Pharmacology Biochemistry & Behavior, Vol. 9, pp. 227–234. Printed in the U.S.A.

Locus Coeruleus and Labile Memory

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(Received 20 March 1978)

ZORNETZER, S. F., W. C. ABRAHAM AND R. APPLETON. *The locus coeruleus and labile memory.* PHARMAC. BIOCHEM. BEHAV. 9(2) 227–234, 1978.—Memory lability is defined as the period of time recently-formed memory remains susceptible to experimental modification. Electrolytic lesions delivered through chronic indwelling electrodes to the locus coeruleus (LC) complex of mice, made shortly after learning, resulted in an extension of memory lability. Mice with unilateral, but not bilateral LC damage became amnesic following electroconvulsive shock (ECS) administered 7 days (168 hr) after memory formation. ECS had no effect on memory in LC-lesioned mice when administered 14 days following training. In a second experiment, the temporal relationship between time of memory formation and time of LC damage was found to be critical to the occurrence of this extended period of lability. In a third experiment, we tested the possibility that prolonged trace lability was the result of weaker memory formation as reflected by decreased persistence (i.e. faster forgetting) of the memory. The results indicated equal rates of forgetting for normal and LC-lesioned mice. Present results support the hypothesis that the locus coeruleus complex normally plays an important role in delimiting the time-course of initially labile stages of memory. By inference, these data suggest further that such a delimiting function of the locus coeruleus is mediated through its noradrenergic modulation of other brain regions.

Locus coeruleus Retrograde amnesia Memory lability Norepinephrine Inhibitory avoidance Brain lesions

THE CONTROVERSY over the years concerning the exact temporal nature of the retrograde amnesia gradient [6,19] can be viewed today, with the wisdom of hindsight, as largely a pseudo-issue. Thus, there is no single and simple temporal gradient of memory lability, i.e. susceptibility to experimental modification. Rather, there is a family of curves describing a number of gradients, each gradient being a function of a host of variables including task difficulty, footshock intensity, time of day, etc. [28]. There is presumably a limit, however, to the normal lability period of a recently formed memory. Thus, although some exceptions have been reported [5,13], it is generally believed that the typical period of memory lability in animals terminates within 3–6 hours of memory formation [3, 8, 9, 21]. This popular belief is in part the reason why multiple-process memory storage models have been proposed [26, 27, 34]. According to such models one or more initial information holding systems mediate recall of labile memory for relatively short intervals following acquisition. Other information holding systems mediate recall at more extended time intervals.

Recent findings [36] from our laboratory suggest that unilateral electrolytic damage to the locus coeruleus (LC) complex in mice, sustained immediately following inhibitory avoidance training, results in a dramatic extension of the labile memory period. However, such LC lesions made immediately following training had no effect, by themselves, upon subsequent inhibitory avoidance performance. This finding is consistent with a large number of reports indicating little or no effect of LC damage on either acquisition or retention of a variety of behavioral tasks [1, 25, 31]. The important difference between our previous results and those reported by other investigators is that we followed posttraining LC damage with yet another posttraining treatment (electroconvulsive shock (ECS)) thereby uncovering a role of the LC in memory processing that had previously gone undetected.

EXPERIMENT 1

In our earlier report [36] mice administered ECS 40 hr but not 168 hr following immediate, posttraining electrolytic lesions to the LC complex were subsequently amnesic for an inhibitory avoidance response. An analysis of the original data reported for the 168 hr LC lesion-ECS interval indicated, interestingly, that although there was no statistically significant performance deficits for control or LC-lesioned groups, an unusual number of unilateral LC-lesioned mice appeared deficient. We therefore decided to repeat and extend our original experiment in order to investigate further the temporal dynamics of retrograde amnesia (RA) following LC damage.

METHOD

One hundred and ten male Swiss ICR mice were stereotaxically implanted bilaterally with twisted, teflon insulated, nichrome electrodes (each strand 125 μm dia.). The target structure was the LC (Coordinates: anterior–posterior=–1.3 mm caudal to lambda, medial–lateral=0.8

[1]This research was supported by Alfred P. Sloan Foundation.
[2]Supported by NSF Predoctoral Fellowship.
[3]The authors gratefully acknowledge the assistance of A. J. Dunn, R. L. Delanoy, M. Gwynn, Ge' Ruigt, in various aspects of this research.

