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Mamillothalamic Tract Transection Blocks Anterior Thalamic Training-induced Neuronal Plasticity and Impairs Discriminative Avoidance Behavior in Rabbits

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Rabbits with bilateral transecting lesions of the mamillothalamic tract, control (tract-sparing and sham) lesions, or no lesions, and chronic, fixed-position anterior ventral (AV) and medial dorsal (MD) thalamic and posterodorsal subicular complex unit recording electrodes were trained to step in an activity wheel in response to a 0.5 sec tone (CS+) in order to avoid a brief foot shock. The rabbits also learned to ignore a different tone (CS−) not predictive of shock. Behavioral acquisition was significantly retarded in rabbits with mamillothalamic tract transection compared to controls. When trained, transected rabbits failed to avoid the shock more often than controls. Mamillothalamic tract transection abolished and control lesions attenuated AV thalamic discriminative training-induced activity (i.e., development with training of greater discharges in response to the CS+ than to the CS−). Transection and control lesions attenuated AV thalamic excitatory training-induced activity (greater elicited activity during training than during unpaired tone-shock presentations before training) as well as AV thalamic "spontaneous" baseline unit activity. CS-elicited discharge magnitude was reduced by control lesions and it was further reduced by tract transecting lesions. Significant lesion-related changes were not found in the subicular or MD thalamic neuronal records. Mamillothalamic tract afferent information flow is thus essential for AV thalamic discriminative training-induced activity, excitatory training-induced activity, tone-elicited discharges and maintenance of conditioned avoidance responses. The effects of the control lesions suggested that afferents which course in parallel with and near the mamillothalamic tract may contribute to AV thalamic spontaneous activity and excitatory training-induced activity.

[Key words: training-induced neuronal activity, dynamic neuronal plasticity, instrumental conditioning, avoidance learning, limbic system, memory, functional brain circuitry,* in vivo *unit recording, significance coding, activity-dependent facilitation, muscarinic M_2 receptors]

Analyses of the neural mediation of learning and memory are advancing in relation to neurobiology of activity-related synaptic strength adjustments (Madison et al., 1991; Massicotte and Baudry, 1991; Bramham, 1992; Johnston et al., 1992; Colley and Routtenberg, 1993), analyses of circuitry and fundamental mechanisms of plasticity underlying invertebrate learning (e.g., Hawkins et al., 1993) and the elucidation of the circuitry and neuronal information processing in a variety of mammalian learning paradigms (Gabriel and Moore, 1990).

The present study continues an ongoing analysis in the third category, of discriminative instrumental avoidance learning, wherein rabbits learn to step (in a large activity wheel) in response to a warning tone (CS+) in order to avoid a foot-shock, and they learn to ignore a different tone (CS−) which does not predict shock. Lesion studies have implicated circuitry of the limbic thalamus [the anterior and medial dorsal (MD) nuclei], the cingulate cortical projection fields of these nuclei, and the hippocampal formation in this learning (Gabriel et al., 1989, 1991), and massive changes of unit activity in response to the CS+ and CS− develop in these areas during avoidance training. This training-induced activity is both excitatory (greater during training with paired CS-US presentations than before training with unpaired tone and US presentations) and discriminative (greater in response to the CS+ than to the CS− in trained rabbits).

Additional work has indicated that thalamic training-induced activity does not require input from cortex, whereas cingulate cortical training-induced activity does require thalamic input. Experimental damage to subicular and cingulate cortical neurons that project to limbic thalamus did not attenuate but rather enhanced the limbic thalamic training-induced activity (Gabriel et al., 1986, 1987, 1991). Lesions of the limbic thalamic nuclei abolished training-induced activity and virtually all tone-elicited activity in cingulate cortex (Gabriel et al., 1989). Therefore, CS-elicited neuronal activation relevant to the performance of the learned behavior flows from thalamus to cortex, not from cortex to thalamus. These results set the stage for a search for the subcortical afferents which are critical for the development of the thalamic training-induced activity.

Microinfusion of 6-hydroxydopamine depleted anterior thalamic nor-epinephrine (NE) to within 4% of control values but did not reduce the thalamic training-induced activity. Indeed, training-induced activity was enhanced by this manipulation (Sparenborg and Gabriel, 1992). Thus, projections of NE-containing brainstem neurons to the anterior thalamus do not appear to be essential for the thalamic training-induced activity (although the depletion of NE did interfere with anterior thalamic neuronal processing of unexpected stimuli).

The ACh-containing fibers which project to the anterior nuclei from the lateral dorsal tegmental nucleus (e.g., Satoh and Fibiger, 1986) may contribute specifically to anterior ventral (AV)

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thalamic excitatory training-induced activity, as the elicited firing of AV thalamic neurons in trained rabbits was greatly attenuated following systemic administration of the muscarinic receptor antagonist scopolamine hydrobromide. Intriguingly, AV thalamic discriminative training-induced activity was not affected by the scopolamine injections (Henzi et al., 1990).

The present study addressed the relevance of the massive mamillothalamic afferent projection system for learning and AV and MD thalamic training-induced activity. Multiunit activity was recorded in these nuclei during avoidance training in controls and in rabbits given electrolytic lesions which transected the mamillothalamic tract. The lesions were made prior to training, during surgery for implantation of recording electrodes.

Materials and Methods

Subjects, electrodes, surgery, and recording procedures. The subjects were 49 male New Zealand White rabbits weighing 1.5–2.0 kg on delivery to the laboratory and maintained on ad libitum water and a regimen of rabbit chow (1 cup daily) restricted to prevent obesity. After a minimum period of 48 hr for adaptation to living cages, each rabbit underwent surgery for implantation of unit recording electrodes. Surgical anesthesia was induced by either injection of chlorpromazine (12.5 mg) in solution followed by sodium pentobarbital (25.0 mg) in solution through the marginal vein of the pinna or subcutaneous injection (1 ml/kg of body weight) of a solution containing 60 mg/ml of ketamine HCl and 8 mg/ml of xylazine followed by hourly injections of 1 ml of the solution. During surgery, bilateral electrolytic lesions of the mamillothalamic tract were attempted in 23 rabbits. The lesions were made by passing a 1.0–1.5 mA cathodal D.C. current for 15 sec through lesioning electrodes made from stainless-steel insect pins coated with an insulating material (Epoxylite). The insulator was removed from the tips to form 0.7–1.0 mm surfaces for passage of current.

