BEHAVIORAL BIOLOGY 22, 275–301 (1978)

Differential Plasticity of Morphologically Distinct Neuron Populations in the Medial Geniculate Body of the Cat during Classical Conditioning[1]

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This study investigated the development of neuronal classical conditioning in the three morphologically distinct major subdivisions of the medial geniculate body (MGB), i.e., the ventral, dorsal, and medial regions. Multiple unit activity was recorded simultaneously from at least two of these regions during classical conditioning of the pupillary dilation response in the cat using acoustic and pawshock stimulation. The training paradigm consisted of sensitization, conditioning, and discrimination periods to control for non-associative factors. The subjects bore chronically implanted electrodes and were trained, following recovery from surgery, under neuromuscular paralysis to prevent changes in effective CS intensity in the ear due to middle ear contractions, head movement, or noise produced by body movement. Neuronal conditioned responses, consisting of an increase in multiple unit activity during presentation of the CS+, developed only in the medial subdivision of the medial geniculate body; this effect was never found in the ventral and dorsal regions. The conditioned neuronal responses in the medial division of the MGB were not due merely to increased light stimulation consequent to pupillary dilation because they were evident during the initial 152 msec of conditioning trials, prior to the onset of pupillary dilation. An analysis of the characteristics of initial responses to the CS+ and US prior to conditioning indicated that responses to the CS+ were not consistently different among the ventral, dorsal, and medial subdivisions of the MGB. However, the responses to the pawshock did relate to conditionability; neurons in the medial division, which exhibited conditioning, were driven only during the duration of US presentation whereas responses in the ventral and dorsal divisions, which did not exhibit conditioning, continued to be prominent after the offset of the US. This preliminary finding suggests that the characteristics of the initial response to the US may be critical in determining whether or not neuronal conditioning develops.

[1] We gratefully acknowledge the histological and technical assistance of Lola Moffitt and Mark Myers, the typing assistance of Karen Henoch and Elaine Hackelman, and the illustrations of Maureen Killackey.

[2] Supported by USPHS Predoctoral Fellowship MH 05424. This work has been submitted in partial fulfillment of the Ph.D. requirements at the University of California, Irvine. Present address: Department of Anatomy, University of Vermont, Burlington, Vermont 05401.

0091-6773/78/0223–0275$02.00/0

The quest for the neural mechanisms underlying behavioral plasticity has implicated sensory systems during the acquisition of a behavioral conditioned response (CR). In particular, the auditory system has been extensively studied, and there are many reports of the enhancement of evoked potentials (Gerken and Neff, 1963; Hearst *et al.*, 1960; Papova, 1969) and single or multiple unit responses (Buchwald *et al.*, 1966; Bures and Buresova, 1967; Cassady *et al.*, 1973; Disterhoft and Olds, 1972; Gabriel *et al.*, 1975; Halas *et al.*, 1970; Olds *et al.*, 1972; Oleson *et al.*, 1975) to an acoustic conditioned stimulus during behavioral conditioning. In spite of the progress that has been made in neurophysiological approaches to behavioral conditioning, the role of the auditory system is still uncertain. Before the role of such sensory changes in the establishment of a behavioral CR can be determined, it is necessary to establish that (a) the neural changes are consistent and develop prior to or at the same time as the behavioral CR and (b) that the modification of auditory system responses is due to the conditioning procedure itself. In order to conclude that the enhanced auditory responses are due specifically to the association between the conditioned stimulus (CS) and the unconditioned stimulus (US), other confounding variables must be ruled out. These include changes in effective stimulus intensity at the cochlea, movement artifact, sensory feedback from movement, and "pseudoconditioning" (see Oleson *et al.*, 1975, for more details). The problem of "pseudoconditioning" is of special importance because Hall and Mark (1967) and Mark and Hall (1967) have shown that enhancement of acoustically evoked potentials occurs during establishment of a conditioned emotional response (CER) whether the acoustic stimulus serves as the conditioned stimulus or as an indifferent background stimulus. Furthermore, Khachaturian and Gluck (1969) reported that responses evoked by background flash stimulation were enhanced due to institution of a conditioning procedure.

