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Modulation of Auditory Cortex Unit Activity during the Performance of a Conditioned Response

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Single-unit activity in primary auditory cortex was studied in unanesthetized, paralyzed cats during the performance of a classical conditioning task. The conditioned stimulus was a 0.5-s white noise (WN) burst paired with tail shock delivered 4.5 s later. Cats habituated to WN without shock served as controls. Overlayed on these tasks was a continuous background of 1/s, behaviorally irrelevant, 100-ms duration tone bursts set to the best frequency and optimal intensity for the particular unit being studied. Spontaneous activity and tone responses following WN were compared with the respective activity preceding WN. The spontaneous or evoked activity of 75% of the cells recorded in the trained animals changed significantly after WN, whereas the activity of 28% of the cells recorded in habituated animals changed. Augmentation and suppression of both spontaneous and evoked activity were found. These results have implications for the encoding of acoustic stimuli in terms of the modulation of lemniscal sensory system activity.

INTRODUCTION

Responses of auditory system neurons are not determined solely by the physical parameters of acoustic signals. They are dependent in part on nonacoustic variables such as the sleep–waking cycle (2, 3, 14, 18–20), experience (4, 6, 9, 13, 16), and anesthesia (7, 8, 10). Such nonsensory variables may be considered to determine the state of the animal. The great majority of those and other similar studies in the auditory system involved

Abbreviations: WN—white noise, CS—conditioned stimulus, US—unconditioned stimulus, SPL—sound pressure levels.

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measurements of altered evoked potentials or multiple-unit activity. Consequently, little is known about the influence of those variables on the tone-evoked and spontaneous activity of individual neurons in auditory cortex.

The present experiment was designed to characterize in the unanesthetized preparation the influence of behaviorally determined changes of state upon the spontaneous and evoked activity of auditory cortex neurons. A classical conditioning pupillary dilation response was selected as a means to control the state of the animal experimentally. The behavior is quickly learned and the time course of the paradigm can be prolonged sufficiently to enable repeated testing of auditory cortex responsiveness during a behavioral trial. The paralyzed cat preparation was adopted because it offered significant advantages over the moving animal for (i) the measurement and control of acoustic signals, (ii) preventing sporadic or stimulus-evoked middle ear muscle contractions that result in uncontrolled modifications of the effective acoustic stimulus, and (iii) the sustained recording of single-unit activity.

The level of spontaneous activity and responses to behaviorally irrelevant, best frequency tone bursts presented repetitively throughout a sequence of behavioral trials were analyzed. Systematic and often complex alterations of both spontaneous and tone-evoked activity were frequently observed during and after the conditioning trial. These data demonstrate that in the unanesthetized cat the pattern and amount of auditory cortex neuron activity are determined to a considerable extent by state-dependent variables.

METHODS

Animal Preparation. Under sodium pentobarbital anesthesia a pedestal was secured to the skull of 10 cats with bone screws and dental cement. The pedestal was used to maintain the head immobile during training and single-unit recording without the need of pressure points on the animal. Training was initiated 5 days after surgery. After pupillary conditioning was established, the animal was prepared for the recording of auditory cortex single-unit activity. Under sodium pentobarbital anesthesia a modified Davies Plexiglas chamber was implanted over the right auditory cortex. The bone enclosed by the recording chamber was removed at this time but the dura was left intact. When recording was not being done, the chamber was filled with sterile saline and closed by a Teflon cap. The first recording session occurred after a 5-day recovery period and was repeated each 3 to 4 days for as long as 3 weeks.

For both training and unit recording the cat was paralyzed with an intraperitoneal injection of gallamine triethiodide, intubated, artificially ventilated, and secured by the pedestal to a modified stereotaxic apparatus

located in a double-wall acoustic chamber. Respirator settings were initially determined from a respiration chart. Subsequent use of a CO_2 monitor during the experiment confirmed that the previously used settings were appropriate. Body temperature was monitored and maintained at 38°C. Supplementary doses of gallamine triethiodide were administered intraperitoneally as needed (approximately 10 mg/kg/h). The corneas were covered with opthalmic ointment to prevent drying. None of the animals used in this experiment showed any signs of increased fear or hostility during the course of training and unit recording.

