Pharmacology Biochemistry & Behavior, Vol. 9, pp. 359–367. Printed in the U.S.A.

Endogenous Norepinephrine and Serotonin Within the Hippocampal Formation During the Development and Recovery from Septal Hyperreactivity

FRED H. GAGE, ROY G. THOMPSON AND JAMES J. VALDES

Department of Psychology, Chemistry of Behavior Program, Texas Christian University, Fort Worth, TX

(Received 14 April 1978)

GAGE, F. H., R. G. THOMPSON AND J. J. VALDES. *Endogenous norepinephrine and serotonin within the hippocampal formation during the development and recovery from septal hyperreactivity.* PHARMAC. BIOCHEM. BEHAV. 9(3) 359–367, 1978.—Fluorometric analysis of serotonin (5-HT) and norepinephrine (NE) content of the hippocampal formation revealed that biogenic amines are distributed heterogeneously in the dorsoventral axis, and that NE also exhibits a heterogeneous distribution in the medial-lateral direction while 5-HT does not. Dissection of the hippocampus into its dorsal and ventral halves shows that both NE and 5-HT exhibit higher concentrations in the ventral hippocampus in comparison to its dorsal counterpart. A dissection which separated the cell fields CA 1 and 2 from CA 3 and 4 and the dentate gyrus showed NE to be the highest in the latter region, while 5-HT was uniformly distributed between the two regions. Taken together, these data indicate that NE is more highly concentrated in the CA 3 and 4 and dentate area of the ventral hippocampus while 5-HT concentration differences are apparent only in a dorsal-ventral dissection. Concentrations of NE and 5-HT in the dorsal and ventral hippocampus were also determined at 1, 3, 6, 11, 16, 24, and 30 days following a lesion to the septal nuclei. The results demonstrate that biogenic amine levels in the dorsal hippocampus achieve maximal depletion earlier than do their ventral counterparts, and that percent depletion is greater for 5-HT than NE in both dorsal and ventral areas. On the first day following septal lesions, 5-HT is increased above normal levels. Sixteen days after septal lesion, 5-HT is substantially depleted below normal levels. In addition, by 30 days, 5-HT shows significant return toward normal levels from its earlier depleted state. Behavioral changes related to sensory reactivity correlate with the relative decreases of NE and 5-HT following septal lesions.

Septal lesions Hippocampal heterogeneity Norepinephrine Serotonin Hyperreactivity Recovery of function

DESTRUCTION of the septal nuclei in rats has long been known to produce a transient period of irritability or hyperreactivity to somatosensory stimulation [5]. Several investigations have also recently shown that the integrity of the hippocampus is essential for the development of septal lesion-induced hyperreactivity [12,29]. In these studies it was shown that serial destruction of first the fornix or dorsal hippocampus, followed by a septal lesion resulted in a much attenuated responsiveness of the animal to the presented stimuli.

The involvement of the noradrenergic and/or serotonergic systems in the development of the septal syndrome has also been suggested by experimental procedures which have depleted norepinephrine (NE) [7,32] or serotonin (5-HT) [14]. In addition, pharmacological manipulations which act to decrease 5-HT concentrations prior to a septal lesion [10, 15, 16] or increase catecholamine concentrations postoperatively [13,25] have been shown to significantly alter the respective development or recovery phase of the septal syndrome.

On the basis of converging histofluorescent, autoradiographic, microiontophoretic, and biochemical assay techniques [28,38], NE and 5-HT have been implicated as putative neurotransmitters within the hippocampal formation. This monoaminergic innervation appears to arise entirely from neurons whose cell bodies lie in the mesencephalic raphe and locus coeruleus nuclei. Axons leaving these pontine nuclear complexes ascend in the tegmental tracts to join the medial forebrain bundle which then projects to the rostral septum. Here, both serotonergic and noradrenergic axons have been visualized to turn dorsally and caudally to enter the hippocampus via the fornix/fimbrial and cingulum bundle pathways [11, 23, 28].

From the anatomical description of their ascending paths then, it is not surprising that the NE and 5-HT content of the hippocampus exhibits various degrees of depletion following forebrain transections or septal lesions [20, 26, 37]. Most investigators to date, however, have analyzed the hippocampus either as a whole or have chosen only the dorsomedial aspect as their representative sample for measuring biochemical changes. Of central importance to the present line of investigation is the accumulating evidence which indi-

cates a high degree of heterogeneity between the dorsal and ventral hippocampus with respect to their afferent structures [33,35], electrophysiological characteristics [6,42], and possible neurotransmitter content [24,37].

On the basis of the existing evidence which implicates both the hippocampus and biogenic amines in modulating septal hyperreactivity, the present experiment was therefore undertaken with three aims in mind. First, to determine whether NE and 5-HT are differentially distributed within the hippocampal formation. Second, to determine the effects of a standard septal lesion—one that reliably elicits the septal syndrome—on these endogenous levels. And, third, to follow the biochemical changes that occur in the dorsal and ventral hippocampus at various times following the septal lesion in an attempt to compare changes in biogenic amine levels with changes in behavior.

METHOD

Animals

The animals were male Sprague-Dawley rats weighing 250–350 grams at the beginning of the study. The rats were individually housed in wire cages and provided with ad lib food and water.

Procedures

Dissection of hippocampal formation. Each animal was taken from its cage, decapitated and the brain rapidly removed from the skull. The cortical tissue was peeled away exposing the full dorsal-ventral extent of the hippocampus. The hippocampus was then teased away from underlying tissue and placed lengthwise on an ice-chilled dissecting plate. Special care was taken to remove all adhering cortical and fimbrial tissue although some fibers remained in most cases. To differentiate between dorsal and ventral hippocampus, the hippocampi were extended in a straight line on the dissection plate and bisected at the approximate genu to yield dorsal and ventral samples. These samples were then pooled for each animal and frozen on dry ice.

The second dissection procedure was the same through and including removal of the hippocampus and separation of extraneous tissue. At this point, the hippocampus was stretched out on dry ice on its lateral fimbrial edge on a glass dissecting plate maintained on dry ice and allowed to harden. A longitudinal section along the entire dorsal-ventral axis effectively separated CA 1–2 from CA 3–4 and dentate gyrus. As would be expected, CA 1–2 tissue samples weighed less than CA 3–4 samples.