In addition to the need for these types of experimental controls, an analysis of the effects of conditioning procedures upon auditory system activity ought to relate such findings to the anatomical substrates of this system. Recent anatomical studies have revealed that the auditory system is much more complexly organized than heretofore acknowledged. Specifically, the medial geniculate body (MGB) has been shown in a number of mammals to contain a number of distinct subdivisions (Fig. 1) on the basis of cyto- and myeloarchitectonics, Golgi analysis, afferents, and efferents (Morest, 1964, 1965a, b; Oliver and Hall, 1975; Ramon y Cajal, 1966; Ryugo and Killackey, 1974; Ryugo and Killackey, 1977b). Three major components are usually recognized, ventral, medial, and dorsal. The ventral division (MGv) is characterized by: (a) neurons with restricted receptive fields (Aitkin and Webster, 1972); (b) tonotopic (Aitkin and Webster, 1972) and laminar (Morest, 1965; Ryugo and Killackey, 1977b) organization of these neurons; (c) a functionally homogeneous and topo-

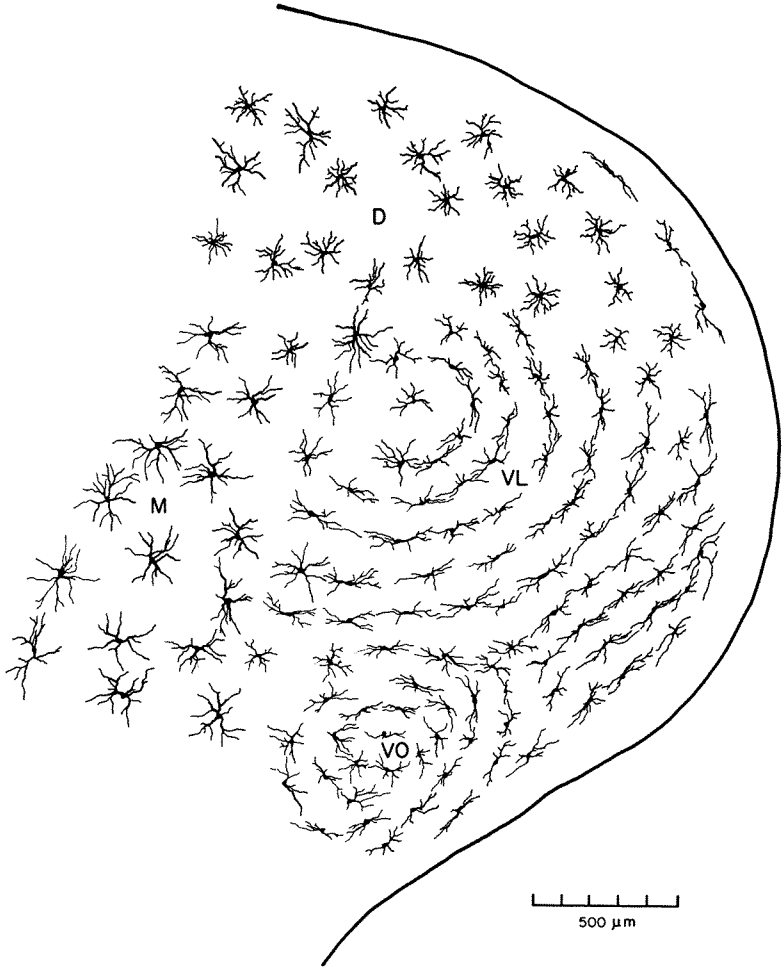

FIG. 1. Camera lucida reconstruction of the MGB from Golgi material. Typical distribution of neuronal types through the middle of the medial geniculate body of the adult cat. Coronal section, Golgi–Cox. Abbreviations: D, dorsal division; M, medial division; VL, ventral division, pars lateralis; VO, ventral division, pars ovoida.

graphic input from the central nucleus of the inferior colliculus (Jones and Rockel, 1971; Ryugo and Killackey, 1977a); (d) neurons with "tufted" dendrites, typical of specific thalamic sensory relay nuclei (Morest, 1964; Scheibel and Scheibel, 1966, 1967, 1970); and (e) a dense and topographic projection to primary auditory cortex (Colwell and Merzenich, 1975; Niimi and Naito, 1974; Ryugo and Killackey, 1977b). In contrast, the medial division (MGm) is characterized by: (a) neurons that demonstrate multimodal as well as wide receptive field properties (Erickson *et al.*,