The fibers of mamillary neurons give rise to two major tracts, the mamillothalamic and the mamillotegmental tracts. The fibers which form the mamillotegmental tract project rostralward in the caudoventral hypothalamus, before they exit from the main tract and project caudally (Shen, 1983). The stereotaxic coordinates used for the mamillothalamic tract lesions (AP = +2.5, L = ±1.5, V = 10.3; Girgis and Shih-Chang, 1981) defined a site rostral to the site at which the mamillotegmental tract separates from the mamillothalamic tract. In four of the rabbits, the lesions were made at the same AP and L coordinates, but two vertical coordinates were used (10.0 and 11.0, 10.5 and 11.5, or 10.3 and 10.8).

Four or six stationary multiunit recording electrodes were implanted in each rabbit through burr holes (diameter = 0.5 mm) drilled through the skull over the target sites. Various target-site distributions in each rabbit were employed to attain an equal number of recordings for the experiment as a whole, in the following sites: the AV thalamic nucleus (AP = +2.0, L = ±2.3 and V = 7.5), the MD thalamic nucleus (AP = +4.6, L = ±1.5, V = 8.25), the dorsoposterior subicular complex (AP = +8.5, L = ±6.75, V = 4.5), the medial mamillary nuclei (AP = 4.5 or 5.5; L = 0.5, V = 14.0 or 14.5), and areas CA1, CA3, and the dentate gyrus of Ammon's horn. The recording electrodes were fabricated as were the electrodes for making lesions, but with smaller exposed tips (10–50 μm; 1 kHz impedance: 500 kΩ to 2 MΩ). Miniature Teflon cylinders (length = 2.5 mm; diameter = 1.5 mm) impaled on stainless steel insect pins were positioned over each burr hole and affixed to the skull using dental acrylic. The pins were removed from the cylinders after hardening of the acrylic. Recording electrodes were then slowly advanced to the recording sites by press fitting them through the holes in the Teflon cylinders. Unit activity was monitored during electrode advancement to enhance placement accuracy.

Behavioral training. Following a minimum period of one week for recovery from surgery, the rabbits received training in an activity wheel designed for the administration of aversive conditioning (Brogden and Culler, 1936). The wheel was contained in a shielding chamber in a room adjacent to that housing the equipment used for data collection. A video camera and monitor permitted viewing of the rabbit during conditioning. An exhaust fan and a speaker in the chamber produced a masking noise of 70 dB re 20 N/m^2, throughout training. The conditional stimuli were pure tones (1 or 8 kHz, free field, 85 dB re 20 N/m^2, rise time = 3 msec) played through a speaker attached to the chamber ceiling directly above the wheel. Onset of the positive conditional stimulus (CS+) was followed after 5 sec by the shock unconditional stimulus (a constant current 1.5–2.5 mA foot shock delivered through the grid floor of the wheel). The shock was terminated by behavioral responses, defined as wheel rotations exceeding 2°. Responses after CS+ onset but prior to shock onset prevented shock administration. The duration of the CS+ was 0.5 sec. The maximum duration of the shock was 1 sec. The CS− was a 0.5 sec 1 kHz or 8 kHz tone, the tone not chosen as CS+. The CS− was never followed by the shock. The interval from the end of a trial (defined as the end of the 5 sec period following CS onset, or of wheel rotation when locomotion occurred) to CS onset for the next trial was 8, 13, 18, or 23 sec. These values occurred in an irregular sequence. Responses during the intertrial interval reset the interval.

The rabbits received daily 60 trials with the CS+ and 60 trials with the CS−, in an irregular sequence, until a criterion of performance was reached. The criterion required that the percentage of trials with behavioral responses to the CS+ (i.e., avoidance responses) exceed the percentage of trials with responses to the CS− by 60 or more, in two consecutive sessions. For example, a rabbit would meet this criterion by performing conditioned responses on 80% of the CS+ trials and on 20% of the CS− trials, or by performing conditioned responses on 90% of the CS+ trials and on 30% of the CS− trials. This criterion yields an asymptote of performance, that is, performance levels achieved at criterion do not change significantly during postcriterial training. Training was terminated if criterion was not attained after 15 sessions.

Prior to training, each rabbit received two preliminary training sessions, in which CS+ and CS− were presented, each 60 times. The intervals between the tones were timed as during training. The tones only were presented in the first of these sessions and the tones with explicitly unpaired shock presentations were given in the second session (Rescorla, 1967), that is, shock was not presented during the tone, within 3 sec prior to the tone, or within 7 sec after the tone. The incidence and distribution over trials of the shock during this session were identical to the average values of these parameters obtained during the initial session of training for a sample of 100 rabbits. Because the CS did not predict the shock in this session, avoidance learning did not occur. This session thus yielded baseline data for detecting associative neural and behavioral changes brought about by pairing the CS+ with the shock during training.

Collection of neuronal data. The neuronal records were fed into field-effect transistors (FETs) attached to the connector which mated with the cranial socket, about 2.5 cm from the brain recording sites. The FET outputs were led from the rabbit through the axle of the rotating wheel and the wall of the conditioning chamber via a shielded cable. Each recording channel was split, one limb entering single-ended preamplifiers with bandwidth appropriate for unit recording (1/2 amplitude cutoffs at 500 and 8000 Hz) the other limb entering preamplifiers for electroencephalographic recording of field potentials (1/2 amplitude cutoff at 0.2 and 60 Hz). The unit activity was subjected to a second stage of active bandpass filtering (1/2 amplitude cutoff at 600 and 8000 Hz, rolloff = 18 dB/octave) to remove all slow field potential frequencies. The filter outputs entered Schmitt triggers, which produced an 80 μsec square wave output pulse when the input voltage exceeded a preset threshold. The thresholds were adjusted under computer control to yield a mean output pulse rate within limits of 95–165 per second. With this criterion, typically, the largest three or four spikes were sampled. In addition, the bandpass filter outputs were half-wave rectified and integrated (see Buchwald et al., 1973). The time constants for the rise and fall of the integrators were 15 and 75 msec, respectively. The Schmitt-trigger data indicated the discharge frequency of the largest spikes on each record. The integrated unit activity measured energy fluctuations of the entire record, including activity below the triggering thresholds.

