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Effects of cingulate cortical lesions on avoidance learning and training-induced unit activity in rabbits

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Summary. This study extends an ongoing analysis of the neural mediation of discriminative avoidance learning in rabbits. Electrolytic lesions encompassing anterior and posterior cingulate cortex (area 24 and 29) or ibotenic acid lesions in area 24 only were made prior to avoidance conditioning wherein rabbits learned to step in response to a tone conditional stimulus (CS+) in order to avoid a brief, response-terminated 1.5 mA. foot-shock unconditional stimulus (US). The US was presented 5 s after CS+ onset, in the absence of a prior stepping response. The rabbits also learned to ignore a different tone (CS−) not followed by the US. Multi-unit activity of the caudate and medial dorsal (MD) thalamic nuclei, projection targets of the cingulate cortex, was recorded during learning in all rabbits. Activity was also recorded in area 29 in the rabbits with area 24 lesions. Learning in rabbits with combined lesions was severely impaired and it was moderately retarded after lesions in area 24. MD thalamic and caudate training-induced neuronal discharge increments elicited by the CS+ were enhanced in rabbits with lesions, suggesting a suppressive influence of cingulate cortical projections on this activity. Early-, but not late-developing training-induced unit activity in area 29c/d was absent in rabbits with area 24 lesions, indicating that area 24 is a source of early-developing area 29 plasticity. These results are consistent with hypotheses of a theoretical working model, stating that: a) learning depends on the integrity of two functional systems, a mnemonic recency system comprised by circuitry involving area 24 and the MD nucleus and a mnemonic primacy system comprised by circuitry involving area 29 and the anterior thalamic nuclei; b) corticothalamic information flow in these systems suppresses thalamic CS elicited activity in trained rabbits; c) corticostriatal information flow is involved in avoidance response initiation. An absence of rhythmic theta-like neuronal bursts in area 29b in rabbits with area 24 lesions is attributable to passing fiber damage.

Key words: Multi-unit activity – Conditioning and learning – Neural basis of learning – Limbic functions – Caudate nucleus – Medial dorsal thalamic nucleus – Behaving rabbits

Introduction

Learning and memory at all levels of mammalian phylogeny depend critically on the circuit interactions of limbic thalamus, cingulate cortex and hippocampus (see reviews by Gabriel et al. 1980; Squire 1987; Mishkin and Appenzeller 1987; and Olton et al. 1979; see also Sutherland et al. 1988; Markowska et al. 1989; Squire and Moore 1979; Valenstein et al. 1987). Yet, little is known of the dynamic neuronal interactions and learning-relevant information flow among these limbic structures. This study continues an analysis of these processes during discriminative avoidance learning, wherein rabbits learn to step in an activity wheel in response to an auditory warning stimulus (CS+) in order to avoid a shock unconditional stimulus (US).

Past work has shown that discriminative avoidance learning depends on the integrity of limbic thalamus, the anterior and medial dorsal (MD) thalamic nuclei. MD thalamic lesions retarded behavioral acquisition. Anterior thalamic lesions did not affect acquisition but significantly impaired avoidance in well-trained rabbits (Gabriel et al. 1983). Learning was virtually abolished in rabbits with combined anterior and MD thalamic lesions (Gabriel et al. 1989).

Cingulate cortical and limbic thalamic neurons are massively and reciprocally interconnected and cingulate cortical cells project extensively to the caudate nucleus (CN) (reviewed by Vogt 1985). These connections and avoidance learning deficits in several species due to cin-

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gulate and CN lesions (reviewed by Gabriel et al. 1980) suggest that neurons of limbic thalamus, cingulate cortex and CN are cooperatively involved in mediating active avoidance learning. A major goal of the present study is to confirm the role of cingulate cortex, and to elaborate the separate contributions of the anterior and posterior cingulate cortex, in discriminative avoidance learning of rabbits.

The CN has been implicated in control of locomotor behavior (reviewed by Mogensen 1987) and learning (e.g., R.L. Thompson 1959; Packard and White 1990; Packard et al. 1989). Cingulate cortical projections to CN represent a possible route whereby the training-induced neuronal activity elicited by the CS+ in cingulate cortex may contribute to the initiation of locomotor avoidance responses. Here, recordings of multiunit activity were made in the dorsal CN in rabbits with cingulate cortical lesions to provide preliminary information on learning- and performance-relevant corticostriatal information transfer.

Previous findings foster the expectation that lesions restricted to either the anterior cingulate (area 24) or to the posterior cingulate (area 29) will produce effects similar, respectively, to the effects of MD or anterior thalamic lesions. This expectation has been confirmed in the case of area 29 lesions, which, like anterior thalamic lesions, did not affect acquisition but impaired avoidance behavior in well-trained rabbits (Gabriel et al. 1987). Here we test the hypothesis that area 24 lesions will mimic MD lesions, thus yielding a moderate retardation of behavioral acquisition. Combined area 24 and area 29 lesions are expected to impair learning as severely as combined MD and anterior thalamic lesions.

In keeping with the idea of cooperative thalamic and cortical involvement in avoidance learning, past studies have demonstrated the development during learning of massive CS+ elicited and pre-avoidance neuronal discharges in limbic thalamus and cingulate cortex (reviewed by Gabriel et al. 1988; see also Peterson 1986; Pirch et al. 1985). The fact that this training-induced neuronal plasticity originates in limbic thalamus and is projected to cingulate cortex during CS+ presentation was demonstrated by the virtual abolition of all CS+ elicited excitation in cingulate cortex in rabbits with limbic thalamic lesions (Gabriel et al. 1983, 1989). The discovery that the training-induced anterior ventral (AV) thalamic discharges were enhanced in rabbits given area 29 lesions or lesions of the dorsal subicular complex of the hippocampal formation (Gabriel et al. 1987) indicates that input from cingulate cortex and/or the hippocampal formation is not necessary for the development of thalamic plasticity. Indeed, the enhancement of thalamic discharges in rabbits with area 29 lesions suggests that area 29 neurons *suppress* AV thalamic activity. To determine whether this relationship holds for area 24 and the MD nucleus, we here recorded MD activity in rabbits with area 24 lesions.

Aforementioned effects of lesions indicate that whereas area 24 is involved preferentially in behavioral acquisition and area 29 is involved preferentially in mediating maintained performance of the well-learned behavior, each area alone is capable of mediating behavioral acquisition and maintained performance. If this hypothesis is correct, area 29 neurons should exhibit learning-relevant plasticity in rabbits with area 24 damage. However, in correspondence with the expected retardation of behavioral learning, one could also expect the neuronal plasticity in area 29 to develop slowly in the absence of benefit from the early-learning neurons of area 24. Here, these hypotheses are tested by recording area 29 multiunit activity in rabbits with area 24 lesions.

Methods

Subjects, electrodes, surgery and lesions

The subjects were 35 male New Zealand White rabbits, weighing 1.5–2.0 kg. on delivery, maintained on ad libitum water and one cup daily of rabbit chow throughout the experiment. We have found this dietary restriction to be compatible with good health, while preventing obesity. After adaptation to living cages, the rabbits were prepared for surgical implantation of fixed-position intracranial electrodes for recording of multi-unit and electroencephalographic (EEG) activity. Three or four electrodes were implanted in each rabbit through burr holes (diameter = 0.5 mm) drilled in the skull over target sites in the brain. The electrodes were made from stainless-steel insect pins (un-insulated shaft diameter = 0.28 – 0.30 mm) coated with an insulating material (Epoxylite) which was removed from the tips to form 10–50 micron recording surfaces with electrical impedance ranging from 500 KΩ to 2 MΩ. Miniature teflon electrode guides (2.5 mm in length) impaled on stainless-steel insect pins were positioned over each burr hole and affixed to the skull using dental acrylic. The pins were removed after hardening of the cement, leaving a teflon guide permanently implanted over each hole. Recording electrodes were press fitted through the 0.013-in. channels in the teflon guides, and pushed to their stereotaxic target sites (Girgis and Shih-Chang, 1981) in the brain using a rod attached to the stereotaxic manipulator. Because the rod was not attached to the electrode the risk that slight movements of the rabbit (e.g., due to respiration) would disrupt the recording of action potentials was minimized. Surgical anesthesia was induced by injection of 18.75 mg of chlorpromazine HCl in solution followed by 37.50 mg of pentobarbital sodium in solution, into the marginal vein of the pinna. Neuronal activity was monitored during electrode lowering as an aid to placement. An assembly consisting of a miniature 9-contact connector, with wires from the electrodes soldered to the contacts, was attached to the skull with screws and dental acrylic. A stainless-steel machine screw threaded into the frontal sinus and connected to one of the contacts served as the reference electrode.

Seven rabbits received bilateral electrolytic lesions in cingulate cortex (areas 24 and 29). Current was delivered via stainless-steel insect pins coated with Epoxylite, which was removed to expose 0.50–0.75 mm at the tips of the pins. The pins were positioned in 9 sites, each 1.5 mm ventral to brain surface and 1.2 mm lateral to midline, from 13 mm anterior to 11 mm posterior to bregma in each hemisphere. The distance between adjacent sites within-hemispheres was 3.0 mm. A 1.0 mA. D.C. cathodal current was passed for 30 sec. at each site. Twelve controls underwent surgery for the implantation of recording electrodes but no lesions were made. Electrodes for lesion induction were not lowered in controls as the large number of penetrations required to make "sham" lesions may have produced actual lesion effects. Two unit recording electrodes were implanted in the dorsal anterior CN in the rabbits with lesions, and either one or two electrodes were implanted in the same region in controls. Projecting axons of cingulate cortical neurons have been indicated in a variety of species to terminate in this region of the CN (Carman et al. 1963; Carman et al. 1965; Domesick 1972; Kemp and Powell 1970; Yeterian and Van Hoesen 1978; Beckstead 1979; Barnes et al. 1980).