1964, 1967; Poggio and Mountcastle, 1960; Wepsic, 1966); (b) a lack of tonotopic (Aitkin, 1973; Love and Scott, 1969) or laminar (Morest, 1965a) organization; (c) a functionally heterogeneous input, including spinothalamic (Lund and Webster, 1967), medial lemniscal (Jane and Schroeder, 1971; Schroeder and Jane, 1971; Walsh and Ebner, 1973), and brachial from the inferior colliculus (Moore and Goldberg, 1963; 1966; Ryugo and Killackey, 1977a); (d) neurons with "radiate" or isodendrites, characteristic of regions receiving heterogenous input, such as the brain stem reticular formation (Morest, 1964; Ramon-Moliner, 1962, 1975; Scheibel and Scheibel, 1966); and (e) projections to auditory cortex in a diffuse and widespread fashion (Rose and Woolsey, 1958; Ryugo and Killackey, 1974, 1977b). The third major component of the medial geniculate body is the dorsal division (MGd). It is characterized by: (a) neurons with evenly spaced radiating dendrites (Morest, 1964); (b) cells which are only mildly responsive to acoustic stimulation (Aitkin and Webster, 1972; Lippe and Weinberger, 1973); (c) input from the diffusely arranged lateral tegmental fiber system (Morest, 1964); and (d) a cortical target area, insular cortex, which is quite distinct from primary auditory cortex (Raczkowski *et al.*, 1976; Rose and Woolsey, 1958).

At least three types of thalamocortical afferents project to auditory-responsive cortex, each originating in adjacent but distinct subdivisions of the medial geniculate body. The projections of MGv and MGm converge upon primary auditory cortex, but preserve their individual integrity by maintaining a pattern of laminar separation (Ryugo and Killackey, 1977b). MGd projects to a separate cortical area. The striking difference between the distribution and manner of terminations of these thalamocortical fiber systems strongly suggests that they underlie different features of cortical physiology.

Given this contrast among MGB subdivisions, it is of interest to determine whether they have different functional roles in relation to plasticity. Neurons of the specific auditory system (MGv), with their restricted receptive fields and rapidly conducting fibers, may furnish the greatest range and accuracy of sensory discrimination. Such neurons apparently endow the system with a high fidelity representation of the acoustic environment. Does a paradox emerge from previous reports of plasticity in this "high fidelity" system (Buchwald *et al.*, 1966; Disterhoft and Olds, 1972; Gabriel *et al.*, 1975; Halas *et al.*, 1970; Olds *et al.*, 1972; Oleson *et al.*, 1975) or would finer analysis of the relationship between morphology and physiology reveal that the specific system provides the high fidelity while the less specific system (MGd or MGm) is more plastic (Disterhoft and Stuart, 1976; Graybiel, 1974)?

Thus, even well-controlled neurophysiological approaches to the role of the auditory system in behavioral plasticity may be incomplete without careful consideration of the system's regional morphology. The acoustic

relay center of the thalamus, the medial geniculate body, is ideal for the investigation of this problem because of the detailed anatomical and physiological information that is already available. The present study attempts to (a) examine the effects of classical conditioning procedures upon neural activity within the medial geniculate body during the acquisition of a behavioral conditioned response with control for non-associative factors and (b) to assess these data within the context of the anatomical subdivisions of this nucleus.

METHODS

Subjects and Surgery

The data were collected from 12 adult cats of either sex, free from ear infection and weighing 2.7 to 3.5 kg. Each animal was initially anesthetized with sodium pentobarbital (Nembutal, 30 mg/kg, IP) and was maintained at a surgical level of anesthesia with supplemental intravenous injections of the short-lasting barbiturate, sodium thiamylal (Surital). The animal was then placed in a stereotaxic instrument for the multiple placement of depth electrodes into the medial geniculate body. Recording electrodes consisted of size 00 stainless steel insect pins, etched to have tips of 2–5 μ and insulated with Epoxylite. All electrodes were referenced to a stainless steel screw that was positioned over the frontal sinus. Recording leads were led into a plastic cylinder that was attached to the skull via a pedestal built with dental acrylic. A screw top enclosed the cylinder except during recording sessions, thus protecting the recording leads. Short lengths of plastic tubing were affixed to the anterior and posterior regions of the pedestal to provide atraumatic fixation of the head during recording sessions. Delagon antibiotic powder was applied to exposed skin surfaces, and the animals were given an intramuscular injection of 300,000 units of Bicillin (Wyeth Laboratories). All animals were allowed at least 2 weeks of postsurgery recovery.

