Exp Brain Res (1990) 79:18–24

Experimental Brain Research

Picture recognition vs. picture discrimination learning in monkeys with medial temporal removals

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Summary. Three monkeys with complete ablations of temporal-lobe limbic structures and three unoperated controls were compared in an automated testing apparatus for their ability to remember pictures presented between 1 and 180 seconds previously, as well as to learn picture discriminations in which successive trials with a given pair were separated by either 20 seconds or 24 hours. The operated animals were not impaired in picture discrimination learning under either condition and they were not impaired in picture recognition memory up to about 10 seconds. At 10 seconds and beyond, however, the operated animals showed rapid deterioration of picture memory. The results demonstrate that the limbic system's selective contribution to learning and retention uncovered initially with objects applies equally to pictures, this contribution being essential for recognition memory but not for discrimination habits. The results demonstrate further that, as in humans, temporal-lobe limbic structures are essential for recognition only when the retention test exceeds the immediate memory span of a few seconds.

Key words: Memory - Habits – Amygdala – Hippocampus – Pictorial stimuli – Macaques

Introduction

Patients with extensive damage to the medial temporal region of the brain tend to forget newly acquired sensory and cognitive information extremely rapidly, within seconds to minutes, even though they can acquire and retain perceptual and motor skills about as well as normal subjects (Milner 1962; Corkin 1968; Warrington and Weiskrantz 1968; Cohen and Squire 1980). This syndrome of rapid but selective loss of retention has been reproduced in nonhuman primates. For example, unlike normal monkeys, monkeys with bilateral ablations of the amygdala, hippocampus, and underlying cortex seem unable to remember for more than about a minute which of two objects has just been presented for familiarization (Mishkin 1978; Murray and Mishkin 1984), and they seem unable to remember for more than a few seconds which of two objects has just been baited (Phillips and Mishkin 1984); yet, paradoxically, the same monkeys learn two-choice visual discriminations as quickly as normal animals, and they can do so even when the successive trials on a given discrimination are separated by 24-hour intervals (Malamut et al. 1984; Murray 1987). These and similar findings (Zola-Morgan and Squire 1984) indicate that in monkeys, just as in humans, memory processes can be divided into qualitatively different types, one of which is largely if not totally dependent on temporal-lobe limbic structures. Reproduction of the syndrome of "temporal-lobe amnesia" in monkeys opens up the possibility of investigating the physiology and chemistry of the limbic-dependent memory process. The present experiment was undertaken with the aim of facilitating that goal.

Until now, the demonstrations of severe anterograde amnesia in monkeys given complete medial temporal ablations have been carried out in the Wisconsin General Testing Apparatus (WGTA), in which the freely moving monkey gains solid food rewards by manipulating and displacing objects (Mishkin 1978; Zola-Morgan et al. 1982; Murray and Mishkin. 1984; Malamut et al. 1984). In the present study we asked whether the same syndrome that had been uncovered with use of the WGTA– i.e. the same constellation of limbic-dependent and

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limbic-independent retention abilities – could be demonstrated with use of an automated apparatus in which the seated monkey gains liquid rewards by pressing panels lit by pictorial stimuli (Overman and Doty 1980; Gaffan et al. 1984, 1986). Confirmation of the earlier findings in this type of testing situation would not only broaden the support for those findings but also validate a method of assessing both limbic-dependent and limbic-independent retention that is compatible with the concurrent application of such neurobiological techniques as single-unit recording, intracerebral drug injections, measurement of glucose utilization, etc.

As reported below, the results obtained with the WGTA were fully replicated with the automated apparatus. In addition, because the automated apparatus, unlike the WGTA, permitted evaluation of immediate memory (i.e. retention over extremely short intervals), it was possible to demonstrate that this form of memory is intact after limbic lesions in monkeys just as it is after limbic damage in man. Thus, this methodology allowed an accurate assessment of when the memory of amnesic monkeys starts to fail.

Method

Subjects

The subjects were six experimentally naive, young adult male rhesus monkeys (*Macaca mulatta*) weighing 3 to 4 kg at the beginning of the experiment. They were housed individually and allowed ad libitum water for an hour following their testing session and ad libitum food throughout the day.