Monoaminergic and cholinergic fibers course through the supracallosal stria of the anterior cingulate cortex enroute to the posterior cingulate cortex (Morrison et al. 1981). In order to minimize damage to these fiber systems, which could disrupt the functioning of the posterior cingulate, fiber-sparing ibotenic acid (IBO, 5 μg/μl) infusions were administered in 13 rabbits along the midline in anterior cingulate cortex at six sites 1.5 mm apart from 1 to 7.5 mm anterior to bregma. The IBO solution (5 μg/μl of ibotenic acid mixed in isotonic saline) was drawn into a 28 gauge injection cannula attached via oil-filled PE tubing to a 25 microliter syringe held in an infusion pump. The cannula was lowered under stereotaxic control to the most ventral region of the midline cortex. Ibotenic acid was infused at a rate of 0.8 μl/min. During the infusion, the cannula was raised 0.3 mm every 15 s and the infusion pump was stopped when the cannula tip reached a position 0.3 mm ventral to brain surface. Recording electrodes were implanted in the dorsal CN (see above), the lateral division of the MD thalamic nucleus, and in two regions of the posterior cingulate cortex (areas 29b and 29c). The stereotaxic target in area 29b was 9 mm posterior to bregma, 2 mm lateral to the midline and 3–4 mm beneath the cortical surface in a region adjacent to the presubiculum. The target site in area 29c was in the hemispheric medial wall 1–4 mm beneath brain surface and 3–4 mm posterior to bregma. Eighteen controls underwent surgery for the implantation of recording electrodes. Four of these received sham (normal saline) injections as indicated above and 14 received no injections.

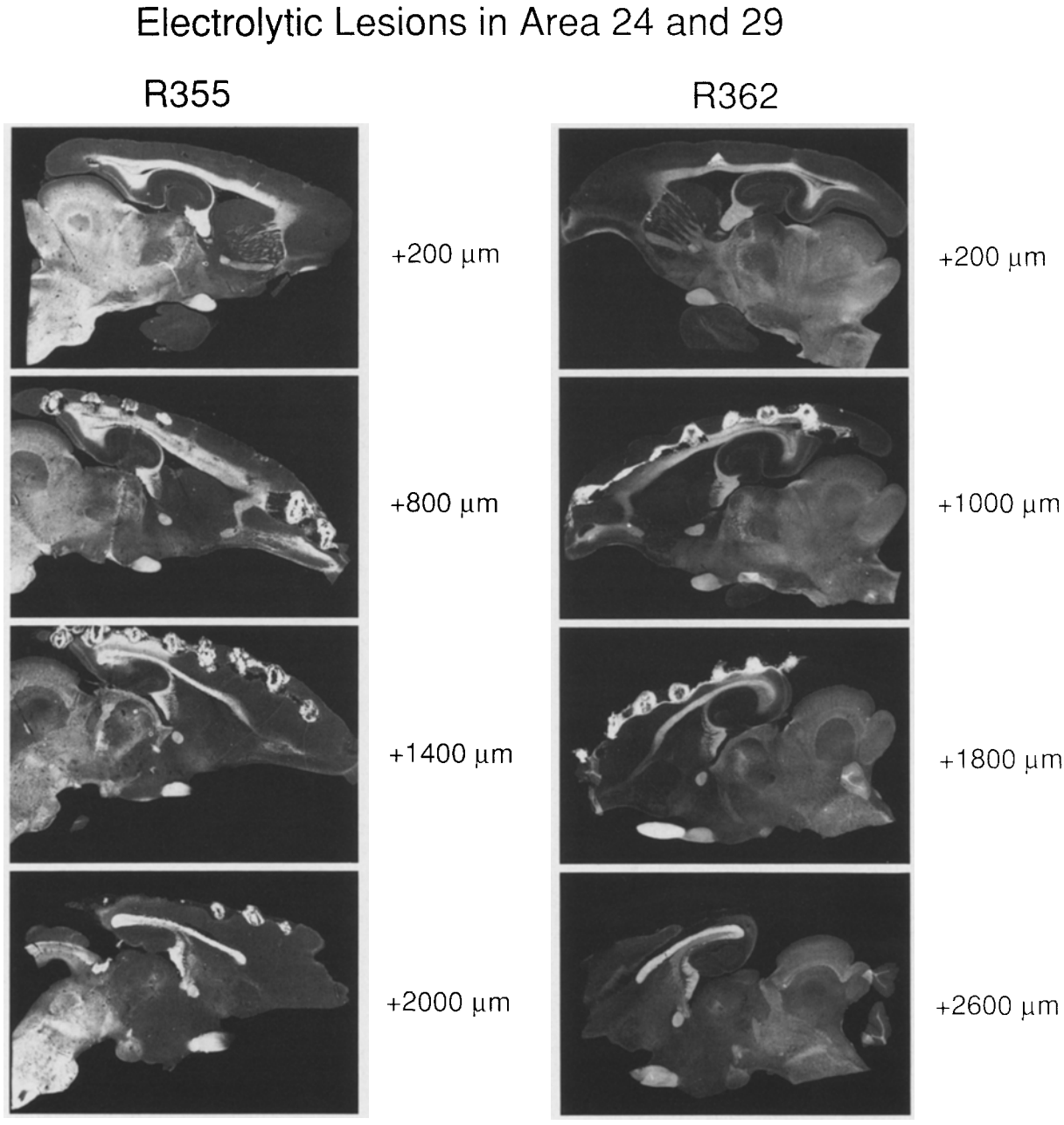

Fig. 1. Each column shows a series of 40-μm saggital sections cut through anterior and posterior cingulate cortex every 400 microns from 0.3 to 1.9 mm lateral to midline. The white areas show the central regions of the largest *(left column)* and smallest *(right column)* cingulate cortical electrolytic lesions

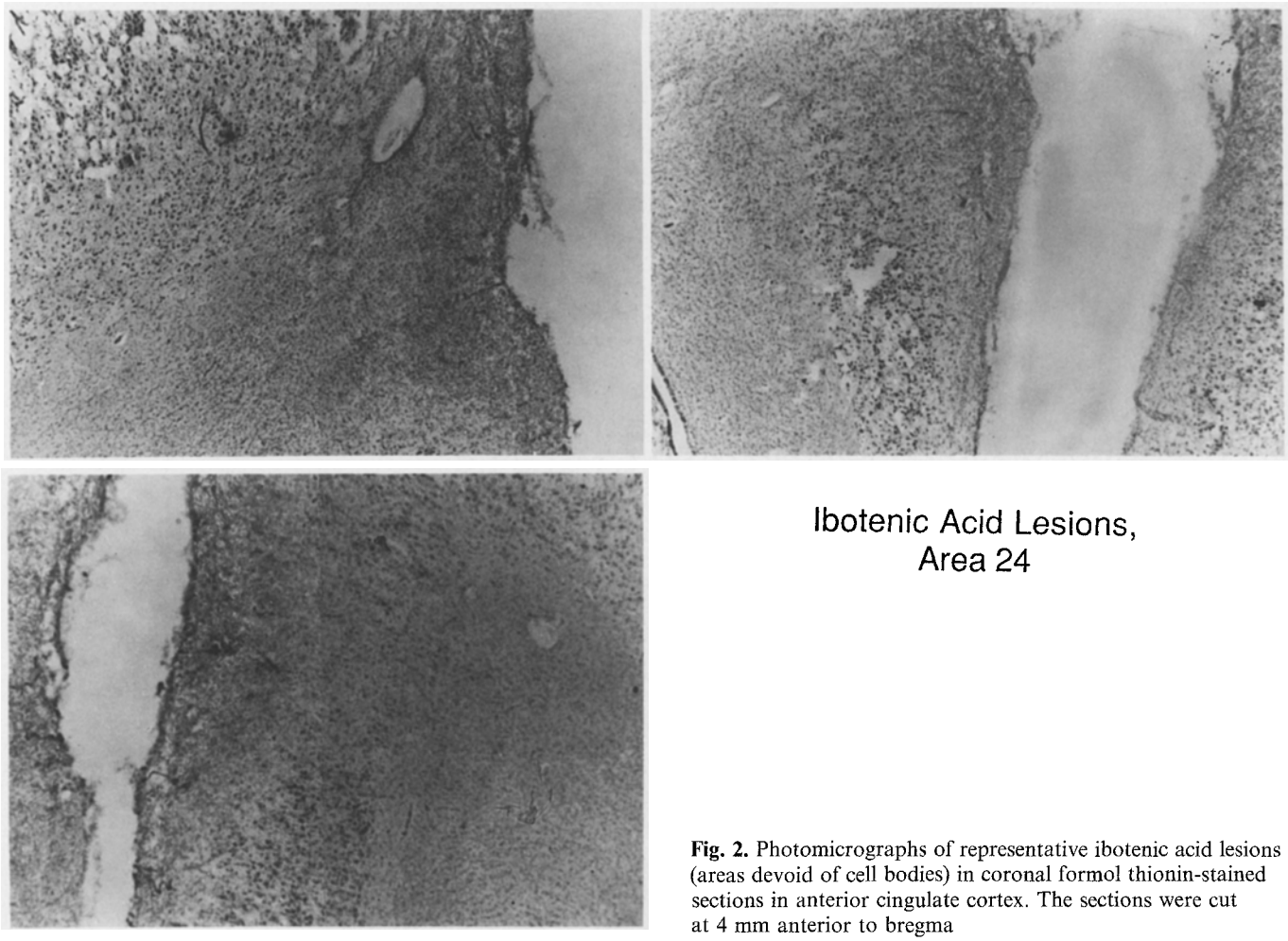

Fig. 2. Photomicrographs of representative ibotenic acid lesions (areas devoid of cell bodies) in coronal formol thionin-stained sections in anterior cingulate cortex. The sections were cut at 4 mm anterior to bregma

Histology

To identify the lesion and recording sites after testing, the rabbits received an overdose of pentobarbital sodium, and transcardiac perfusion with normal saline followed by formalin. The brains were frozen and sectioned at 40 microns and the sections were photographed while still wet (Fox and Eichman 1959). After drying, the sections were treated with a metachromatic Nissl and myelin stain using formol-thionin (Donovick 1974).

Lesions and experimental conditions

The amount of damage produced by electrolytic lesions was quite similar for all eight of the rabbits that received combined area 24 and area 29 lesions (see Fig. 1).

