Nucleus cuneiformis lesions: Amnestic effects on visual pattern discrimination in the rat*

TED L. PETIT†

University of Florida, Gainesville, Florida 32611

and

ROBERT THOMPSON

Louisiana State University, Baton Rouge, Louisiana 70803

Rats were trained on a brightness and pattern discrimination problem following removal of the left posterior half of the neocortex. Electrolytic lesions of the rostral and basolateral portions of the nucleus cuneiformis of the mesencephalic reticular formation on the right side caused serious retention losses of the pattern problem (three animals were unable to relearn), while lesions of similar magnitude to other brainstem areas on the right side did not lead to significant impairments in retention. The animals subjected to transverse lateral midbrain knife-cuts showed a slight but significant loss in retention. These results were interpreted as supporting the view that an occipitoreticular pathway is necessary for the performance of pattern discrimination problems in the albino rat.

A great deal of research has been generated within the last decade in search of a subcortical system involved in visually guided responses. In 1965, Thompson proposed two different anatomical pathways for mediating such visual discriminations: one for brightness discriminations (an indirect occipito-preecto-tegmental pathway) and a second for pattern discriminations (a direct occipito-mesencephalic tract terminating in the nucleus cuneiformis). The anatomical data supporting this latter pathway is contained in the study by Valverde (1962), who described an occipitoreticular pathway in the albino rat that descends from the visual cortex through the lateral portion of the cerebral peduncle and, at approximately the level of the substantia nigra, begins to leave the peduncle at right angles to terminate in the nucleus cuneiformis of the mesencephalic reticular formation and the nucleus reticularis pontis oralis. All areas of this pathway have been recently studied using the lesion technique, with the results pointing to its significance in mediating visual pattern discriminations in the rat. Bilateral damage to the lateral pedunculo-nigral area (Howze & Thompson, 1972; Thompson & Craddock, 1972), basolateral portion of the nucleus cuneiformis (Thompson, 1965; Thompson & Thorne, 1973), or nucleus reticularis pontis oralis (Thompson & Henderson, 1971) have been found to seriously impair the retention of a horizontal-vertical (pattern) discrimination habit.

The current study was undertaken to evaluate the foregoing conceptualization of Valverde's occipitoreticular pathway by first determining whether damage to the nucleus cuneiformis (NC) would drastically impair the capacity of rats to reacquire a

pattern discrimination. In an effort to more exactly delineate the area critical for pattern discriminations, a larger number of animals than used in previous studies (Thompson, 1965; Thompson & Thorne, 1973) were subjected to lesions situated in the area of the nucleus cuneiformis. Preliminary work in our laboratory disclosed that rats with extensive bilateral damage to the NC invariably failed to survive for more than a few days. (These results were obtained whether or not the lesions were induced by two-stage surgical procedures.) Since rats readily survive extensive unilateral damage to the NC, it was decided to construct an experimental design that would utilize this capability. The design that was finally settled upon was based on the neuropsychological findings that bilaterally occipitotomized rats, while being able to relearn a white-black (brightness) discrimination problem, cannot relearn a horizontal-vertical (pattern) discrimination problem (Horel, Bettinger, Royce, & Meyer, 1966; Lashley, 1950; Thompson, 1969) and upon the neuroanatomical findings that the occipitoreticular pathway described by Valverde is largely homolateral. If the occipitoreticular pathway is indeed critical for the performance of visual discrimination habits in the rat, then it would follow that rats trained on a brightness habit and a pattern habit *with only the right occipital cortex intact* would show considerable retardation in relearning the pattern habit after damage to the *right* NC, but would have little difficulty relearning the pattern habit after damage to the *left* NC. On the other hand, damage to either the right or left NC should have little effect on relearning the brightness habit. These expectations have been confirmed in a study recently completed in our laboratory (Thompson, Howze, & Petit, in preparation) using the same experimental design and the appropriate control lesions.

The second purpose of this study was to investigate

*This study was supported in part by Grant MH-08377 from the National Institute of Mental Health.