The Schmitt trigger events were counted and the integrator outputs and field potentials digitized in 100 10 msec intervals, 0.3 sec before and 0.7 sec after CS-onset. A digital value was stored for each measure and electrode, every 10 msec during the sampling interval. Only the spike frequency and integrated unit activity data are presented in this report.

Due to the smoothing effect of the time constants, the integrated unit activity yields somewhat less accurate CS-elicited neuronal discharge profiles than are yielded by the spike frequency data. However, the integrated unit activity measure is more sensitive than the spike frequency measure and on occasion reveals significant relationships not seen or seen only as nonsignificant trends in the spike frequency data.

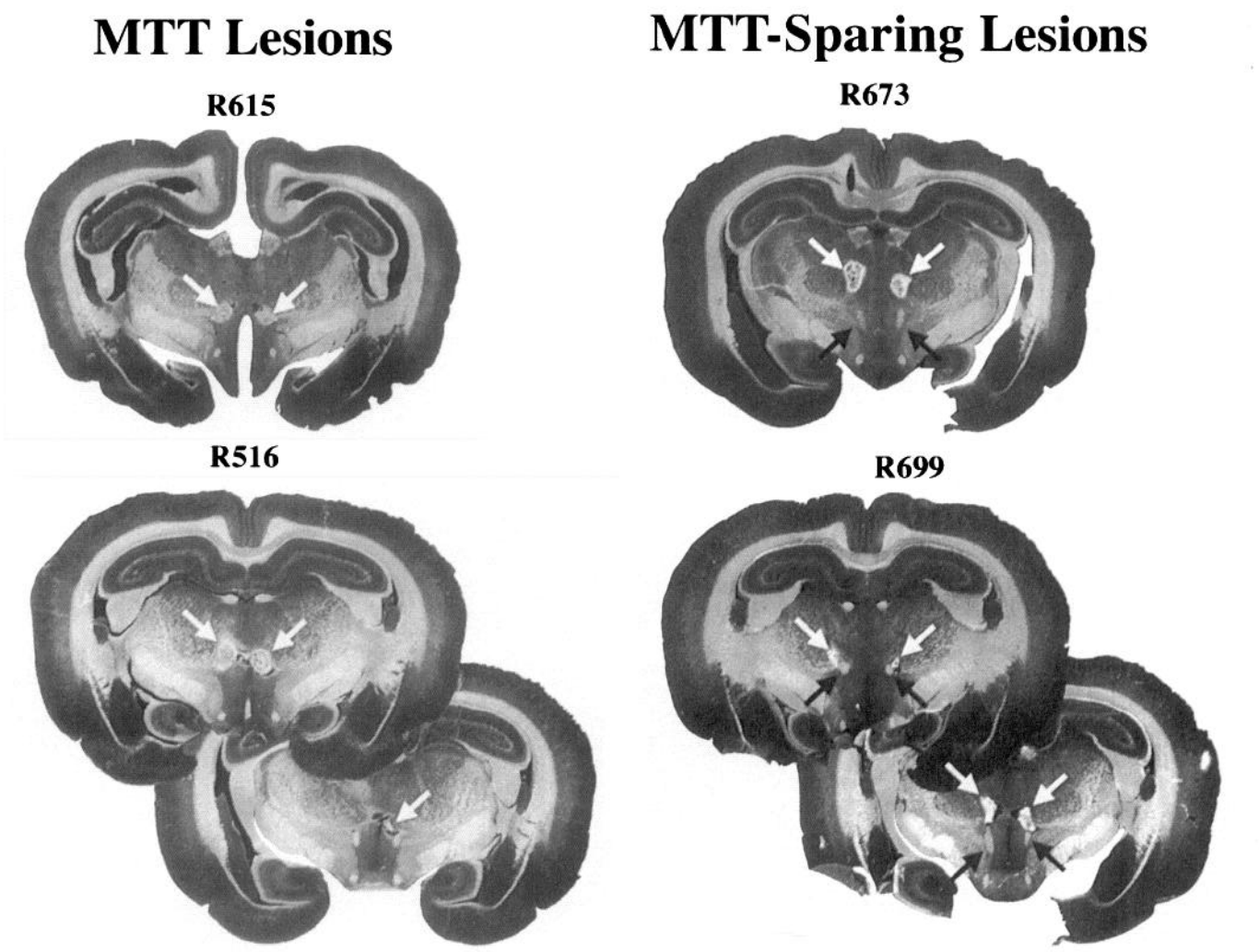

Figure 1. Coronal sections with typical bilateral electrolytic lesions in the mamillothalamic tract (MMT, *left*) and lesions which spared the mamillothalamic tract (*right*). Lesions are indicated by *white arrows*, and spared mamillothalamic tract by *black arrows*. Sections from a rabbit with small lesions (*top*) and one with large lesions (*bottom*) are shown for each lesion type.

Here, the spike frequency data are presented for all analyses in which the same significant relationships were revealed by both measures. The statistically significant effects obtained in analyses of the integrated unit activity are given for instances in which the effects were not significant but present as nonsignificant trends in the analysis of the spike frequency data.

Several computer- and experimenter-controlled methods were used for exclusion of data samples containing movement artifacts (see Gabriel et al., 1983).

Data analysis. Varied numbers of training sessions were required for criterion attainment. The analysis of the neuronal data focused on behaviorally defined stages of training common to all rabbits. Each stage was represented by the data of a single training session. The stages were (1) preliminary training with explicitly unpaired presentations of the CSs and foot shock, (2) the first session of avoidance training, (3) the session in which the first significant behavioral discrimination was performed, (4) the session in which the acquisition criterion was attained. The session of the first significant behavioral discrimination was the avoidance training session in which the percentage of locomotions performed in response to the CS+ first exceeded by 25% or more the percentage of locomotions performed in response to the CS−.