The ibotenic acid lesions were more variable than the electrolytic lesions. The percentage of anterior cingulate cortical volume exhibiting ibotenic acid-induced cell loss (Figs. 2 and 3) was estimated for each rabbit by counting the number of 0.5-mm grid squares covering the lesion and the number covering spared tissue in each of six coronal sections spaced 1.0–1.5 mm apart from bregma to 6 millimeters anterior. A single damage score was obtained for each rabbit by averaging the percentages of damage for each section. The percentage of damage in rabbits given ibotenic acid injections varied from 94.2% to 17.7% (Mean = 48.86%). Two rabbits that received ibotenic acid injections had less than 10% damage.

Eleven rabbits with ibotenic acid lesions were assigned to the experimental group. The two rabbits with damage scores under 10% were assigned to the control group, which also contained 4 rabbits given saline injections, and 14 rabbits with relevant recording electrode placements that received no injections. Contrasts of controls and rabbits with lesions in the statistical analyses employed the large group combining non-injected and injected controls ($N = 20$), and, in certain instances, the injected controls alone ($N = 6$). The large group is referred to henceforth as the control group whereas the smaller, injected group is referred to as the injected controls.

Distribution of neuronal records

After discarding multi-unit records exhibiting peak-to-peak amplitudes less than 25 μV, and records exhibiting no significant CS-elicited discharges, a total of 92 records were analyzed. The recording sites in CN and the MD nucleus are shown in Figs. 4 and 5, and the numbers of records obtained in each group are shown in Table 1.

Behavioral training

One to two weeks after surgery, the rabbits underwent differential conditioning 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 data collection equipment. 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,

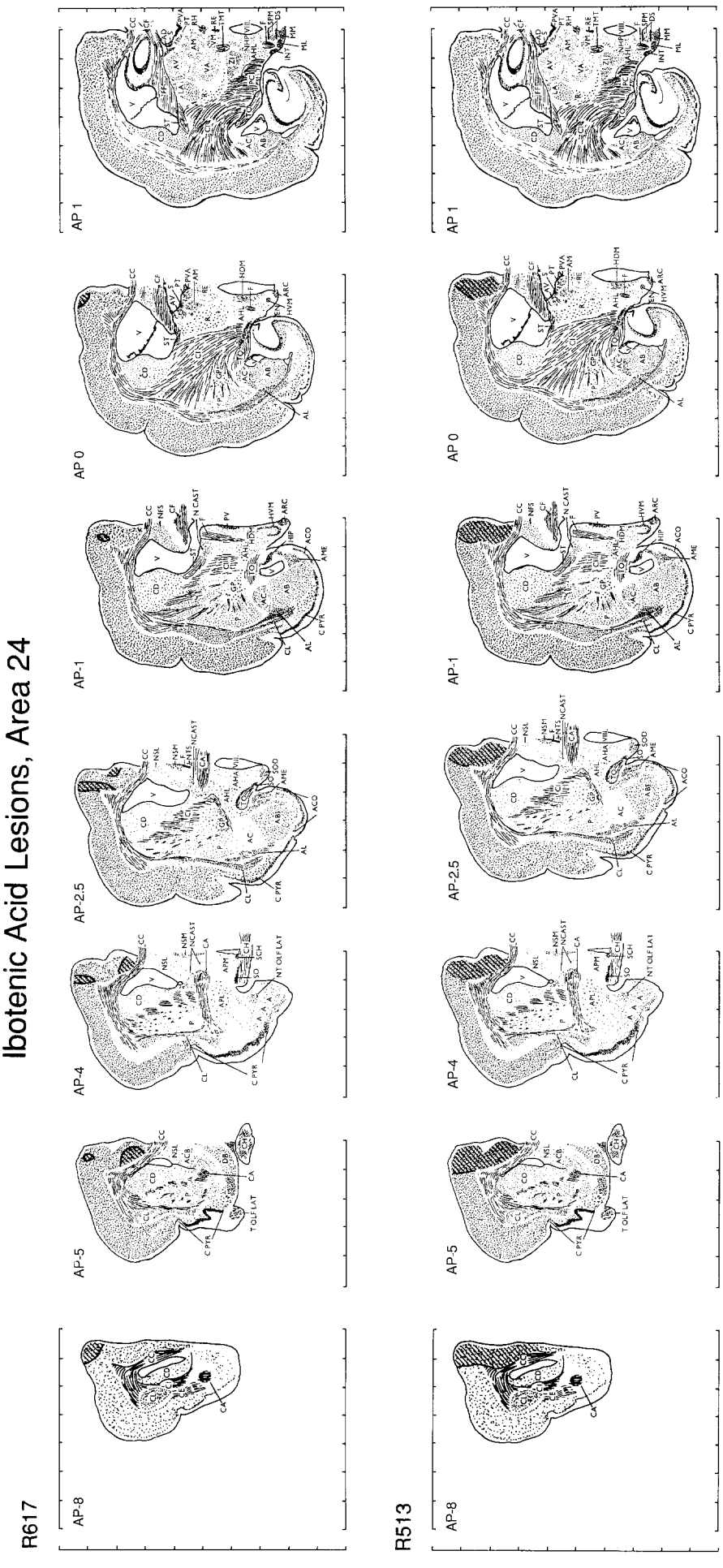

Fig. 3. The largest (R513) and smallest (R617) ibotenic acid lesions in coronal sections through the anterior cingulate cortex (area 24). The sections were based on drawings in the stereotaxic atlas of Fifkova and Marsala, presented in Bures et al. (1967). The position in the anterior-posterior dimension is indicated at the upper left of each section, in millimeters from bregma. Negative numbers indicate sections anterior to bregma. The percentage of damage due to the lesions was estimated by counting the number of 0.5-mm grid squares covering lesion and the number covering spared tissue in each of six coronal sections spaced 1.0–1.5 mm apart from bregma to 6 mm anterior. A single damage score was obtained for each rabbit by averaging the percentages of damage for each section. The percentage of damage in rabbits given ibotenic acid injections varied from 94.2% to 17.7%. Two rabbits with less than 10% damage were placed in the injection control group

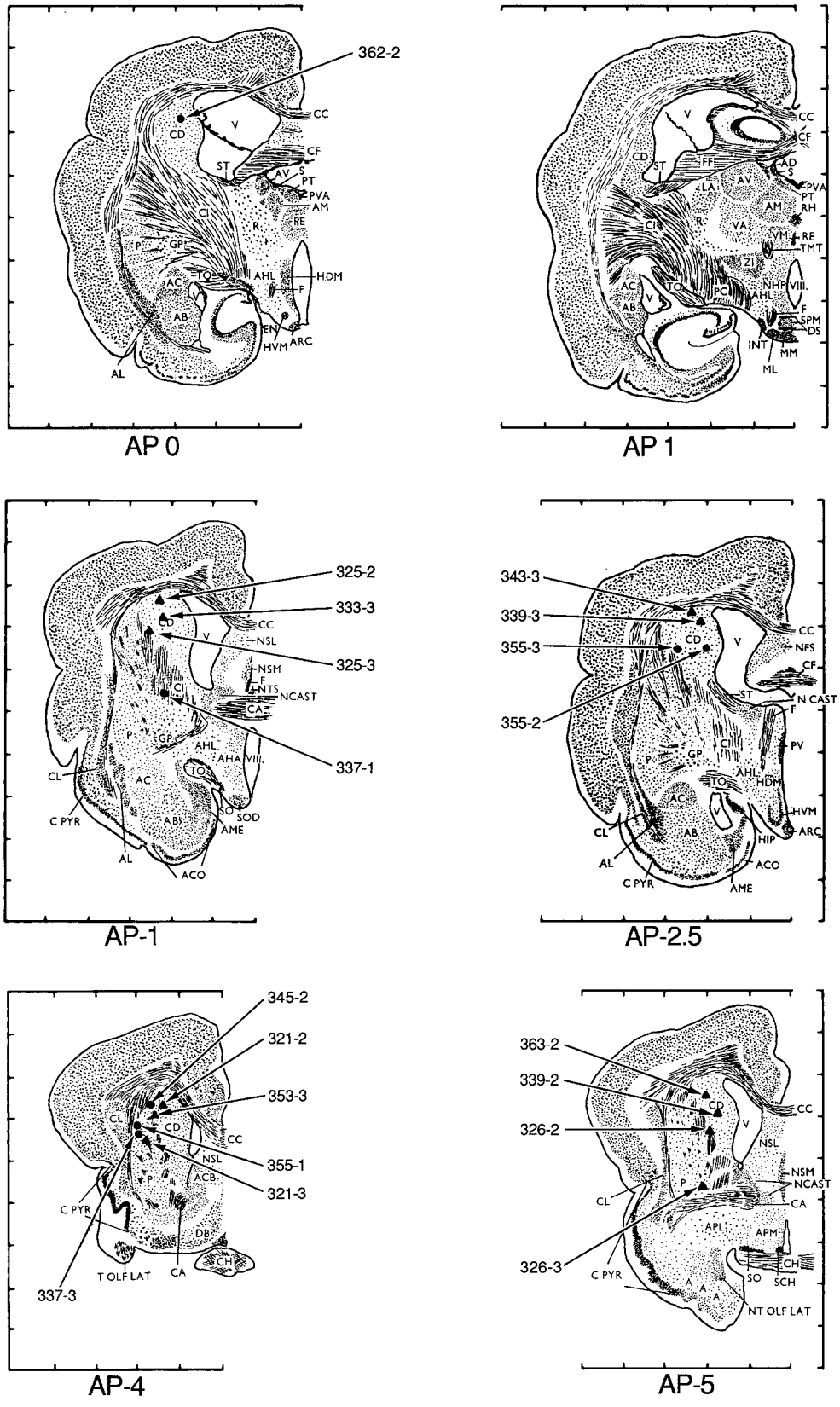

Fig. 4. The recording sites in the caudate nucleus (CN) are shown on drawings of coronal sections at six anterior-posterior (AP) levels in millimeters from bregma, for rabbits with anterior and posterior cingulate cortical lesions *(circles)* and controls *(triangles)*. Two of the electrodes were located in the putamen, a paleostriatal area. No significant differences were found in a control analysis that compared the activity recorded with these electrodes to that recorded with neostriatal electrodes. The drawings of the coronal sections are based on the rabbit stereotaxic atlas of Fifkova and Marsala presented in Bures et al. (1967). The scale marks on the ordinate and abscissa represent 2-mm intervals. The distribution of CN recording sites in rabbits with area 24 lesions and in the controls for these lesions was highly similar to that shown here, except that none of the electrodes were located in the putamen