†Requests for reprints should be sent to Ted L. Petit, Department of Psychology, University of Florida, Gainesville, Florida 32611.

the possibility that lesions of the NC produced visual discrimination deficits due to interruption of ascending reticular and/or lemniscal fibers (Sprague, Levitt, Robson, Liu, Stellar, & Chambers, 1963), rather than destruction of cells within the NC. If the retention losses noted from NC lesions are due to interruption of these ascending fibers, then a transverse knife-cut through the NC should cause losses of retention similar to those induced by electrolytic lesions. If, however, the lesion deficits are due to destruction of cells and/or damage to fibers described by Valverde (1962) as leaving the peduncle at right angles coursing toward the NC, then such a transverse knife-cut should not produce retention losses of pattern discriminations to the same extent as electrolytic lesions.

METHOD

Subjects

Thirty-eight adult male albino rats of the Wistar strain were used. Two weeks prior to the onset of experimental training, all animals were subjected to extensive unilateral (*left hemisphere*) posterior neocortical ablations. In most cases, the adjoining cingulate, entorhinal, and pyriform areas on the left side were also destroyed, but the hippocampus remained largely intact.

Apparatus

All animals were trained in a two-choice Thompson-Bryant (1955) visual discrimination box, with escape (or avoidance) from footshock as the motive. Details of the apparatus and preliminary training procedures have been presented elsewhere (Thompson, 1969).

Procedure

All animals were first required to learn a brightness discrimination habit (Problem WB). A response to the unlocked white card (positive) admitted the animal to the goalbox, whereas a response to the adjacent locked black card (negative) was automatically punished by mild shock to the feet. Eight trials were given daily, with an intertrial interval of 60 sec. The position of the positive card was switched from the right to the left window in a strict double-alternation sequence. The criterion of learning consisted of no more than one error in 2 successive days. Subsequently, the animal was required to learn a pattern discrimination habit (Problem HV: horizontal black- and white-striped card positive, vertical black- and white-striped card negative). The training procedure was the same as that described for Problem WB.

After completion of training on Problem HV, 19 animals sustained unilateral subcortical electrolytic lesions of the right brainstem (side of the intact occipital cortex), 11 animals received unilateral brainstem knife-cuts on the right side, and 8 animals served as the control group.

Following a 2-week recovery (or rest) period, all animals were required to relearn the two discrimination problems in the order in which they were originally mastered. The training procedure was the same as that used in original learning.

Surgery and Histology

All operations were unilateral and were performed with the animals under deep chloral hydrate anesthesia. Neocortical lesions were produced by aspiration, while subcortical lesions ipsilateral to the intact cortex were made stereotactically. Electrolytic lesions were made by passing a 2.0-mA constant current for 10-15 sec through an implanted stainless steel electrode with 1.75 mm of the tip uninsulated. In order to assure extensive brainstem damage, two separate lesions were made by repositioning the electrode laterally.

The NC was divided into four different sectors relative to the critical region for visual pattern discriminations as reported by Thompson and Thorne (1973), and different groups of rats received lesions to a given sector. Group ANC was subjected to lesions which destroyed the NC anterior to the level of the third nerve. Group LNC received lesions at the level of the third nerve which destroyed the NC lateral to the red nucleus. Group DNC suffered lesions at the level of the third nerve which destroyed the NC dorsal to the red nucleus. Group PNC sustained lesions which destroyed the NC posterior to the level of the third nerve.

Transverse subcortical knife-cuts ipsilateral to the intact cortex were accomplished by attaching a small ophthalmic knife (1.75 mm wide) to the stereotaxic instrument and twice (repositioning the knife laterally) inserting it vertically through the neocortex to the desired depth. The knife-cuts were divided into those positioned anterior (Group KANC), posterior (Group KPNC), or at the level of the third nerve (Group KLNC).

After postoperative testing, each S was sacrificed with an overdose of Nembutal, its vascular system perfused with 10% Formalin, and the brain removed and allowed to harden for 48 h in a 10% Formalin solution. After the neocortical lesions were sketched, each brain was blocked, frozen, and sectioned either frontally (electrolytic lesion group) or sagittally (knife-cut group) at 90 microns with a freezing microtome. Every third section through the area of the lesion was photographed at 10x with a Leitz enlarger. Description of the electrolytic lesions in the text will be made only in terms of the central necrotic zone (vacuolated and severely coagulated areas), which is readily delineated in photographs of unstained sections.