Experimental design and procedure

At the beginning of the training day, the animals were given a 10 mg/kg ip injection of gallamine triethiodide (Flaxedil) to produce paralysis. An endotracheal tube, coated with a local anesthetic (Xylocaine) was inserted into the trachea with the aid of a laryngoscope, and artificial respiration was initiated with a Harvard respirator. Once immobilized, the animal was positioned in the atraumatic head holder that attached to a stereotaxic instrument. All experimental procedures were carried out with the animal enclosed in an acoustic room (IAC 1202). Body temperature was maintained at approximately 37° C by a thermostatically-controlled circulating warm water pad. Paralysis was maintained throughout the experiment with supplemental doses of Flaxedil (20 mg/45 min). Expired carbon dioxide levels were not routinely monitored because we have found that

pupillary size and motility are equally, if not more, sensitive indices of the animal's state. All subjects had motile pupils and pupillary dilation could easily be induced by incidental stimuli, as well as by the stimuli used during training.

Acoustic stimulation was delivered to the ear contralateral to the side of the recording electrodes by a TDH 39 earphone, mounted on a hollow earbar that was positioned without pressure into the external auditory meatus. Two types of acoustic stimuli were delivered: 2000 Hz tones (CS−, 85 dB) produced by a Hewlett–Packard oscillator, and white noise (CS+, 75 dB) produced by a Grason–Stadler white noise generator. The bandwidth of the white noise was 20–20 kHz at the output of the generator, but the spectrum of white noise delivered to the animal was limited by the frequency response characteristics of the earphone (frequency response linear to 6 kHz). Stimulus intensities are expressed as decibels above a reference of 0.0002 dynes/cm^2 and were controlled by Hewlett–Packard decade attenuators. Intensities were measured with a Bruel and Kjaer sound level meter with a ¼–inch condenser microphone. The intensity was set at the beginning of each experiment with the animal and earphone in place, by measuring the intensity through a probe in the hollow earbar that was led to the sound level meter. Stimulus intensity was constantly monitored over the course of the experiment, and was found to be constant (i.e., varied less than 1 dB). The tone intensity was set higher to compensate for the greater potency of white noise in eliciting pupillary dilation.

Needle electrodes were inserted into the subcutaneous tissue of the forelimb ipsilateral to the stimulated ear for delivery of the shock (US). The shock was produced by a Grass SD-5 stimulator with parameters set to deliver a 0.5 sec train of 5 msec pulses of 20–25 V, presented at a rate of 50/sec.

The left eyelid was retracted wih a pediatric speculum, and an infrared pupillometer was positioned in front of the pupil for continuous monitoring of pupillary diameter. The eye was illuminated by a 12 V DC lamp, and the corneas were protected from drying by the application of terramycin ophthalmic ointment. The output of the pupillometer was amplified, written out on a Grass model 7 polygraph, and also recorded on an FM channel of a Crown–Vetter tape recorder. Multiple unit discharges were amplified by means of Tektronix 122 preamplifiers, whose output was led into the polygraph for additional amplification and then into highpass filters (0.6 kHz) and recorded on direct channels of the tape recorder.

The experimental session consisted of three phases: A sensitization phase, which provided a measure of the pupillary dilation and neural responses to the random presentation of acoustic and somatic stimuli; a conditioning phase, which provided a measure of the change in pupillary dilation and neural responses to a previously unpaired acoustic stimulus

during pairing with somatic shock; and a discrimination phase, in which a measure of the differential pupillary and neural responsiveness to the paired acoustic stimulus and the unpaired acoustic stimulus was obtained and used as an index of acoustic stimulus specificity for the elicitation of a conditioned response. In all three phases, the acoustic stimuli were 1 sec in duration and the somatic shock was 0.5 sec in duration. The trials were randomly presented with an intertrial interval of 30–90 sec, and presentation was programmed by BRS Digibits.

The experimental session began with a sensitization phase. A total of 70 sensitization trials were given, 35 each of white noise and tone, randomly intermixed. Interspersed among the acoustic stimuli were 35 shock presentations. The sensitization phase served as the baseline for the assessment of the effects of subsequent conditioning and discrimination treatments.

Following sensitization, 40 conditioning trials were initiated without pause between the last sensitization trial and the first conditioning trial. White noise was designated as the conditioned stimulus (CS+) and was always followed, without delay, by shock, the unconditioned stimulus (US). Oleson *et al.* (1975) have shown discrimination reversal using white noise and tone in a training procedure very similar to that used in this experiment. Therefore, the choice of white noise as the CS+ should not affect the results in any significant manner. Immediately following the completion of the conditioning series, discrimination training was initiated. Thirty-five white noise-shock (CS+) trials were randomly intermixed with 35 tone but no shock (CS−) trials, with the restriction that no more than three trials of one type occur consecutively.