Biochemical determinations. NE and 5-HT were assayed according to the fluorometric procedure of Shellenberger and Gordon [34]. All reagents were freshly prepared on the day of the assay and refrigerated. An internal standard, blank and control tissue were included in each assay for comparison with previously calculated standard concentration curves. Percent recovery was determined to be 88 and 75% for NE and 5-HT, respectively. The values reported are not corrected for recovery. Dopamine was also determined by the assay procedure but will not be reported here because the levels were found to be less than 150 ng/g, and its presence may only reflect a role as the immediate precursor for NE [8]. All assays were performed at approximately the same time of day to preclude effects attributable to circadian variations in hippocampal 5-HT content [19].

Surgical procedure. For those rats receiving septal lesions, the animals were first injected with 0.2 cc of atropine followed by an anesthetic dose (1 cc/kg) of a mixture of Ketamine and chloral hydrate. The animal was placed in a stereotaxic instrument and its scalp incised and retracted. Two overlapping holes were trephined in the skull at approximate locations and the electrode, insulated to within 0.5 mm of the tip, was lowered to the desired depth. The coordinates for electrode tip placement were A–1.9, L–0.5 and V–5.0 mm according to the rat brain atlas by Pellegrino and Cushman [31]. A 12 mA current of 12 sec duration, generated by a Grass LM-4 radiofrequency lesion maker, produced the bilateral lesion. Those animals receiving sham operations were subject to the same surgical procedure but current was not passed through the electrode.

Histological procedure. Prior to dissecting the hippocampus from the brain, a coronal section made through the anterior fornix separated that part of the forebrain containing the septal nuclei. This tissue was placed in 10% Formalin for two weeks, dehydrated, embedded in celloidin, sectioned at 40 microns, stained with thionin and cleared in cedarwood oil. The sections were then mounted on slides and the lesion reconstructed on diagrams of the rat brain [31].

Behavioral apparatus and testing. An open-field activity arena was used to assess exploratory and general motor activity levels. Its dimensions were 117×117×50 cm with 25 squares, 25×25 cm, marked off on the floor with white tape. Six 35 W fluorescent bulbs evenly illuminated the test room. Data recorded from the open-field included the latency-to-exit from the animal's home cage, number of squares crossed, and the number of rears. The animal's cage was placed on its side in the arena and when the rat was oriented towards the front, the cover was removed and the latency-to-exit was recorded. The cage was then removed and the number of squares traversed and number of rears were recorded over a 2 min period. The animal was then placed back in its cage and tested for reactivity.

Each animal was tested for reactivity in its home cage which was placed within a hardboard cylinder, 25×36×94 cm, to prevent the animal from escaping. A detailed account of the behavioral rating scale will not be presented here but may be found in Gage and Olton [12]. In general, four different stimuli were presented to the animal: a puff of air to the back, a light tap on the snout and back with a glass rod, and handling. Reactivity scores were given the numerical assignment of 0, 1, 2, or 3 representing an increasing dimension of responsiveness. Stimuli were presented to each animal several times at spaced intervals and the weakest response was recorded. Only those animals that obtained a combined reactivity score of 7 or more were retained as experimental subjects, since one of the major concerns of the present experiment was to examine the changes in biogenic amine concentrations following a fully developed septal syndrome. Only those animals from which complete biochemical data was obtained, i.e., both dorsal and ventral NE and 5-HT, were included in the behavioral analysis. Thus, the behavioral data reported for each of the postoperative test days represent only the behavior of those rats which were killed and assayed on that day.

Behavioral testing was conducted for at least two consecutive days prior to surgery to insure a low baseline level of reactivity, and at 1, 3, 6, 11, 16, 24, and 30 days following surgery.

RESULTS

Regional Differences in NE and 5-HT Concentrations

The results from the experiments on the differences in concentrations of NE and 5-HT in the dorsal and ventral hippocampus are presented in Fig. 1.

Both NE and 5-HT show a significantly higher concentration in the ventral hippocampus when compared to the dorsal hippocampus. As mentioned in the Method section, extra care was taken to remove cortical or amygdala tissue attached to the ventral hippocampus. However, accidental inclusion of cortical tissue with samples of ventral hippocampus would serve to decrease rather than increase NE concentrations, since cortical NE has been reported to be only 150–250 ng/g [40].

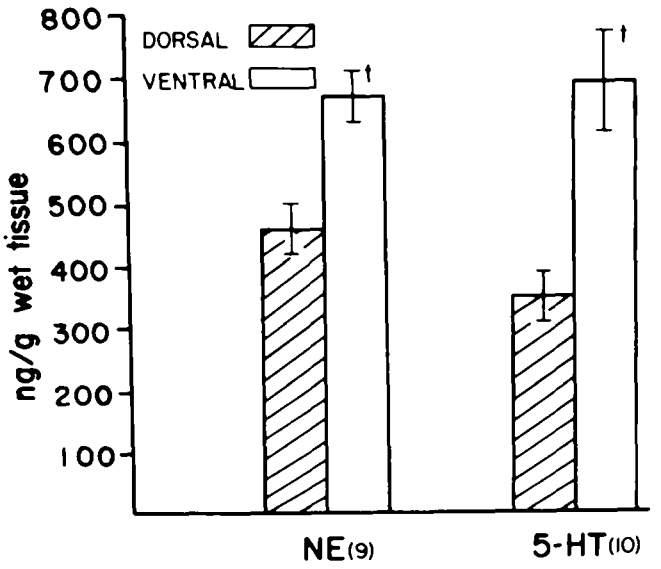

FIG. 1. Concentrations of norepinephrine and 5-hydroxytryptamine in dorsal and ventral hippocampus. Number in parentheses equals number of animals. Vertical lines mark standard deviation. †=$p<0.01$, two-tailed t test.

The results of the experiments on differences in NE and 5-HT concentrations between regions CA 1–2 and CA 3–4 dentate gyrus are presented in Fig. 2. This set of experiments shows a clear difference only in NE concentrations. Specifically, there is a significant increase in the amount of NE in the CA 3–4 and dentate gyrus relative to CA 1–2. This difference is not reflected in the concentration of 5-HT between the two areas.