When ranking overall size of lesions for correlation purposes, the photograph of the brain section showing the largest extent of the lesion was selected for each animal. The size of the lesion indicated on the photographs was then ranked from the greatest amount of damage to the least.¹ All other estimates of lesion size were based on inspection of several photographs derived from each operated animal.

Measures of Performance

Two different error scores were calculated from the learning and relearning records. "Criterion run" errors were defined as the number of errors committed prior to the attainment of the criterion (at least 15 correct responses within 2 consecutive days). "Significant run" errors referred to the number of errors committed prior to the first attainment of a series of correct responses that had a probability of occurrence of less than .05 (see Thompson, 1969). From these data, two error savings scores were determined: a criterion run savings score and a significant run savings score. Since the latter almost invariably yields a greater percentage of savings than the former, it represents a more sensitive measure of retention. In the text, reference will be made to the significant run savings score when it suggests a different interpretation from that based upon the criterion run savings score.

RESULTS

Original Learning

It is important to note that Problem WB was considerably easier to master than Problem HV. In terms of criterion run measures, the former was learned in an average of 4.9 days and 15.8 errors, as compared with 6.5 days and 21.2 errors for the latter. The Wilcoxon test for related samples (Siegel, 1956) disclosed that the differences in days as well as errors were significant ($p < .05$).²

Retention

Table 1 summarizes the retention scores for all groups

Fig. 1. Four unstained sections showing multiple lesions of the nucleus cuneiformis in R12. (This animal failed to relearn Problem HV after having earned a criterion run savings score of 70% on Problem WB.)

on the two visual discrimination problems. It is of some interest to note the performance of the control group. Not only did these animals show excellent retention of both habits, but also they revealed that Problem HV was as easy to relearn as Problem WB—the differences in savings scores were far from statistical significance. Thus, although acquisition of Problem WB and Problem HV may be impaired by unilateral ablation of the posterior neocortex (see Footnote 2), retentiveness of these problems (at least for the period of 2 weeks) is not.

Electrocoagulation Groups. A total of 19 animals were subjected to lesions of the right brainstem involving varying degrees of damage to the NC.

Table 1
Mean Retention (Percentage of Error Savings)
Scores for All Groups

Group	N	Problem WB		Problem HV	
		CR	SR	CR	SR
Control	8	90.6	90.6	99.3	99.6
Lesions					
ANC	2	-75.8*	-62.5*	-8.3*	-0.8*
LNC	10	17.8*	36.3*	-128.3*	-89.5*
DNC	3	41.5*	74.3	68.7	86.4
PNC	4	80.8	83.3	78.8	83.9
Knife-Cuts					
KANC	1	36.6	80.0	77.7	77.2
KPNC	7	57.3	59.0	79.1	76.6
KLNC	3	32.7	44.1	88.6	87.8

*Differed from the controls at least at the .05 level.

(1) *Group ANC.* Two animals sustained lesions of the right midbrain anterior to the level of the third nerve; the damage extended rostrally to the di-mesencephalic juncture involving damage primarily to the central gray and subcollicular area (Fig. 2A). This group performed significantly ($p < .05$) worse than the controls on both Problem WB and Problem HV. The criterion run savings scores were 8% and -160% for Problem WB and 21% and -37% for Problem HV.

(2) *Group LNC.* A total of 10 animals were subjected to lesions of the right midbrain at the level of the third nerve. The damage was located lateral to the red nucleus and within the ventral portion of the brainstem involving extensive damage to the substantia nigra. Although 7 of the 10 animals relearned Problem WB with positive criterion run savings scores, 7 of the 10 earned negative savings scores on Problem HV (mean score of -128%). Not only were the criterion run savings scores on the pattern discrimination significantly less than those earned by the controls ($p < .05$), but also three animals in this group were unable to relearn the habit within the allotted 20 days. Those animals failing to relearn the pattern discrimination sustained right ventral midbrain lesions at the level of the third nerve, involving extensive damage to the substantia nigra and slight damage to the red nucleus, although the primary locus of all lesions was lateral to the red nucleus extending laterally to the cerebral peduncle (see Fig. 1).