After a training or recording period, which were limited to 7 h, the cat was given antibiotics, and after recovery from the paralysis, was returned to its home cage. Care was taken to maintain the inside of the Davies chamber, surgical tools, electrodes, and chamber cap as sterile as possible. No evidence of infection was observed. After a number of electrode penetrations had been made, the animal was killed with an overdose of sodium pentobarbital and the brain was prepared by conventional histological means for the reconstruction of the electrode tracks. To facilitate this reconstruction, small lesions were placed at the bottom of the majority of the penetrations.

Behavioral Paradigm and Training Procedures. The behavioral paradigm was a modification of the pupillary conditioning task elaborated in the paralyzed cat by Weinberger *et al.* (16, 17). White noise was the conditioned stimulus, tail shock was the unconditioned stimulus, and the behavioral response was pupillary dilation. An infrared pupillometer was positioned in front of the left eye. Dilation of the pupil was examined by digitizing the output of the pupillometer at 33-ms intervals, beginning 5 s before presentation of the conditioned stimulus and ending 5 s after the unconditioned stimulus. Each conversion was stored in the computer for later averaging and registration with the digitized unit data. The unconditioned stimulus was a 250-ms duration train of 15-V, 2-ms pulses presented at 100/s and delivered via needle electrodes inserted in the tail.

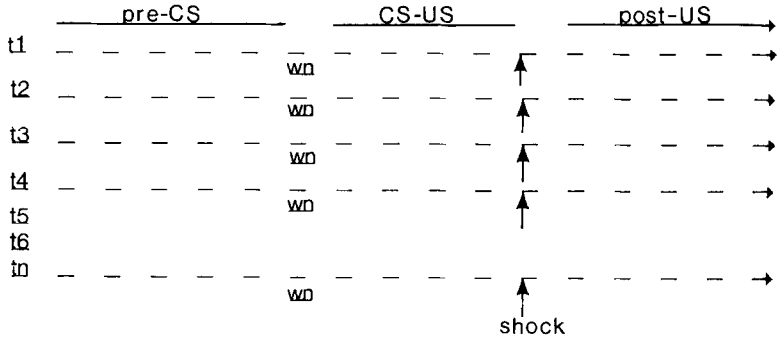
Acoustic signals were presented through a 2.0-cm-long earpiece placed in the left external auditory meatus, contralateral to the auditory cortex to be studied. Two 4-cm-long plastic tubes connected the earpiece to the ends of two 6-cc metal containers, each housing a Beyer DT-48 earphone. Acoustic signals generated by a manual/programmable oscillator and a white noise generator were shaped by a dual audio switch (rise/fall time 5 ms). Stimulus level was controlled by a dual manual/programmable attenuator. White noise and tonal signals were independently controlled and delivered via the separate earphones to the earpiece. Sound pressure levels (SPL) of tonal signals between 20 Hz and 30 kHz were measured by coupling the earpiece by a short plastic tube to a 1.25-cm condenser

microphone connected to a sound level meter. These values were used during the course of the experiments to present signals of known intensity. All stimulus levels expressed in decibels are referenced to 0.0002 dynes/cm^2.

Initial responses to the white noise stimulus, 75 dB, were first habituated by presenting 5-s duration signals at intervals varying between 30 and 90 s. A series of 30 shocks to the tail and 30 white noise stimuli was then presented in a random temporal sequence to obtain an estimate of the effect of shock presentation upon the pupillary response to the white noise signal independent of conditioning. This sequence provided a measure of sensitization of pupillary dilation that was sufficiently stable to serve as a baseline for evaluating the subsequent conditioning.

Conditioning was begun with a 5-s duration, 75-dB white noise (WN) conditioned stimulus followed immediately by shock to the tail. To preclude temporal conditioning the intertrial interval varied randomly between 30 and 90 s. Reliable anticipatory pupillary dilation responses to WN presentation usually developed within 40 trials. Subsequently, WN was progressively shortened in 500-ms steps until a conditioning trial consisted of a 500-ms WN burst followed 4.5 s later by tail shock (Fig. 1).