Following septal lesions, the concentrations of hippocampal NE and 5-HT exhibit a gradual and time-dependent decrease. Figure 3 shows the changes which occur in the noradrenergic content of the hippocampus following a septal lesion. Of particular note is the finding that neither dorsal nor ventral NE is greatly reduced until 6 days after the lesion. Furthermore, the ventral hippocampus never shows the pronounced decrease in NE exhibited by the dorsal hippocampus. On Day 16, when NE levels appear maximally depleted, dorsal NE is depleted to 45% of control values while ventral NE remains at 73% of control concentration.

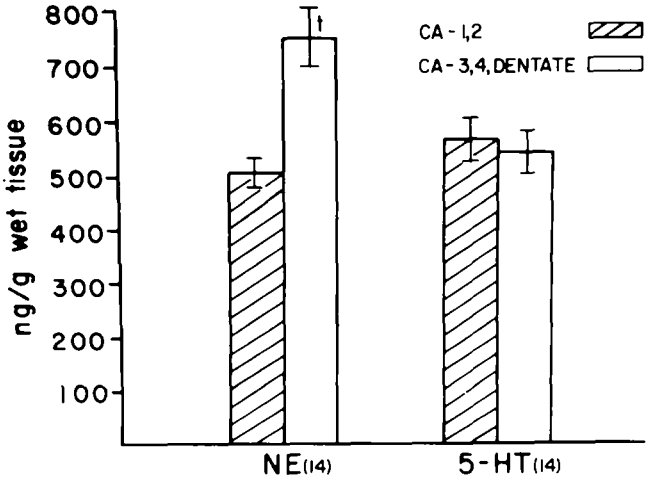

FIG. 2. Concentrations of norepinephrine and 5-hydroxytryptamine in CA 1-2 vs. CA 3–4 and dentate gyrus. Number in parentheses equals number of animals. †=$p<0.01$, two-tailed t test.

Figure 4 shows the effects of septal lesions on the concentration levels of 5-HT in the dorsal and ventral hippocampus. On the first day following the lesion, there is a significant increase in 5-HT content with the dorsal hippocampus. A similar increase can be seen in the ventral hippocampus although it does not reach statistical significance. By Day 3, however, 5-HT levels drop significantly below control values in the dorsal hippocampus, and by Day 6 ventral 5-HT also is significantly depleted. 5-HT levels appear to reach their maximum state of depletion by Day 24, at which time 5-HT is depleted by 98 and 78% in the dorsal and ventral hippocampus, respectively. In contrast to NE concentrations, however, 5-HT levels exhibit a significant return toward normal levels from their maximally depleted levels. Specifically, on Day 30 the concentration of 5-HT in the ventral hippocampus is significantly greater than Day 16 and dorsal hippocampal 5-HT is significantly elevated over that found on Day 24. Day 16 was used for the ventral hippocampus and Day 24 was used for the dorsal hippocampus, because these were the postoperative days of greatest depletion for the respective areas.

Histology

The histological evaluation revealed that the lesions were large and bilateral, typically destroying both the medial and lateral septal nuclei. The lesion generally extended dorsally to the ventral surface of the corpus callosum, ventrally toward the anterior commissure, and in some cases extended into the lateral ventricles destroying the most medial extent of the caudate nucleus. The lesions were generally of similar size and location of those reported in previous studies [12,29].

Behavior

Prior to the septal lesions, all of the animals responded with little within animal variability on the behavioral measures, though between animal variability was high for latency to emerge in an open field, lines crossed and rears. Thus

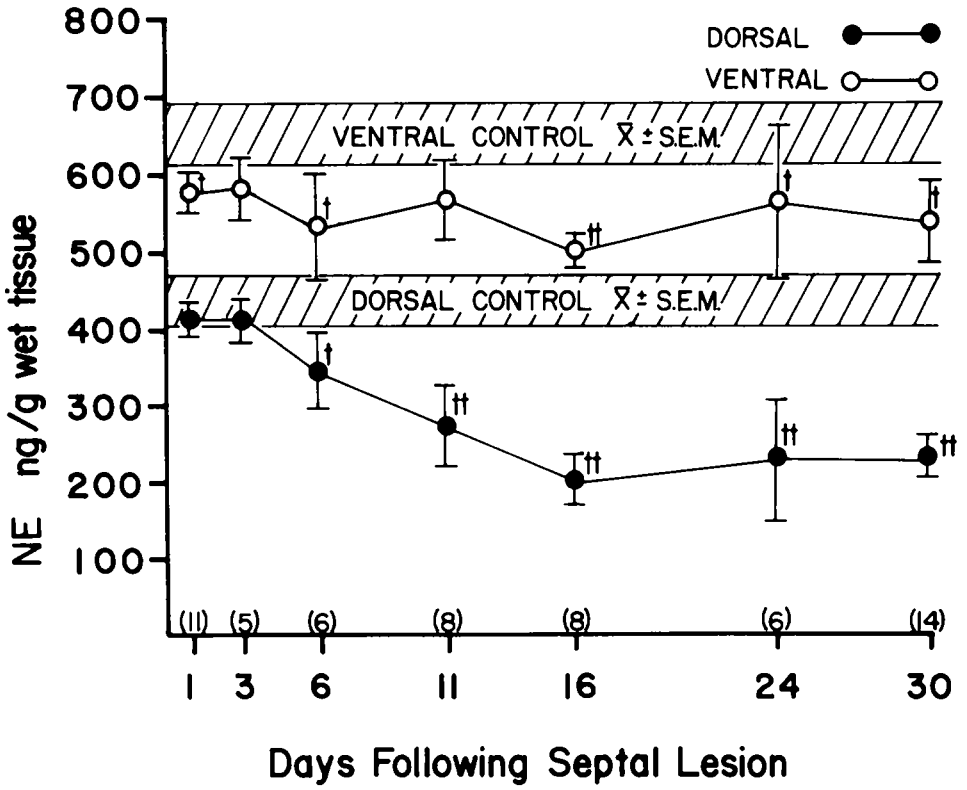

FIG. 3. Concentration of norepinephrine in the dorsal and ventral hippocampus following septal lesions. Number in parentheses equals number of animals. †=$p<0.05$, two-tailed t test; ††=$p<0.01$, two-tailed t test.