(3) *Group DNC.* Three animals sustained damage to

Fig. 2. Four unstained sections showing lesions in one animal of Group ANC (A), one animal in Group DNC (B), and two animals in Group PNC (C and D). The animal in Section A earned -38% criterion run savings score on Problem HV, while the remaining three animals showed perfect retention.

the right midbrain at the level of the third nerve but dorsal to the red nucleus, involving little or no damage to the substantia nigra (Fig. 2B). All three animals earned positive criterion run savings scores on Problem WB as well as on Problem HV. This group was not significantly different ($p > .05$) than the controls with regard to pattern discrimination performance: One animal earned perfect savings, the other two had scores of 50% and 53%.

(4) Group PNC. Four rats were subjected to lesions of the right brainstem which were located posterior to the level of the third nerve (Figs. 2C and 2D). There was no significant difference ($p > .05$) between this group and the control on either discrimination problem. On Problem HV two animals earned perfect criterion run savings scores, and the scores of the other two animals were 87% and 28%. All lesions began posterior to the level of the third nerve, extending caudally through the dorsal or lateral portions of the pontine reticular formation, causing slight damage to the inferior colliculus or central gray, although the primary locus of the lesions was ventral to the inferior colliculus. The nucleus reticularis pontis oralis was not damaged by any of the lesions.

Knife-Cut Groups. A total of 11 rats received deep transverse knife-cuts through the right lateral midbrain area. The incisions began approximately 1.0 mm from the midline and extended laterally to approximately 2.5 mm, with several of the incisions cutting through the

lateral extent of the brainstem. All cuts extended ventrally at least to the level of the substantia nigra, with the incisions in four animals extending through the base of the brainstem (see Fig. 3). The Ss were divided into those sustaining knife-cuts anterior (Group KANC), posterior (Group KPNC), and at the level of the third nerve (Group KLNC), and their criterion run savings scores analyzed with the use of Kruskal-Wallis one-way analysis of variance (Siegel, 1956). All Hs fell short of statistical significance ($p > .05$), indicating that the groups were not reliably distinguishable from each other or the control on either Problem WB or Problem HV. Although when analyzed with the use of a Mann-Whitney (1947) nonparametric test a difference ($p < .05$) is found between the control and the combined knife-cut groups, the mean criterion run savings scores for the knife-cut group as a whole was 49% on Problem WB and exceeded 81% on Problem HV. It is also important to note that all knife-cut animals succeeded in relearning the pattern discrimination within 3 days or less.

Performance and Lesion Size. In order to investigate the relationship between the size of the lesions and the pattern retention deficits, a rank-order correlation was computed for the combined groups between the size of the necrotic zone and the individual criterion run savings score. Rho was found to be .158, falling far short of statistical significance.

Although the correlation between pattern

Fig. 3. Six unstained sections showing the lateral (L) and medial (M) extent of transverse brainstem knife-cuts of three animals. The animal in the top sections (Group KPNC) showed the poorest retention of the knife-cut Ss (WB retention = 87%, HV retention = 22%). The animal in the middle sections is of Group KLNC (WB retention = 40%, HV retention = 86%). The animal in the bottom sections is of the Group KPNC (WB retention = 100%, HV retention = 100%).

performance and amount of damage for those animals in Group LNC was higher ($\rho = .377$), it was still not significant.

Summary. Figure 4 summarizes the major findings of this experiment. The left side of each section shows the distribution of brainstem lesions in those rats failing to relearn Problem HV within 20 days. It will be noted that the region of common damage in those three rats failing to relearn Problem HV occupies the paralateral portion of the midbrain reticular formation. The right side of each section of Figure 4 shows the distribution of brainstem lesions critical for visual pattern discriminations as outlined by Thompson and Thorne (1973).

Other Behavioral Observations

During the first few days following the initial posterior cortical ablation of the left hemisphere, the animals showed some tendency to circle to the left. Although this circling locomotor pattern was not visible at the time of original learning, virtually all animals exhibited a mild to moderate left position preference; the majority of errors made on Problems WB and HV occurred when the positive card appeared on the right.

