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Training-stage related neuronal plasticity in limbic thalamus and cingulate cortex during learning: a possible key to mnemonic retrieval

Michael Gabriel[1], Brent A. Vogt[2], Yasuo Kubota[1], Amy Poremba[1] and Eunjoo Kang[1]

[1]*Department of Psychology and Beckman Institute, University of Illinois, 405 North Mathews Avenue, Urbana, IL 61801; (U.S.A.) and*
[2]*Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest University, 300 South Hawthorne Road, Winston-Salem, NC 27103 (U.S.A.)*

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This study is part of an ongoing project concerned with the analysis of the neural substrates of discriminative avoidance learning in rabbits. Multi-unit activity was recorded in 5 anterior and lateral thalamic nuclei and in 4 layers of 2 posterior cingulate cortical areas (29c/d and 29b) during learning. The rabbits learned to step in response to a warning tone to avoid a foot-shock, and to ignore a different tone not followed by shock. Excitatory training-induced unit activity (TIA, increased tone-elicited activity during training relative to a pretraining session with unpaired tone-shock presentations) and/or discriminative TIA (greater discharges to the warning than to the safe tone) developed during training in 11 of the 13 areas. Discriminative TIA in the thalamic nuclei increased monotonically as learning occurred. Anterodorsal (AD) thalamic excitatory TIA peaked in an early stage (the first session of training), laterodorsal thalamic and parvocellular anteroventral (AVp) excitatory TIA peaked in an intermediate stage (the session of the first behavioral discrimination), and magnocellular anteroventral (AVm) and anteromedial (AM) thalamic excitatory TIA peaked in a late stage (the session in which asymptotic behavioral discrimination first occurred). The excitatory TIA in these nuclei declined as training continued beyond the stage in which the peak occurred. Peaks of excitatory TIA developed in area 29c/d of posterior cingulate cortex in the early (layer IV), intermediate (layers I–III and V) and late (layer IV) training stages, as just defined. Only layer IV in area 29b of posterior cingulate cortex exhibited a peak of excitatory TIA, which occurred in the early and intermediate training stages. As in limbic thalamus, discriminative TIA increased monotonically over training stages in layers V and VI of areas 29c/d and in layer VI of area 29b. However, layers I–III and IV in area 29c exhibited peak discriminative TIA in the intermediate and late training stages, respectively. Lesion studies indicate that limbic thalamus and cingulate cortex are essential for learning. The peaks represent a unique topographic pattern of thalamic and cortical excitation elicited by the CS+. It is proposed that the peaks constitute a retrieval pattern, i.e. a unique topographic array of excitation. This pattern encodes the spatio-temporal context which defines the learning situation and is necessary for recall and output of the learned response.

INTRODUCTION

A prevalent strategy in analyses of the neural basis of learning and memory involves the study of neuronal plasticity, defined as persistent change in neuronal activity induced by experience or by artificial stimulation. Recently, important advances have been made in relation to the neurochemical bases of training-induced neuronal plasticity in invertebrate preparations[4] and in relation to plasticity such as long-term potentiation (LTP), studied in vivo and in vitro[14]. Yet because LTP and other forms of plasticity in mammalian preparations are embedded in a complex, interactive circuitry, or in the case of in-vitro studies, are isolated from the surrounding circuitry, little direct information is available on their behavioral relevance. One approach to this issue involves the analysis of neuronal plasticity in mammalian brain circuits that have a well-specified behavioral relevance.

Past research has indicated that brain circuitry involving neurons of the anterior and medial dorsal (limbic) thalamic nuclei and neurons in the cingulate cortical projection fields of these nuclei, is essential for discriminative avoidance learning in rabbits[7]. In these

Correspondence to: M. Gabriel, The Beckman Institute, University of Illinois, 405 North Mathews Avenue, Urbana, IL 61801, U.S.A. Fax: (1)(217)244 8371.

studies, rabbits acquired a conditioned response (CR, stepping in a rotating-wheel apparatus) to a warning tone (a positive conditional stimulus or CS +) in order to avoid a foot-shock unconditional stimulus (US). A different tone, the negative conditional stimulus or CS − , was never followed by shock and thus did not call for a response. Bilateral lesions in either cortex or thalamus severely impaired CR acquisition, and unit recordings in these areas during learning in behaving rabbits demonstrated massive training-induced neuronal activity (TIA). Excitatory discharges in response to both CS + and CS − became greater during conditioning, relative to a preliminary session in which the CSs and US were presented in an explicitly unpaired, noncontingent manner. In addition, neuronal discrimination, or greater firing in response to the CS + than to the CS − , developed during conditioning.

An intriguing property of the TIA in these areas is that it attained maximum amplitude in different stages of behavioral acquisition, depending on the recording site. Rapidly-forming discriminative activity occurred in the pooled activity of deep layers V and VI in posterior cingulate cortex, and a prevalence of late-developing discriminative activity occurred in the pooled activity of superficial layers I–IV and in the AV nucleus[7]. Since these observations were made, many more conditioning-related multi-unit records have been obtained in these areas, permitting an independent replication of the earlier findings, finer neuroanatomical resolution within the areas previously studied, and extension of the observations to related areas not previously studied. The present data indicate distinct stage-related patterns of TIA in the anterior dorsal (AD), anterior medial (AM), lateral dorsal (LD), parvocellular anterior ventral (AVp) and magnocellular anterior ventral (AVm) thalamic nuclei and in layers I–III, IV, V, and VI of posterior cingulate cortical areas 29c/d and 29b. These data and related findings foster the hypothesis that the stage-related activity constitutes an excitation pattern in the brain that gives rise to mnemonic retrieval and consequent output of the learned response. In addition, the distinct stage-related patterns of physiological plasticity documented here invite a search for correlated biochemical changes of possible causal relevance to the plasticity. Indeed, specific correspondences between TIA, in particular limbic thalamic and cingulate cortical areas with training-induced changes in muscarinic acetylcholine receptor binding have already been noted[30]. These results afford an important step toward the identification of cellular mechanisms underlying neurophysiological plasticities in mammalian learning-relevant brain circuitry.