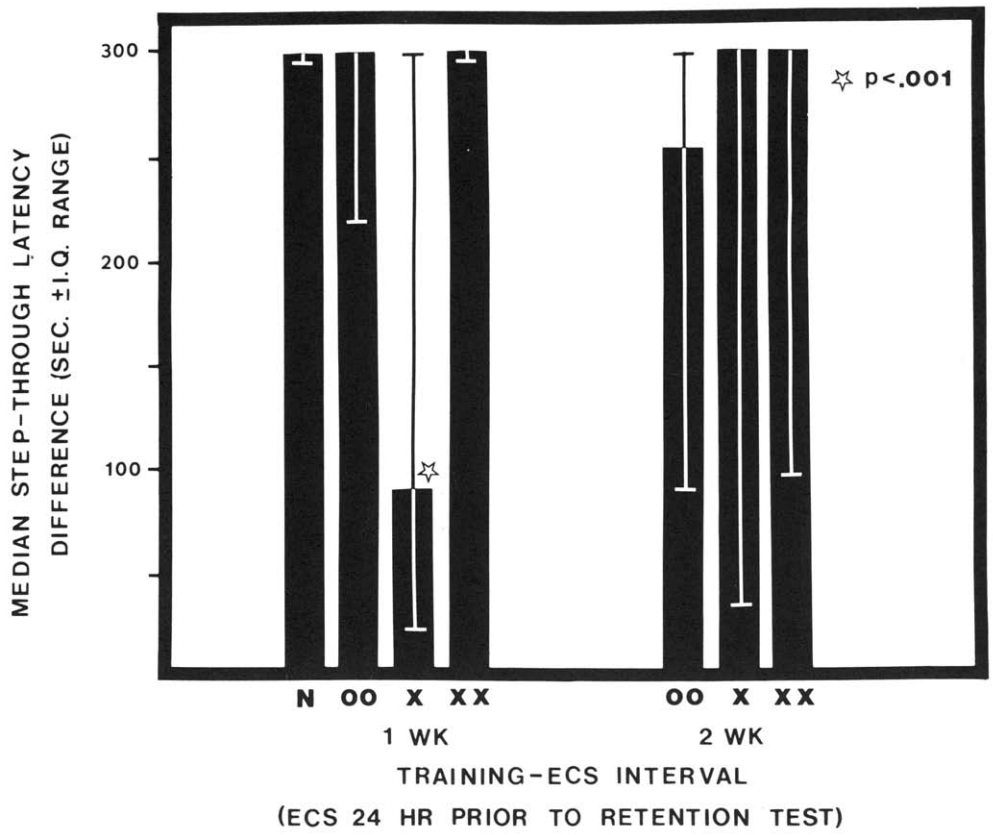

FIG. 1. Median step-through latency (STL) difference scores and interquartile ranges for mice in Experiment 1. Long latency indicates good retention. N=normal unoperated mice (N=15); LC-00=mice bilaterally implanted with neither electrode producing locus coeruleus damage; LC-X=mice receiving unilateral damage to the locus coeruleus complex; LC-XX=mice receiving bilateral damage to the locus coeruleus complex.

mm from midline, dorsal–ventral=3.6 mm below brain surface). Animals were anesthetized with Nembutal (50 mg–kg) during surgery. The electrodes were soldered to small gold-plated contacts and firmly secured to the skull with dental acrylic cement. Following surgery the mice were placed in individual housing and allowed a 7 day postoperative recovery period. Additionally, 15 mice served as unoperated controls. Except for anesthesia and surgery, those mice were treated identically to experimentals. At the end of the recovery period all mice were trained on the single-trial inhibitory avoidance step-through task [20]. Training and testing procedures are reported in detail elsewhere [35]. Briefly, mice were placed into the brightly-illuminated clear Plexiglas outer chamber of a two-chamber apparatus. All mice were placed into this outer chamber so that they faced away from a small hole which opened into a larger trough-shaped, darkened Plexiglas chamber. Upon completely stepping through this hole and into the larger compartment, mice automatically received a 300 μA footshock until they escaped to the outer chamber. Mice that did not enter within 100 sec were discarded from the experiment. Solid state programming equipment was used to automatically record the initial step-through latency and to control the administration of the footshock.