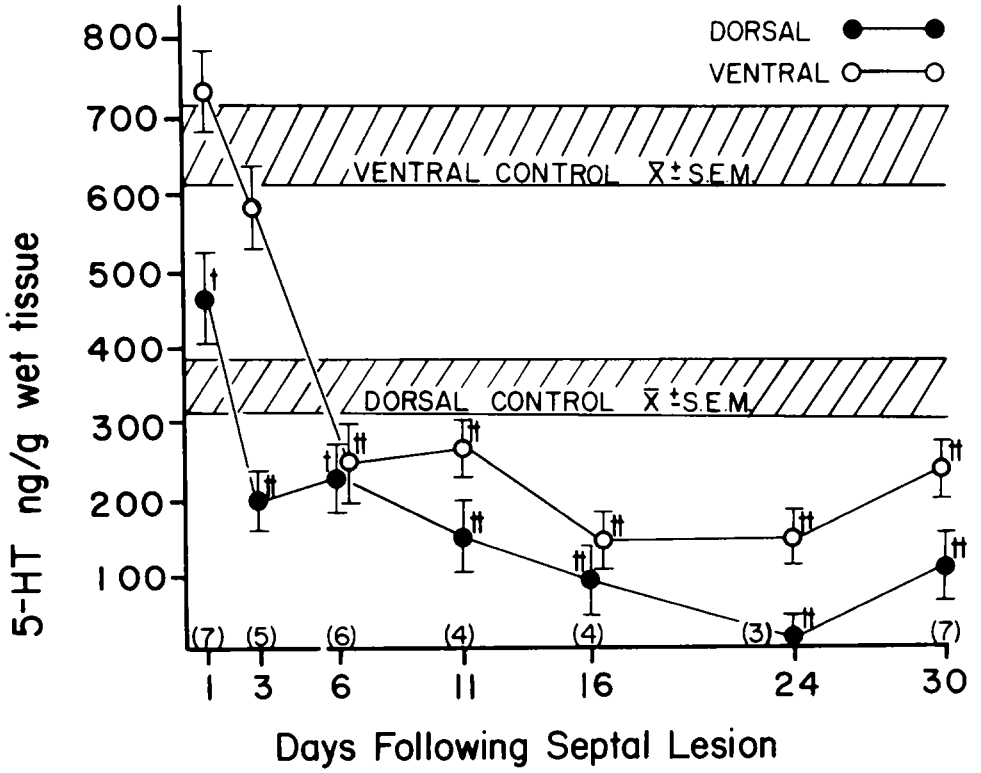

FIG. 4. Concentrations of 5-hydroxytryptamine in the dorsal and ventral hippocampus following septal lesions. Number in parentheses equals number of animals. Day 30 concentrations are significantly greater than Day 16 and Day 24 for ventral and dorsal hippocampus, respectively. †=$p<0.05$, two-tailed t test; ††=$p<0.01$, two-tailed t test.

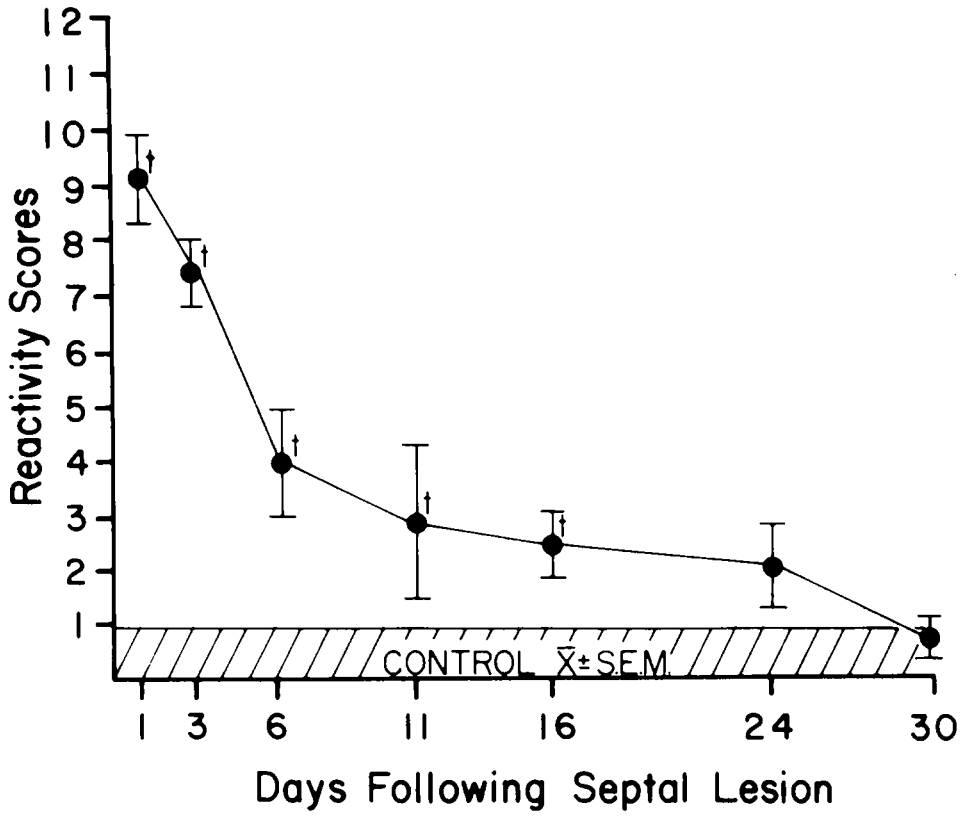

FIG. 5. Means and SEM's of reactivity scores following lesions to the septal area. † = $p<0.01$, Mann Whitney U Test.

difference scores were used to evaluate postoperative differences between sham and lesioned animals on these measures. They did not show any appreciable degree of reactivity to somatosensory stimulation and emerged into a novel environment with little hesitation. In addition, they exhibited both horizontal and vertical exploring activity in the open-field as evidenced by the number of squares crossed and the number of rears. An analysis of variance using repeated measures was performed on each of the four behaviors in the control-sham operated animals. Since no differences were found across days for reactivity, latency to emerge into an open field, and rears, the scores were pooled and a single mean and standard error of the mean was obtained to which the septal lesioned animals could be compared. A significant increase in lines crossed for the control group over days was revealed by the analysis of variance. These control scores are therefore presented for each day.