METHODS

Subjects, electrodes, target areas and surgical procedures

The subjects were 93 1.5–2.0 kg male, New Zealand white rabbits obtained from a professional rabbit breeder and maintained on ad libitum water and rabbit chow. After a minimum of 48 h for adaptation to living cages, each rabbit was prepared for surgical implantation of fixed-position metal electrodes for recording of multi-unit neuronal activity and intracerebral EEG. The electrodes were made from stainless-steel pins (uninsulated shaft diameter, 0.28–0.30 mm) insulated with epoxylite. The recording surfaces were formed by removing insulation from the pin tips to expose a region from 10 to 50 μm in length. So fabricated, the electrodes had electrical impedances ranging from 500 kΩ to 2 MΩ. The surgeries were performed aseptically using facilities and procedures approved by the American Association of Laboratory Animal Care (AALAC).

Surgical anesthesia was induced using an intramuscular 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. Four or six electrodes were implanted in each rabbit. The head of the anesthetized rabbit was positioned in a Kopf rabbit head-holder, the skull exposed and prepared, and holes for the electrodes drilled. An assembly consisting of a miniature 9-contact connector, with wires from each electrode soldered to one contact, was attached to the skull with screws and dental acrylic. Miniature teflon guides with 0.013-inch holes drilled through their centers were impaled on uninsulated pins and positioned over each electrode hole. After cementing each guide to the skull, the pins were removed and the electrodes press-fitted through the guides to the brain targets. The guides held the electrodes firmly, permitting them to remain unattached to the stereotaxic electrode carrier as the electrodes were advanced. Thus, slight movements due to respiration did not affect the neuronal record. Neuronal activity was monitored during electrode advancement, to supplement stereotaxic criteria. A stainless-steel machine screw threaded into the frontal sinus and connected to one of the contacts served as a reference electrode.

The rabbits were subjects in a variety of specific projects and thus had recording electrodes in areas not dealt with in this report. The present results are based on recordings from electrodes in two cytoarchitecturally defined[28] posterior cingulate cortical areas (29c/d or 29b), four cortical layer groups (layers I–III, IV, V and VI) and five limbic thalamic nuclei containing neurons that project to posterior cingulate cortex: the anterodorsal (AD), parvocellular division of the anterior ven-

tral (AVp), magnocellular division of the anterior ventral (AVm), anterior medial (AM) and the lateral dorsal (LD) nuclei. The stereotaxic electrode targets in the cortical areas were 3–5 mm posterior to bregma, 0.2–1.5 mm lateral from the midline and 0.2–4.0 mm ventral to the brain surface (area 29c/d), and in the ventral underbank of retrosplenial cortex 9 mm posterior to bregma, 2.0–2.5 mm lateral to the midline and 3.5–4.5 mm ventral to brain surface (area 29b).

Histology

To localize electrode tracks after testing each rabbit received an overdose of sodium pentobarbital, followed by transcardial perfusion with normal saline and formalin. Each brain was frozen and sectioned at 40 μm, and the sections containing the electrode tracks were photographed while still wet[6]. After photography, the sections were stained for Nissl substance and myelinated axons using formol–thionin[5]. Records from misplaced electrodes or records that did not exhibit clear tone-evoked discharges throughout training were discarded. The numbers of records used were as follows: area 29c/d layers I–III (7), area 29c/d layers IV (9), area 29c/d layer V (7), area 29c/d layer VI (5), area 29b layers I–III (7), area 29b layers IV (6), area 29b layer V (11), area 29b layer VI (10), AD (6), AVp (9), AVm (9), AM (5), and LD (11).

Behavioral training

Following ten days for recovery from surgery the rabbits received conditioning in a wheel apparatus[3] located in a shielding chamber in a room adjacent to that containing apparatus for controlling the experiment. An exhaust fan and a speaker in the chamber produced a masking noise of 70 dB which remained present throughout conditioning. The conditional stimuli (CSs) were pure tones (1 or 8 kHz, 85 dB with a rise time of three ms) played through a speaker located directly above the wheel. The unconditioned stimulus (US) was a constant-current foot-shock (1.5–2.5 mA) delivered through the grid floor of the wheel. A response was defined as any wheel rotation exceeding two degrees. During conditioning, onset of the positive CS (CS+) was followed after five seconds by the US. The CS+ duration was 500 ms. Both CS+ and US were terminated by responses. With these procedures the rabbits typically performed one or two steps forward in the wheel in response to the US. The US never elicited vocalization, defecation or other signs of severe distress. A response during a CS before US onset (i.e. a CR) prevented US delivery. The maximum duration of the US, if the rabbit did not respond, was one second. The duration of the CS− was 500 ms, but the CS− was not followed by the US. The interval between the end of the trial (6 s after CS onset or after wheel-rotation when locomotion occurred) to the onset of a new trial (CS onset) was 10, 15, 20, or 25 s. These values occurred in a random sequence. Locomotion reset this interval. The CS+ and CS− were presented 60 times in each daily conditioning session, in an irregular quasi-random sequence.

Daily training sessions were administered until a criterion of behavioral discrimination was met. The criterion required that the proportion of wheel-rotations made in response to the CS+ exceed the proportion in response to the CS− by 0.60 or more, in two consecutive training sessions. Prior to training each rabbit received two pretraining (PT) sessions, in which the tones to be used as CSs were presented, each 60 times, as in training. One pretraining session involved presentation of the tones only, and the other involved presentation of the tones, with the US interspersed in a non-contingent, explicitly unpaired[21] manner. The PT sessions provided control data for the assessment of possible pre-wired differential unit responses to the tones. Also, PT with non-contingent CS–US presentation provided essential baseline information permitting the detection of associative neuronal changes (i.e. TIA) specific to paired CS–US presentations on the next and subsequent training days.