85 dB re 20 N/m^2, rise time = 3 ms) played through a speaker located above the wheel. Onset of the positive conditional stimulus (CS+) was followed after five seconds by onset of the unconditional stimulus (US, a constant current [1.5–2.5 mA.] foot-shock delivered through the grid floor of the wheel). The CSs, presented in an irregular order, and the US were terminated by behavioral responses, defined as wheel rotations exceeding two degrees. Responses after CS onset but prior to US onset prevented US administration. In the study of the effects of combined lesions the maximum CS duration was 6 s on trials without responses. In the

Table 1. Distribution of neuronal records. The numbers and sites of the neuronal records

Caudate neuronal activity in rabbits with combined area 24 and area 29 lesions	
Lesions	7
Controls	12

Activity in rabbits with ibotenic acid lesions in area 24				
	MD nucleus	Area 29b	Area 29c/d	Caudate nucleus
Lesions	9	7	7	10
Controls	12	13	11	4

study of the effects of ibotenic acid lesions in area 24, the maximum CS duration was 0.5 s. The maximum duration of the US, given failure of response, was 1 second. The CS− (the frequency, 1 KHz or 8 KHz, not chosen for the CS+) was also response-terminated, but it was not followed by the US. The interval from the end of a trial (defined as the end of the 5 s period following CS onset, or of wheel-rotation when locomotion occurred) to CS onset defining the beginning of the next trial was 8, 13, 18, or 23 s. These values occurred in an irregular sequence. Responses reset this interval.

The rabbits received 60 trials with the CS+ and 60 trials with the CS− daily, until a criterion was reached. The criterion required that the proportion of trials with behavioral responses to the CS+ (i.e., avoidance responses) exceed the proportion of trials with responses to the CS− by 0.60 or more, in two consecutive sessions. This criterion yields an asymptote of discriminative performance, i.e., the performance levels yielded by this criterion do not change significantly if further training is given. Training was terminated if criterion was not attained after 15 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, with the same timing and ordering as in training. In the first pretraining session, the tones only were presented. In the second, US presentations were interspersed among the tones in a noncontingent, explicitly unpaired manner (see Rescorla 1967). The frequency and temporal distribution of the US during these sessions was identical to the average values of these parameters obtained during the initial session of conditioning for a sample of 100 rabbits. The only differences between the PT session and actual training were that the US was not presented during a tone or within three seconds prior to or following a tone in PT. Because the CS did not predict the US during PT, US avoidance was not learned. The PT procedures provided baseline data for detecting associative neural and behavioral changes brought about by pairing the CS with the US during training.

Collection of neural data

Multi-unit records were fed during training into field-effect transistors (FETs) attached to the connector that mated with the cranial socket, about 2.5 cm from the recording sites within the brain. The FET outputs, conducted via a 3 ft. shielded cable, were split, one limb entering single-ended preamplifiers with bandwidth appropriate for unit recording (gain = 20,000, 1/2 amplitude cutoffs as 500 and 8000 Hz) the other limb entering preamplifiers for EEG ("field potential") recording (gain = 4000, 1/2 amplitude cutoffs at 0.2 and 60 Hz). The unit activity was subjected to a second stage of active bandpass filtering (1/2 amplitude cutoffs at 600 and 8000 Hz, roll-off = 18 dB/octave) to remove all slow EEG frequencies. The unit records were then fed to Schmitt triggers, which were adjusted by the data collection computer to yield a mean rate of outputs (80

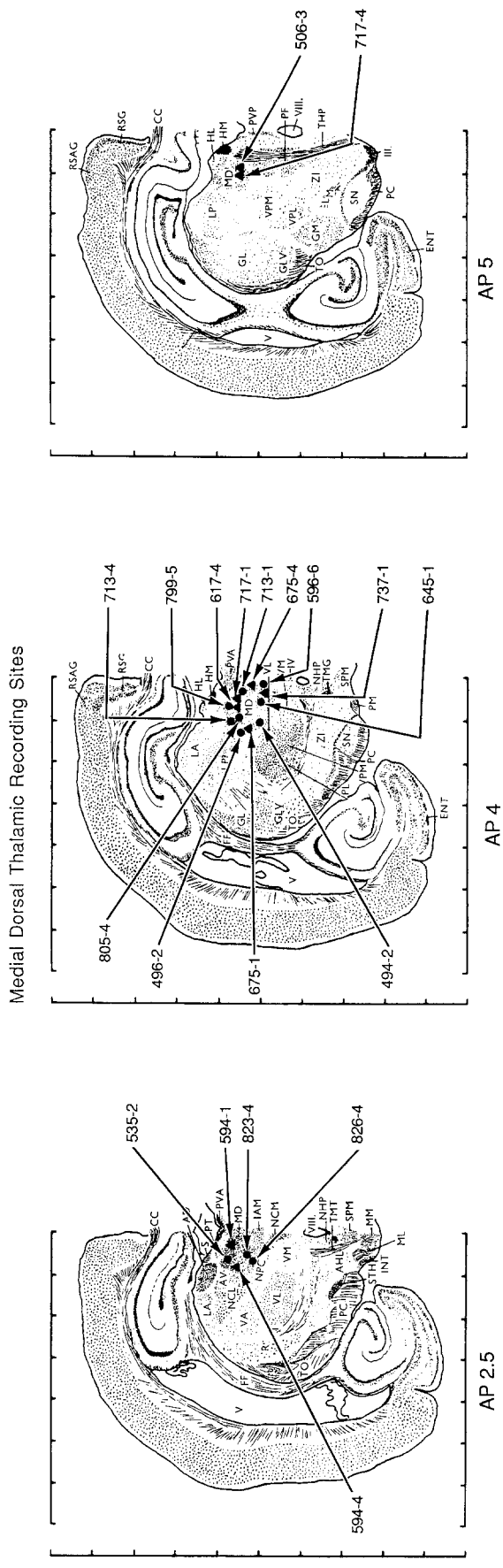

Fig. 5. The medial dorsal (MD) thalamic recording sites are shown on drawings of coronal sections at various anterior-posterior (AP) levels in millimeters from bregma, for rabbits with anterior cingulate (area 24) cortical ibotenic acid lesions *(circles)* and controls *(triangles)*. The drawings of the coronal sections are based on the stereotaxic atlas of Fifkova and Marsala presented in Bures et al. (1967). The ordinate and abscissa scale marks represent 2-mm intervals

micro-second square wave pulses) within limits of 110–190 pulses 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 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 ms respectively. The Schmitt-trigger data provided an index of the discharge frequency of the largest spikes on each record, whereas the integrated unit activity measured energy fluctuations of the entire record, including activity below the triggering thresholds.

The Schmitt trigger pulses were counted and the integrator and field potentials 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.

The digital values obtained on each trial were averaged to form peristimulus histograms and average evoked responses for a given day's training. Separate histograms were made for CS+ and CS− trials, and these were subjected to statistical analysis as described below. The several computer and experimenter-controlled methods used to eliminate movement artifacts from the neural data are described elsewhere (e.g., Gabriel et al. 1983).

Data analysis

Because the number of training sessions required for criterion attainment varied, the analysis focussed on four behaviorally-defined stages of training shared by all rabbits. Each stage was constituted by the data of a single training session. The stages were: a) pretraining with the CSs and noncontingent foot-shock (PT); b) the first exposure to conditioning (FE, the first session of conditioning with paired CS–US presentation); c) the first significant behavioral discrimination (FS, the first session in which significant behavioral discrimination occurred); d) the session in which the learning criterion (CRIT) was attained. The first significant behavioral discrimination was defined as the first session in which the proportion of avoidance responses exceeded the proportion of responses to CS− by 0.25 or more. This value approximates the minimum required to produce a significant chi-square ($P<0.05$) for a difference between correlated proportions (Walker and Lev 1953, p. 101).

The proportion of trials with behavioral conditioned responses was submitted to factorial repeated measures analysis of variance (mixed model) with a single between-subject factor, representing the comparison of rabbits with lesions and controls. In addition, there were two orthogonal repeated measure factors, training stage (four levels: PT, FE, FS and CRIT) and CS (two levels: CS+ and CS−). Additional analyses were computed for the latency and duration of conditioned responses, the number of intertrial responses and the number of sessions required to reach the acquisition criterion.

A similar analysis of variance model was used for the neuronal data in the form of z-scores. These analyses involved a between-subject (lesion/control) factor and orthogonal repeated measures factors of training stage, CS, and interval after CS onset (40 consecutive 10-ms intervals). The significances of the differences between mean neuronal responses were evaluated using planned comparisons (two-tailed *t*-tests) of the average neuronal activity in response to the CS+ with the average response to the CS− at each training stage, for each interval after CS onset (Winer 1962). Also, planned comparisons of activity during pretraining with activity during the various training stages were made at each interval.

Results

Acquisition of the avoidance behavior

The rabbits with combined area 24/area 29 lesions required an average of 12.7 training sessions to complete the acquisition criterion, as compared to 6.1 sessions taken by the controls. Five of the seven rabbits with lesions failed to reach the criterion after 15 training sessions. A significant difference between the group means ($F[1/17]=11.96$, $P<0.005$) demonstrated a severe retardation of conditioned response acquisition in rabbits with lesions. In addition, the mean frequency of conditioned response performance was significantly reduced during training in the rabbits with lesions, as indicated by a significant main effect of the group factor ($F[1/17]=8.07$, $P<0.02$) as well as a significant three-way interaction of group, CS and training stage ($F[4/68]=4.57$, $P<0.003$). Individual comparisons indicated no group differences in response to the CS− in any of the training stages. However, the rabbits with lesions performed conditioned responses to the CS+ significantly less often than controls in all stages of avoidance conditioning ($P<0.01$) after pretraining (Fig. 6). The lesions did not significantly affect the frequency of inter-trial responses. Thus, rabbits with lesions initiated spontaneous locomotion equally as often as controls.