Immediately following training all mice (including unoperated controls) were lightly anesthetized with diethyl ether (anesthesia period=45–75 sec) and the animals with previously implanted electrodes received bilateral electrolytic lesions. Lesions were made by passing an anodal current (rectal cathode) of 500 μA for 10 sec through the uninsulated tip of the indwelling electrodes aimed at the LC. Experimenter bias was prevented during the postlesion ECS and retention testing periods since there was no way of knowing which mice had received accurately placed LC lesions. Following lesioning the mice were returned to their home cages and divided into two groups: one receiving ECS after a 7 day delay and the other receiving ECS 14 days after training. ECS parameters were 15 mA for 200 msec administered transcorneally. Twenty-four hr following ECS administration mice were tested for retention of the inhibitory avoidance response. Again a step-through latency score was determined. A step-through latency (STL) difference score was then calculated by subtracting the initial step-through latency from the test step-through latency. A difference score of 300 sec was arbitrarily chosen as the maximum score. Such a score represents good retention of the learned response. Following retention testing all mice were transcardially perfused with 0.9% saline followed by 10% Formalin for subsequent histological localization of lesion sites. Frozen sections (30 μm) were taken through the area of the lesion and stained with cresyl violet.

RESULTS AND DISCUSSION

On the basis of histological analysis three groups were

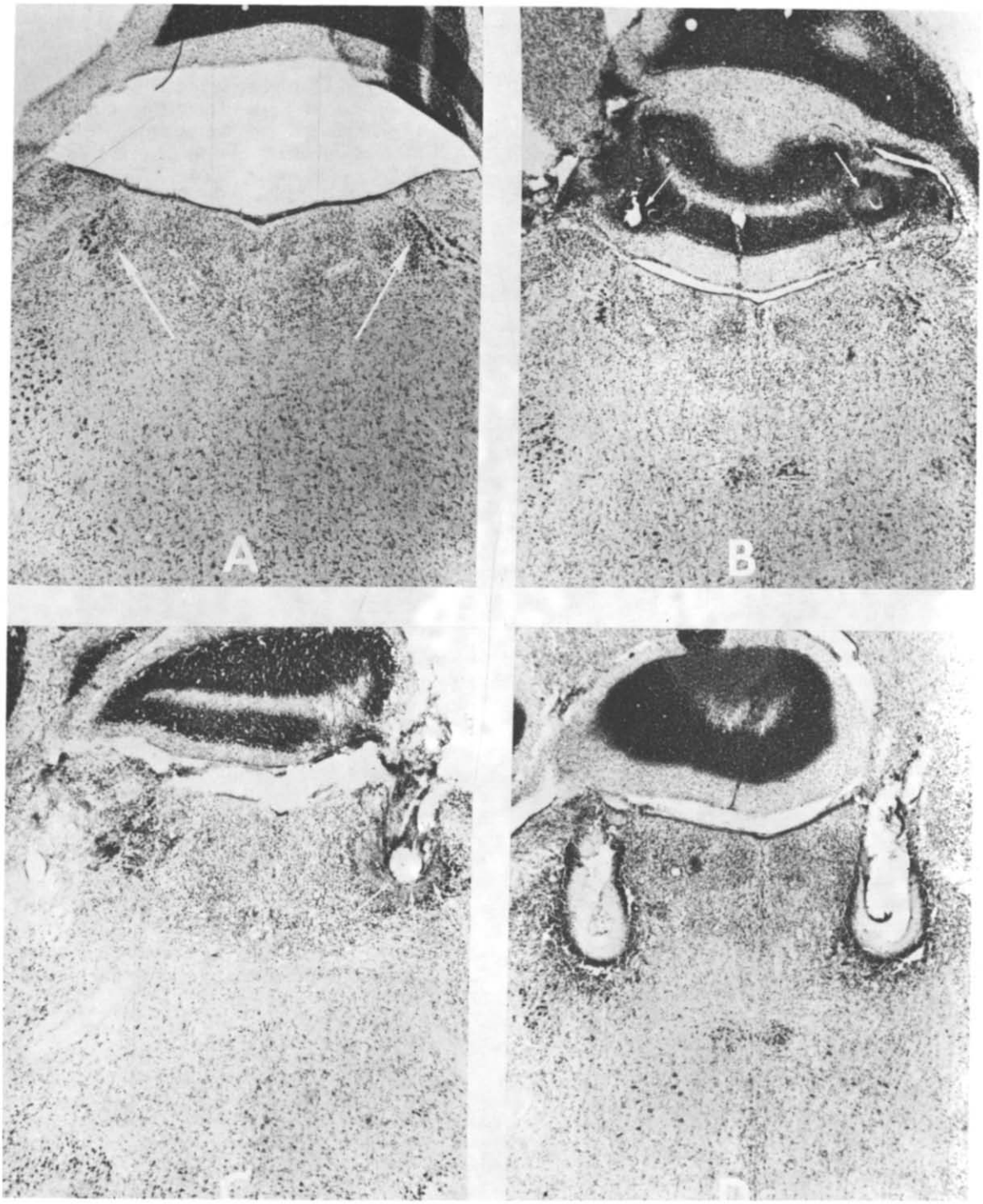

FIG. 2. Photomicrographs of mouse dorsal pontine region. A) Arrows indicate intact locus coeruleus complex (LC) in a normal mouse. B) Arrows indicate electrolytic lesion damage in the cerebellum overylying the LC. C) Arrows indicate a successful LC lesion (right side) and a lesion which missed the LC (left side). D) Bilateral destruction of the rostral portions of the LC. Cresyl violet stain, 20X.