In order to obtain a measure of the unit activity elicited by the CS+ and CS−, the difference between the mean sampled activity values in the 30 pre-CS baseline 10 msec intervals and the values in each of the first 40 intervals after CS onset was calculated. This procedure differs from our standard z-score calculation, which involves an additional step of dividing the differences by the standard deviation of the baseline values. However, use of the standard deviation was precluded for the present data as the standard deviations were found to be systematically affected by the lesions, as reported below.

The neuronal and behavioral data were submitted to factorial, repeated measure analysis of variance (ANOVA) using the P2V program (BMDP Statistical Software, Inc.). The alpha level for all testing was set at 0.05. The analyses had factors of groups [three levels: mamillothalamic tract transected, control (sham and tract-sparing) lesions, and no lesion as described below], training stages (four levels as described above), stimulus (2 levels: CS+/CS−), and, for the neuronal data, post-CS interval (40 levels). Factors yielding significant overall F ratios in the analysis of variance were further analyzed using simple effect tests following procedures outlined by Winer (1962, Chap 7). Correction of the F test due to violations of the sphericity assumption of these analyses was performed as needed following the procedure of Huynh and Feldt (1976). The uncorrected F ratios reported in the text represent only those effects which were significant after correction.

Histology, lesion assessment, and experimental groups. After the completion of training, a lethal dose of pentobarbital sodium was followed by transcardiac perfusion with normal saline and 10% formalin. The brains were removed, frozen and sectioned at 40 μm, and the sections were photographed while still wet (Fox and Eichman, 1959). The dried sections were treated with a metachromatic nissl and myelin stain using formol-thionin (Donovick, 1974).

Nine of the rabbits in which lesions were attempted had complete

Table 1. The number and location of neuronal records in the three experimental groups

Recording site	Lesion	Control lesion	No lesion
AV Nucleus	14	12	18
MD nucleus	5	5	5
Posterodorsal subiculum	4	3	—
Presubiculum	3	5	—

bilateral transection of the mamillothalamic tract (Fig. 1), four had partial bilateral mamillothalamic tract damage and three had unilateral mamillothalamic tract transection. These rabbits were included in a "lesion group" ($N = 16$) for assessment of the behavioral effects of the lesions. Because partial and unilateral mamillothalamic tract lesions which affected behavior may have failed to alter particular recordings of thalamic neuronal activity, only the neuronal records of rabbits with complete bilateral transection of the mamillothalamic tract ($N = 9$) were used for analyses of the neuronal data.

The lesions missed the mamillothalamic tract, but did damage areas surrounding the mamillothalamic tract in seven rabbits. The data of these rabbits were pooled with data of five rabbits given sham lesions (electrode insertion without current passage) to form a "control lesion" group ($N = 12$). Preliminary analyses comparing the behavioral and neuronal data of the rabbits with sham and tract-sparing lesions did not yield significant differences, or any trends suggesting that differences would be found with larger samples. In order to assess the effects of the control lesions, neuronal records of rabbits that served as controls in other studies, with identical parameters of behavioral training and AV thalamic recording electrodes, were assigned to a group with no lesions ($N = 16$). The data of the no-lesion rabbits were obtained concurrently, over a period of several years, with the data of the rabbits given lesions. A separate no-lesion group ($N = 5$) with MD thalamic recording electrodes was used in the analyses of the MD thalamic data. The photographs of the wet sections containing electrode tracks indicated 44, 15, 7, and 8 recording probes in the AV nucleus, MD nucleus, posterodorsal subiculum and presubiculum, respectively (see Table 1). No significant effects of the lesions were found on MD thalamic or subicular neuronal activity, and thus the records of these areas are not considered further in this report. Insufficient data were obtained from the mamillary and Ammon's horn recording sites. This report thus focuses exclusively on the effects of the lesions on the neuronal activity of the AV thalamic nucleus.

Results

Mamillothalamic lesions and avoidance behavior

Discriminative avoidance learning was significantly retarded in rabbits with mamillothalamic tract lesions. These rabbits required an average of 8.44 training sessions to meet the criterion, whereas the rabbits with control lesions and no lesions met the criterion in 6.17 and 4.94 sessions, respectively. The analysis yielded a significant main effect [$F(2,41) = 3.26$, $P < 0.048$] of the grouping factor. Individual comparisons indicated that the mean number of sessions taken by rabbits with lesions to reach the criterion exceeded significantly the mean for the rabbits with no lesions ($P < 0.05$). The rabbits with control lesions did not differ significantly from either the rabbits with no lesions or the rabbits with lesions.

The incidence of successful avoidance responses during criterion attainment in rabbits with lesions was significantly lower than that exhibited by rabbits with control lesions and no lesions (Fig. 2).

An analysis was computed on the percentage of trials (CS presentations) in which locomotory responses were performed, in each of four stages of behavioral acquisition (pretraining, the first conditioning session, the session of the first significant discrimination and the session in which criterion was attained).

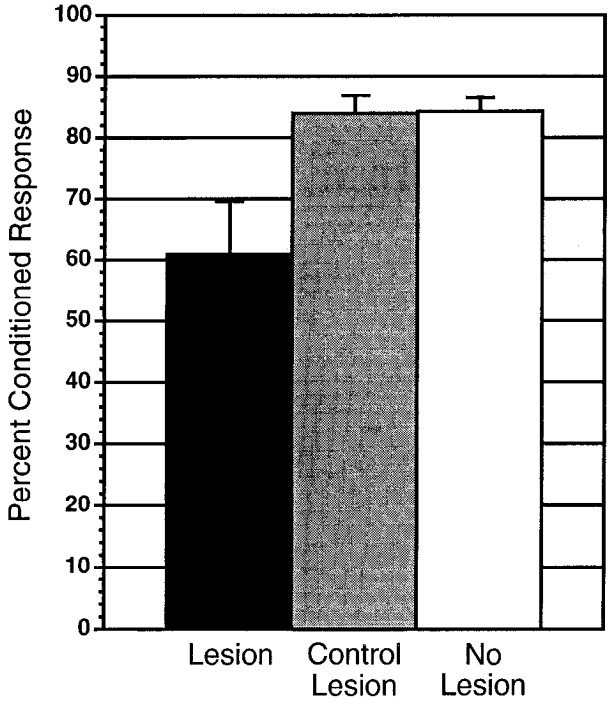

Figure 2. The average percentage of avoidance responses performed by rabbits with mamillothalamic tract lesions, rabbits with control lesions and no-lesion controls in response to the CS+ presentations during the session in which the acquisition criterion (described in Materials and Methods) was attained.