Following the septal lesion, there was a marked change in the animal's behavior when compared to their individual preoperative scores. On the first day following the lesion, the rats exhibited an exaggerated response to the previously non-noxious somatosensory stimuli (Fig. 5). Although the septal-lesioned rats remained more reactive than their preoperative baseline scores for up to 24 days, by Day 6 their reactivity had dramatically subsided and by Day 11 the differences between pre- and postoperative scores were very small.

The septal-lesioned animals also exhibited a marked change in their behavior in the open-field arena. On the first and third postoperative days, the septal animals showed a pronounced increase in their latency to emerge into the open-field from their home cage (Fig. 6). Over the following days of testing, the latency difference between pre- and postoperative scores decreased to control animal levels. On Day 30, however, there was another significant increase in latency. After the animals had emerged into the open field, the septal animals showed a significantly greater decrement in their exploratory activity than did controls (Fig. 7). This decrement, observed as a decrease in the number of squares crossed, was significant for all postoperative days. As was the case with emergence time, the septal animals once again exhibited another change in behavior on Day 30, reflected in a further decrease in the number of squares crossed. The number of rearing responses made in the open-field decreased significantly immediately following the septal lesion (Fig. 8) and remained depressed throughout all of the testing days.

DISCUSSION

The results from the experiments in non-lesioned animals provide clear evidence for differences in concentration of the biogenic amines, NE and 5-HT, in two different planes of the hippocampal formation. First, NE and 5-HT concentrations are greater in the ventral than dorsal hippocampus. Second, the concentration of NE is higher in the CA 3-4 and dentate gyrus than CA 1-2, as opposed to the equivalent distribution of 5-HT between these two regions. These

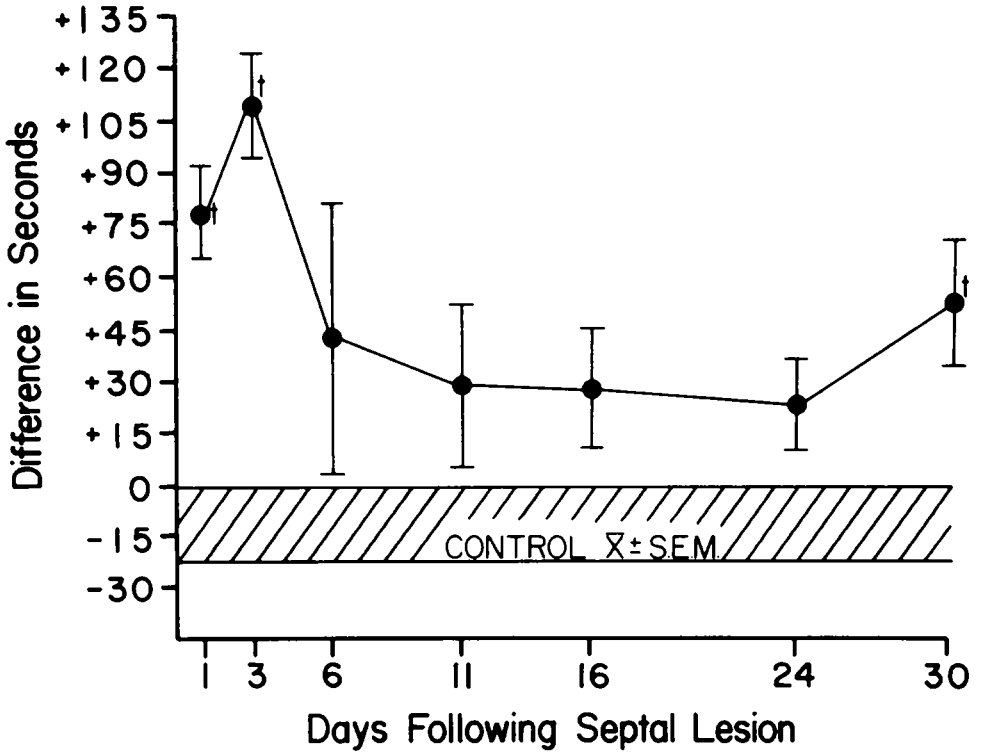

FIG. 6. Latency to emerge into an open field: Means and SEM's of difference scores for individual animals between preoperative and postoperative behavioral testing. $^{\dagger}=p<0.05$, two-tailed t test.