formed for each of the two training-ECS intervals. Thus, for both the 7 day and 14 day ECS delay periods the following groups emerged: unilateral LC-lesioned mice (LC-X, N=21, for the 7 day group and N=14 for the 14 day group); bilateral LC-lesioned mice (LC-XX, N=34 for the 7 day group and N=9 for the 14 day group); and bilateral lesion control mice (LC-00, N=11 for the 7 day group and N=6 for the 14 day group).

Figure 1 shows the results of this experiment. For the 7 day training/lesion-ECS delay period a Kruskal-Wallis nonparametric analysis of variance indicated a significant lesion effect, $H=13.08$, $p<0.01$. Individual Mann-Whitney U tests (two-tailed) revealed that mice in group LC-X had significantly shorter STL difference scores than mice in either group LC-XX ($p<0.001$) or mice in the lesion control group ($p<0.02$). Groups LC-XX and LC-00 were not significantly different. ECS administered to unoperated control mice at this time point was also ineffective in producing RA.

For the 14 day training/lesion-ECS delay period the Kruskal-Wallis nonparametric analysis of variance indicated no significant treatment effect, $H=0.50$. Figure 2 shows photomicrographs of representative lesions for each group. Structures surrounding the LC which were occasionally damaged included the cerebellum, inferior colliculus, dorsal tegmental nucleus of Gudden, superior cerebellar peduncle, surrounding central gray, and dorsal portions of the pontine reticular formation. No consistent relationship between damage to any of these structures and the behavioral data was found.

The results of this experiment indicate that unilateral LC damage, sustained shortly after learning, significantly extends to at least 7 days, but not 14 days, the susceptibility period of a newly formed memory to ECS-produced retrograde amnesia. It is unclear why we were unable to establish this phenomenon in our previous study [36]. The only procedural difference was the use of slightly larger lesions (500 μA for 15 sec) in the earlier work. However, we have now obtained this 7 day lesion effect on two different occasions and are confident of its reliability.

At least two interpretations of the present results can be made. First, the performance deficit seen seven days after training and LC lesion may indicate an exaggerated extension of the memory lability period. Alternatively, this performance deficit may result from nonspecific proactive effects of the combination of the LC lesion and subsequent ECS. This second interpretation, if correct, would be less interesting from a memory consolidation point of view, but perhaps more believable given the conventional wisdom that lability of newly-formed memory to ECS-produced disruption generally lasts for only a few hours in most experimental paradigms.

EXPERIMENT 2

This experiment was designed to distinguish between the two possible explanations given above for the performance deficit found in Experiment 1. Thus, we sought to determine if posttraining LC damage followed by ECS results in a true retrograde amnesia, or in a proactive disruption of performance in the inhibitory avoidance task possibly due to interactions between LC damage and subsequent ECS.

METHOD

Forty mice were surgically prepared with LC-targeted electrodes as described in Experiment 1. Following the 7 day postoperative recovery period, mice were trained in the step-through apparatus. Immediately following training, the mice were returned to their home cages for 6 hr. At the end of this 6 hr posttraining interval all mice were lightly etherized and received electrolytic lesions as described earlier. Following lesioning, mice were returned to their home cage for 18 hr (a total of 24 hr after initial training) at which time they received ECS, as described for Experiment 1, and again returned to their home cages. Twenty hours after ECS administration, or a total of 48 hr after training, all mice were tested for inhibitory avoidance performance. We reasoned that if a proactive effect due to the LC lesions and subsequent ECS was occurring, such an effect should be greater at the shorter lesion-ECS interval (i.e. 18 hr) used in this experiment as compared to the 7 day interval used in Experiment 1, or the 24 hr interval reported previously [36]. Therefore, this experiment was designed to try to maximize finding such a proactive effect. On the other hand, if the LC lesion effect is mediated in some way through abnormal memory trace formation, then by delaying the lesion for 6 hr after training, a time when it is generally believed consolidation into long-term memory should have normally occurred, we would expect the LC lesion to have no effect upon subsequent susceptibility to ECS-produced RA.