Collection and analysis of neuronal data

The neuronal records were fed into field-effect transistors that served as high-impedance source-followers located on the connector that mated with that on the rabbit a short distance (about 2.5 cm) from the brain recording sites. The FET outputs fed via shielded cable, were split, one limb entering single-ended preamplifiers with band-width appropriate for unit recording (gain = 4000, 1/2 amplitude cut-offs as 500 and 8000 Hz), the other limb entering pre-amplifiers for field potential recording (gain = 2000, 1/2 amplitude cut-offs at 0.2 and 60 Hz). The unit activity records were subjected to a second stage of active band-pass filters (1/2 amplitude cut-offs at 600 and 8000 Hz, roll-off = 18 dB/octave) to remove all EEG frequencies. The records were then fed to Schmitt triggers, that were automatically adjusted to yield a mean rate of output pulses within limits of 110–190 per second. With this criterion, typically, the largest three or four spikes on each record were sampled. In addition, the band-pass filter outputs were full-wave rectified and integrated. The time constants for the rise and fall of the integrators were 15 and 75 ms respectively. The Schmitt trigger data provide an index of the discharge frequency of the largest spikes on each record, whereas the integrated unit activity

measured the energy fluctuations of the entire record, including activity below the triggering thresholds. The Schmitt trigger pulses were counted and the integrator and field potential signals digitized on each trial (CS presentation) for 1.0 s, 0.3 s before CS onset and 0.7 s after CS onset. A digital value was stored for each measure and electrode every 10 ms during the sampling interval. These data were written to magnetic tape after each trial. In addition, averages of the behavioral data, unit histograms, integrated activity and field potentials were stored on disc and displayed on a VT100 graphics terminal continuously as they were being formed during the training sessions. The details of several procedures employed to insure that the neuronal records were not influenced by movement artifacts are reported elsewhere (e.g. ref. 8).

Statistical treatment of the data

The neuronal data in four stages of behavioral acquisition were analysed: (a) pretraining, the session in which the CSs and US were presented in an unpaired manner; (b) the first session of conditioning, in which the CS + was paired with the US (always the day after pretraining); (c) the session in which the first significant behavioral discrimination occurred; and (d) the session in which the criterion of behavioral learning was attained. The session of the first significant discrimination was defined as the first session in which the percentage of CRs to the CS + exceeded the percentage of responses to the CS − by at least 25%. The criterion for the completion of training required that this difference be 60% or more in two consecutive sessions. For each rabbit, separate peri-stimulus histograms based on 60 trials with the CS + and 60 trials with the CS − were constructed from the individual trial data, indicating the cumulated spike frequency and the total integrated unit activity in each of the four training stages. Standard scores (Z-scores) were computed for each histogram as follows: the total spike frequency or digital value (for the integrated activity) in the 30 pre-CS (baseline) intervals was subtracted from the mean frequency or digital value in each of 40 intervals following CS onset. The difference in each case was divided by the standard deviation of the pre-CS intervals. These scores for each brain area, representing the magnitude of the CS-elicited neuronal response normalized with respect to the pre-CS baseline, were submitted to multi-factor repeated-measure analysis of variance (ANOVA) with factors of CS type (CS + /CS −), training stage (4 levels as just described) and 10-ms interval after CS onset (40 levels). Separate analyses were performed for the data of the AD, AV, AM, and LD nuclei. The analysis of the AV data had a subdivision factor (2 levels, AVp and AVm) in addition to the CS, training-stage and interval factors. A separate analysis was performed on the posterior cingulate cortical data with area (29c/d or 29b), CS, training-stage and interval factors, and an additional 4-level factor for comparison of the data in layers I–III, IV, V and VI. Individual comparisons in the form of two-tailed t-tests ($\alpha \leq 0.05$) were carried out following significant ANOVAs.

The field-potential recordings are not presented in this report as this report focuses on cytoarchitectural and neural circuit correlates of training-induced neuronal activity, and field potential data are not well-localized to the recording sites. The field potential data are available on request.

RESULTS

Training-induced activity in limbic thalamus

All but two of the monitored areas exhibited excitatory TIA (increased magnitude of the CS elicited activity during training, compared to pretraining), and discriminative TIA (significant neuronal discrimination between CS + and CS − during conditioning, not present during pretraining). In addition, there were striking differences between the areas in relation to the training stage in which maximal excitatory TIA occurred. The AD nucleus showed maximal excitatory TIA during the first session of conditioning and declining TIA as training progressed, LD and AVp exhibited maximal excitatory TIA during the session of the first significant behavioral discrimination and declining TIA in the next stages, and AVm and AM did not exhibit maximal excitatory TIA until the session in which criterion was attained (Figs. 1 and 2). Additional data, not shown here, indicated that the late-developing TIA in AVm and AM declined with the progress of training beyond criterion.

These conclusions were based on significant F ratios for the interactions of the training-stage and post-CS 10-ms interval factors for the analyses of the data of all of the thalamic areas except the AV nucleus (AD: $F_{156,780} = 1.64, P < 0.0001$; LD: $F_{117,1170} = 1.89, P < 0.0001$; AM: $F_{312,1248} = 1.55, P < 0.0001$). These interactions indicated significant covariation of the CS elicited neuronal activity (pooled for the two CS types) and the training stage. In addition, the analyses of activity in the AM nucleus yielded a marginally significant interaction of the training-stage, CS type and interval factors ($F_{312,1248} = 1.15, P < 0.06$), and the analysis of the data of the AV nucleus yielded a significant interaction of the training-stage, CS type, subdivision (AVm and AVp) and interval factors ($F_{117,2106} = 1.37,$