Apparatus

Training was conducted in a sound attenuating chamber equipped with a one-way observation window. The monkeys were seated in an adjustable chair that allowed free arm and leg movement, and positioned 15–20 cm in front of three 7 × 7 cm opalescent plastic panels, hinged at the top and aligned horizontally 1 cm apart. Standard 35-mm colored slides of many different objects, e.g. coffee cup, shoe, screwdriver, car keys, Christmas tree ornament, etc., were rear-projected onto the panels by three carousel projectors, one per panel. Each projector was equipped with a 180-mm lens to yield high resolution color images. The monkey was rewarded for correct panel presses with approximately 0.5 ml of orange juice (Tang) delivered through a solenoid to a metal drinking spout positioned so as not to obscure the animal's view of the panels.

Surgery and histology

Following extensive preoperative training, three of the animals received bilateral ablations of the amygdala, hippocampus, and subjacent cortex (Group AH), the three others being retained as unoperated controls (Group N). Surgery was performed aseptically in one stage with the animal secured in a head holder while under Ketamine (10 mg/kg) and Nembutal (20-30 mg/kg) anesthesia. Heart rate, respiration rate, and temperature were monitored throughout. The ablations were made by aspiration of tissue under visual control with the aid of an operating microscope. Following surgery the wounds were closed in anatomical layers, after which the animal was placed in an incubator until it awakened from anesthesia. Dexamethasone phosphate (0.4 mg/kg) was administered for one day preoperatively and several days postoperatively to prevent edema, and gentamicin sulfate (5.0 mg/kg) for several days postoperatively to prevent infection.

The ablation of the amygdala was accomplished through an orbitofrontal approach and included not only the amygdaloid complex but also the periamygdaloid and entorhinal cortices medial to the rostral half of the rhinal sulcus. Ablation of the hippocampus was performed through an occipitotemporal approach and included not only the hippocampal formation but also the parahippocampal gyrus medial to the occipitotemporal sulcus, as well as the entorhinal cortex medial to the caudal half of the rhinal sulcus.

At the conclusion of the study the operated monkeys were given a lethal dose of Nembutal and perfused intracardially with saline followed by 10% formalin. The brains were then removed, embedded in celloidin, and sectioned in the coronal plane at 25 um, and every 10th section was stained with thionin. Following microscopic examination, the boundaries of the lesions were transferred to drawings of standard sections, and these were used to reconstruct ventral surface views of the brain. Representative cross sections through the lesion and the surface reconstruction for each animal are illustrated in Fig. 1, where they are compared with illustrations of the intended removal. The lesions were generally as planned, except for sparing of the caudal 2–3 mm of the hippocampal formation in each case. In addition, Case AH1 sustained bilateral infarction of ventral temporal cortex lateral to the occipitotemporal sulcus, amounting to approximately 15% of cytoarchitectonic area TE and 5% of area TEO. Also, case AH3 sustained a left unilateral infarction affecting the posterior dorsal portion of the tail of the caudate nucleus and adjacent optic radiations, and the latter resulted in turn in degeneration within the medial third of the left lateral geniculate nucleus.

Behavioral procedure

Picture recognition with progressively increasing delays. Preliminary training consisted of rewarding the animal with juice for pressing any of the three panels as soon as it was illuminated. They were then trained on a picture recognition task, delayed nonmatching-to-sample (DNMS), with a 1-sec intratrial delay. A trial began with the presentation of a stimulus (the sample) on the central panel. To encourage the monkey to view the sample for an extended period, juice reward was progressively delayed during early training until it took place approximately 3 seconds after the animal initiated a panel press. As a result, the animal typically kept the panel depressed continuously until picture offset, which occurred 5 seconds after picture onset. One second later the sample reappeared on one of the lateral panels together with a comparison stimulus on the other lateral panel. The animal was rewarded for choosing the comparison stimulus, i.e. the one that had not appeared on the central panel one second earlier. A panel press to the comparison stimulus resulted in the immediate delivery of a juice reward, the offset of both stimuli, and the start of a 20 sec intertrial interval. There was no correction or punishment for an error other than the immediate offset of both stimuli and the start of the next intertrial interval.