RESULTS

Histological analysis of lesion damage resulted in the formation of three groups as described earlier. Groups LC-X (N=15), LC-XX (N=14), and LC-00 (N=11) all had median STL difference scores of 300 sec. No statistical differences were found among these groups.

DISCUSSION

The data reported here indicate that proactive nonspecific performance effects due to possible interactions between LC lesions and subsequent ECS can not account for the performance deficit described in Experiment 1 above. LC lesions, delayed six hr after memory formation, appear ineffective in altering the susceptibility of the previously-formed memory. These data are consistent with a time dependency hypothesis of a decaying susceptibility gradient. Thus, in agreement with the majority of existing literature, administration of treatments affecting the brain after memory consolidation has occurred (i.e. after the normal labile period terminates), does not retroactively alter memory processes. These data suggest further that the normal role of the LC in mediating the stabilization or consolidation of a newly formed memory is accomplished shortly (i.e. less than 6 hr) after memory formation.

EXPERIMENT 3

It is well-documented that the susceptibility of a recently formed memory to ECS-produced disruption varies with the strength of the trace [10, 29, 33]. We designed this final experiment to test the possibility that LC lesions in some manner alter memory trace strength, a fact that may have been obscured by use of a ceiling on step-through latency difference scores. We chose to index trace strength in LC-lesioned animals by examining the long-term forgetting functions for the inhibitory avoidance response. We also wished to confirm that the lesions actually damaged norepinephrine-containing neurons. Thus at the conclusion of behavioral testing, the animals were sacrificed and their brains prepared for cortical and hippocampal norepinephrine assays.

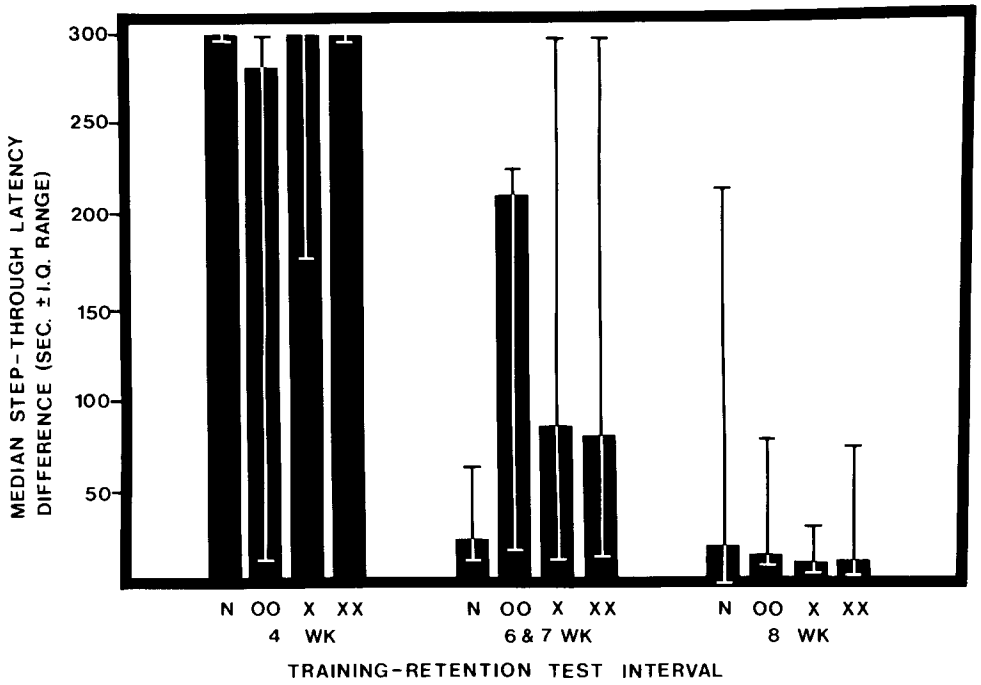

FIG. 3. Median STL difference scores and interquartile ranges for mice in Experiment 3. Control mice were bilaterally implanted with electrodes that did not produce LC damage. LC-X and LC-XX mice received unilateral and bilateral LC damage respectively. See text for details of experimental design.

METHOD

One hundred thirty-two mice were used in the experiment. Electrodes were implanted bilaterally and aimed stereotaxically at the locus coeruleus. Twenty-eight mice did not receive surgery and served as normal controls. The procedures for surgery, recovery and training in the step-through apparatus were identical to those used in Experiment 1. Immediately following training, mice were lightly etherized and lesioned with 300 μA anodal current for 10 sec. Mice were then returned to individual housing. No amnestic treatments were administered during the interval between training and testing. Retention testing occurred 4, 6, 7, or 8 weeks after training. Step-through latency difference scores again served as the behavioral measure.