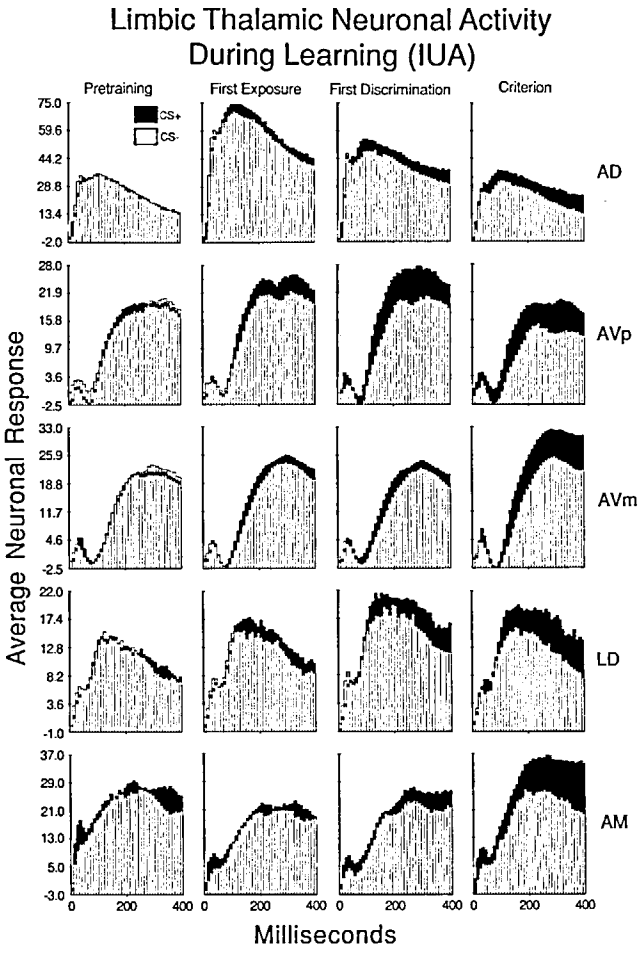

Fig. 1. Average magnitude of the integrated unit activity elicited by the CS + (dark bars) and CS − (light bars) in 40 consecutive 10-ms intervals from CS onset to 400 ms thereafter. The plotted values are average standard scores normalized relative to the 300-ms pre-stimulus baseline. Data are shown for each of five areas of limbic thalamus (AD, the anterior dorsal nucleus; AVp, parvocellular anterior ventral (AV) nucleus; AVm, magnocellular AV nucleus; LD, lateral dorsal nucleus; and AM, anterior medial nucleus). The activity in each area is shown for each of four stages of conditioned response acquisition as defined in the caption of Table I.

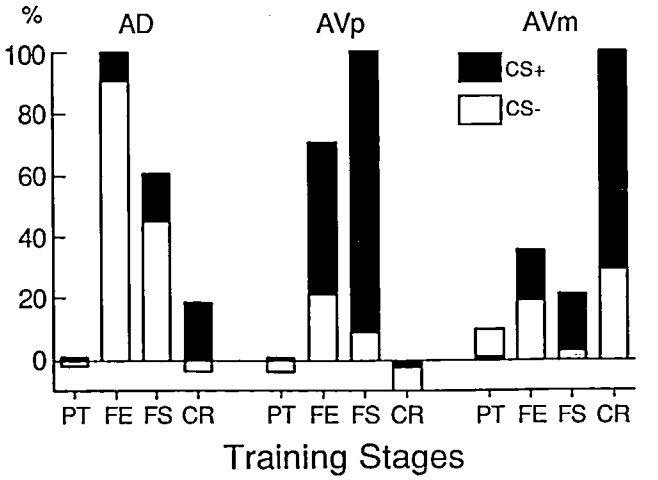

Fig. 2. The average magnitude of integrated unit activity in various nuclei of the anterior thalamus 250–350 ms after CS onset. The plotted values are average standard scores normalized relative to the 300-ms pre-stimulus baseline. The maximum discharge in a given area was set at 100% and the value obtained during pre-training (PT) was set at 0%. Data are shown for the AD, AVp and AVm records in four stages of behavioral acquisition (PT, FE, FS and CR or CRIT) as defined in the caption of Table I. These results illustrate training-stage-related peaks of training-induced neuronal plasticity in the anterior thalamic subdivisions.

$P < 0.007$). These interactions indicated that training-stage-related activity changes that occurred in response to the CS + were different from the changes that occurred in response to the CS − .

Two-tailed t-tests were computed at each interval after CS onset, to determine whether, at each stage of training: (a) the average discharge magnitude after CS onset differed significantly from the average in the corresponding interval during pretraining; and (b) the average discharge elicited by the CS + differed significantly from the average discharge elicited by the CS − . These two comparisons assess, respectively, excitatory and discriminative TIA, as defined previously. In the AD and LD, the activity elicited by the two CS types was pooled because only the training-stage and interval factors, but not the CS type factor, interacted significantly for these areas. Thus, the comparisons assessed only excitatory TIA. For the remaining areas (the analyses of which yielded interactions of training-stage, CS type and interval), the comparisons assessed discriminative TIA and excitatory TIA at each training stage, but the excitatory TIA was assessed only for the activity elicited by the CS + .

The results (Table I) indicated that the average discharge magnitudes in many 10-ms intervals were significantly greater during one or more training stages, compared to the average discharge magnitudes in the corresponding interval during pretraining. The particular training stages exhibiting the increases depended on the thalamic area. Thus, for the AD nucleus significant average discharge increments relative to pretraining occurred in virtually every 10-ms interval during the first session of conditioning (i.e. the first CS/US pairing). Significant increments occurred in somewhat fewer intervals during the session of the first significant behavioral discrimination, and no significant increments occurred during the stage of training in which the criterion of acquisition was met. Thus, the first session of conditioning was the session in which peak excitatory

TABLE I

Number of post-CS ten-millisecond intervals with training-induced neuronal activity (TIA)