Simple effect tests following a significant interaction of the factors of lesion, acquisition stage and stimulus [CS+/CS−; $F(6,123) = 2.98$, $P < 0.01$] showed that the performance levels of the three groups did not differ during preliminary training, the first conditioning session or during the session in which the first significant behavioral discrimination occurred. However, the rabbits with control and no lesions exhibited significantly greater average percentages (83.9% and 84.2%, respectively) of trials with avoidance responses than did the rabbits with lesions (60.9%) during the session in which the criterion was attained ($P < 0.01$). No significant differences were found between the groups in the incidence of responses to the CS−.

In summary, the mamillothalamic tract lesions retarded but did not prevent behavioral acquisition. In addition, the lesions reduced the incidence of successful avoidance responses by trained rabbits.

Additional analyses did not indicate significant effects of the lesions on the latencies or durations of conditioned and unconditioned (shock-elicited) responses or on the numbers of intertrial responses performed during acquisition.

The aforementioned effects of the lesions were very similar when the analysis was restricted to the reduced subset of rabbits ($N = 9$) with bilaterally transected mamillothalamic tracts. Upon repeating these analyses using for the lesion condition the behavioral records of only the bilaterally transected rabbits, a significant loss of performance efficiency in trained rabbits ($P < 0.01$) was again found. In addition, there was a trend indicating retardation of acquisition in this group: the number of sessions needed to attain criterion by the transected rabbits was 8.00 compared with 6.17 and 4.94 in the control lesion and no-lesion groups. Nevertheless, these values did not yield a significant F

due to the slight drop of the mean from 8.44 to 8.00 in the lesion condition, and the reduction of the sample size.

Mamillothalamic tract lesions and neuronal activity

Baseline activity. Analyses were performed on the integrated unit activity recorded during the 30 10 msec intervals comprising the baseline period prior to each CS presentation. The mean AV thalamic baseline integrated activity was 32.1 (3.85 μV), 41.6 (5.08 μV), and 66.5 (8.12 μV) for the lesion, control-lesion and no-lesion groups, respectively, yielding a significant main effect in the analysis of variance [$F(2,41) = 12.01$, $P < 0.0002$]. Individual comparisons demonstrated that the baseline means yielded by transected rabbits and by rabbits with control lesions were significantly reduced relative to the means of the rabbits with no lesions ($P < 0.01$). The transected rabbits did not differ significantly from those with control lesions.

The lesions also reduced the variability of the AV thalamic baseline activity, as indicated by an analysis of the SD of the activity in the 30 10 msec pre-CS intervals [$F(2,46) = 11.22$, $P < 0.0002$]. The average values of the SDs in rabbits with lesions, control lesions and no lesions were 0.30, 0.33, and 0.71, respectively. Again, the means of the transected rabbits and rabbits with control lesions did not differ significantly, but were significantly reduced relative to the means of rabbits with no lesions ($P < 0.01$). These results indicated that mamillothalamic tract afferents and/or nonmamillothalamic tract afferents in the vicinity of the mamillothalamic tract, which were disrupted by the mamillothalamic tract and control lesions, are essential for maintenance of the level of ongoing baseline activity of AV thalamic neuronal ensembles. Also, as indicated in Materials and Methods, the lesion-related alterations of the baseline variation called for the use of simple subtraction, rather than the standard z-score calculation, to normalize neuronal activity which followed CS onset.

Sensory discharges elicited by the conditional stimuli. As in past studies (Gabriel, 1993) the CS+ and CS− elicited *triphasic average neuronal discharges* in the AV thalamic nucleus, consisting of (1) excitation, reaching maximum 20–30 msec after CS onset, (2) an inhibitory pause from 40 to 70 msec, and (3) excitation from 90 msec until the end of the 400 msec sampling epoch. The triphasic profile is shown in the second and third row of panels in Figure 3.

The triphasic AV thalamic histogram profiles were attenuated in rabbits with transected mamillothalamic tracts and control lesions, relative to the profiles of rabbits with no lesions (compare the panels in the first row to those in the second and third rows of Fig. 3). In addition, the histogram profiles of rabbits with lesions were significantly attenuated relative to the profiles of rabbits with control lesions (compare the panels in the first row to those of the second row in Fig. 3). Moreover, the inhibitory pause after CS onset present in the rabbits with control and no lesions was abolished in transected rabbits (compare the panels in the first row to those in the second and third rows of Fig. 3). These changes were indicated by significant interactions of the Lesion and post-CS Interval factors [$F(78,1521) = 3.29$, $P < 0.0001$, spike frequency; $F(78,1521) = 9.06$, $P < 0.0001$, integrated activity]. These interactions did not include the factors of training stage or stimulus, indicating that the lesion-related changes were not specific to particular training stages or eliciting stimuli.