Following lesions to the midbrain on the right side, the circling locomotor pattern to the left was reinstated and tended to be more marked and of longer duration than before, sometimes lasting for several weeks. Despite this motorial disturbance, the majority of the animals were excellent runners in the apparatus, requiring only occasional footshocks to force escape responses, showing frequent VTEs, and making adaptive correction responses following an error. While the majority of the animals showed aphagic-adipsic disorders for 1-5 days following surgery, these disturbances disappeared by the time the retention test was administered.

Those animals subjected to subcortical knife-cuts of the right side showed no noticeable behavioral changes after the operation. No obvious changes in emotionality were observed in any of the brain-damaged animals.

DISCUSSION

The findings of this study further add to the evidence demonstrating the importance of the NC of the mesencephalic reticular formation for the performance of a visual pattern discrimination problem in the white rat. It is of particular interest to note the striking

Fig. 4. Semidiagrammatic composites of lesions at four levels of the brainstem. The enclosed areas on the left side of each section indicate placement of lesions in those rats in this study that failed to relearn Problem HV. The right side of each section represents the distribution of brainstem lesions critical for pattern discriminations as outlined by Thompson and Thorne (1973). Abbreviations: CG = central gray, IC = inferior colliculus, IP = interpeduncular nucleus, MB = mamillary bodies, MG = medial geniculate nucleus, ON = oculomotor nerve, P = pons, RF = mesencephalic reticular formation, RN = red nucleus, SC = superior colliculus, SN = substantia nigra.

similarity between the findings of this study and previous studies by Thompson and Thorne (1973) and Thompson (1965) using bilaterally placed lesions of the midbrain reticular formation. While the results of this study confirm the finding of Thompson and Thorne (1973) that the rostral extension of the NC (Thompson's 1965 study did not investigate this midbrain area) is crucial for the retention of visual pattern discrimination habits in the rat, all three studies point to the necessity of the basolateral portion of the NC for such performance, and the diagrammatic overlap between the three papers is impressive (see Fig. 4).

The resulting deficits in visual pattern discrimination performance by animals in Group LNC cannot be reasonably explained in terms of emotionality, motivation, set, or motricity, since all animals in this group previously reacquired a brightness discrimination. Similarly, poor retention of the pattern discrimination cannot be readily attributed to poor general performance, because the correlation between performance on Problem WB and Problem HV was far from significant at the .05 level ($\rho = .275$). It should also be emphasized that the results cannot be reasonably explained in terms of a "mass action" effect, since a correlation between lesion size and performance for all lesioned animals on the pattern discrimination was also far from significant ($\rho = .158$). It must also be noted that lesions dorsal (Group DNC) or posterior (Group PNC) to the critical region did not produce a significant deficit on Problem HV. The finding that the integrity of the ventral half of the midbrain as opposed to the dorsal half is considerably more important for the execution of discriminative responses to visual cues has been reported in several previous studies (McNew, 1968; Thompson, 1963, 1969; Thompson & Massopust, 1960).

Lateral midbrain lesions that resulted in learning deficits in cats have been investigated previously by Sprague et al (1963). The authors of that paper suggested the possibility that the deficits were caused by interruption of lemniscal pathways. Those animals in this study subjected to transverse knife-cuts through the lateral midbrain area were used to test this hypothesis. These knife-cuts interrupted the ascending lemniscal-reticular pathways but not the occipitoreticular pathways (since the latter enters the NC at right angles from the cerebral peduncle). All knife-cut animals relearned the pattern discrimination within 3 days or less, with a mean criterion savings score of 81% on Problem HV, showing significantly greater retention than the lesion group (mean criterion run savings score of -128%).