All data were analyzed with the assistance of a PDP/8 computer. Pupillary data were led into an analogue-digital converter. Multiple unit data were played back through Schmitt triggers which produced a single pulse each time their thresholds were exceeded, and led to pulse detectors in the computer. The thresholds were set with the aid of a computer program to pass approximately 125 pulses/sec during a period of no stimulation which preceded the onset of the sensitization period. Using this procedure, the range of trigger settings varied from 30–70 μV (peak-to-peak). Threshold detectors were set according to the same rate rather than amplitude to preclude possible bias if there were a systematic relationship between amplitude and subdivision of the MGB. No such relationship was found however, so that this precaution proved unnecessary.

The computer counted the number of pulses occurring during the 500 msec immediately preceding trial onset, and during the 1000 msec of acoustic stimulation, excluding the US which followed. Data were additionally analysed for the first 152 msec of a trial, the interval preceding the beginning of pupillary dilation. The number of pulses were stored in consecutive 4 msec bins. Thus, there were 125 pretrial bins and 250

pertrial bins. The effect of acoustic stimulation on the rate of multiple unit activity was calculated by subtracting the mean pretrial rate from the mean pertrial rate, yielding a difference score for each trial at each recording site. Using this mean difference score obtained during the sensitization period as baseline, the percentage difference was calculated for each trial of sensitization, conditioning, and discrimination.

Histology

Following the completion of behavioral training, each animal was given an overdose of a barbiturate (Nembutal) and small electrolytic lesions were made with anodal current. The head was perfused with saline followed by 10% formalin, and the brain was removed and stored in 30% sucroseformalin. Electrode tips were histologically verified on sections stained with cresyl violet. Critical sections were then traced at a × 20 magnification. Golgi material was prepared from three adult cats by a modified Golgi–Cox procedure. The Golgi tissue was cut into 90 μm sections. Figure 1 is a camera lucida reconstruction of the MGB from three adjacent Golgi sections, drawn at × 100. The resultant montage was then photographically reduced.

RESULTS

Histology

Recording loci are depicted in Fig. 2. Electrode tip placements were histologically verified in MGv (n = 16), MGm (n = 8), and MGd (n = 10).

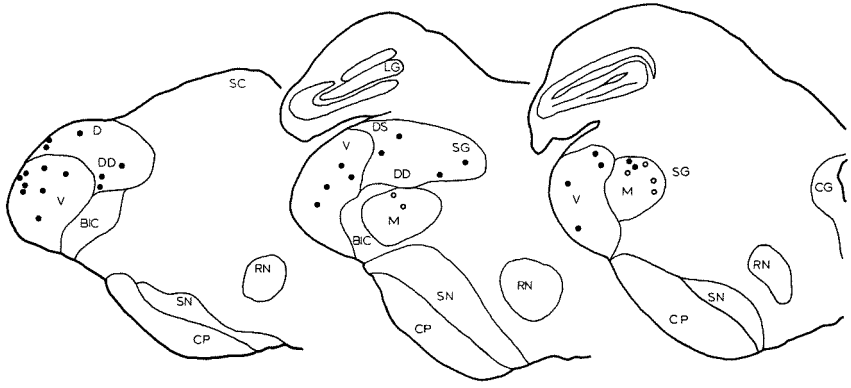

FIG. 2. Histological verification of microelectrode placements in MGB. Coronal sections taken at A3, A4, and A5 (left to right, respectively). Solid circles indicate electrode placements where conditioned changes were not detected. Open circles indicate sites of conditioned change. All of the loci which exhibited conditioned changes were confined to MGm; the two unconditionable loci in MGm occurred in cats that failed to demonstrate behavioral conditioning. Abbreviations: BIC, brachium of inferior colliculus; CG, central gray; CP, cerebral peduncles; D, dorsal division; DD, deep dorsal nucleus; DS, superficial dorsal nucleus; LG, lateral geniculate body; RN, red nucleus; SC, superior colliculus; SG, suprageniculate nucleus; SN, substantia nigra; V, ventral division.

Data obtained from electrode sites located outside the medial geniculate body are not included in the analysis.

Pupillary Behavior

Pupillary dilation responses were recorded from all 12 subjects. At the beginning of sensitization, both white noise and tone elicited dilations which usually lasted for 1 sec or more. By the end of sensitization, dilation responses were reduced in both amplitude and duration. The pawshock (US) produced consistently large pupillary dilation throughout the experimental session. The onset latency of dilation to both acoustic and somatic stimulation was consistent with the 170–220 msec latency reported previously (Oleson *et al.*, 1975). There was no consistent difference in potency of the acoustic stimuli across subjects.