Simple effect tests carried out for the spike frequency data demonstrated that the average AV thalamic neuronal discharges of transected rabbits were significantly reduced, compared to the discharges of rabbits with no lesions, in virtually all 10 msec intervals from 20 to 400 msec after CS onset. The only exception occurred for intervals 5 through 11, from 40 to 110 msec after CS onset, wherein the average discharges of transected rabbits exceeded significantly those of the rabbits with no lesions, this due to the abolition of the inhibitory pause in the histogram profiles of transected rabbits. Also, the average discharges of transected rabbits were significantly reduced, relative to those of rabbits with control lesions. For this comparison, the differences were confined to intervals from 220 to 400 msec after CS onset. However, again, the average activity from 30 to 90 msec after CS onset present in transected rabbits exceeded the activity in rabbits with control lesions during these intervals, due to the absence of the inhibitory pause in the transected rabbits. Finally, the activity in the rabbits with control lesions was significantly reduced relative to the activity in the rabbits with no lesions at all intervals from 160 to 400 msec after CS onset. There were, however, two intervals (from 20 to 40 msec after CS onset, representing the initial brief-latency excitatory component of the triphasic discharge profile) at which the activity of rabbits with control lesions exceeded significantly the activity of rabbits with no lesions. The probability levels for these comparisons varied from the criterion level of $P < 0.05$ to $P < 0.0001$.

Training-induced neuronal activity

Excitatory activity. Excitatory training-induced activity refers to enhancement of the neuronal activity elicited by the conditional stimuli during training relative to the elicited activity during preliminary training with unpaired CS and shock presentations. An effect of the lesions on AV thalamic excitatory training-induced activity was indicated by significant interactions of the factors of lesion, stimulus and training stage [$F(6,117) = 2.68$, $P < 0.02$, spike frequency; $F(6,117) = 3.00$, $P < 0.0093$, integrated activity].

Individual comparisons carried out for the spike frequency data indicated that the expected AV thalamic excitatory training-induced activity did occur in rabbits with no lesions, in the form of a significantly greater average discharge in response to the CS+ during the criterial session of acquisition (Fig. 3, third row, second panel, solid bars) compared to the average discharge during the preliminary training session (Fig. 3, third row, first panel, solid bars; $P < 0.01$). However, excitatory training-induced activity did not occur in any training session in transected rabbits or in rabbits with control lesions. Indeed, the average excitatory discharges elicited by the CS+ and by the CS− in the transected rabbits were significantly attenuated during the criterial stage of acquisition (Fig. 3, top row, second panel), relative to the preliminary training session ($P < 0.05$; Fig. 3, top row, first panel).

Similarly, the average discharges elicited by the CS− in rabbits with control lesions were significantly attenuated ($P < 0.05$) in the criterial session (Fig. 3, second row, second panel, open bars), relative to preliminary training (Fig. 3, second row, first panel, open bars). The discharges elicited by the CS+ of rabbits with control lesions did not change during training (Fig. 3, second row, solid bars).

Discriminative activity. Discriminative training-induced activity refers to the development of significantly different neuronal discharge profiles in response to the CS+ than to the CS− during training. None of the groups exhibited significant