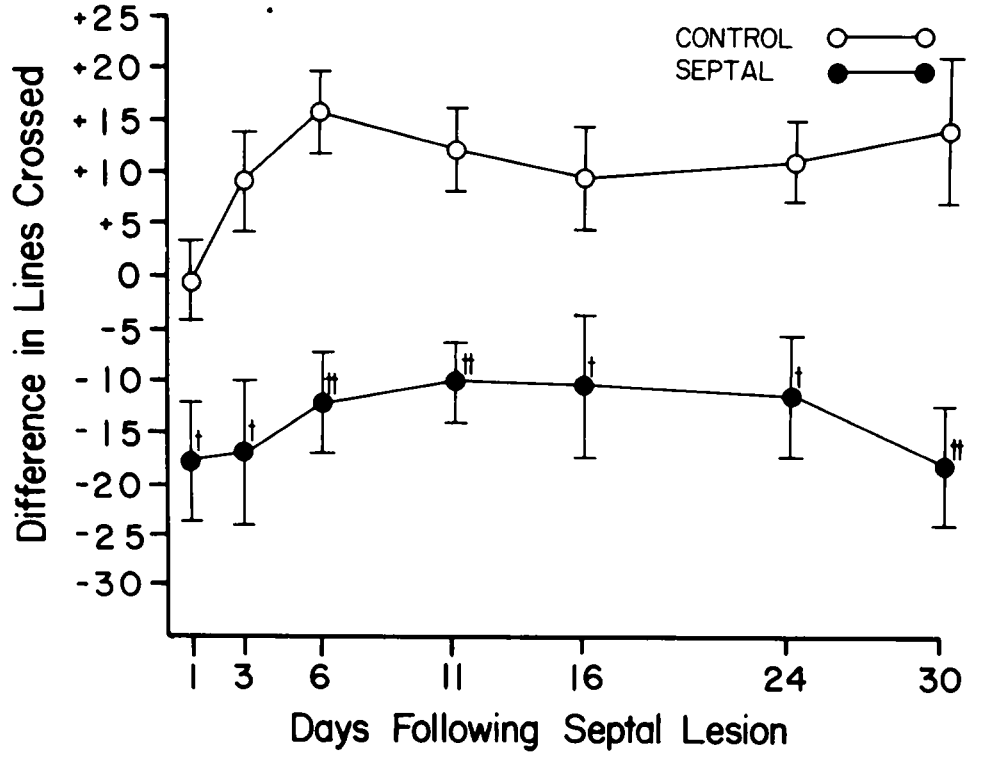

FIG. 7. Open Field Activity: Means and SEM's of difference scores for individual animals between preoperative and postoperative behavioral testing. $^{\dagger}=p<0.05$, two-tailed t test; $^{\dagger\dagger}=p<0.01$, two-tailed t test.

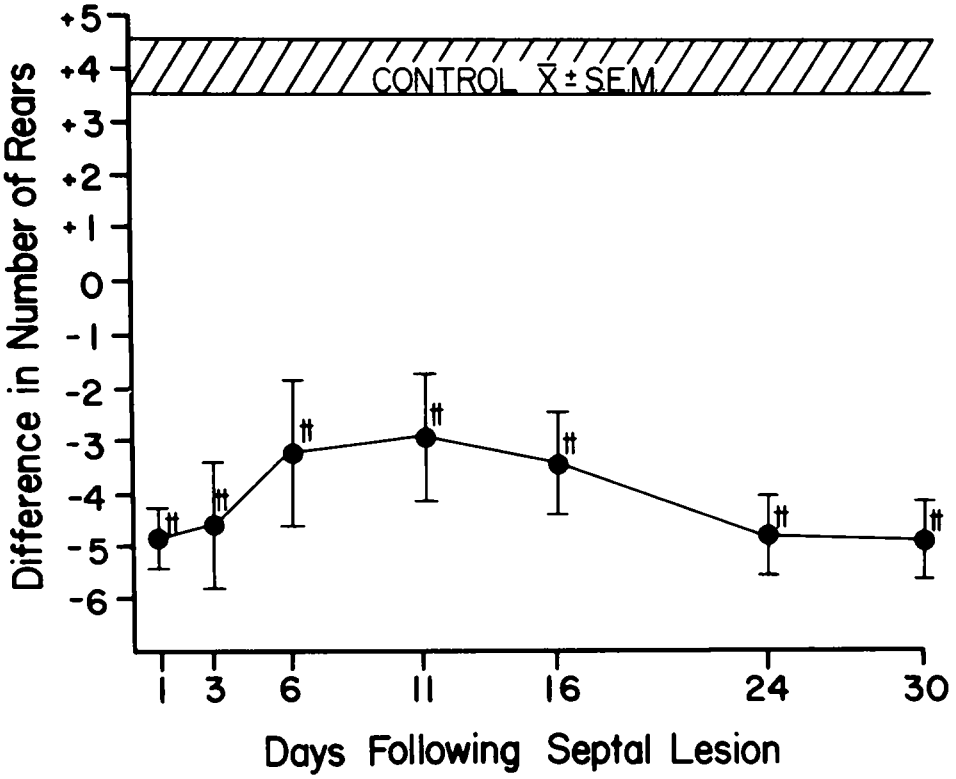

FIG. 8. Rearing behavior: Means and SEM's of difference scores for individual animals between preoperative and postoperative behavioral testing. $^{\dagger}=p<0.05$, two-tailed t test; $^{\dagger\dagger}=p<0.01$, two-tailed t test.

quantitative results are interpretable in light of the existing anatomical evidence which suggests a differential innervation of dorsal and ventral hippocampus [17, 23, 28, 30] as well as a differential distribution of 5-HT and NE terminals between particular cell fields [4, 27, 28, 39].

Following a septal lesion, rats typically exhibit a number of behavioral changes which tend to dissipate over time. In the present instance, the responsiveness to somatosensory stimulation, and latency to exit into the open field reflect the refractory nature of these behavioral alterations. That these behavioral indices exhibit their greatest deficit immediately following the lesion and eventually return to control values suggests that they may be mediated by a common neural mechanism.

The observation that septal lesions have a less deleterious effect on hippocampal NE than on 5-HT content is in accordance with the finding of other investigations [20, 26, 37], and with the suggestion that a significant proportion of hippocampal NE is contributed by a ventral pathway [37]. The eventual, almost total depletion of 5-HT levels, on the other hand, suggests that the vast majority, if not all, of the ascending serotonergic fibers which terminate in the hippocampus course through the septal area.

The elevated levels of 5-HT seen on the first day following the lesion are similar to those reported in other neurotransmitter systems following preterminal injury [1, 18, 20, 22]. The phenomenon may thus represent a general neural reaction of the synaptic terminal to mechanical injury. In the present instance, however, NE levels did not exhibit this initial elevation. Since not all investigators who have looked at acute changes in neurotransmitter levels following neural damage report this elevation in all systems [1, 3, 36], the effect may depend on one or more uncontrolled factors; such as the time interval between injury and sacrifice, the baseline levels of the neurotransmitter present in control tissue, and the percent damage to the particular system within the tissue being assayed. Alternatively, the presence of synaptic mechanisms responsible for the increased levels, i.e., postsynaptic feedback, may be different between systems [2].