At this time 100-ms duration probe tones were presented continually at 1-s intervals for the remainder of the training period. White noise bursts and shocks were time locked to the onset of appropriate test tones. These tone bursts would be used during single-unit recording to probe the changing response properties of auditory cortex cells and could, therefore, have



BEHAVIORAL PARADIGM

FIG. 1. Behavioral paradigm. Classical conditioning trials consisted of a 500-ms duration white noise stimulus (WN) followed 5 s later by tail shock. Probe tones: 100-ms duration, presented 1/s, normally set to the best frequency of the cell under study. Unit activity was summed across behavioral trials. Pre-CS: 5-s period preceding WN. CS–US: 4-s period beginning at onset of first tone after WN. Post-US: period beginning at first tone after tail shock.

no behavioral significance themselves. Consequently, the frequency and intensity of the probe tones were varied during training. This lack of cue value permitted the tones to be used subsequently in searching for responsive units without perturbing the conditioned responses. Similarly, when a cell was isolated the frequency of the probe tones could be fixed at the best frequency of the particular cell under study without disturbing the behavior. The essential aspect of the paradigm is that the frequency, intensity, and duration of the probe tone stimuli used in the study of a cortical unit were constant from presentation to presentation within a set of conditioning trials. Variation of responses to the probe tones would consequently depend on the temporal relation of a given tone to a behavioral trial, i.e., whether the tone occurred before or after presentation of the conditioned stimulus.

Three control animals were subjected to the same habituation and training series as were the seven experimental animals except that tail shock was not presented. Data obtained from these animals provided a measure of the influence of a 500-ms white noise burst upon responses of single auditory cortex cells to subsequent probe tones, independent of conditioning. This control was sufficient because the intent of the experiment was to characterize the modulation of cortical unit responsiveness accompanying the reliable evocation of a behavioral response, rather than examining which particular aspects of the behavioral context contributed to the modulation of single unit activity.

Conditioned Behavior. The seven experimental cats learned the conditioned pupillary dilation response within a single training session. In each case conditioned responses were larger than the responses at the conclusion of the habituation and sensitization phases of training. Four examples of averaged pupillary responses to the conditioned WN stimulus recorded during the study of cells are shown in Fig. 2A. Although minor variations are apparent, the responses began during WN and were maintained throughout the 5-s interval between WN onset and shock. Tail shock evoked a transient increased dilation followed by a gradual constriction of the pupil to the pre-WN level over the next second or two. For the habituated animals either no systematic dilation or a small transient dilation occurred in response to WN presentation (Fig. 2B). For both groups of animals, pupillary responses were not evoked by the periodic background tonal signals. As conditioned responses were stereotyped and consistent for each of the experimental and control animals over several recording sessions, additional specification of the behavioral response is omitted.

Single-Unit Recording and Analysis. After the animal was mounted in the modified stereotaxic frame, a series of conditioning trials was given to

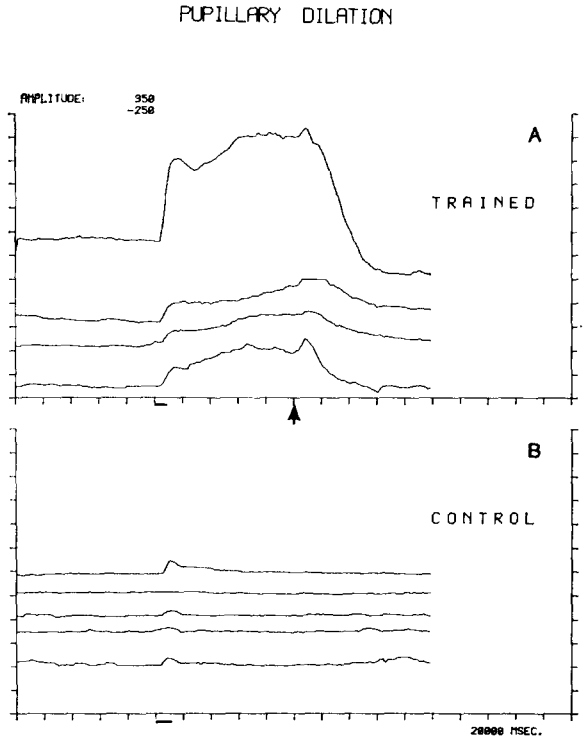


FIG. 2. Behavioral responses. A—pupillary dilation behavior representative of the largest and smallest conditioned responses to WN. The two middle traces are from the same animal on different unit recording days. B—pupillary dilation responses to WN recorded from habituated animals. The traces in A and B are displaced for ease of presentation. Duration of abscissa, 20 s. Ordinate: output of pupillometer in mV averaged over 30 trials. Each tic mark on abscissa indicates onset of probe tone. Horizontal line indicates WN presentation; shock presentation at arrow.

confirm the asymptotic level of conditioning attained earlier. Blocks of 10 conditioning trials were occasionally given to maintain asymptotic performance while searching for units with the on-going 1/s probe stimuli.