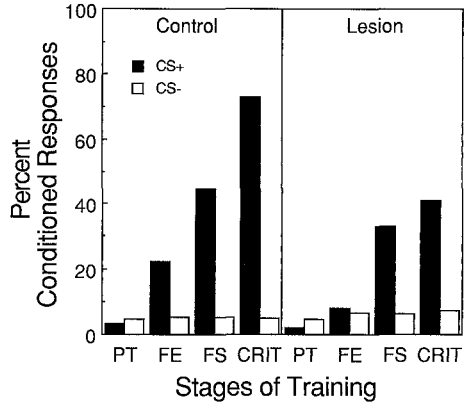

Fig. 6. The average percentage of conditioned avoidance responses performed by rabbits with anterior and posterior cingulate cortical electrolytic lesions and controls. Conditioned response performance is shown during four stages of training, PT, FE, FS and CRIT, as defined in the text. Training of 5 of the 7 rabbits with lesions that failed to attain the acquisition criterion was terminated after 15 training sessions. Conditioned response frequencies entered for the CRIT stage for these rabbits were those attained in the 14th and 15th training sessions

The rabbits with ibotenic acid lesions in area 24 (Fig. 3) required 7.2 sessions and controls required 5.3 sessions to meet the acquisition criterion. This difference approached significance ($F[1/29]=3.17$, $P<0.09$), suggesting a mild retardation of acquisition in the rabbits with lesions. The damage scores and numbers of sessions to attain criterion were positively correlated ($r=0.39$, $P<0.09$) and the correlation would have been significant were it not for a single aberrant case: the rabbit with the largest damage score reached criterion very rapidly. There were no significant effects of the lesions on the percentage of conditioned responses in the various stages of acquisition.

The only other behavioral measure significantly affected by the ibotenic acid lesions was the latency of the unconditioned response (UR), i.e., locomotion elicited by the shock US. The data showed that "escape learning" as indicated by a progressive decrease of UR latency over training stages, occurred in controls but not in rabbits with lesions. Individual comparisons carried out follow-

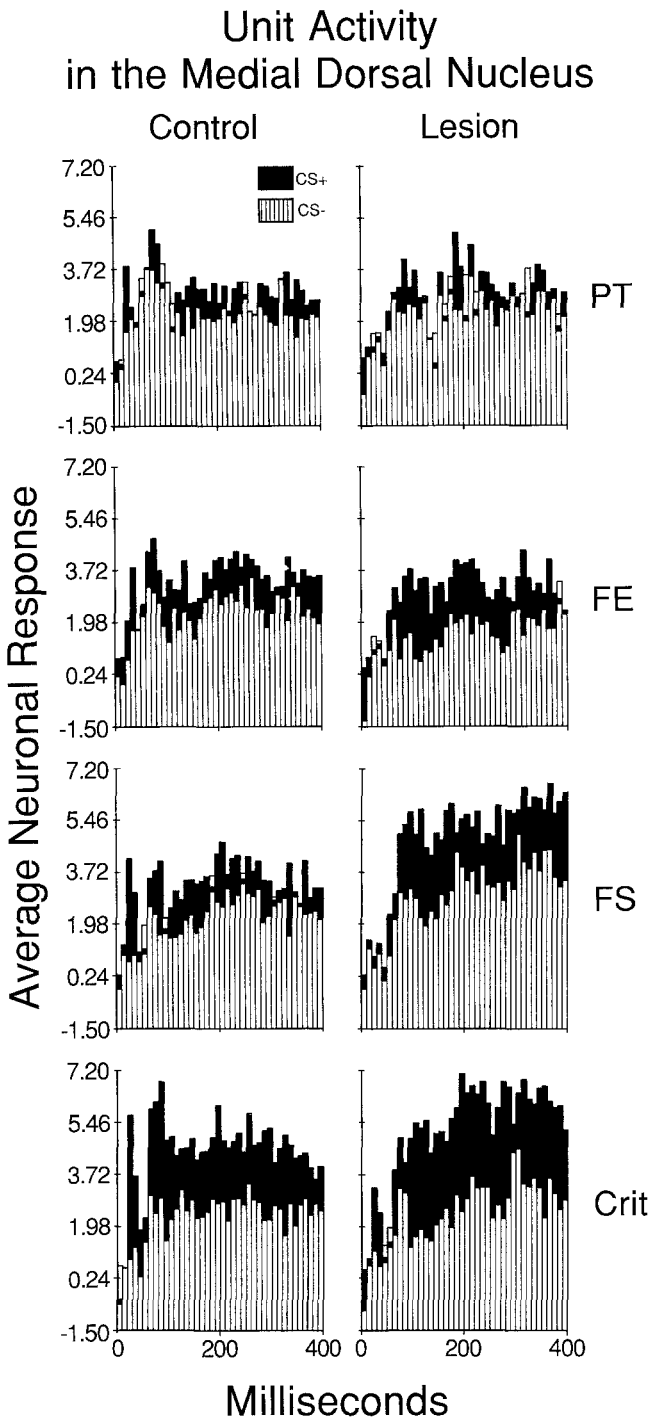

Fig. 7. Average medial dorsal (MD) thalamic multi-unit spike frequency profiles associated with presentation of the CS+ *(black bars)* and CS− *(white bars)* in rabbits with anterior cingulate (area 24) cortical ibotenic acid lesions (*right column* of panels) and in controls (*left column* of panels) during four stages of discriminative avoidance training. Each panel shows discharge frequency in 40 consecutive 10-ms intervals after CS onset, in four stages of training (PT, FE, FS and CRIT). A description of the training stages is provided in the text. The data are in the form of z-scores normalized with respect to a 300-ms pre-CS baseline. The onset of the CS is at the beginning of the first interval. The activity elicited by the CS+ and CS− in rabbits with lesions exceeded significantly the activity in controls during the FS and CRIT stages of training

ing a significant interaction of the lesion and training stage factors ($F[2/58] = 7.65$, $P < 0.002$) indicated that the average UR latencies in rabbits with lesions (216 ms) did not differ significantly from the control average (235 ms) in the first session of conditioning. However, the average latency in controls during the session in which the criterion of learning was attained (177 ms) was significantly reduced ($P < 0.01$) relative to the average (251 ms) in the rabbits with lesions. Moreover, the average latency drop from the first to the criterial session was significant in the controls ($P < 0.05$) but not in the rabbits with lesions.

Activity of MD thalamic neurons

The analyses of spike frequency and integrated unit activity of MD thalamic neurons in 40 consecutive 10-ms intervals (Fig. 7) yielded highly significant interactions of the training-stage, CS and post-CS interval factors, indicating that: a) the average spike frequency elicited by the CS+ increased significantly during the conditioning stages, relative to the frequency during pretraining with tone and unpaired shock presentations; b) significantly greater spike frequencies were elicited by the CS+ than by the CS− during conditioning, but not during pretraining.

Evidence that the lesions affected the training-induced activity of MD neurons was provided by the analysis of the spike frequency data, which yielded significant in-

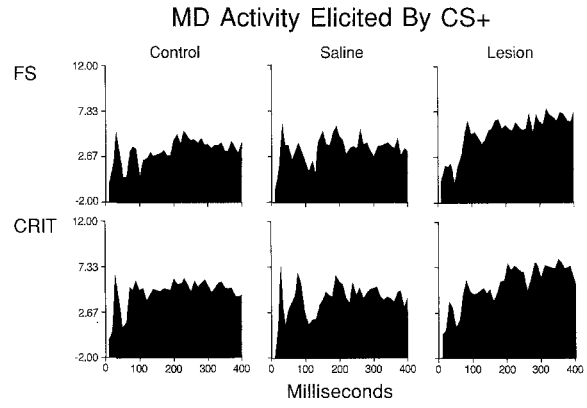

Fig. 8. Average medial dorsal (MD) thalamic multi-unit spike frequency elicited by the CS+ in three groups of rabbits: 1) the no-injection controls that received surgery for electrode implantation but no lesions ($N = 9$); 2) the injection controls that received surgery plus saline ($N = 2$) or ibotenic acid ($N = 2$) injections yielding less than 10% cell loss, and; 3) rabbits that received ibotenic acid injections that induced cell loss magnitudes ranging from 17.2% to 92.2% ($N = 9$). Each panel shows the average spike frequency during 40 consecutive 10-ms intervals after CS+ onset. Data are shown for the two training stages in which the MD neuronal discharges were enhanced in the rabbits with lesions, the session of the first significant (FS) behavioral discrimination (*upper row* of panels) and the session in which the criterion (CRIT) of behavioral discrimination was attained (*lower row* of panels). The data are in the form of z-scores normalized with respect to a 300-ms pre-CS baseline. These data demonstrate MD neuronal discharge enhancement in the rabbits with lesions relative to the injection and no-injection controls. There were no differences between the control groups, indicating that the enhanced discharges in the rabbits with lesions were not due to cannula insertion per se