Pupillary Conditioning

The effects of CS–US pairing produced a relatively rapid and pronounced increase in pupillary responses to the white noise CS+, compared to the white noise sensitization value. The magnitude of pupillary dilation grew through repeated pairings, and became maximal during discrimination training. Figure 3 is a trial-by-trial percentage difference plot of pupillary responses from animal Tas 16 over the entire training period. During the sensitization period, pupillary responses to both white noise and tone stimulation fluctuated about the mean sensitization value. During CS–US pairing, there was a systematic growth of the pupillary response. This increase in the response was maintained during conditioning. Given that there is enhancement of the response during the conditioning session, the critical issue emerges of determining a reliable and valid criterion for an assessment of such enhancement. The acquisition criteria used in this study required that the following two conditions be met: (a) five consecutive trials during conditioning in which responses to the CS+

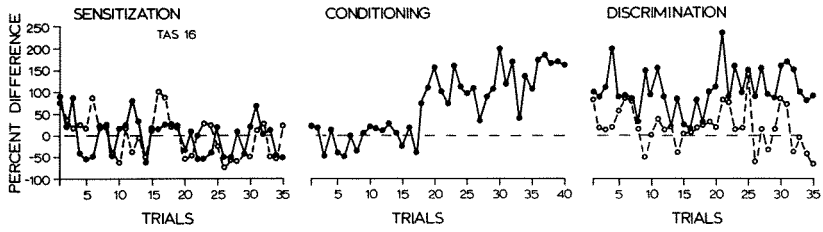

FIG. 3. Trial-by-trial plot of the pupillary response changes during training. Each point represents the normalized pupillary response magnitude expressed as a percent difference score for each trial, relative to the mean value of pupillary response magnitude obtained during sensitization (dashed line) for subject Tas 16. Response to the CS+ (solid circles) increased during conditioning and was maintained during discrimination. Responses to the CS− (open circles) were consistently smaller than the responses to the CS+ during discrimination, and often fell below mean sensitization levels.

were greater than the mean response to that same stimulus during sensitization (trials required to reach criterion); (b) significantly larger ($P < 0.05$, two-tailed binomial sign test) pupillary dilation responses during the last 15 conditioning trials compared to the mean sensitization response. Satisfaction of this criterion indicated that a change in responsiveness had occurred and was maintained over the course of conditioning. Three of the 12 cats failed to meet both of these criteria (Table 1).

Pupillary Discrimination

Discrimination training was employed to evaluate whether the increase in pupillary response magnitude was specific to the US-contingent CS+ or merely due to an indiscriminate response enhancement to any stimulus (CS−). In order to compare responses to the CS+ and CS−, the mean responses to white noise and tone during sensitization were subtracted, respectively, from each response to the CS+ and CS− during discrimination. This procedure yielded the changes in response magnitude to the CS+ and CS− relative to the mean sensitization, which were then subjected to the Wilcoxin test. The discrimination criterion required a statistically significant difference between responses to the CS+ and CS− ($P < .05$, Wilcoxin test). Eight of the nine cats that demonstrated pupillary conditioning additionally met the criterion for pupillary discrimination. (The three cats that failed to condition also failed to discriminate.) Discrimination between the CS+ and CS− was evident within the first few trials of discrimination training in four cats (e.g., Fig. 3) and developed more slowly in the other subjects. The demonstration of discrimination indicates that the increased pupillary dilation responses were specific to the shock-contingent auditory stimulus.

Neural Data

The establishment of conditioned responses at the behavioral level enabled an examination for possible accompanying neural response alterations within the auditory thalamus. In the absence of such a behavioral control, it would be difficult to interpret negative results from the neural data.

During sensitization, the white noise and tone stimuli both evoked multiple unit activity in each of the three major subdivisions of the medial geniculate body. These responses generally consisted of a short latency (<20 msec) onset response which was often followed by a sustained increase in activity above background level during the 1 sec presentation of the stimuli (Figs. 4 and 5). A sustained decrease in activity was observed in a few cases (Fig. 4, "MGV Tn"). The three subdivisions did not yield consistently different poststimulus histograms to acoustic stimuli; however, the onset response in MGv often exhibited the shortest latency (8–12 msec).