Glass-coated platinum–iridium microelectrodes were advanced through the dura to auditory cortex by a hydraulic microdrive secured to the recording chamber and controlled from outside the acoustic room. Unit activity was amplified by conventional means. Visual inspection of the spike waveform displayed at a fast sweep speed on an oscilloscope ensured that only one unit was being studied. The time interval between the beginning of a set of conditioning trials and the occurrence of each unit discharge, tone onset, WN onset, and shock was digitized and stored in the computer for subsequent analysis.

When a unit was isolated the tones were used to determine its best

frequency. Next, a set of conditioning trials was begun in which the frequency and intensity of the probe stimuli were fixed, respectively, at the best frequency of the unit and at a level that most reliably evoked activity. Best frequency stimuli were used to provide a common reference point for comparisons between cells. Single-unit activity was combined across behavioral trials, e.g., discharges occurring during the 1-s periods in which WN was presented were summed together, as were discharges occurring during the 1-s periods in which shock was presented, etc.

The modulation of auditory cortex single-unit activity was evaluated by comparing the mean spontaneous and tone-evoked activity occurring before each trial (pre-CS) with the respective activity occurring during (CS–US) and after the trials (post-US). Spontaneous activity was defined as those discharges occurring between tone-evoked responses. Long-term changes that may occur during the study of a cell were controlled because the pre-CS unit activity sampled during the entire sequence of trials constituted the baseline data. If the spontaneous or evoked activity occurring during the pre-CS period did not vary significantly around its mean value (χ^2 analysis), the mean pre-CS period activity was usd as the "expected value" in χ^2 analyses of the respective activity occurring during the CS–US and post-US periods. In the case of units recorded in control animals, the analyses were performed upon the activity in the pre-CS period and the 4-s period beginning at the first probe tone after WN. The activity of a unit was considered unstable and was consequently excluded if the pre-WN activity varied significantly around its mean value as determined by the χ^2 test.

RESULTS

Modulation of Unit Activity. Sufficient data were obtained from 75 units in the 10 animals to permit statistical analyses of evoked and spontaneous activity during the classical conditioning trials. A summary of the χ^2 analyses indicating the number of cells whose activity changed significantly ($P < 0.05$) during the CS–US period is presented in Table 1.

Of the 43 available cells in the control animals, significant changes of either evoked or spontaneous activity occurred in 12 cases (28%). In the trained animals, however, either the evoked or spontaneous activity of 24 of the available 32 cells (75%) changed significantly during the classical conditioning trials. In the conditioned animals, the spontaneous activity of 16 units (73%) changed significantly after WN relative to the mean pre-CS levels. Of the 27 available comparisons of tone-evoked responses in the trained animals, 17 units (63%) changed significantly during the conditioning trials. In contrast, of the 39 available comparisons of spontaneous activity in the control animals, the discharge rate of only 10 units

TABLE 1

Modulation of Auditory Cortex Responsiveness

	Trained	Control
Spontaneous or evoked activity		
Number of units tested	32	43
Significant modulation	24	12
Percentage changed	75%	28%
Spontaneous activity		
Not testable[a]	10[b]	4
Increased	12	6
Decreased	4	4
No change	6	29
Percentage changed	73%	26%
Evoked activity		
Not testable[a]	5	3
Increased	11	7
Decreased	6	0
No change	10	33
Percentage changed	63%	18%

[a] Activity prior to conditioning trials either not present or too variable to serve as baseline.

[b] Spontaneous activity not available from eight units studied prior to use of computer system in experiment. Tone-evoked discharges counted on a digital counter.

(26%) changed significantly. Furthermore, only 7 of the 40 units (18%) in the control animals showed significantly altered tone-evoked responses after WN presentation. The activity of each of the seven modulated units was augmented.