In order to verify the effectiveness of the lesions in damaging the LC, hippocampal and cortical norepinephrine levels were determined. Several hours following retention testing, animals were decapitated and their brains removed rapidly over ice. The cerebral cortices and hippocampi were dissected and pooled for each hemisphere. Tissue was homogenized in 2 ml of 0.4 N $HClO_4$ and centrifuged at 11,000 rpm for 10 min. The supernants were saved and frozen at -50°C for the subsequent assay. The hindbrains were saved and subjected to the previously described histological procedures (see Experiment 1) for lesion localization.

In preparation for purification procedures, alumina was prepared by the method of Anton and Sayre [2] and mixed with 0.1 M EDTA, after which the EDTA was aspirated off. Samples were thawed and added to 125 mg of the prepared alumina. The supernatant-alumina suspension was adjusted to pH 7.9 using 3 M Trizma base. The suspension was then transferred to a Pasteur pipette which had been packed at the bottom with glass wool. Retained alumina particles were washed with 6 ml of 0.1 M sodium acetate, 1% NA_2EDTA solution and eluted with 2 ml of 0.2 M acetic acid. Two 0.9 ml aliquots were then taken for the norepinephrine assay performed using the procedures of Laverty and Taylor [22].

RESULTS

Histological analysis revealed that accurately placed lesions produced extensive damage to the locus coeruleus complex. Based on this analysis animals were divided into three main groups for each retention test time point. Thus, at 4 weeks the groups consisted of normal mice (N=6), LC-X mice (N=14), LC-XX mice (N=13), and LC-00 mice (N=4). At 6 and 7 weeks the data were combined (see below). The groups at this combined time point consisted of normal mice (N=14), LC-X mice (N=22), LC-XX mice (N=44), and LC-00 mice (N=5). Finally, at the 8 week time interval, groups consisted of normal mice (N=8), LC-X mice (N=9), LC-XX mice (N=11) and LC-00 mice (N=10).

Figure 3 depicts the behavioral results. The 6 and 7 week time points were combined for statistical purposes due to the high variability in performance at these time intervals and to the limited number of lesion control animals in each group alone. There were no statistically significant performance differences among the various groups within any given time point as determined by a Kruskal-Wallis one-way analysis of variance. Thus, the groups within each time point were combined. A Kruskal-Wallis analysis of variance across time points indicated a statistically significant change in performance, $H(2)=22.2$, $p<0.001$.

Figure 4 presents the results of the norepinephrine assay obtained from a sample of each main group above. For each time point hemispheric NE levels from the cerebral cortex and hippocampus were pooled. These data were combined across animals on the basis of histological determination of LC damage for each particular hemisphere. Thus, hemis-