The number of 10 ms intervals after CS onset in which significant training-induced integrated neuronal activity occurred (maximum = 40), as indicated by significant individual comparisons following analysis of variance. Data are shown for four stages of behavioral acquisition: PT, the pretraining session in which the CSs and US were presented non-contingently; FE, the session of the first exposure to conditioning, wherein paired presentations of the CS + and US were given; FS, the session in which the first significant behavioral discrimination occurred; CRIT, the session in which the criterion of behavioral discrimination was attained. The first column shows the number of intervals in which discriminative activity (significantly greater neuronal discharge to the prospective CS + than to the prospective CS –) occurred during PT. This activity is due to pre-existing differences in responsiveness to the CSs. Entries in the columns labeled Disc. TIA indicate the number of intervals in which the average neuronal activity elicited by the CS + exceeded the activity elicited by the CS – . This measure is referred to as *discriminative TIA* in the text. Entries in the columns labeled Excit. TIA indicate the number of intervals in which the average neuronal activity elicited by the CS + exceeded the activity elicited by the CS + during PT. This measure is referred to as *excitatory TIA* in the text. In two nuclei (AD and LD) the CS was not a significant factor in the interaction of training-stage and post-CS interval. Thus, no comparisons of CS + to CS – elicited activity were performed, and the table entries in the column for excitatory TIA represent the number of intervals in which excitatory TIA occurred for data pooled with respect to the CS + and CS – . Only a single number appears in the column labelled PT as only discriminative activity, not excitatory activity could be measured during PT. The asterisks (*) indicate instances in which the discharges elicited by the CS – exceeded those elicited by the CS + .

Area	*PT*	*FE*		*FS*		*Crit*	
	Discriminative Activity	*Disc. TIA*	*Excit. TIA*	*Disc. TIA*	*Excit. TIA*	*Disc. TIA*	*Excit. TIA*
AD	0	0	37	0	33	0	0
AVm	0	10	19	14	7	28	28
AVp	1	29	30	24	29	26	2
AM	9	2	39	14	22	39	34
LD	0	0	0	0	27	0	16
29 c/d Layer							
I–III	0	0	25	20	34	0	0
IV	0	2	30	1	31	32	33
V	0	15	21	31	21	34	29
VI	0	21*	30	0	33	16	0
29 b Layer							
I–III	6	6*	0	0	0	2	0
IV	0	6*	11	0	25	1	0
V	0	0	0	0	0	3	0
VI	0	4*	0	24	0	27	0

TIA occurred for AD neurons. Application of this testing strategy to the data of the other nuclei indicated that the peak of excitatory TIA occurred in AVp and LD during the session of the first significant behavioral discrimination, and the peak in AVm and AM occurred during the session of criterion attainment.

Significant discrimination between CS + and CS – reached maximal magnitude in the criterial stage of training in all of the nuclei (AVp, AVm, AM) in which the CS type factor interacted significantly with the training-stage factor (see Table I). Also, even though the CS type factor did not interact significantly with training stage for LD and AD, the neurons in these nuclei did exhibit some discriminative TIA as indicated by a marginally significant main effect of the CS factor ($P < 0.06$) for the AD data, and by a significant interaction of the CS and interval factors for LD data ($P < 0.0002$). The discrimination in these two nuclei also attained its greatest magnitude during the criterial stage of training. These observations coupled with the finding that excitatory TIA peaks occurred in different training stages indicate that discriminative and excitatory thalamic TIA were not directly coupled during training. The greatest magnitude of discriminative TIA occurred in all nuclei during criterial performance whereas the excitatory TIA changes reached peaks in different training stages and declined as training continued beyond those training-stages. The results suggest independent control of excitatory and discriminative TIA in limbic thalamus.

Spike frequency. The integrated unit activity and the spike frequency data exhibited similar relationships to the experimental variables employed in this study. However, the integrated activity as reported above was more sensitive than spike frequency, and thus yielded more robust effects in the statistical analyses. Thus, for

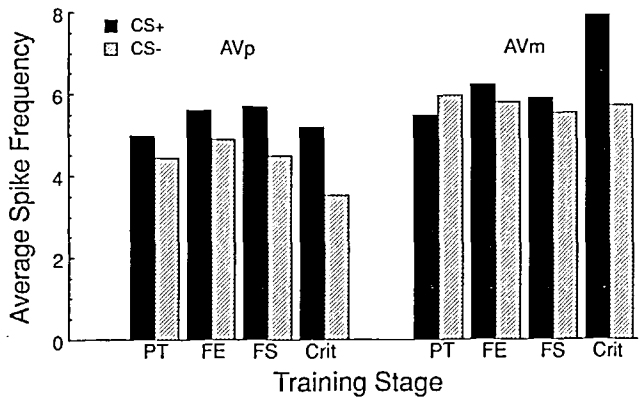

Fig. 3. The average multi-unit spike frequency in the parvocellular anterior ventral (AVp) and magnocellular anterior ventral (AVm) nuclei during the first 400 ms after CS onset. The plotted values are standard scores normalized relative to the 300-ms pre-stimulus baseline, and averaged with respect to subjects and the 40 10-ms intervals comprising each post-stimulus histogram. Data are shown separately for the CS + (dark bars) and CS − (light bars) in four stages of behavioral acquisition (PT, FE, FS and CRIT) as defined in the caption of Table I.

example, none of the interaction terms involving the factors of subdivision, CS and training stage in the analysis of the AVp and AVm integrated unit activity reached an acceptable significance level in the analysis of spike frequency. The interaction of subdivision, CS and training stage did approach significance ($F_{3,54}$ = 1.92, $P < 0.14$), and a plot of the mean values of this interaction (Fig. 3) indicates an ordering of the average spike frequencies similar to the average discharge magnitudes shown in Fig. 1 for AVp and AVm integrated unit activity. Orderings of the mean values basically similar to those shown in Fig. 1 were also found for AD, LD and AM but the relevant terms in the analyses did not approach significance.