TABLE 1

Conditioning and Discrimination Indices, Showing the Number of Trials to Acquisition Criterion, Significance of Response Changes during Conditioning for both 1000 msec and 152 msec CS+ Intervals, and Significance of Response Differences during Discrimination

Subject	CONDITIONING														DISCRIMINATION			
	Pupil		MGv				MGm				MGd				Pupil	MGv	MGm	MGd
			1000		152		1000		152		1000		152					
	TC[a]	P[b]	TC	P	TC	P	TC	P	TC	P	TC	P	TC	P	P[c]	P	P	P
Tas 2	14	.01	40	NS	19	NS	—	—	—	—	2	NS	40	NS	.001	NS	—	NS
	—	—	40	NS	40	NS	—	—	—	—	—	—	—	—	—	NS	—	NS
Tas 11	40	NS	40	NS	40	NS	—	—	—	—	40	NS	40	NS	.01	NS	—	NS
	—	—	40	NS	40	NS	—	—	—	—	—	—	—	—	—	NS	—	—
Tas 12	18	.01	40	NS	40	NS	—	—	—	—	—	—	—	—	.001	NS	—	—
	—	—	40	NS	40	NS	—	—	—	—	—	—	—	—	—	NS	—	—
	—	—	40	NS	40	NS	—	—	—	—	—	—	—	—	—	NS	—	—
Tas 13	13	.01	—	—	—	—	—	—	—	—	40	NS	40	NS	.01	—	—	NS
	—	—	—	—	—	—	—	—	—	—	40	NS	40	NS	—	—	—	NS
	—	—	—	—	—	—	—	—	—	—	40	NS	40	NS	—	—	—	NS
Tas 3	23	.05	40	NS	40	NS	24	.05	32	.05	—	—	—	—	.001	NS	NS	—
Tas 8	14	.01	40	NS	40	NS	27	.05	27	.05	—	—	—	—	NS	NS	NS	—
Tas 9	16	.01	40	NS	40	NS	14	.01	14	.01	40	NS	40	NS	.01	NS	.05	NS
Tas 10	6	.01	40	NS	40	NS	12	.05	40	NS	—	—	—	—	.001	NS	.05	—
	—	—	40	NS	40	NS	—	—	—	—	—	—	—	—	—	NS	—	—
Tas 16	9	.01	40	NS	40	NS	16	.05	29	.05	40	NS	1	NS	.001	NS	.001	NS
	—	—	—	—	—	—	—	—	—	—	34	NS	40	NS	—	—	—	NS
Tas 18	21	.01	40	NS	40	NS	5	.05	5	.01	40	NS	40	NS	.001	NS	.01	NS
Tas 7	40	NS	40	NS	40	NS	40	NS	40	NS	40	NS	40	NS	NS	NS	NS	NS
Tas 15	40	NS	2	NS	40	NS	22	NS	40	NS	40	NS	40	NS	NS	NS	NS	NS

[a] Trials to criterion. If criterion was not met, the total number of conditioning trials (i.e., 40) is given.

[b] Probability value for two-tailed binomial sign test for conditioning.

[c] Probability value for Wilcoxin test for discrimination.

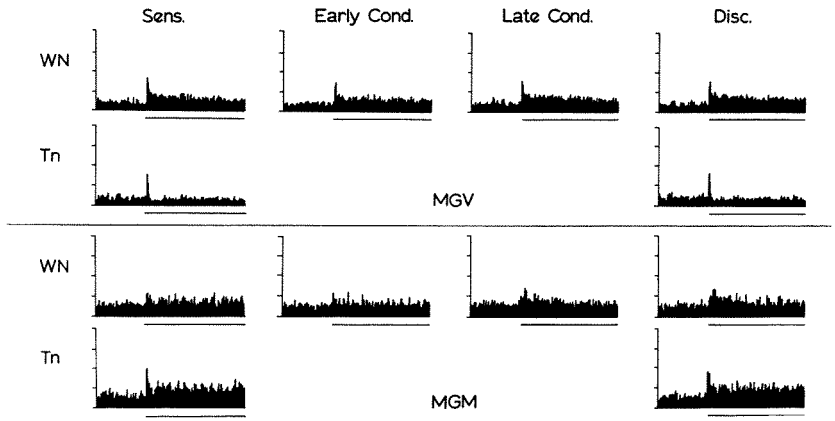

FIG. 4A. Histograms of multiple unit activity in MGv and MGm during training of animal Tas 16. Neuronal responses to CS+ (WN) and CS− (Tn) are illustrated during sensitization (trials 26–35), early conditioning (trials 1–10), late conditioning (trials 31–40), and discrimination (trials 26–35). Multiple unit responses in MGv remain relatively constant to both WN and Tn during training; in contrast, increased multiple unit responses to WN are evident in MGm by late conditioning and are maintained through discrimination. Responses to Tn do not change. Horizontal lines at bottom represent the onset and 1.0 sec duration of acoustic stimulation. Calibration: 24 spikes per division.