Animals received 40 trials per day, each trial consisting of a new pair of sample and comparison stimuli. Each day, however, the same 80 stimulus slides (set A) were randomly rearranged and thus were trial-unique only within each session. The left-right position of the correct (comparison) stimulus followed a predetermined pseudorandom sequence. Animals were trained

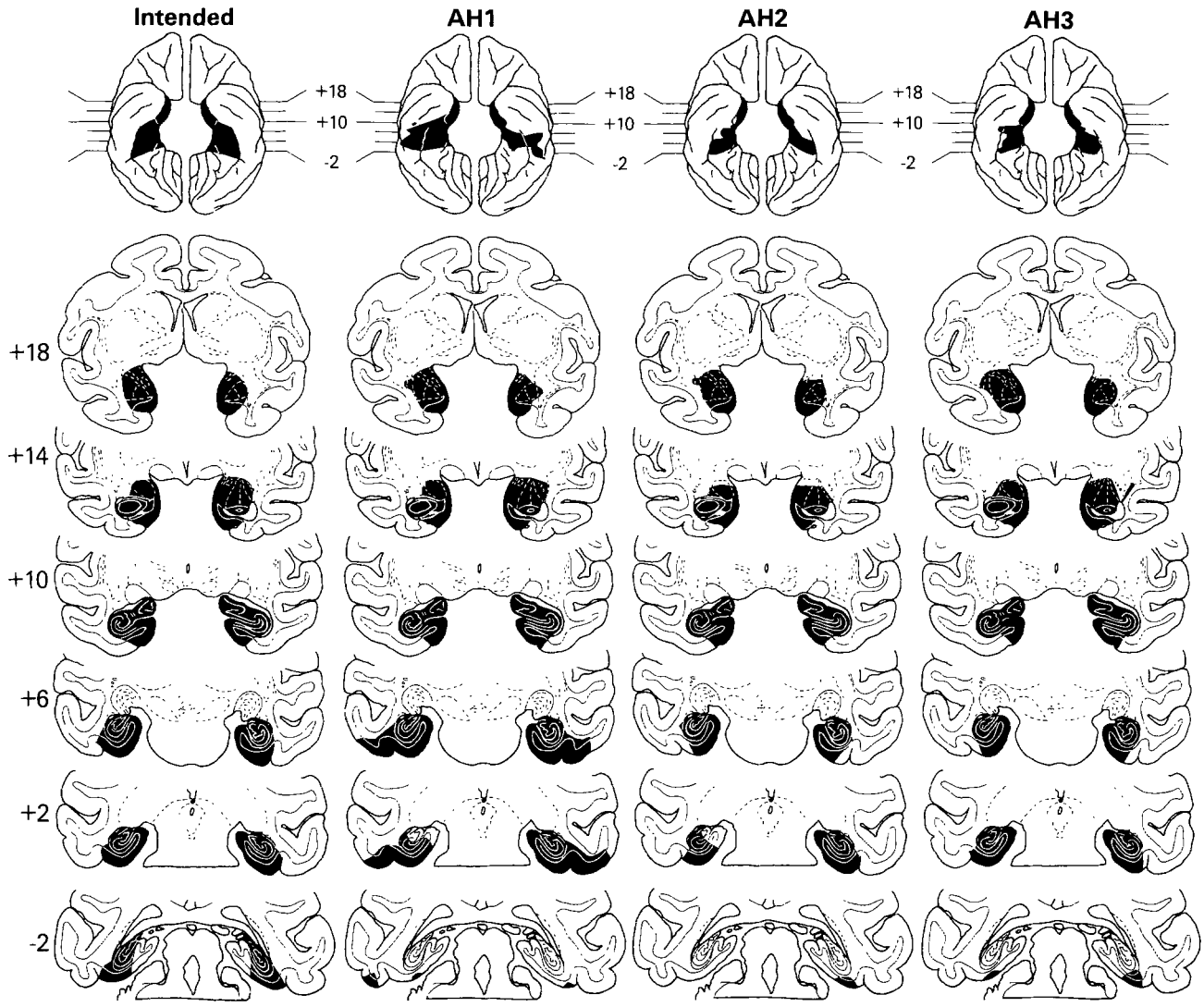

Fig. 1. Comparison of intended with actual lesions transferred to standard cross sections and ventral surface views of the rhesus monkey brain

to a criterion of 90 per cent correct responses for two consecutive days, after which they were trained to the same criterion at 5-sec and then 10-sec delays.

The animals were next tested on DNMS at delays of 30, 90, and finally 180 sec, but now with a new set of 80 stimulus slides at each delay (sets B, C, and D, respectively). At the 30- and 90-sec delays, animals received 40 trials per day for 5 days each, whereas at the 180-sec delay, they received 20 trials per day for 10 days. On completion of testing, surgery was performed on Group AH.