Training-induced activity in posterior cingulate cortex

Overview. As shown in Figs. 4 and 5, neuronal records in the various layer groups of the posterior cingulate cortex exhibited different patterns of excitatory and discriminative TIA. These observations were based on a significant interaction of the four-level layer factor with the CS type, training-stage and interval factors ($F_{351,6318}$ = 1.78, $P < 0.0001$), as well as a significant interaction of the factors of area (area 29c/d vs 29b), layer-group, training-stage and CS type ($F_{9,162}$ = 2.82, $P < 0.005$). The fact that the area factor interacted significantly with the other factors indicated that the laminar pattern of training-stage-related activity in area 29c/d differed from that in area 29b.

Area 29c/d. Two-tailed *t*-tests (Table I) indicated that peak excitatory TIA in different layers of area 29c/d occurred in various stages of acquisition (Figure 4). The

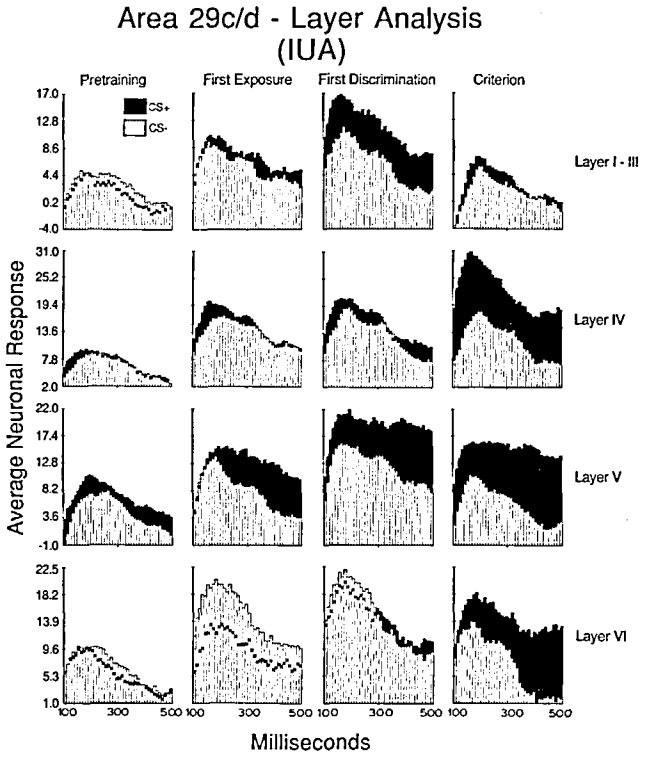

Fig. 4. Average magnitude of cingulate cortical area 29c/d integrated unit activity elicited by the positive conditional stimulus (CS +, dark bars) and by the negative conditional stimulus (CS −, light bars) in 40 consecutive 10-ms intervals after CS onset. The plotted values are average standard scores normalized relative to the 300-ms pre-stimulus baseline. Data are shown for each of four cortical layers (I–III, IV, V and VI). Because the neuronal response in the initial 100 ms in these areas is not influenced by training, the intervals for which data are plotted are the 10th through the 50th intervals, which exhibited training-induced plasticity. The activity in each layer is shown for each of four stages of conditioned response acquisition as defined in the caption of Table I.

peak of excitatory TIA occurred during the first conditioning session and in the session of the first significant behavioral discrimination in layer VI, the session of the First significant behavioral discrimination in layers I–III and V and during the session of criterial performance in layer IV. Maximal discriminative TIA occurred during the criterial session in all of the layers excepting layers I–III. In layer I–III maximal excitatory and discriminative TIA occurred in the session of the first significant behavioral discrimination, whereas both excitatory and discriminative TIA in these layers were greatly reduced in the criterial session.

Area 29b. Discriminative TIA was absent or negligible in all layers of area 29b excepting layer VI, wherein it developed monotonically during learning, reaching maximal magnitude in the session of criterion attainment, as in area 29c/d (Fig. 5). However, clear discrimination between CS + and CS − occurred in the first

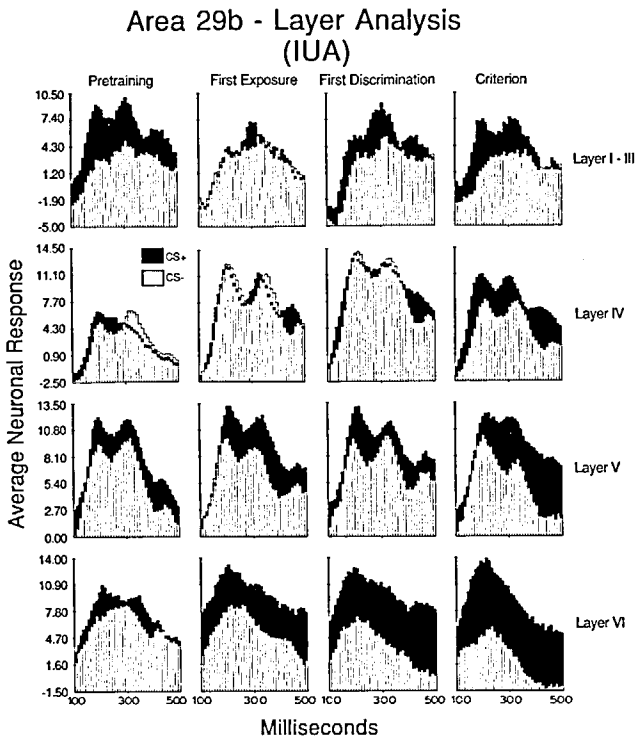

Fig. 5. Average magnitude of cingulate cortical area 29b integrated unit activity elicited by the CS + (dark bars) and CS − (light bars) in 40 consecutive 10-ms intervals after CS onset. The plotted values are average standard scores normalized relative to the 300-ms pre-stimulus baseline. Data are shown for each of four layer groups (I–III, IV, V and VI). Because the neuronal response in the initial 100 ms in these areas is not influenced by training, the intervals for which data are plotted are the 10th through the 50th intervals, which exhibited training-included plasticity. The activity in each area is shown for each of four stages of conditioned response acquisition (PT, FE, FS and CRIT) as defined in the caption of Table I.