The suggestive positive correlation between the time course required for maximal neurotransmitter depletion and dissipation of the hyperreactivity lends itself to one of several interpretations. First, the overt behavior observed following the lesion may be attributable to the immediate loss of afferent nerve impulses which are necessary for the normal integrative function performed by the remaining intact structures. In this respect, the later occurring changes in NE and 5-HT are merely indicative of latent metabolic degradation in the degenerating axons. Studies which have reported an irritability syndrome, similar to that induced by a septal lesion, following 6-hydroxydopamine [7] or 6-hydroxydopa [32] injections may lend credence to this interpretation. The resultant irritability has been shown to persist for up to several months, at which time the decrease in reactivity is correlated with the recovery of telencephalic NE levels. Loss of a functional noradrenergic system as one factor leading to hyperreactivity is further extended by observations that a single injection of L-dopa permanently eliminates septal lesion-induced hyperreactivity [13,25].

While this interpretation and its supportive evidence

would suggest that the observed hyperreactivity is due to the functional loss of catecholamines, they do not provide an adequate explanation for the behaviors' spontaneous dissipation. Other compensatory or adaptive mechanisms, such as collateral sprouting or sparing of function, which have been invoked to explain the behavioral recovery observed in other paradigms, would need to be elucidated to fully account for the transitory nature of the septal syndrome.

On the basis of the biochemical and behavioral data reported here, a second interpretation may be considered which would reflect a more intimate or functional link between the reported biochemical and behavioral changes. In the peripheral nervous system, a concomitant contraction of the target tissue has been commonly observed during the degeneration phase of the prejunctional axon [21]. It has been suggested that this post-degeneration contraction reflects the increased spontaneous release of neurotransmitter from the degenerating terminals. If the central nervous system exhibits a similar degenerative reponse (see [41]), the interpretive extension to the present data would propose that the transient behavioral effects of septal lesions may reflect the release of neurotransmitter substances whose axons or cell bodies had been destroyed. From this standpoint, the initial deficits and subsequent recovery would be subserved by the same neural mechanism. Data which would tend to support this interpretation include the extended time course required for CNS degeneration [22], the toxic effects of excessive 5-HT levels [9], and the findings that PCPA pretreatment attenuates the subsequent septal lesion-induced hyperreactivity [10, 15, 16].

Although the present experiment has demonstrated a temporal relationship between the depletion of NE and 5-HT in the hippocampus following a septal lesion, it is premature at this point to conclude that the hippocampal amines are solely responsible for the behavioral effects observed here. Given that a number of ascending and descending septal projections are destroyed by septal lesion, it becomes clear that the contributory role of each to the development and recovery of the septal syndrome remains to be clarified. We are currently investigating both acute and chronic effects of septal lesions on the neurochemical events which occur at various brain sites receiving septal projections. It is proposed that by assessing these changes within identified neurotransmitter systems, valuable information will be gained as to the cellular mechanics underlying the transient nature of the septal syndrome and to their generalizability to the brain's capacity to functionally compensate for neural damage.

ACKNOWLEDGEMENT

This research was supported in part by a research grant from the Texas Christian University Research Foundation to Fred H. Gage.

REFERENCES

1. Andén, N.-E., P. Bédard, K. Fuxe and U. Ungerstedt. Early and selective increase in brain dopamine levels after axotomy. *Experentia* **28**: 300–301, 1972.
2. Andén, N.-E. Shortlasting increase in the synthesis and utilization of noradrenaline due to axotomy-induced irritation. *Acta physiol. scand.* **100**: 51–55, 1977.
3. Bédard, P., A. Carlsson and M. Lindquist. Effect of a transverse cerebral hemisection on 5-hydroxytryptamine metabolism in the rat brain. *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **272**: 1–15, 1972.
4. Blackstad, T. W., K. Fuxe and T. Hökfelt. Noradrenaline nerve terminals in the hippocampal region of the rat and guinea-pig. *Z. Zellforsch.* **78**: 463–473, 1967.
5. Brady, J. V. and W. S. H. Nauta. Subcortical mechanisms in emotional behavior: affective changes following septal forebrain lesions in the albino rat. *J. comp. physiol. Psychol.* **46**: 339–346, 1953.
6. Brust-Carmona, H., F. J. Alvarez-Leefmans and L. Arditti. Differential projections of septal nuclei to ventral and dorsal hippocampus in rabbits. *Expl Neurol.* **40**: 553–566, 1973.
7. Coscina, D. V., J. Seggie, D. D. Godse and H. C. Stancer. Induction of rage in rats by central injection of 6-hydroxydopamine. *Pharmac. Biochem. Behav.* **1**: 1–6, 1973.
8. Costa, E., A. R. Green, S. H. Koslow, H. F. LeFevre, A. V. Revuelta and C. Wang. Dopamine and norepinephrine in noradrenergic axons: A study in vivo of their precursor-product relationship by mass fragmentography and radiochemistry. *Pharmac. Rev.* **24**: 167–190, 1972.
9. Costa, J. L., V. Ho, M. Spatz, I. Klatzo and C. Demirjian. 5-Hydroxytryptamine accumulation in cerebrovascular injury. *Nature* **248**: 135–136, 1974.
10. Dominguez, M. and V. G. Longo. Effects of p-chlorophenylalanine, α-methyl-paratyrosine and of other indole- and catecholamine depletors on the hyperirritability syndrome of septal rats. *Physiol. Behav.* **5**: 607–610, 1970.
11. Fuxe, K., T. Hökfelt and U. Ungerstedt. Morphological and functional aspects of central monoamine neurons. *Int. Rev. Neurobiol.* **13**: 93–126, 1970.
12. Gage, F. H. and D. S. Olton. Hippocampal influences on hyperreactivity induced by septal lesions. *Brain Res.* **98**: 311–325, 1975.
13. Gage, F. H. and D. S. Olton. L-dopa reduces hyperreactivity induced by septal lesions in rats. *Behav. Biol.* **17**: 213–218, 1976.
14. Geyer, M. A., A. Puerto, D. B. Menkes, D. Segal and A. J. Mandell. Behavioral studies following lesions of the mesolimbic and mesostriatal serotonergic pathways. *Brain Res.* **106**: 257–270, 1976.
15. Harrell, L. E. and S. Balagura. Septal rage: Mitigation by presurgical treatment with p-chlorophenylalanine. *Pharmac. Biochem. Behav.* **3**: 157–159, 1975.
16. Jones, A. B., J. D. Barchas and B. Eichelman. Taming effects of p-chlorophenylalanine on the aggressive behavior of septal rats. *Pharmac. Biochem. Behav.* **4**: 397–400, 1976.
17. Jones, B. E. and R. Y. Moore. Ascending projections of the locus coerulus in the rat. II. Autoradiographic study. *Brain Res.* **127**: 23–53, 1977.
18. Korf, J., R. H. Roth and G. K. Aghajanian. Alteration in the turnover and endogenous levels of norepinephrine in cerebral cortex following electrical stimulation and acute axotomy of cerebral noradrenergic pathways. *Eur. J. Pharmac.* **23**: 276–282, 1973.
19. Krieger, D. T. Food and water restriction shifts corticosterone, temperature, activity and brain amine periodicity. *Endocrinology* **95**: 1195–1201, 1974.
20. Kuhar, M. J., V. H. Sethy, R. H. Roth and G. K. Aghajanian. Choline: selective accumulation by central cholinergic neurons. *J. Neurochem.* **20**: 581–593, 1973.
21. Langer, S. Z. Denervation supersensitivity. In: *Handbook of Psychopharmacology, Vol. 2*, edited by L. L. Iverson, S. D. Iverson and S. H. Snyder. New York: Plenum Press, 1975, pp. 245–272.
22. Lidbrink, P. Noradrenaline nerve terminals in the rat cerebral cortex following lesion of the dorsal noradrenergic pathway: A study of the time course of their disappearance. In: *Dynamics of Degeneration and Growth in Neurons*, edited by K. Fuxe, L. Olson and Y. Zetterman. New York: Pergamon Press, 1974, pp. 141–149.