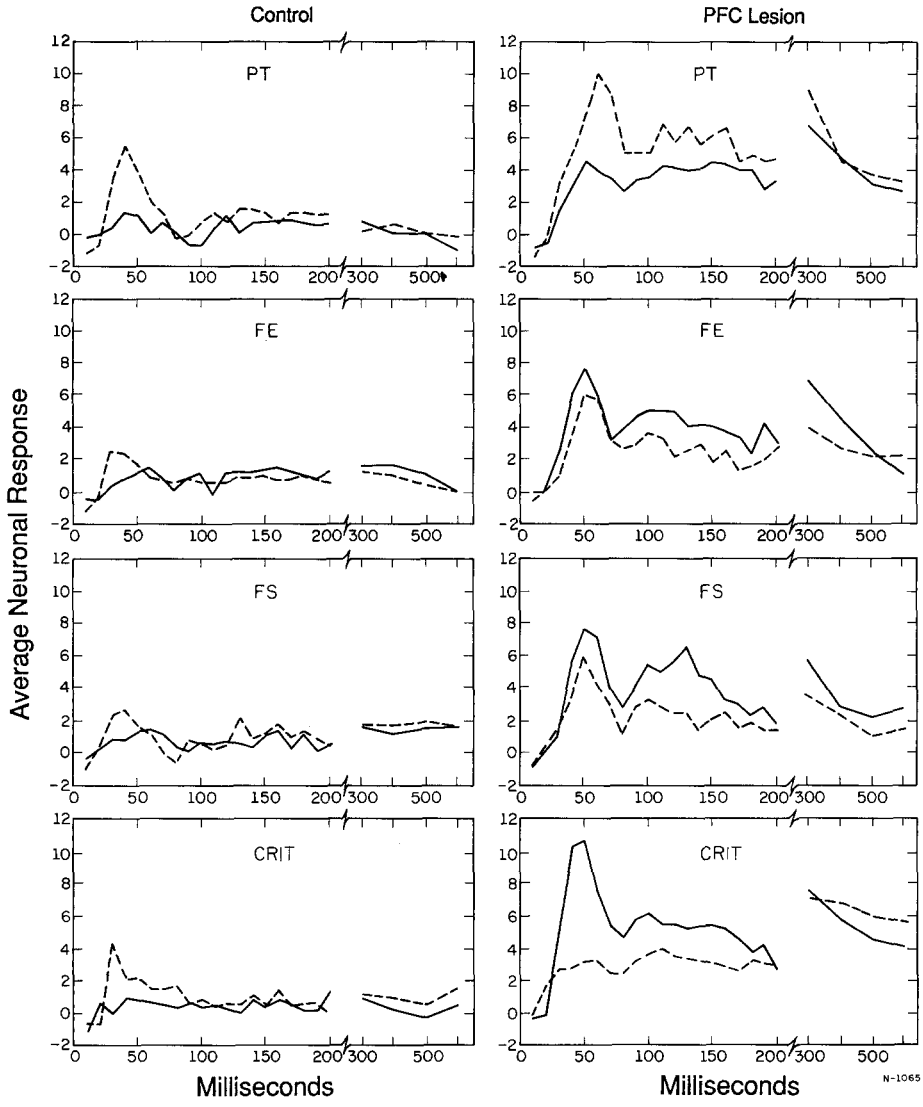

Fig. 9. Average multi-unit firing frequency of caudate nucleus (CN) neurons associated with presentation of the CS+ (solid lines) and CS− (dashed lines) in rabbits with bilateral electrolytic lesions *(right column)* in anterior and posterior cingulate cortex and in controls *(left column)* during four stages of discriminative avoidance conditioning (PT, FE, FS and CRIT), as defined in the text. Each panel shows the average discharge frequency in the initial 20 consecutive 10-ms intervals after CS onset and in the succeeding 4 consecutive intervals of 100 ms after CS onset. The data are in the form of z-scores normalized with respect to a 300-ms pre-CS baseline. The onset of the CS is at the beginning of the first interval. The excitatory and discriminative training-induced discharges in the rabbits with lesions were significantly enhanced relative to controls

teractions of the lesion and training stage factors ($F[3/57]=3.29$, $P<0.03$) and a marginally non-significant interaction of the lesion, training-stage and post-CS interval factors ($F[117/2223]=1.2$, $P<0.08$). The analysis of the integrated unit activity in 40 consecutive 10-ms intervals yielded a significant four-way interaction ($F[117/2223]=1.40$, $P<0.004$). The planned comparisons indicated that the elicited discharges were enhanced in the rabbits with lesions, relative to controls: greater average spike frequencies occurred in response to the CS+ in rabbits with lesions than in controls in 18 10-ms intervals after CS onset (10–12, 17–18, 27, 29–40) during the session of the first significant (FS) behavioral discrimination (third pair of panels in Fig. 7). Greater average discharges in response to the CS− in this session occurred in four intervals (31, 34, 36, 37). During the criterial (CRIT) session 8 intervals (22, 28, 31–33, 35–36, 38–39) showed enhanced activity relative to controls, in response to the CS+ and the enhancement occurred in one interval (30) in response to the CS− (fourth pair of panels in Fig. 7). In addition to the enhancements of MD discharges in rabbits with lesions, the brief-latency excitatory "on" discharge from 20–40 ms after CS onset in controls was absent in rabbits with lesions (all panels of Fig. 7).

In order to examine the possibility that the enhancement of MD activity may have been due to fiber damage in the anterior cingulate cortex an analysis was performed which compared MD activity in the no-injection controls to the activity of the injected controls in the FS and CRIT sessions. No enhancements of the average MD discharges or any trend toward enhancement occurred in the injection controls (Fig. 8). Indeed, the average discharges in FS and CRIT were moderately attenuated in the injection controls, as indicated by a significant interaction of the lesion condition and interval factors (integrated activity only, $P<0.04$). Thus, the enhanced discharges in the rabbits with lesions were not attributable to damage induced by the multiple introductions of the injection cannula.

Neuronal activity in the caudate nucleus

Single area 24 lesions and combined area 24 and area 29 lesions had similar albeit unexpected effects on CN neuronal activity: training-induced activity in CN only occurred in rabbits with lesions. This observation was indicated for the rabbits with combined lesions (Fig. 9) by an analysis of the multi-unit spike frequency in 20 consecutive 10-ms intervals after CS onset, and by an analysis of six consecutive 100-ms intervals. For the 10-ms data, the main effect of the lesions ($F[1/15]=8.15$, $P<0.02$), the interaction of lesions and 10-ms intervals ($F[19/285]=2.46$, $P<0.001$), and the three-way interaction of lesions, acquisition stages and CSs ($F[3/45]=3.08$, $P<0.04$) were significant. For the 100-ms data the main effect ($F[1/15]=12.50$, $P<0.003$) and the four-way interaction of lesions, acquisition stages, CSs and intervals ($F[15/225]=2.92$, $P<0.001$) were significant. The planned comparisons indicated no significant CN neuronal discrimination between CS+ and CS− in controls in any training stage. However, significant discrimination did occur in the rabbits with lesions. The CS− elicited greater neuronal discharges than the CS+ during pretraining, in the first two 100-ms intervals after CS onset ($P<0.05$). This result reflected a sensory bias of the neurons to the particular auditory frequencies used as CS− in the rabbits with lesions. However, significantly greater neuronal firing in response to the CS+ than to the CS− occurred during the remaining three stages of behavioral acquisition, again, in the first two 100-ms intervals after CS onset ($P<0.01$ in all cases).

Inspection of histogram profiles (Fig. 10) suggested that excitatory and discriminative CN unit activity developed during conditioning in rabbits with area 24 lesions but not in controls. In addition, the data in Fig. 10 suggested that an excitatory discharge present at brief latency (20–40 ms) in controls was not present in rabbits with lesions. Significant interactions of the lesion factor with the 10 ms interval factor for the spike frequency measure ($F[39/468]=1.50$, $P<0.03$) and for the integrated unit activity ($F[39/468]=1.54$, $P<0.023$) supported the conclusion that the discharge magnitudes in the rabbits with lesions exceeded those in controls. The planned comparisons carried out for the spike frequency data indicated that significant discrimination between CS+ and CS− occurred in three intervals during FS (5, 22, 23) and the CRIT session (12, 16, 22) in rabbits with lesions ($P<0.05$). No significant discrimination occurred in controls.

The lesions did not affect the average discharge magnitudes in pretraining, the FE or the FS sessions. However, the average discharge magnitude in response to the CS+ during the CRIT session in rabbits with lesions exceeded significantly the average in controls in 12 intervals ($P<0.05$ in all cases). Also, loss of the brief-latency excitatory discharge in rabbits with lesions was indicated by significantly greater discharges ($P<0.05$) from 20–40 ms after CS onset in controls than in rabbits with lesions during the pretraining, FE and FS sessions. Essentially the same results were obtained from similar comparisons carried out for the integrated unit activity.

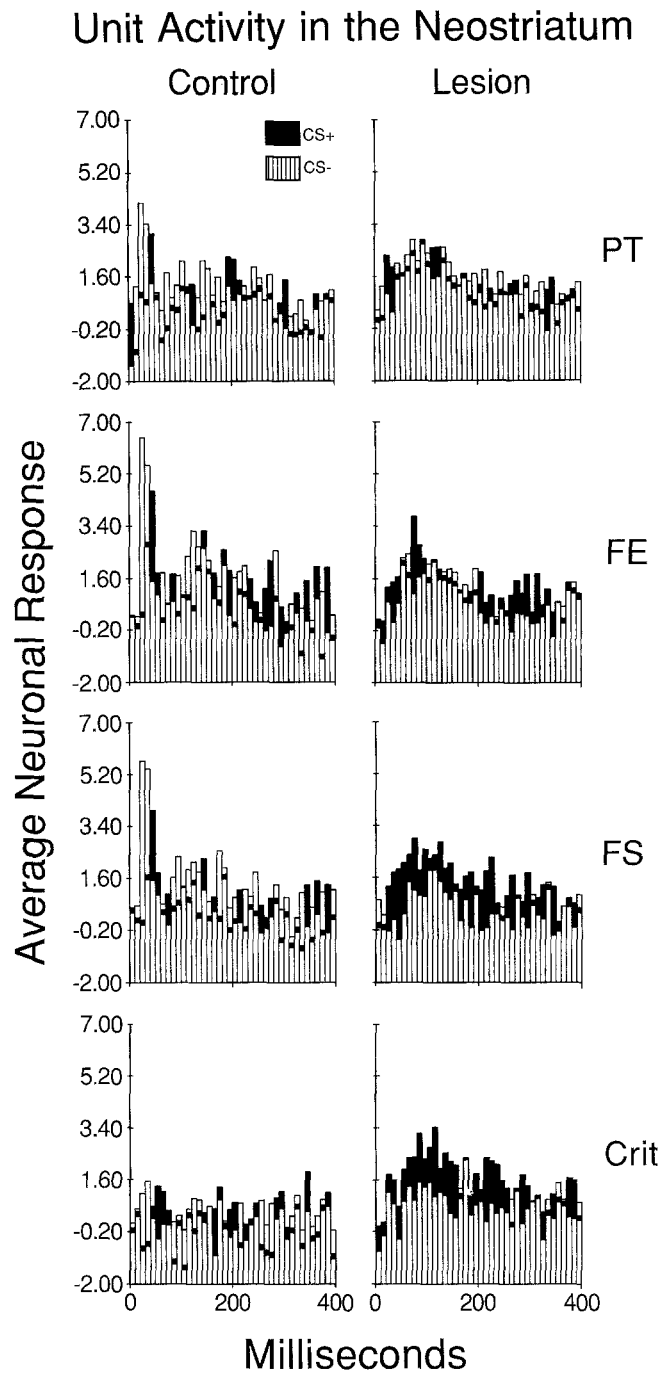

Fig. 10. Average multi-unit firing frequency of caudate nucleus (CN) neurons associated with presentation of the positive conditional stimulus (CS+, *black bars*) and the negative conditional stimulus (CS−, *white bars*) in rabbits with bilateral ibotenic acid lesions in the anterior cingulate cortex (area 24) and controls during four stages of discriminative avoidance conditioning (PT, FE, FS and CRIT) as defined in the text. Each panel shows the average spike frequency in the initial 40 consecutive 10-ms intervals after CS onset. The data are in the form of z-scores normalized with respect to a 300-ms pre-CS baseline. The onset of the CSs is at the beginning of the first interval