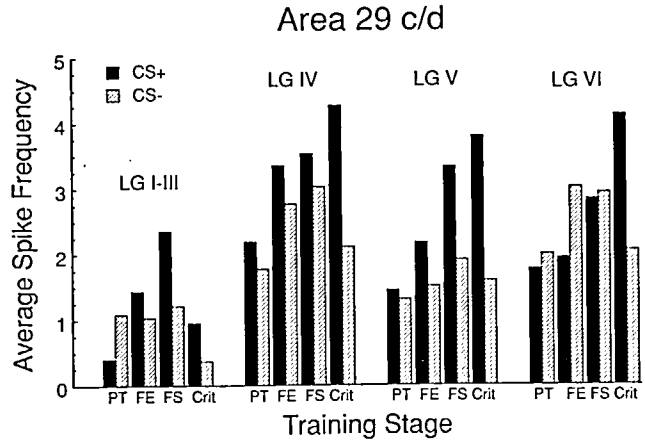

Fig. 6. Average magnitude of cingulate cortical area 29c/d spike frequency elicited by the CS + (dark bars) and CS − (light bars). The plotted values are average standard scores normalized relative to the 300 millisecond pre-stimulus baseline. Data are averaged over the 40 10-ms intervals after CS onset and shown separately for each of four layer groups (I–III, IV, V and VI) and in each of four stages of conditioned response acquisition (PT, FE, FS and CRIT) as defined in the caption of Table I.

conditioning session in area 29b layer VI, whereas the CS − elicited significantly greater average discharges than the CS + during the first conditioning session in area 29c/d. The only other layer in area 29b to exhibit effects of training was layer IV wherein excitatory TIA reached maximal magnitude in the first conditioning session and in the session of the first significant behavioral discrimination. This outcome stands in contrast to that in layer IV of area 29c/d which exhibited a peak of excitatory TIA in the criterial session (Table I).

In addition to the laminar distribution of excitatory and discriminative TIA, other differences between areas 29c/d and 29b should be noted. The CS + elicited greater average discharges than the CS − during pretraining in layers I–III and V in area 29b. Thus, by chance, the frequencies used as CS + were more effective, before learning, in exciting the involved neuronal populations than the frequencies selected as CS − . This pre-learning differential response was significant in a small number of intervals (see Table I). No significant training-induced increment in discrimination occurred in either of the layers exhibiting this effect. Finally, as in other studies, the neurons in area 29b exhibited rhythmic bursts of neuronal activity that were time-locked to CS onset, as indicated by the succession of excitatory peaks characterizing the histogram profiles of layers I–III, IV and V. Although not measured in the present sample, the average frequencies of the first four bursts in response to CS + and CS − in all layers of area 29b in a similar but somewhat larger sample of records obtained in trained rabbits were 7.80 and 6.92 Hz, respectively (Navarre, Kubota and Gabriel, unpublished observations, 1990). No rhythmic bursts occurred in area 29c/d.

Spike frequency. As in the analyses of the thalamic data, the effects of the experimental variables were not as robust in the analyses of the cingulate cortical spike frequency data as in the analyses of the integrated unit activity. For spike frequency, the interaction of layer group, CS, training stage and interval ($F_{351,6318} = 1.10$, $P < 0.10$), and the interaction of area (29c/d vs 29b), layer group, CS, training-stage and interval ($F_{351,6318} = 1.09$, $P < 0.11$) approached but did not attain significance. Again, the ordering of the mean values was similar to the ordering obtained in the analysis of the integrated unit activity (Fig. 5).

DISCUSSION

This study demonstrates TIA in 5 nuclei of limbic thalamus and in 4 layers of cingulate cortex during discriminative avoidance learning in rabbits. The TIA

reflects learning-relevant information processes of the brain, as opposed to changes due to general arousal engendered by the training situation. Learning-relevance is indicated by the associative character of the TIA: both the excitatory and discriminative forms of plasticity depended on the pairing of the CS + with the US. In addition, the excitatory TIA in each of the areas was specific to particular stages of CR acquisition. Neither the pairing-, or stage-specificity of the TIA can be explained as responses to stress or as a reflection of general arousal.

Origins and modulations of TIA

Thalamic TIA develops robustly in rabbits with cingulate cortical and subicular lesions[8] and thus does not depend critically on efferents from these areas. Since these areas are the only areas of cerebral cortex that project to the limbic thalamus, limbic thalamic TIA must arise exclusively as a result of subcortical neural circuit interactions. Anterior ventral thalamic TIA is essentially unaffected by a 95% depletion of anterior thalamic nor-epinephrine (NE)[25] and thus does not seem to depend on brainstem nor-adrenergic afferents. The development of AV thalamic excitatory TIA is blocked and CR performance impaired by transection of the mammillothalamic tract and by systemic administration of scopolamine hydrobromide[10]. A prolonged enhancement of anterior thalamic synaptic responsiveness to mammillary body stimuli was induced by stimulation of dorsal tegmental cholinergic cell groups, and this enhancement was also blocked by muscarinic antagonists[20]. In addition, muscarinic acetylcholine receptor binding increased in correspondence with the development of excitatory TIA in AD, AVp and AVm. Moreover, there was a rather precise correspondence between the receptor binding increases and the excitatory TIA. Maximal muscarinic acetylcholine receptor binding in AD, AVp and AVm occurred respectively in the first session of conditioning, the session of the first significant behavioral discrimination and in the session of criterial performance[30]. The foregoing findings in concert suggest that mammillothalamic tract afferents and cholinergic afferents from the midbrain tegmentum cooperate to induce AV thalamic excitatory TIA. Since a portion of the muscarinic acetylcholine receptors in anterior thalamus are likely to be located presynaptically on axon terminals of mammillary body neurons[24], TIA could be produced as follows. Up-regulated muscarinic acetylcholine receptors presynaptic on mammillothalamic tract axon terminals enhance the release of excitatory neurotransmitter from mammillary body neurons and so elevate neuronal discharges in the anterior nuclei. The relevance of this model to excitatory TIA in limbic thalamic nuclei other than the AV nucleus remains to be determined.