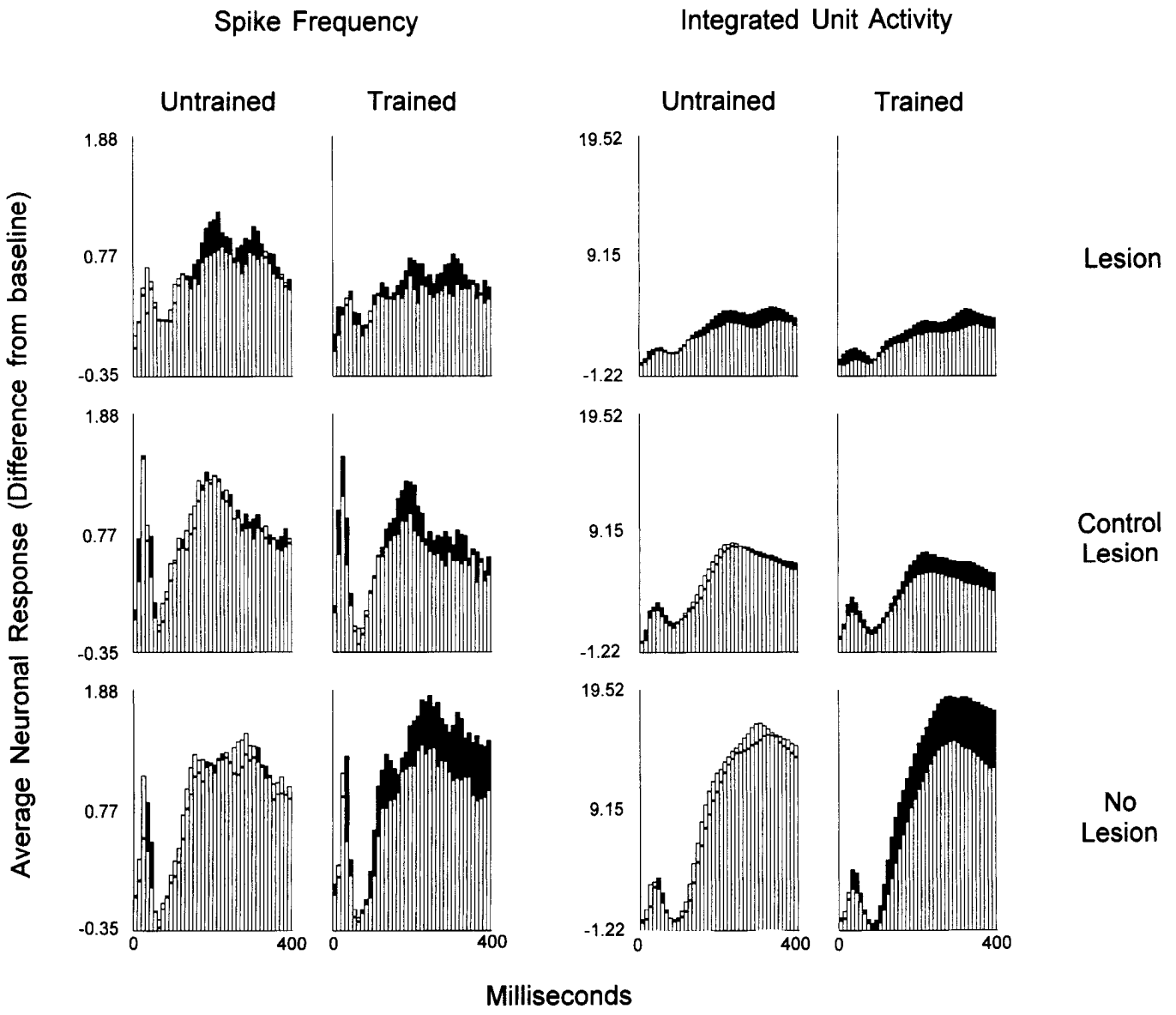

Figure 3. Average multiunit spike frequency (*left panels*) and integrated activity (*right panels*) profiles in the AV nucleus in response to CS+ (*solid bars*) and CS− (*open bars*), in rabbits with mamillothalamic tract lesions, rabbits with control lesions and no-lesion controls. Each panel shows the unit activity during the first 40 consecutive 10 msec intervals following CS onset. The plotted values are difference scores: the result of subtracting the discharge magnitude in each 10 msec interval after CS onset from the mean score of the 30 pre-CS intervals. The records were obtained during the preliminary training session (*Untrained*) and the session in which the acquisition criterion was attained (*Trained*), as described in Materials and Methods.

discriminative training-induced activity during preliminary training (Fig. 3, first and third columns). As in past studies, significantly greater excitatory discharges in response to the CS+ than to the CS− occurred during the criterial session of acquisition in the rabbits with control lesions ($P < 0.05$) and no lesions ($P < 0.01$; Fig. 3, second and third rows, second panel). However, this effect did not occur in rabbits with bilateral transection of the mamillothalamic tract (Fig. 3, first row, second panel). Also, as is characteristic of AV thalamic neuronal activity, significant discriminative training-induced activity did not occur during the first session of conditioning or during the session of the first significant behavioral discrimination. This finding replicates previous findings of exclusively "late-developing" discriminative activity in the AV nucleus (Gabriel et al., 1977, 1980; Foster et al., 1980) of intact rabbits.

The occurrence of discriminative training-induced activity during criterial performance in the no-lesion and control-lesion rabbits, and its nonoccurrence in transected rabbits, were corroborated by separate analyses of the data of each group. The interactions of the factors of training stage and stimulus, which assessed discriminative training-induced activity, were significant for the no-lesion and control-lesion groups [$F(3,51) = 8.79$, $P < 0.0002$, no lesion, spike frequency; $F(3,51) = 6.71$, $P < 0.0008$, no lesion, integrated activity; $F(3,33) = 5.19$, $P < 0.0049$, control lesion, spike frequency; $F(3,33) = 5.92$, $P < 0.0025$, control lesion, integrated activity] but not for the transected rabbits [$F(3,39) = 0.51$, $P < 0.69$, spike frequency; $F(3,39) = 1.42$, $P < 0.26$, integrated activity].

Discussion

Neuronal populations of the anterior ventral (AV) thalamic nucleus in rabbits exhibit training-induced excitation, and discrimination between CS+ and CS− during discriminative avoidance learning. These thalamic nuclei receive major afferent input, via the mamillothalamic tract, from the mamillary nuclei of the hypothalamus. This study investigated the contribution of mamillothalamic tract fibers to behavioral learning and to thalamic training-induced neuronal activity.

The behavioral and thalamic neuronal data of rabbits with bilateral transection of the mamillothalamic tract were compared to data of rabbits with control lesions (sham and attempted mamillothalamic tract lesions which missed their targets). A second comparison group contained rabbits not given lesions.

The mamillothalamic tract transection slowed the rate of behavioral acquisition and reduced the efficiency of avoidance performance in trained rabbits. When trained, the transected rabbits exhibited an average success rate of avoidances on 61% of CS+ trials, significantly less than the success rates (84% and 83%) exhibited respectively by rabbits with control and no lesions. A similar performance loss occurred previously in trained rabbits given avoidance training after bilateral anterior thalamic lesions (Gabriel et al., 1983). Thus, either direct destruction or deafferentation eliminated the specific contribution to avoidance behavior made by anterior thalamic neurons: maintenance but not original acquisition of the behavior. Moreover, deafferentation eliminated the neuronal changes (excitatory and discriminative training-induced activity) found in the AV nucleus of intact animals during behavioral acquisition. These results indicated that afferent mamillothalamic tract information received by the anterior thalamic nuclei is essential both for AV thalamic training-induced activity and for the behavioral contribution to learning made by anterior thalamic neurons. The fact that the mamillothalamic tract transections were of such different size and location compared to the anterior thalamic lesions, yet nevertheless yielded a similar and quite specific behavioral deficit, indicates that the behavioral deficit was not due to nonspecific or remote effects of the lesions.

Lesion-related changes of the neuronal activity occurred both in transected rabbits and in rabbits with control lesions. However, certain lesion-induced changes were of significantly greater magnitude in the transected rabbits. They included loss of AV thalamic tone-elicited neuronal activation and loss of training-induced discriminative neuronal activity. The fact that these changes were greater in transected rabbits than in rabbits with control lesions suggests that the mamillothalamic tract damage was primarily responsible for them, and that the control lesions were partially effective because they disrupted mamillothalamic tract transmission to a certain extent. However, a possible contribution of nonmamillothalamic tract fiber systems to these changes cannot be ruled out. Nonmamillothalamic tract fiber system damage may have been involved as well in the losses of AV thalamic spontaneous background neuronal activity and excitatory training-induced activity (increments of elicited activity during avoidance training compared with activity during preliminary training), which occurred equivalently in transected and control lesion rabbits, relative to the levels of these activities in the no-lesion group.

In summary, the effects that were specific to mamillothalamic tract transection (i.e., were absent or significantly less robust in rabbits with control lesions) included reduction of avoidance responding, abolition of *discriminative* training-induced activity in limbic thalamus and reduction of the magnitude of CS-elicited AV thalamic neuronal discharges. These transection-specific effects suggested that the mamillothalamic tract is involved specifically in avoidance-relevant processing of the CS+.