Five months after surgery, or after an equivalent rest interval for Group N, all animals were retested just as before. That is, they were retrained to criterion at delays of 1, 5, and 10 sec, in succession, with stimulus set A, and then given the performance test at delays of 30, 90, and 180 sec, in succession, for 200 trials at each delay with stimulus sets B, C, and D, respectively.

Picture discrimination learning. All animals were trained next on three consecutive two-choice picture discriminations, each at the rate of 35 trials per day to a criterion of 90 percent correct responses on each of two successive days. The stimulus pairs were chosen to be easily discriminable. In each discrimination, one stimulus was arbitrarily designated the positive and the other the negative, and they were presented pseudorandomly on the left and right lateral panels. Intertrial intervals were 20 seconds.

Concurrent picture discrimination learning with 24-hour intertrial intervals. All animals were then tested on a concurrent picture discrimination task for 20 trials per day, each trial with a different pair of stimuli, one of which was arbitrarily designated the positive and one the negative. The set of 20 pairs was presented in the same order once daily until the animals reached the criterion of 90 percent correct responses on each of two consecutive days. The positive and negative stimuli in a pair appeared randomly on the left and right panels, and trials within a session were separated by 20-second intervals. Because a given pair was presented only once daily, however, successive trials on each pair were separated by 24-hour intervals.

Picture recognition with randomly intermixed delays. Finally, all animals were retested on the picture recognition task for 10 days at the rate of 25 trials per day but with pseudorandomly intermixed delays. A new set of 50 stimulus slides (set E) was used,

and, in each test session, delays of 5, 10, 30, 90, and 180 sec were presented for 5 trials each pseudorandomly intermixed with the constraint that there were no more than two consecutive trials at a given delay.

Results

Picture recognition with progressively increasing delays. Preoperatively, the animals that were to receive the ablations attained criterion more quickly than the control animals at the 1 and 5 sec delays. Thereafter, however, the two groups were fairly well matched (Tables 1 and 2 and Fig. 2). Specifically, there were no preoperative group differences at 30, 90, or 180 sec delay, nor was there a significant interaction between group and delay ($F=0.24$; $df=2,8$; $P<.05$), but there was a significant effect of delay ($F=8.31$; minimum $df=1,4$; $P<.05$). Paired comparisons indicated that performance at 180 sec was worse than that at either 30 or 90 sec (Sign test, $P=.016$ in both cases), though the difference between the latter two was not significant.

After the five-month hiatus between the end of preoperative training and the beginning of postoperative testing, every animal required between one and five sessions to reattain the DNMS rule at the 1-sec delay, but, interestingly, there were no significant group differences. Indeed, the animals with amygdalo-hippocampal lesions showed a small though nonsignificant advantage (Table 1). Postsurgical impairment in DNMS appeared for the first time at the 5-sec delay, and then only in case AH1, the animal that sustained the bilateral infarction of inferior temporal cortex. An impairment in Group AH as a whole appeared for the first time only at the 10-sec delay (Mann-Whitney U test, $P=.05$). In short, up through the 5-sec delay, amygdalo-hippocampal lesions uncomplicated by inferior temporal damage caused no difficulty with picture recognition, impairment becoming manifest though not yet severe with delays of 10 seconds.

With delays of 30 seconds and above, on the other hand, the group impairment did become severe, Group AH scoring on the average 22% below that of Group N across the three longer delays (Table 2 and Fig. 2). This difference reflects the combined effects of two different preoperative-to-postoperative trends, with Group N having gained an average of 5% (means of 80% pre and

Table 1. Trials (and errors) preceding criterion on DNMS at each of three delays

	Preoperative scores			Postoperative scores		
	Delay (sec)			Delay (sec)		
Subjects	1	5	10	1	5	10
N1	1200 (451)	120 (26)	40 (6)	120 (13)	80 (11)	40 (5)
N2	1200 (366)	200 (44)	0 (0)	200 (46)	120 (19)	40 (7)
N3	920 (422)	400 (65)	0 (0)	160 (35)	0 (0)	0 (0)
$\bar{X}$	1107 (413)	240 (45)	13 (2)	160 (31)	67 (10)	27 (4)
AH1	960 (318)	0 (0)	40 (8)	80 (20)	1360 (242)	160 (23)
AH2	960 (305)	40 (12)	0 (0)	40 (12)	120 (22)	280 (53)
AH3	720 (268)	0 (0)	80 (17)	120 (26)	40 (7)	1360 (304)
$\bar{X}$	880 (297)	13 (4)	40 (8)	80 (19)	507 (90)	600 (127)