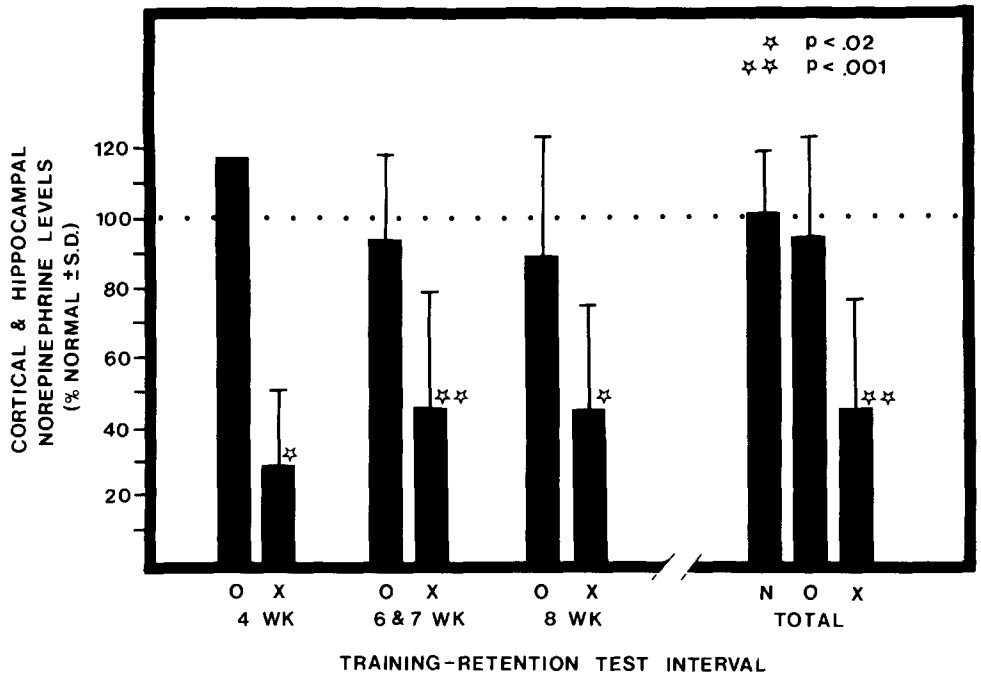

FIG. 4. Mean cortical and hippocampal levels of norepinephrine expressed as percentage of normal values. Standard deviations are shown for all groups except LC-0 at 4 weeks which only had an N=1. Group designations are as previously indicated. Times indicate interval between training/lesion and retention testing/decapitation. Total number of hemispheres analysed for normal mice=36, for LC-X mice=124, and LC-0 mice=32.

pheric NE levels ipsilateral to LC damage and hemispheric NE levels ipsilateral to control lesions were separately averaged. The assay results confirmed the histological analysis of LC damage. At each time point, lesions determined histologically to include regions of the LC produced significant forebrain NE depletion compared to controls as determined by Student's t test (4 weeks: $t(4)=4.26$, $p<0.02$; 6 and 7 weeks: $t(34)=4.804$, $p<0.001$; 8 weeks: $t(29)=3.29$, $p<0.02$. When the data for each time point were pooled (right-hand side of Fig. 4), lesion controls showed NE levels 93% of normal, a difference not statistically significant, $t(68)=0.8$. LC lesions produced NE levels approximately 45% of normal, a highly significant reduction, $t(154)=7.898$, $p<0.001$). Within the LC-X group the mean NE depletion suggests that some NE-containing neurons were spared. Such sparing is a function of the histological analysis we employed. Thus, any lesion involving the LC, regardless of extent, was classified in the LC-X group. In this way, LC-X included both extensive lesions of the LC, producing at least 90% depletion, and marginal involvement of the LC, producing little NE depletion. This wide range in LC destruction is reflected in the standard deviations. In a similar way, the standard deviations for the lesion control groups may indicate that some damage to NE axons occurred without involvement of the nucleus locus coeruleus proper. Indeed, many of the lesions outside of the LC were rostrally located in the general region of the dorsal noradrenergic bundle [24]. As a result of this within group heterogeneity, we attempted to correlate individual animal's rates of forgetting with their individual levels of NE depletion. No significant correlations could be established.

DISCUSSION

The data from this experiment demonstrate that LC damage per se, either unilateral or bilateral, has little effect on the long-term retention of an inhibitory avoidance response. All groups of animals displayed similar retention functions, i.e. good performance 4 weeks posttraining, somewhat impaired performance at the 6 and 7 week points, and essentially no retention of the task 8 weeks after training. The extreme variability at the 6 and 7 week time point may, however, obscure small differences between groups. With this caveat in mind we cautiously conclude that, inasmuch as duration of trace persistence is an indication of trace strength, LC damage does not affect the strength of the long-term memory.

The finding that accurately placed LC lesions can produce up to 90% depletion of NE in the ipsilateral cortex and hippocampus, confirms the findings of many other investigators [16, 25, 30].

GENERAL DISCUSSION

Taken together, the results of these three experiments demonstrate that unilateral damage to the LC complex results in a significant extension of the lability period associated with recently-formed memory. Thus, while control or bilaterally lesioned mice were not susceptible to ECS-produced retrograde amnesia 7 days after memory formation, unilaterally LC-lesioned mice showed a marked memory disturbance up to and including this delay period. Extended susceptibility to ECS-produced amnesia was not permanent, but disappeared sometime between one and two

weeks posttraining. Importantly, the effects of LC lesions cannot be explained by simple performance or proactive effects. LC lesions delayed by 6 hr after training did not result in altered performance when ECS was administered 24 hr after training. In a previous report [36] we found that ECS delivered 24 hr after training and immediate posttraining LC damage resulted in significant memory disruption. The results of the present delayed LC lesion experiment are viewed as crucial if we are to consider that the LC lesion effect is acting in some way upon the dynamics of the recently-formed memory trace.