Neither tone-evoked nor spontaneous activity recorded during the pre-CS periods from the trained animals differed significantly (t-test) from the respective activity recorded in the control animals. Therefore, the observed differences between the percentages of modulated units are not likely to have resulted from differences in pre-CS baseline levels.

Charactristics of Altered Activity. The predominant type of cortical unit modulation was an augmentation of activity. For example, the responses of unit 17C001 to the probe tones during the pre-CS period consisted of an initial increased discharge rate during the 100-ms tones and a much larger burst of activity after the stimulus (Figs. 3, 4). Responses to the probe tones presented with WN were completely suppressed. A marked increase in the spontaneous discharge rate occurred immediately after the termination of WN that surpassed the pre-CS tone-evoked responses. That this increase in spontaneous activity was not merely an off-response to WN

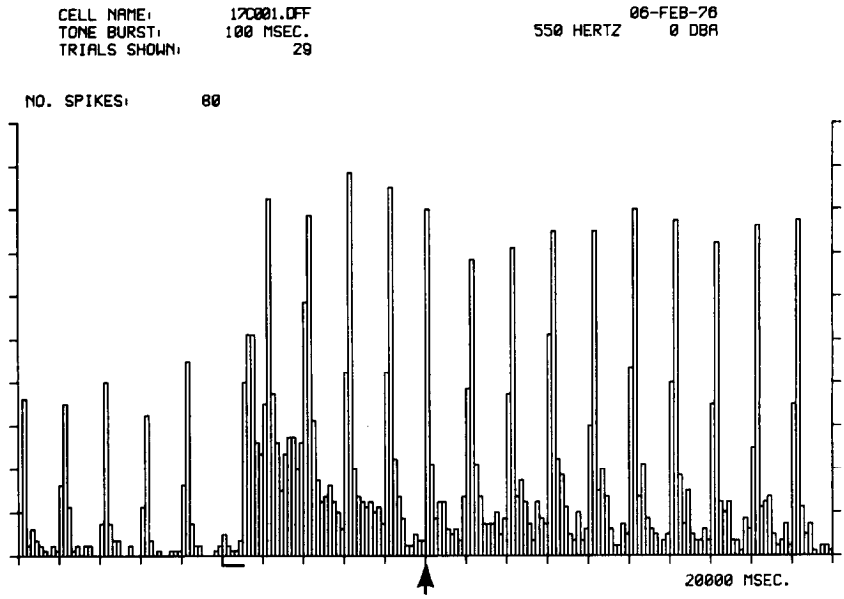


FIG. 3. Augmentation of spontaneous and tone-evoked activity of unit 17C001 during and after 29 conditioning trials. Each figure of this type is a 20-s duration "peristimulus" time histogram (PSTH) consisting of 200 consecutive 100-ms time bins. The origin of each PSTH, time zero, is the beginning of the pre-CS period. Each tic mark on the abscissa indicates the onset of a tone burst, there being 10 bins, i.e., 1 s, between tic marks. The number of discharges occurring in each time bin, summed over the set of conditioning trials, is plotted on the ordinate with the maximum value of the ordinate indicated at the top of the axis. The horizontal line below the abscissa indicates the occurrence of the 500-ms WN conditioned stimulus and the arrow indicates the occurrence of the unconditioned shock stimulus. Probe tone frequency and intensity in dB attenuation are shown at the top of each figure. Note prolonged time course of augmentation: evoked responses 14 s following WN were about twice the amplitude of pre-CS period responses. Note increased spontaneous activity. Probe tone: 550 Hz delivered at 88 dB SPL.

is suggested by the systematic diminution of the spontaneous discharge rate during the subsequent 3.5 s to a plateau of spontaneous activity that was more than three times greater than the pre-CS rate and lasted 11 additional seconds. Responses to the 14 probe tones after the occurrence of WN were augmented above the pre-CS tone-evoked rate by a factor of more than 250%.