Table 2. Percent correct responses on DNMS with progressively increasing delays

	Preoperative scores			Postoperative scores		
	Delay (sec)			Delay (sec)		
Subjects	30	90	180	30	90	180
N1	86	84	71	88	86	84
N2	80	80	74	90	82	73
N3	89	82	76	90	94	84
$\bar{X}$	85	82	74	89	87	80
AH1	96	81	78	78	62	62
AH2	93	78	76	76	62	62
AH3	82	92	72	72	64	62
$\bar{X}$	90	84	75	75	63	62

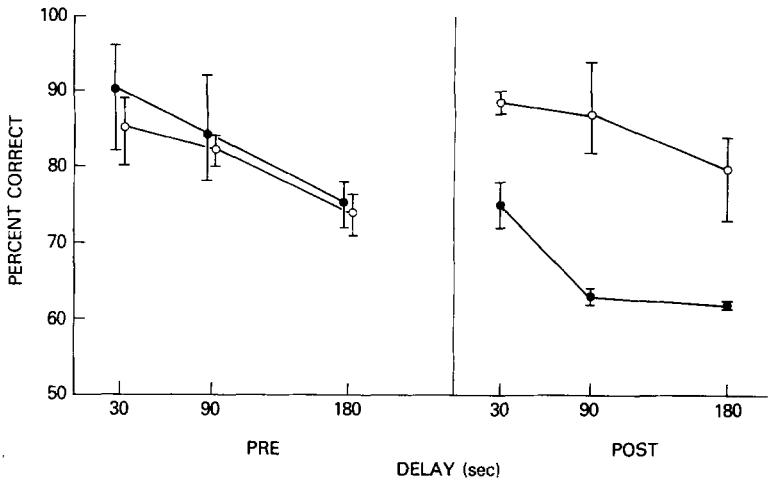

Fig. 2. Average scores on DNMS as a function of progressively increasing delays. Vertical bars indicate range of scores ○ group N, ● group AH

85% post), and Group AH having lost an average of 17% (means of 83% pre and 66% post). Each of these differences was significant (Sign test, $P = .05$). Statistical analysis of the postoperative data revealed a significant effect of lesion ($F = 81.06$; $df = 1,4$; $P < .01$) and of delay ($F = 16.58$; minimum $df = 1,4$; $P < 0.5$) but no interaction between them.

Picture discrimination learning. Unlike the results for DNMS, those for discrimination learning failed to yield any clearcut evidence of impairment in the animals with amygdalo-hippocampal lesions (Table 3). Both groups learned the three discriminations in a total of about 200 trials and 50 errors.

Concurrent picture discrimination learning with 24-hour ITIs. As in the learning of single discriminations under conditions of massed practice, i.e. 35 trials per day with 20-second ITIs, the learning of a large set of discriminations with spaced practice, i.e. 1 trial each per day with 24-hour ITIs, proceeded just as quickly in Group AH as in Group N (Table 4). In this case, both groups learned each discrimination pair in an average of about 5 errors, which is approximately the rate they had attained on the last of the three single discriminations.

Picture recognition with randomly intermixed delays. The second DNMS procedure, in which the delays were pseudorandomized, probably provides a more accurate estimate of forgetting rates than the initial procedure, in which the delays were increased progressively. With the second procedure, the faster forgetting of the operated animals is again clearly evident, with the impairment beginning to appear, as before, only at the 10-sec delay (Table 5 and Fig. 3). Statistical analysis revealed a significant effect not only of the lesion ($F = 27.6$; $df = 1,4$; $P < .01$) and of the delay ($F = 71.25$; minimum $df = 1,4$; $P < .01$) but also of their interaction ($F = 11.81$; minimum $df = 1,4$; $P < .05$).