Neuronal activity in the posterior cingulate cortex

The lesions in area 24 removed early developing training-induced activity in area 29, but they did not affect later developing activity. As in several past studies (e.g., Gabriel et al. 1987) the magnitude of the area 29c/d neuronal discharges elicited by the CS+ and CS− in controls was enhanced, relative to the pretraining magnitude, during FE and FS sessions. This enhancement was absent in rabbits with area 24 lesions. Rabbits with lesions and controls exhibited significantly greater discharges in the asymptotic (CRIT) training session relative to pretraining, but the discharges in rabbits with lesions during criterion were greater than the discharges in controls (Fig. 11). (Rabbits with lesions and controls exhibited robust and equivalent neuronal discrimination between CS+ and CS− during training). The foregoing observations were indicated by the analysis of the integrated unit activity in area 29c/d, which yielded a significant interaction of the lesion, training stage and interval factors ($F[117/1872]=1.46$, $P<0.002$), and by an interaction of the lesion, training stage, CS and interval factors ($F[117/1872]=1.82$, $P<0.0001$). Individual comparisons demonstrated greater discharges in controls than in rabbits with lesions during the FE and FS sessions in virtually every post CS 10-ms interval after the 12th interval. The average discharges in rabbits with lesions exceeded those in controls in intervals 8–19 during the criterial session. No significant differences were found during pretraining.

No significant effects of the area 24 lesions were observed in the analysis of area 29c/d spike frequency data. These results indicate that the foregoing effects of the lesions were specific to the integrated activity measure. They were not reflected by the firing of relatively large amplitude multi-unit spikes.

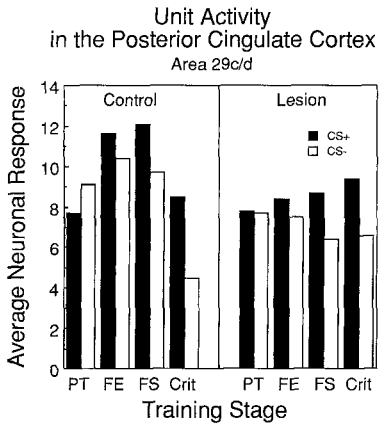

Fig. 11. Average posterior cingulate cortical area 29c/d integrated unit activity elicited by the CS+ *(black bars)* and CS− *(white bars)* in controls and in rabbits with bilateral ibotenic acid lesions (Figs. 2 and 3) in the anterior cingulate cortex during the stages of discriminative avoidance conditioning (PT, FE, FS and CRIT) as defined in the text. Each bar represents the average of 40 z-scores (from 0–400 ms after CS onset), normalized with respect to a 300-ms pre-CS baseline. These results indicate that the area 24 lesions eliminated the early-developing training-induced activity elicited by the CS+ and CS−

The area 29b neuronal records in controls exhibited characteristic rhythmic, theta-like bursts of multi-unit firing not seen in area 29c/d (Mignard et al. 1987). That this activity was time-locked to the onset of the CSs is indicated by the fact that the bursts are evident in the average spike frequency histogram profiles (Fig. 12, left column). The bursts were absent from area 29b in the rabbits with area 24 lesions (Fig. 12, right column). The analyses of these data yielded a marginally non-significant interaction of the lesion condition, training stage and interval after CS onset ($F[117/2106]=1.22$, $P<0.06$), and individual comparisons showed that the average discharges in rabbits with lesions from 70–130 ms after CS onset were of greater magnitude in all training stages ($P<0.05$ in all cases) than those in the intact controls. During this interval, the average control records exhibited the first theta-like burst and the first post-burst inhibitory pause. These effects were replaced in rabbits with area 24 lesions by average non-rhythmic firing that exceeded the average firing in both the burst and pause phases of the control record. Although there were no significant differences in the later intervals, inspection of Figure 12 reveals that the loss of the first theta-like burst in rabbits with lesions carried through the later intervals, as little or no theta-like activity in rabbits with lesions occurred during these intervals. Analysis of area 29b integrated unit activity yielded a significant interaction of the lesion, training stage, CS and interval factors ($F[117/1872]=1.35$, $P<0.01$), and individual comparisons indicated greater excitatory discharges at many intervals after CS+ onset in trained rabbits with lesions than in controls. Because the integration procedure results in a smoothing of the discharge profiles, these data did not allow a clear interpretation of the effects of the lesions on the rhythmic discharge bursts. However, it is likely that the increased magnitudes of the integrated discharges were due to the loss of the firing pauses as documented in the spike frequency data.

The electrodes were concentrated in layers III–V in area 29c/d and in area 29b. Chi-square tests did not indicate significantly different laminar distributions in the rabbits with lesions than in controls.

An analysis comparing the neuronal activity of the injection controls to that of the no-injection controls for area 29b showed a loss of rhythmic, theta-like bursts in the injection controls. Thus, the injection procedure per se and possible consequent damage to passing fibers in area 24 would appear to be sufficient to account for the loss of rhythmic activity in area 29. It is likely that the loss of rhythmic bursting was due to disruption of passing norepinephrine (NE) containing fibers (see Morrison et al. 1981) as single injections of six-hydroxydopamine into the region of the area 24/area 29 border depleted NE in area 29b and resulted in a loss of rhythmic activity (Sparenborg and Gabriel 1987). However, neither the 6-OHDA injections or lesions of the diagonal band of Broca that yielded cholinergic deafferentation in area 29 (Kubota et al. 1990) abolished area 29c/d early-developing training-induced excitation as seen after area 24 lesions in the present study. It is thus likely that this loss was due to disruption of neurotransmission from area 24.

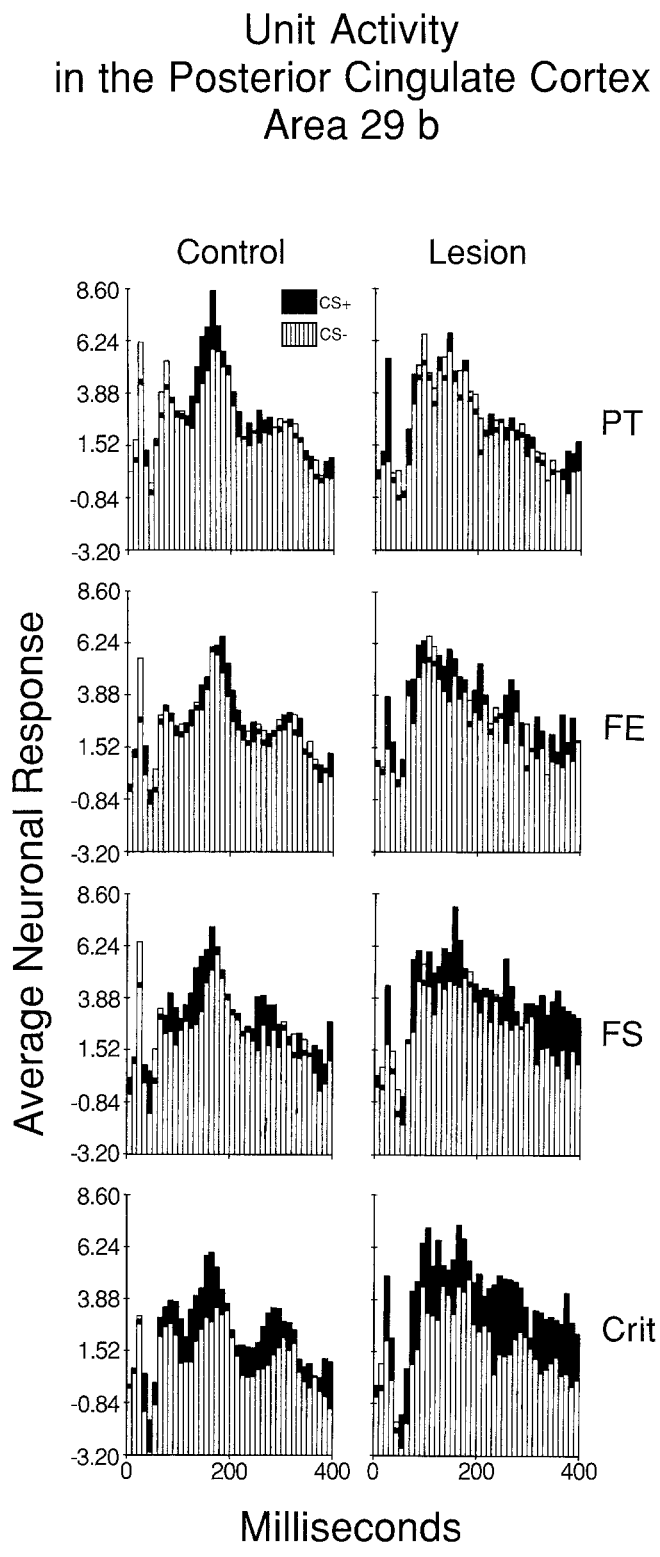

Fig. 12. Average posterior cingulate cortical area 29b multi-unit spike frequency profiles associated with presentation of the positive conditional stimulus (CS+, *black bars*) and the negative conditional stimulus (CS−, *white bars*) in rabbits with bilateral ibotenic acid lesions in anterior cingulate cortex and in controls during four stages of discriminative avoidance conditioning (PT, FE, FS and CRIT) as defined in the text. The data are in the form of z-scores normalized with respect to a 300-ms pre-CS baseline. The onset of the CSs is at the beginning of the first interval. The lesions eliminated the conditional stimulus elicited rhythmic theta-like bursts of unit firing present in area 29b records in controls