These results cast doubt on the proposed explanation that disjoining lemniscal-reticular fibers are at the base of retention deficits of visual discrimination problems caused by lesions of the lateral midbrain areas. Conversely, they tend to strengthen the concept that the integrity of the emerging occipitoreticular fibers from the lateral cerebral peduncle described by Valverde as coursing toward the NC are essential for visual pattern discriminations. This becomes particularly evident when considering that bilateral *longitudinal* knife-cuts through this area, cutting occipitoreticular but sparing lemniscal-reticular fibers, cause significant deficits in retention of a horizontal-vertical discrimination problem (Howze & Thompson, 1972). Conceivably, this pathway may function in attention to visual cues, visio-motor integration, and/or the formation of visual engrams (see Thompson, 1965). When considered in light of other recent data collected in our laboratory (Thompson & Henderson, 1971; Thompson & Craddock, 1972; Howze

& Thompson, 1972), an ever strengthening picture seems to be emerging as to the significance of the pathway, described by Valverde (1962), coursing from the occipital cortex, through the internal capsule, lateral cerebral peduncle, and terminating in the NC and the nucleus reticularis pontis oralis as an integral part of the visual memory system of the white rat.

REFERENCES

- Horel, J. A., Bettinger, L. A., Royce, G. J., & Meyer, D. R. Role of neocortex in the learning and relearning of two visual habits by the rat. *Journal of Comparative & Physiological Psychology*, 1966, 61, 66-78.
- Howze, M. A., & Thompson, R. Disconnecting occipitoreticular projections: Amnesic effects on a visual habit in the rat. *Psychonomic Science*, 1972, 27, 143-145.
- Lashley, K. S. In search of the engram. *Symposia of the Society for Experimental Biology*, 1950, No. IV, 454-482.
- Mann, H. B., & Whitney, D. R. On a test of whether one or two random variables is stochastically larger than the other. *Annals of Mathematical Statistics*, 1947, 18, 50-60.
- McNew, J. J. Role of the red nucleus in visually guided behavior in the rat. *Journal of Comparative & Physiological Psychology*, 1968, 65, 282-289.
- Siegel, S. *Nonparametric statistics for the behavioral sciences*. New York: McGraw-Hill, 1956.
- Sprague, J. M., Levitt, M., Robson, K., Liu, C. N., Stellar, E., & Chambers, W. W. A neuroanatomical and behavioral analysis of the syndromes resulting from midbrain lemniscal and reticular lesions in the cat. *Archives Italiennes de Biologie*, 1963, 101, 225-295.
- Thompson, R. Cortical and subcortical structures mediating visual discrimination habits in the rat. *Boletín del Instituto de Estudios Médicos y Biológicos, Universidad Nacional de México*, 1963, 21, 451-466.
- Thompson, R. Centrencephalic theory and interhemispheric transfer of visual habits. *Psychological Review*, 1965, 72, 385-398.
- Thompson, R. Localization of the "visual memory system" in the white rat. *Journal of Comparative & Physiological Psychology Monograph*, 1969, 69, Part 2, 1-29.
- Thompson, R., & Bryant, J. H. Memory as affected by activity of the relevant receptor. *Psychological Reports*, 1955, 1, 393-400.
- Thompson, R., & Craddock, S. N. Lateral cerebral peduncle lesions: Amnesic effects on a visual habit in the rat. *Psychonomic Science*, 1972, 27, 140-142.
- Thompson, R., & Henderson, T. C. Pontine reticular formation lesions: Amnesic effects on learned habits in the rat. *Psychonomic Science*, 1971, 25, 169-170.
- Thompson, R., & Massopust, L. C. The effect of subcortical lesions on retention of a brightness discrimination in rats. *Journal of Comparative & Physiological Psychology*, 1960, 53, 488-496.
- Thompson, R., & Thorne, B. M. Brainstem reticular formation lesions: Amnesic effects on learned habits in the rat. *Physiological Psychology*, 1973, 1, 61-70.
- Valverde, F. Reticular formation of the albino rat's brain stem, cytoarchitecture and corticofugal connections. *Journal of Comparative Neurology*, 1962, 119, 25-53.

NOTES

1. Test-retest reliabilities after a 2-week interval for all estimates reported in this study ranged from .85 to .95.
2. A comparison of these performance measures with those earned by normal rats in our laboratory indicates that unilateral ablation of the posterior neocortex retards acquisition of both the white-black and horizontal-vertical discrimination habits.

(Received for publication February 12, 1974;
accepted February 21, 1974.)