Table 3. Trials (and errors) preceding criterion on three consecutive two-choice discriminations

Subjects	Pair 1	Pair 2	Pair 3
N1	175 (36)	70 (22)	35 (5)
N2	70 (18)	70 (11)	35 (6)
N3	175 (39)	70 (14)	0 (0)
$\bar{X}$	140 (31)	70 (16)	23 (4)
AH1	35 (12)	70 (23)	35 (14)
AH2	105 (21)	70 (19)	35 (5)
AH3	70 (14)	70 (15)	35 (10)
$\bar{X}$	70 (16)	70 (19)	35 (10)

Table 4. Sessions (S), errors (E), and errors/pair (E/P) preceding criterion on twenty concurrent two-choice discriminations with 24-hour ITIs

Subjects	S	E	E/P
N1	19	(109)	(5.4)
N2	14	(82)	(4.1)
N3	18	(105)	(5.2)
$\bar{X}$	17	(99)	(4.9)
AH1	18	(104)	(5.2)
AH2	11	(72)	(3.6)
AH3	12	(100)	(5.0)
$\bar{X}$	14	(92)	(4.6)

Conclusion

The evidence that medial temporal ablations produce severe yet selective memory loss is extended by the present data in two ways. First, they show that the impairment discovered originally in object recognition as distinct from object discrimination

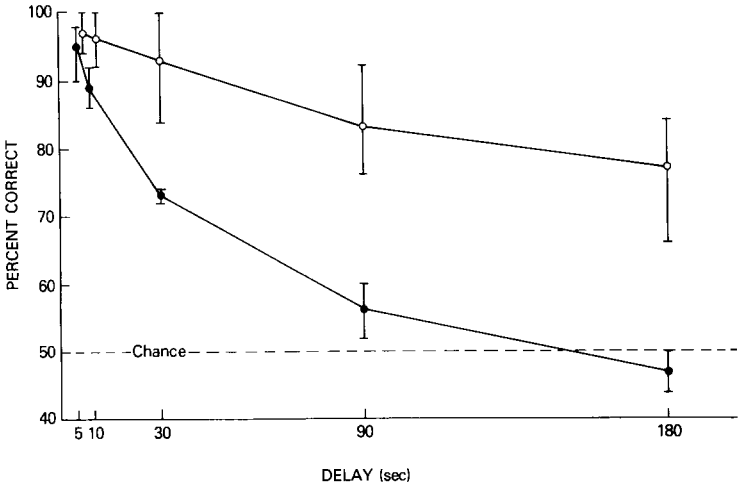

Fig. 3. Average scores on DNMS as a function of randomly intermixed delays. Vertical bars indicate range of scores. ○ group N, ● group AH

Table 5. Percent correct responses on DNMS with randomly intermixed delays

	Delay (sec)				
Subjects	5	10	30	90	180
N1	98	92	84	76	66
N2	94	96	96	80	84
N3	100	100	100	92	80
$\bar{X}$	97	96	93	83	77
AH1	96	90	72	56	50
AH2	98	86	74	52	48
AH3	90	92	72	60	44
$\bar{X}$	95	89	73	56	47

(Mishkin 1978; Malamut et al. 1984) occurs also in picture recognition as distinct from picture discrimination. The results thus establish that automated testing with pictorial stimuli, useful for conducting behavioral neurobiological studies, is in fact a valid method for the selective assessment of limbic-dependent memory processes. Second, the present results provide the first unambiguous evidence that in monkeys, as in humans (Milner 1962), recognition memory after combined amygdalo-hippocampal lesions in *not* impaired at short delays. Our results suggest that, following such lesions, picture recognition in monkeys starts to deteriorate only after about 10 seconds, though the deterioration becomes severe (i.e. performance drops close to chance levels) within a minute or two.

Despite this abnormally rapid forgetting of pictorial stimuli, the operated monkeys learned picture discriminations at a normal rate, whether the successive trials on a given pair were separated by 20 seconds (single discriminations) or 24 hours (concurrent discriminations). The negative finding on the latter task, in which the animals were confronted with the same type and number of choice stimuli per session as in DNMS, but with far longer retention intervals (hours as opposed to minutes), provides a particularly powerful demonstration that the limbic-dependent retention process is a highly selective one. The neuroanatomical system that is responsible for the limbic-independent retention process remains to be positively identified (see Phillips et al. 1988).

Acknowledgements. We thank Leon Dorsey and Linton Stokes for their technical assistance and Jay Weinstein for examining and illustrating the histological material. A preliminary report of this material has appeared (Overman, et al 1983).

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Received February 11, 1989/Accepted July 27, 1989