Neural responses had to meet two criteria in order to be classed as neural conditioned responses: (a) activity evoked by the CS+ had to be larger than the mean response to the same stimulus during sensitization for five consecutive trials during conditioning—this measure defined "trials to criterion"; (b) neural responses to the CS+ had to be significantly larger ($P < 0.05$, two-tailed binomial sign test) during the last 15 trials of conditioning than the mean response for the sensitization period.

During conditioning, there was a difference among the three subdivisions in neural responsiveness to the white noise CS+. Specifically, the

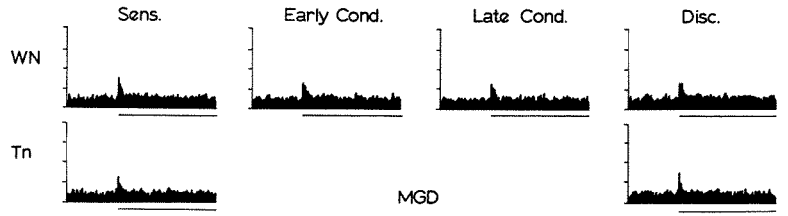

FIG. 4B. Histograms of multiple unit activity in MGd during training of animal Tas 16. Neuronal responses of CS+ (WN) and CS− (Tn) are illustrated during sensitization (trials 26–35), early conditioning (trials 1–10), late conditioning (trials 31–40), and discrimination (trials 26–35). Multiple unit responses in MGd remain relatively constant to both WN and Tn during training. Horizontal lines at bottom represent onset and 1.0 sec duration of acoustic stimulation. Calibration: 24 spikes per division.

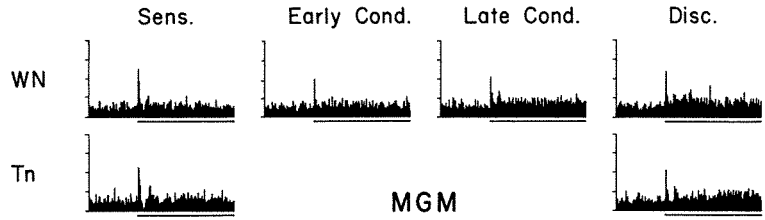

FIG. 5. Histograms of multiple unit activity in MGm during training of animal Tas 3. Neural responses are illustrated for the end of sensitization (trials 26–35), early conditioning (trials 1–10), late conditioning (trials 31–40) and discrimination (trials 11–20). Note the enhanced response late in conditioning, in comparison with sensitization, which is further augmented during discrimination. The response to the Tn (CS−) is slightly increased, relative to sensitization, but unlike responses to the WN (CS+), not statistically significant. Horizontal lines at bottom represent onset and 1.0 sec duration of acoustic stimulation. Calibration: 24 spikes per division.

medial subdivision exhibited statistically significant response enhancement relative to responses to white noise during sensitization. This effect was found in six of eight placements in the MGm. In marked contrast, such neural conditioned responses were never found in the ventral and dorsal subdivisions of the medial geniculate nucleus (Table 1). Histograms of neural activity for all three subdivisions are presented in Fig. 4A and 4B. For the sake of continuity, these data are taken from animal Tas 16, whose pupillary data were given in Fig. 3. Inspection of Fig. 4A reveals a small enhanced neural response in MGm which is not present during the initial portion of conditioning (trials 1–10) but is evident later (trials 30–40). This effect continues to be present, or even larger, during discrimination training. In contrast, responses to the CS− are not potentiated during discrimination training with reference to responses to the tone stimulus during the sensitization period. Conditioned neural changes are seen in neither the ventral subdivision (Fig. 4A, MGv) nor the dorsal subdivision (Fig. 4B). Significant decreases in neural activity were not found in any subdivision.

Figure 5 presents histograms from animal Tas 3. These data reveal a more dramatic increase in the medial subdivision response to the CS+ during conditioning than that seen in Fig. 4A. Note also the lack of any effect of the conditioning procedure upon the neural data from the ventral subdivision in this subject (Table 1).

It might be argued that the increased responsiveness in MGm was due to sensory feedback from the pupillary dilation response (i.e., increased light stimulation as the pupil becomes larger). Although this is unlikely in view of the report that MGm is unresponsive to flash stimulation (Lippe and Weinberger, 1973), an additional analysis was performed for the first 152 msec of each trial, prior to the onset of the pupillary dilation response.