The results of Experiment 3 provide evidence counter to the view that prolonged memory trace lability is due to a drastic change in trace strength. This is of particular interest and concern since our performance measure for memory is potentially biased by the use of an arbitrary 300 sec ceiling. Thus, in this experiment we reasoned that significant differences in the strength of the trace (produced by LC damage) would be reflected by its diminished persistence. In this experiment, LC lesions had no effects on the dynamics of memory decay, i.e., the rate of forgetting. These data suggest that the prolonged susceptibility gradient of recently-formed memory to ECS-produced RA following LC damage is not due to the formation of an "inferior" or weakened trace. Rather, we suggest that this extended susceptibility is due to a fundamental change in the course of events normally associated with the conversion of labile to stable memory.

Other investigators have also been able to extend the labile memory period by administering either protein synthesis inhibitors [14], dopamine-β-hydroxylase inhibitors [12], or REM sleep deprivation [11]. It is significant that such treatments cause central noradrenergic abnormalities. The present experiments represent a more direct manipulation of noradrenergic activity and as such our findings are consonant with the following hypothesis: The noradrenergic LC complex normally functions to promote consolidation of recently formed memory. Damage to the LC complex results in ineffective consolidation but not in loss of labile memory. Further, this labile memory can persist over relatively long periods of time and can be utilized (i.e. retrieved) even though stable memory may not yet have been formed. We do know that stable memory eventually is formed. However, it is not yet clear when consolidation occurs relative to the existence of the labile trace.

The hypothesis stated above is consistent with, and an extension of, the suggestion by Crow [7] and Kety [17] that catecholaminergic modulation of widespread forebrain regions [24], produced by learning-associated stress and nonspecific phasic arousal, may represent an essential component of the specific information to be consolidated. Recently, Gold and McGaugh [15] also suggested that such nonspecific arousal may play a key role in memory processes.

An interesting finding in this study is that unilateral, but not bilateral lesions of the LC appear to be effective in extending the susceptibility of newly formed memory. This finding is reminiscent of earlier data [32] indicating that contralateral superior colliculus lesions in cats reduced a visual deficit resulting from a prior contralateral neocortical lesion. These results were interpreted with the suggestion that balance and interhemispheric coordination of visual information processing was more important than the absolute loss of tissue involved in the visual information processing. Balance, in the case of LC function, might be related to a balance in hemispheric norepinephrine levels. The extent to which equivalent NE function between the hemispheres is important in memory processing is not understood. One intriguing hypothesis, however, is that the conversion of labile to stable memory may normally require a coordinated harmonious processing of information between the two cerebral hemispheres (no specific intrahemispheric location hypothesized).

Recently, Buda *et al.* [4] reported that in addition to a depletion of norepinephrine in ipsilateral forebrain following a LC lesion, the undamaged contralateral LC showed a large increase in tyrosine hydroxylase activity. These data suggest that the effects of a unilateral LC lesion on forebrain catecholamine balance may be two-fold: first, there is an ipsilateral decrease in forebrain catecholamine levels and second, there may be an increase in contralateral catecholamine activity in the region of the contralateral LC thus exacerbating the putative neurotransmitter imbalance. The fact that the duration of the increased turnover of contralateral tyrosine hydroxylase from Buda's *et al.* study matched the duration of the labile trace in the above experiments (i.e., approximately 7 days) may be of importance and should be pursued further.

One of the few widely accepted operational definitions in contemporary experimental memory research is the equation of short-term memory with labile memory and the similar equation of long-term memory with stable memory. The temporal transition from labile to stable memory does not appear to be an absolute all-or-none phenomenon, but rather a graded time-dependent process [19]. In actuality, the temporal definition of STM in animals derives primarily from evaluating the time course of susceptibility of the memory to experimental modification. The period of normal susceptibility to modification usually ends by 3–6 hr [28].

The finding that labile memory can persist over long periods of time creates problems for the conventional wisdom concerning short- and long-term memory and raises some interesting questions. To what extent does the generally accepted 3–6 hr time period of memory susceptibility represent a true biological marker indicative of fundamental changes in the underlying neurobiological events mediating the storage of information? To what extent can we be certain that persistent memories identifiable in time well beyond this 3–6 hr period are really stored in (i.e., retrieved from) LTM (stable memory) as opposed to being stored in (i.e., retrieved from) STM (labile memory)?

The data described above suggest that the conversion from labile to stable memory appears critically dependent upon the normal functioning of the locus coeruleus complex and its noradrenergic projections. Further, the data indicate that the role of the LC in this conversion process is time-dependent. A new concept emerging from the present experiments is that labile memory may both persist and be retrieved after extended time periods. Our data raise serious questions concerning the heuristic value of such terms as short- and long-term memory.

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