Discussion

The avoidance learning of rabbits with combined lesions of anterior and posterior cingulate cortex (area 24 and area 29) was severely impaired. It is unlikely that the impairment resulted from motivational or motor deficiency (e.g., elevated pain thresholds or inability to locomote) as the latencies and durations of behavioral responses to the shock in rabbits with lesions were indistinguishable from those in controls, and the rabbits with lesions initiated spontaneous (intertrial) locomotions as frequently as controls. It is quite likely that the lesions interfered with passing monoaminergic and cholinergic fibers in the cingulum bundle and supracallosal stria enroute to cingulate cortex and the hippocampal formation (e.g., Morrison et al. 1981). However, disruption of these fibers cannot account for the observed behavioral deficits, as deliberate depletion of NE fibers in cingulate cortex and hippocampal damage per se do not disrupt avoidance learning (Sparenborg and Gabriel 1987; Gabriel et al. 1987). These results thus extend to wheel-running avoidance in rabbits the conclusion based on results obtained with rats, cats and dogs that cingulate cortex is importantly involved in mediating the acquisition of active avoidance behavior.

A statistically marginal retardation of learning, and normal asymptotic performance, occurred in rabbits that received ibotenic acid lesions in area 24. As previously noted (see Introduction) similar results followed MD thalamic electrolytic lesions (Gabriel et al. 1989) suggesting that area 24 and MD neurons facilitate acquisition, but are not necessary for learning or for maintenance of the avoidance behavior. Area 29 and related anterior thalamic neurons contribute preferentially to the maintenance of avoidance behavior after acquisition is complete. For convenience the thalamocortical systems represented by area 24 and area 29 will be referred to as the anterior and posterior systems. Despite the preferential involvement of these systems respectively in behavioral acquisition and in performance maintenance in well-trained rabbits, respectively, it is clear that processes relevant to acquisition and maintenance are not strictly localized, as the combined lesions at the cortical level reported here, and combined lesions at the thalamic level (Gabriel et al. 1989) virtually abolished behavioral acquisition. It is thus likely that each thalamocortical system is sufficient, in the absence of the other system, to mediate behavioral acquisition *and* maintenance of the behavior. This conclusion is similar to a conclusion derived from studies of primates (see Mishkin and Appenzeller 1987) indicating that combined lesions in two distinct sets of limbic structures yield substantially more profound memory impairment than separate lesions in either set. The two sets in the case of the primate studies, as in the present studies with rabbits, are distinguished respectively by their close anatomical association with the anterior and MD thalamic nuclei.

It is interesting to consider the unique functions of the anterior and posterior systems. A variety of seemingly disparate empirical results can be accounted for by the hypothesis that the anterior and posterior systems perform, respectively, the mnemonic functions of recency

and primacy encoding (Gabriel 1990). The concept of a mnemonic recency system is quite similar to the concept of a working memory system as elaborated by Olton et al. (1979). It denotes a functional neural memory system in which recent task events and associated environmental features are stored with high fidelity for relatively brief periods of time. In addition to the findings in rabbits, studies of anterior system mnemonic processes in primates and rats are compatible with this view (Funahashi et al. 1989; Passingham 1985; Stokes and Best 1988, 1989). In contrast, a mnemonic primacy system gradually forms enduring mnemonic representations of persistent and repeating task events and associated features. These representations are maintained in the primacy system even when older information is displaced by newer information in the recency system.

One realm of the data that has been difficult to reconcile with this conception concerns the detailed electrophysiological results obtained from recordings made in the two cortical areas during learning. If the posterior cingulate is, as proposed, a part of a mnemonic primacy system which mediates gradual mnemonic encoding, then the neuronal plasticity in this system should develop gradually, whereas plasticity should develop rapidly in area 24 recency networks. However, the data indicate that rapid and gradual plasticity development occurs in each area, depending on the neuronal record and (in area 29) the cortical layer being monitored (see Gabriel et al. 1980; Orona and Gabriel 1983). The present finding that rapid plasticity in area 29c/d is lost in rabbits with anterior cingulate lesions helps to clarify this issue: the rapid plasticity may represent the projection of area 24 plasticity to area 29c/d. This projection could facilitate the area 29 primacy-encoding process, and its absence in the rabbits with lesions could account for their delayed criterion attainment. By the same token, projection of the late-developing plasticity from area 29 to area 24 could inform area 24 of the status of the primacy code, thus freeing area 24 networks for engagement in new learning problems.

The present findings demonstrated enhancement of MD thalamic training-induced discharges during the session of the first significant behavioral discrimination and during criterial conditioned response performance in rabbits with area 24 lesions. These results are similar to a previously observed enhancement of the firing of AV thalamic neurons in rabbits previously given area 29 or dorsal subicular complex lesions (see Introduction). They suggest that the neurons in both cingulate cortical areas suppress the firing of the thalamic cells to which they project. It should be noted, however, that the effects of the lesions were not identical. The maximal enhancement of AV thalamic activity following cingulate cortical lesions occurred during the last of three post-criterial overtraining sessions, whereas the maximal enhancement of MD activity occurred as shown here, during a much earlier training stage, the session of the first significant behavioral discrimination. This difference is reminiscent of the several findings mentioned above, indicating a preferential involvement of the anterior and posterior systems respectively in original task acquisition and in maintenance of the well-learned behavior. However, these considerations do not illuminate the functional relevance of the suppression of limbic thalamic activity by cingulate cortical neurons implied by the observed enhancements.

Past research has demonstrated training-stage dependence of the limbic thalamic activity, i.e., maximal or peak CS elicited discharges were attained in different stages of acquisition, depending upon the particular limbic thalamic nucleus being monitored. Training given beyond the stage of peak excitation brought about a decline of the neuronal discharges. Stage-specific peaks of excitation were observed in the anterior dorsal (AD), parvocellular AV, magnocellular AV, lateral dorsal (LD) and anterior medial (AM) nuclei (Gabriel et al. 1991), and in the MD nucleus (Orona and Gabriel 1983). Cingulate cortical and hippocampal subicular lesions eliminated the peak of training-induced activity normally seen in the AV nucleus (Gabriel et al. 1987): AV discharges in rabbits with lesions continued to increase in magnitude as training continued past criterion, whereas they declined progressively after criterion in controls. We recently found that AV thalamic multi-unit activity is massively activated, and conditioned response performance is suppressed, by the presentation of unexpected stimuli to trained rabbits. This massive activation of AV neurons is dependent on the presence of NE in the anterior thalamic nuclei (Gabriel and Sparenborg 1987) and recent unpublished results indicate that it is also dependent on the integrity of the hippocampus. These results provide the background for a possible explanation of the functional relevance of the cingulate cortex-engendered suppression of limbic thalamic training-induced neuronal activity. We offer the suggestion that cingulate cortical and hippocampal influences act in concert to suppress the training-induced excitation in limbic thalamus because such suppression is needed to produce unique topographic distributions of CS-driven thalamocortical excitation in cingulate cortex. The unique topographic distributions of excitation in cortex are caused directly by unique distributions of excitation among the various limbic nuclei, which have different (though overlapping) cingulate cortical projection fields. The distribution patterns are stable within any given training stage but they change gradually as training continues. Thus, in the early stages of training, inputs to cingulate cortex from the AD nucleus predominate whereas inputs from LD, parvocellular AV and MD predominate during the first discrimination, and inputs from the magnocellular AV and AM predominate during criterion attainment. The input distribution at a particular training stage constitutes the retrieval condition, i.e., the condition which identifies the eliciting stimulus as the CS+ and thus calls for the avoidance response. Unexpected stimuli acting through hippocampal circuits to release limbic thalamic NE massively activate most or all of the limbic nuclei, creating an unfamiliar input distribution and thereby blocking subsequent activities that lead to behavioral output.

Substantial empirical work is needed to confirm this interpretation, but it does provide a framework for inter-

preting the existing data and the extensive literatures demonstrating: a) animals with hippocampal damage fail to suppress the performance of learned behaviors in response to unexpected task events (reviewed by Gabriel et al. 1980), and; b) noradrenergic depletion frequently mimics the behavioral effects of hippocampal lesions (reviewed by Gabriel et al. 1990).

The foregoing analysis raises the question of how neuronal processes of cingulate cortex come to bear on the execution of locomotor avoidance responses. The present demonstration of altered CN neuronal activity in rabbits with area 24 lesions is consistent with the hypothesis that neurotransmission from the cingulate cortex to the CN occurs during CS presentation in intact rabbits. This neurotransmission could be involved in the initiation of avoidance responses. Yet, the particular form of the alteration of CN unit activity found in the present study was not predicted. Training-induced excitatory and discriminative unit activity occurred in the CN in rabbits with cingulate cortical lesions, but was absent in intact controls. As an aid to the comprehension of this unexpected result it is suggested that CS-driven cingulate cortical afferents suppressed the spontaneous firing of a particular class of CN neurons. This, in combination with CS-driven firing increases in other neurons, may have resulted in little or no detectable multi-unit discharges in the controls. Enhanced discharges in the rabbits with lesions could have been produced by removing the suppressive cortical inputs while sparing the excitatory inputs. Consistent with this interpretation, inhibitory intracellular and extracellular CN neuronal responses have been reported in response to electrical stimulation of cortex (Toan and Schultz 1985; Wilson et al. 1982).

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