23. Lindvall, O. and A. Bjorklund. The organization of the ascending catecholamine neuron systems in the rat brain as revealed by the glyoxylic acid fluorescence method. *Acta. Physiol. Scand.*, Suppl. **412:** 1–48, 1974.
24. Malo, P., M. D. Lavina, E. L. Rodriquez Echandia and A. Machado. Distribution of acetylcholinesterase along the dorsoventral axis of the hippocampal formation in the rabbit. *J. Neurochem.* **29:** 729–733, 1977.
25. Marotta, R. F., N. Logan, M. Potegal, M. Glusman and E. L. Gardner. Dopamine agonists recovery from surgically-induced septal rage. *Nature* **269:** 513–515, 1977.
26. McGeer, E. G., J. A. Wada, A. Terao and E. Jung. Amine synthesis in various brain regions with caudate or septal lesions. *Expl Neurol.* **24:** 277–284, 1969.
27. Moore, R. Y. and A. E. Halaris. Hippocampal innervation by serotonin neurons of the midbrain raphe in the rat. *J. comp. Neurol.* **164:** 171–183, 1975.
28. Moore, R. Y. Monoamine neurons innervating the hippocampal formation and septum: Organization and response to injury. In: *The Hippocampus, Vol. 1*, edited by R. L. Isaacson and K. H. Pribram. New York: Plenum Press, 1975, pp. 215–237.
29. Olton, D. S. and F. H. Gage. Role of the fornix in the septal syndrome. *Physiol. Behav.* **13:** 269–279, 1974.
30. Pasquier, D. A. and F. Reinoso-Suarez. Differential efferent connections of the brain stem to the hippocampus in the cat. *Brain Res.* **120:** 540–548, 1977.
31. Pellegrino, L. S. and A. J. Cushman. *A Stereotaxic Atlas of the Rat Brain*. New York: Appleton-Century-Crofts, 1967.
32. Richardson, J. S. and D. M. Jacobowitz. Depletion of brain norepinephrine by intraventricular injection of 6-hydroxydopa: a biochemical, histochemical, and behavioral study in rats. *Brain Res.* **58:** 117–133, 1973.
33. Segal, M. and S. Landis. Afferents to the hippocampus of the rat studied with the method of retrograde transport of horseradish peroxidase. *Brain Res.* **74:** 1–15, 1974.
34. Shellenberger, M. K. and J. H. Gordon. A rapid, simplified procedure for simultaneous assay of norepinephrine, dopamine, and 5-hydroxytryptamine from discrete brain areas. *Analyt. Biochem.* **39:** 536–572, 1971.
35. Siegel, A. and J. P. Tassoni. Differential efferent projections of the lateral and medial septal nuclei to the hippocampus in the cat. *Brain Behav. Evolut.* **4:** 201–219, 1971.
36. Stock, G., T. Magnusson and N.-E. Anden. Increase in brain dopamine after axotomy or treatment with gammahydroxybutric acid due to elimination of the nerve impulse flow. *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **278:** 347–361, 1973.
37. Storm-Mathisen, J. and H. C. Goldberg. 5-hydroxytryptamine and noradrenaline in the hippocampal region: Effect of transection of afferent pathways on endogenous levels, high affinity uptake and some transmitter-related enzymes. *J. Neurochem.* **22:** 793–803, 1974.
38. Storm-Mathisen, J. Localization of transmitter candidates in the brain: The hippocampal formation as a model. *Prog. Neurobiol.* **8:** 119–181, 1977.
39. Swanson, L. W. and B. K. Hartman. The central adrenergic system. An immunofluorescence study of the location of cell bodies and their efferent connections in the rat utilizing dopamine-β-hydroxylase as a marker. *J. comp. Neurol.* **163:** 467–505, 1975.
40. Thierry, A. M. and J. Glowinski. Existence of dopamine nerve terminals in the rat cortex. In: *Frontiers in Catecholamine Research*, edited by E. Usdin and S. H. Snyder. New York: Pergamon Press, 1973, p. 216.
41. Ungerstedt, U. Functional dynamics of central monoamine pathways. In: *The Neurosciences, Third Study Program*, edited by F. O. Schmitt and F. G. Worden. Cambridge: MIT Press, 1974, pp. 979–988.
42. Vinogradova, O. S. Functional organization of the limbic system in the process or registration of information: Facts and hypothesis. In: *The Hippocampus, Vol. 2*, edited by R. L. Isaacson and K. H. Pribram. New York: Plenum Press, 1975, pp. 3–69.