appear to be related to changes in irritability or to the expression of irritability prior to decapitation for assay of brain serotonin.

Although food and water intake were not specifically measured, slight postoperative decreases in body weight were seen in animals with caudate lesions (— 13 per cent), lateral pontine tegmentum lesions (— 14 per cent), and lateral hypothalamic lesions (— 17 per cent) on the fourth and seventh postoperative days. However, all of the other lesioned groups also demonstrated a slight fall in body weight during this period (5 to 8 per cent). After the seventh postoperative day all the operated animals showed increase in body weight approaching that of the sham-operated controls. The slight postoperative decreases in body weight seen over the first seven days after the lateral hypothalamic lesions are similar to the recent findings of Morgane,[14] who obtained persistent aphagia and adipsia only with lateral hypothalamic lesions having a more dorsolateral placement than those in the present study. Animals with medial hypothalamic lesions did not demonstrate weight gains differing from sham-operated controls over the 35-day postoperative survival period. The lesions in these animals, while involving a large extent of the ventromedial nucleus, were almost invariably confined to this nucleus and tissue medial and ventral to it. The lesions were purposely placed near the midline to avoid the lateral hypothalamus and provide a control for the destruction of hypothalamic tissue. The absence of hyperphagia and obesity in these animals is consistent with the findings of Hetherington and Ranson[15] who felt that extension of ventromedial nucleus lesions into the area lateral to the nucleus was necessary to produce this effect. It can be seen, therefore, that there was no correlation between brain serotonin levels and transient postoperative changes in body weight.

Further, the body and brain weights at the time of serotonin assay showed no correlation with levels of serotonin in brain.

DISCUSSION

This study has demonstrated that lesions of the central nervous system in the rat can produce a fall in brain serotonin levels, and that such changes in brain serotonin levels are not correlated with irritability, body weight, or brain weight. The relationship between the changes in emotionality and brain serotonin levels produced by these lesions is, however, under further investigation with the use of more refined behavioral techniques. Histologic examination of the lesions suggests that it is damage to one particular system of fibers that is responsible for the effect on brain serotonin: the fibers of the medial forebrain bundle within the lateral hypothalamus. The greatest fall in brain serotonin level (36 per cent) was produced by lesions in the lateral hypothalamus at the infundibular level. This is a fiber-rich, cell-poor area containing the medial forebrain bundle. It should be noted that no effect occurs with lesions in the medial hypothalamus. Lesions in the septal region or in the dorsomedial midbrain tegmentum, areas known to contribute significant numbers of fibers to this region of the medial forebrain bundle, also produced a significant, though less marked, fall in brain serotonin levels (12–14 per cent). Small lesions in the ventral midbrain tegmentum, which damaged few cell bodies in this region but transected a number of fibers of passage that enter the medial forebrain bundle, also produced a significant fall (15 per cent). The failure of lesions in the hippocampus to alter significantly brain serotonin levels is of special interest. Though this area contributes precommissural fibers to the medial forebrain bundle, they are few and do not extend caudally beyond the preoptic region. Severing other fiber systems did not cause a decrease in brain serotonin levels. Thus lesions in the caudate nucleus transecting many of the fibers of the internal capsule, and lesions in the lateral pontine tegmentum, which severed a considerable number of fibers belonging to specific sensory systems, did not produce a fall in brain serotonin levels.

All the lesions with the exception of those in the cortex were placed in areas reported to be high in serotonin, in the cat and dog.[1–3] However, to account for the change in brain serotonin levels on the basis of tissue destroyed by the lesions* requires a concentration of 17 μg/g in the septal region, 68 μg/g in the lateral hypothalamus, 28 μg/g in the dorsomedial midbrain tegmentum, and 210 μg/g in the ventral midbrain tegmentum. It is unlikely that these areas contain such high levels of serotonin, since the highest reported concentration for any local area of brain is only 3 μg/g.[1, 2] It is therefore clear that the destruction of the medial forebrain bundle within the lateral hypothalamus, or of areas contributing fibers to this region, results in a fall in brain serotonin levels which is 5 – 70 times greater than can be accounted for by the destruction of local concentrations of serotonin alone.

The fall in serotonin level may be secondary to some effect of the lesions on cerebral metabolism, and this possibility is under further investigation. An alternative hypothesis is that the fall is a result of a central denervation secondary to section of fibers in the medial forebrain bundle. It is known

* These values were obtained by measurement of the length and cross-sectional area of the largest lesion in each group. The volume of tissue destroyed was calculated with a standard 40 per cent shrinkage factor to correct for histologic preparation of the tissue.

from the studies of Cannon and Lissak,[17] Goodall,[18] and von Euler[19] that sectioning of peripheral adrenergic nerves is accompanied by a fall in the extractable catecholamines of the denervated organs. This finding has been interpreted as evidence that the source of such tissue amines is the adrenergic nerve. If there is a similarity between the sectioning of peripheral and central nerve fibers, and if the fall in brain serotonin levels is a consequence of degeneration of fibers producing serotonin, then these findings represent evidence for "serotonergic" fibers within the central nervous system. The lesions reported in this experiment to affect brain serotonin levels may be viewed as "central" denervations transecting fibers of the medial forebrain bundle and denervating regions supplied by it. In this regard we are currently investigating the effects of transection of the medial forebrain bundle on the local concentration of serotonin in regions of the brain supplied by this fiber tract. Investigations are also in progress on the effects of these lesions in the rat on brain levels of other amines to determine whether fiber systems can be delineated which show selective specificity for the various biogenic amines.

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