The systemic administration of scopolamine hydrobromide, which eliminated excitatory TIA, did not alter significantly the discriminative TIA in the AV nucleus[10]. These results and the present findings that thalamic excitatory and discriminative TIA are dissociated, suggest that discriminative and excitatory TIA have separate origins. Quite possibly, the discriminative TIA originates in neurons of the auditory thalamus (the medial and dorsal divisions of the medial geniculate nucleus), as these neurons exhibit discriminative TIA during avoidance conditioning and in other conditioning paradigms[1,9,22].

Findings reviewed above indicating that inputs from the subiculum or cingulate cortex are not essential for limbic thalamic TIA do not rule out an important modulatory influence of efferents from these areas upon limbic thalamic TIA. Lesions of the subiculum or the CA sectors of the hippocampus, induced before conditioning, increased the magnitude of excitatory and discriminative TIA in the AV nucleus in asymptotically trained rabbits. Excitatory TIA in the AV nuclei increased progressively during training to criterion but failed to show the expected decline as training continued beyond criterion. In other words, the AV thalamic peak of TIA was abolished in rabbits with these lesions. Lesions of the posterior cingulate cortex or the AD nucleus had the same effects and in addition such lesions increased the magnitude of AV thalamic excitatory TIA in the first session of conditioning, again, disrupting the patterning that results in peaks of excitatory TIA in intact rabbits[8,12,13]. Thus, whereas the development of excitatory and discriminative TIA in limbic thalamus does not depend on inputs from the hippocampal formation or cingulate cortex, efferents from these areas are important and perhaps essential sources of the AV thalamic training-stage-related peaks of TIA. It remains to be determined whether these sources also modulate the peaks of TIA in limbic thalamic nuclei other than the AV nucleus.

Functions of the training-stage-related peaks

The associative character of TIA in limbic thalamus and cingulate cortex, the build-up of neuronal firing in these areas that occurs in anticipation of CR output and the severe impairments of CR acquisition due to limbic thalamic or cingulate cortical lesions have fostered a theoretical model which posits that the TIA in these areas is an essential precursor of the learned response. Specifically, it is proposed that the TIA gives rise to the pre-CR activity in cingulate cortex, i.e. the putative

command volley projected from cingulate cortex to the motor system which triggers the output of the learned response. In the light of this general model, it is interesting to consider the specific mnemonic function of the stage-related peaks of TIA.

The peaks of TIA collectively result in a distinctive topographic distribution of CS elicited excitation in cingulate cortex. For example, in a well-trained rabbit, maximal excitation occurs in layers and cortical projection fields accessed by axon terminals of AVm and AM neurons, whereas minimal excitation occurs in the partially overlapping but distinct layers/fields accessed by AD and AVp neurons. The distribution of excitation is quite different in moderately trained rabbits, and the distribution in moderately trained rabbits differs from that in novices. We offer the tentative working hypothesis that the unique topographic excitation patterns in various training stages code the spatio-temporal context which defines the learning situation. It follows that the unique topographic pattern should be elicitable only by the CS + presented to a trained subject in the conditioning environment.

Although spatial cues do not initiate or direct the locomotor avoidance behavior, spatial cues are important elements of the experimental context which prepare or 'prime' relevant brain circuitry for the avoidance task. Evidence that the avoidance behavior and the peaks of TIA are dependent on the spatial context is available from data demonstrating that the peaks and CR performance are disrupted by changes in background contextual stimuli (including visuo-spatial and olfactory components) of the training environment. Excitatory AV thalamic TIA is enhanced in trained animals when the background illumination level is reduced and odor in the conditioning chamber is altered[12]. Moreover, a role of circuitry involving posterior cingulate cortex and anterior thalamus in spatial encoding processes is supported by recent studies indicating disturbed acquisition and performance in learning tasks that require spatial encoding for their solution[15,17,23,27]. The fact that efferent outflow of the hippocampal formation is importantly involved in spatial information processing[19], and in modulation of the peaks (see above), is consistent with the hypothesis that the peaks reflect neural processing of spatial information. In concert, these findings indicate that spatial information processing and the modulations of the peaks are products of interactions of hippocampal structures with posterior cingulate cortex and limbic thalamus. The encoding of temporal information by the peaks is evidenced by the very nature of the peaks, i.e. the topographic pattern of excitation represented by the peaks changes systematically with time and/or with conditioning trials.

Given that they encode the spatio-temporal context the peaks thus represent a candidate brain pattern for the mnemonic retrieval of the CR. That is, the excitation pattern defined by the peaks may be the 'key' that unlocks the mechanism of CR performance. Suppression of CRs and the accompanying widespread activation of limbic thalamic neurons consequent upon alterations of the training environment[12] can be explained as a result of novelty-induced disruption of the retrieval pattern.

The involvement of the peaks in retrieval processes is in all likelihood not limited to avoidance learning. Instead, we propose that it is characteristic of all learning situations governed by interactions of hippocampal, cingulate cortical and limbic thalamic circuitry. The peaks are thus implicated putatively in the operation of mnemonic processes variously referred to as memory[16], working memory[18], declarative memory[26], cognitive mapping[19] and others, i.e. processes believed to be dependent on the integrity of medial temporal lobe structures. The hypothesis offered here is not in conflict with these theories. It is however, preferentially compatible with theories which maintain that: (a) the mnemonic functions of this system emerge from the interactions between medial temporal lobe, cingulate cortical and diencephalic limbic circuits[16]; and (b) these interactions are fundamentally (though not exclusively) important for context-based retrieval of stored information[11,31]. The particular contribution of the present data is the step that they foster toward the specification of the neural patterns and circuit interactions which define the retrieval process.

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