Other findings suggested that the system of cholinergic fibers which originate in the lateral dorsal tegmental (LDT) nucleus (Hoover and Baisden, 1980; Satoh and Fibiger, 1986; Woolf and Butcher, 1986; Hallanger et al., 1987; Cornwall et al., 1990; Shibata, 1992; Poremba et al., 1994) is also involved in the mediation of training-induced activity. However, this system appears to be involved only in the production of excitatory training-induced activity in the AV nucleus, as suggested by the finding that blockade of muscarinic receptors by systemic and local intracranial administration of scopolamine hydrobromide eliminated the excitatory CS-elicited activity of AV thalamic and posterior cingulate cortical neurons in trained rabbits (Henzi et al., 1990; Kubota et al., 1993) but did not affect discriminative training-induced activity.

These results considered along with the present findings suggested that excitatory training-induced activity in limbic thalamus may require jointly, inputs from both the mamillothalamic tract and the cholinergic LDT fiber systems. The cellular mechanism for excitatory training-induced activity could be analogous to "activity-dependent facilitation" (see Carew et al., 1984; Hawkins, 1991), which might be mediated by anterior thalamic M_2 ACh receptors that have been shown to increase in number in correspondence with excitatory training-induced activity during discriminative avoidance conditioning (Vogt et al., 1991).

Other studies indicated that cholinergic fibers which originate in the LDT nucleus run a parallel course in close proximity to the mamillothalamic tract (Satoh and Fibiger, 1986). These results are compatible with the hypothesis that the lesions in transected and control lesion rabbits disrupted these fiber systems, accounting for the nearly equivalent losses of excitatory training-induced activity in these two groups.

The loss of discriminative training-induced activity in transected rabbits could be interpreted as an indication that the anterior thalamic discriminative training-induced activity found in intact rabbits is intrinsic to the AV nucleus but requires mamillothalamic tract afferent input for its development. The logical alternative is that the discriminative training-induced activity is projected to the AV nucleus via direct neurotransmission from the mamillary nuclei. The latter hypothesis is supported by recent findings indicating that discriminative training-induced activity develops in multiunit recordings made in the mamillary nuclei (Kubota et al., 1994). This training-induced activity was exclusively of the late-developing variety (i.e., it did not develop until the criterial session of acquisition), the very same variety of discriminative training-induced activity which is characteristic of the AV nuclei of intact rabbits (Gabriel et al., 1977, 1980; Foster et al., 1980).

These considerations raise the question of whether the late-developing discriminative training-induced activity develops de novo in the mamillary nuclei or is projected there from upstream structures and relayed onward to the anterior nuclei.

The dorsal and posterior subicular complex is a principal cerebral cortical site of origin of axonal projections to the AV thalamic and mamillary nuclei (Meibach and Siegel, 1977; Swanson and Cowan, 1977; Köhler, 1990; Witter and Groenewegen, 1990). Bilateral electrolytic subicular complex lesions enhanced AV thalamic discriminative training-induced activity

(Gabriel et al., 1987) indicating that the subicular afferents are not essential for training-induced activity production but may instead provide an inhibitory modulation of the training-induced activity.

Bilateral lesions of the amygdaloid complex (Poremba and Gabriel, 1991), or of the medial geniculate nucleus (Poremba and Gabriel, 1993), an origin of fibers which project to amygdaloid and periamygdaloid regions (LeDoux et al., 1985), eliminated behavioral acquisition as well as discriminative and excitatory training-induced activity in the AV and MD thalamic nuclei. Medial geniculate and amygdaloid neurons exhibit discriminative training-induced activity (Gabriel et al., 1975; Pascoe and Kapp, 1985; Maren et al., 1991). This training-induced activity was of the early-developing variety. That is, the discriminative training-induced activity in the basolateral amygdaloid nucleus attained maximum magnitude in the session of the first significant behavioral discrimination and subsequently decreased in magnitude as criterion was reached (Maren et al., 1991). If the amygdaloid and/or the geniculate training-induced activity induced mamillary and anterior thalamic training-induced activity by direct synaptic neurotransmission, then the latter training-induced activity should also appear at maximum magnitude during the session of the first significant behavioral discrimination. However, as mentioned, the mamillary and anterior thalamic training-induced activity is of the late-developing variety and therefore not a product of direct neurotransmission from amygdaloid and/or medial geniculate neurons.

Lesion studies indicate that several nuclei of the amygdala are essential for acquisition of aversively motivated conditioned responses (Kapp et al., 1990; LeDoux, 1990; Davis, 1992; Hatfield et al., 1992; Helmstetter, 1992). Yet, amygdaloid neurons are apparently not involved in maintenance of aversively motivated behavior in well-trained subjects, as indicated by studies which have shown that learned behaviors dependent on the integrity of the amygdala nevertheless survive amygdaloid lesions induced after the behavior is acquired (reviewed by Sarter and Markowitsch, 1985; see also Miserandino et al., 1990; Roozendaal et al., 1993). We offer the suggestion that the mamillothalamic and anterior thalamic system is the system which supports the *maintenance* of aversively motivated responding, this based on the striking ubiquity of late-developing discriminative activity in this system and, as shown here and previously, the attenuation of discriminative behavior in the late training stages in animals with mamillothalamic tract and anterior thalamic lesions. It is further suggested that the early-developing discriminative training-induced activity found in the amygdala may act as a *precipitating event* or *trigger* which initiates the late-developing mamillary and anterior thalamic training-induced activity. Perhaps this triggering function requires as a precondition, the projection of discriminative training-induced activity from the amygdala to the mamillary and anterior nuclei during the early training stages. The afferents which originate in the amygdala and which initiate the late-developing discriminative training-induced activity in the mamillary nuclei could gain access to the mamillary nuclei via amygdalofugal fibers which project to various hypothalamic areas including lateral hypothalamic area, premamillary nucleus, and supramamillary nucleus (Hopkins and Holstege, 1978; Krettek and Price, 1978; Price and Amaral, 1981; Caffé et al., 1987; Canteras et al., 1992) and thence forward to the mamillary nuclei (Gonzalo-Ruiz et al., 1992).

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