Response modulation need not be uniform during the entire course of the evoked activity. The extent of the modulation shown by unit 17C001, for example, differed for the portions of the responses occurring during the 100-ms tones and during the subsequent 100-ms epochs. Mean activity during the first 100 ms of the responses rose from 10 discharges in the

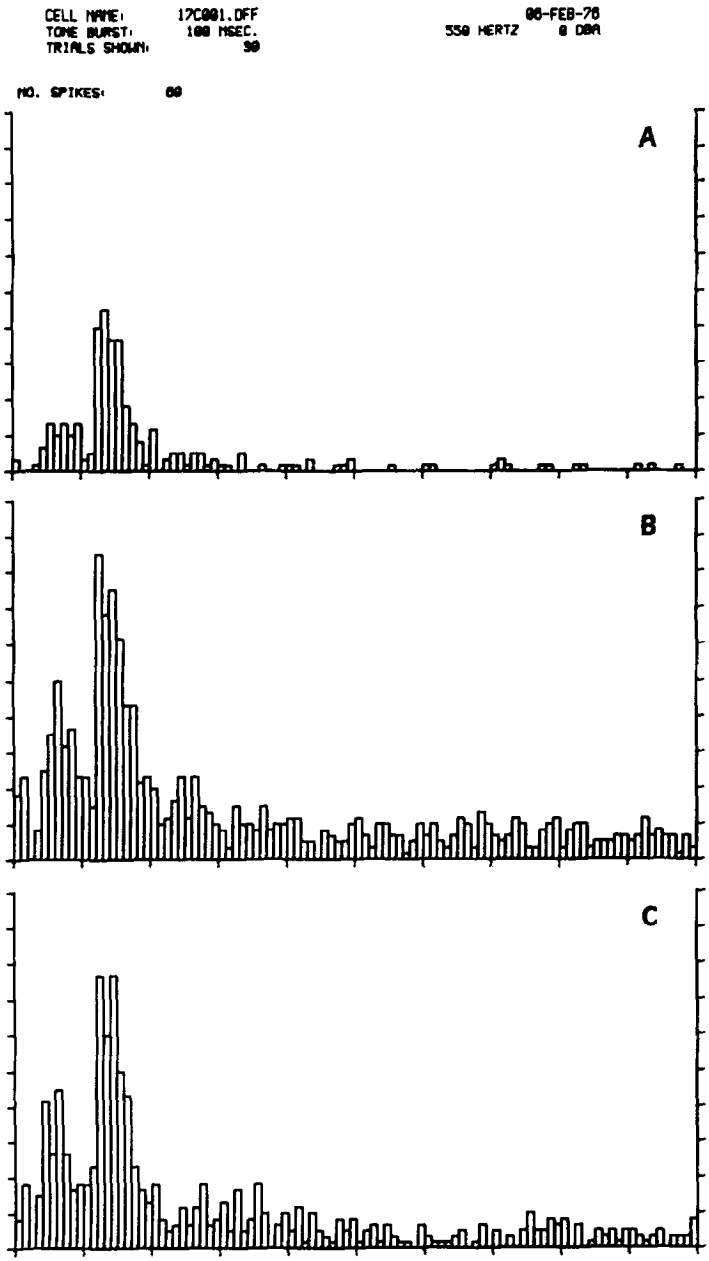


FIG. 4. Average discharge pattern of tone-evoked and spontaneous activity of unit 17C001 during the pre-CS (A), CS–US (B), and 4-s post-US (C) periods of the conditioning trials. PSTH duration, 1 s. Bin width, 10 ms, 10 bins between tic marks. Probe tones occurred during first 10 bins in each histogram. Maximal value of ordinate in each panel is 60 discharges. These data derive from the activity shown in Fig. 3. Pre-CS, CS–US, and post-US activity are separately averaged, respectively in A, B, and C. Note the augmented discharge rate during and after the 100-ms tones and the increased spontaneous activity in B and C.

pre-CS period to 36 discharges in the CS–US period (an increase of 360%); the mean activity during the next 100-ms epochs rose from 30 discharges to 67 discharges (an increase of 220%). Evoked responses during the post-US period shown in Fig. 3 exhibited a similar disparity between the magnitude of the increase of the first and second portions of the responses. Thus, although the absolute augmentation of the second part of the responses of unit 17C001 was always greater than that of the first, the relative increase in discharge rate during the tones was invariably larger.

Augmented spontaneous discharge rates were not always accompanied by significantly altered tone-evoked responses. Tone-evoked responses of unit 17C012 (Fig. 5A) during the CS–US period did not differ significantly from those occurring in the pre-CS period, although the portions of

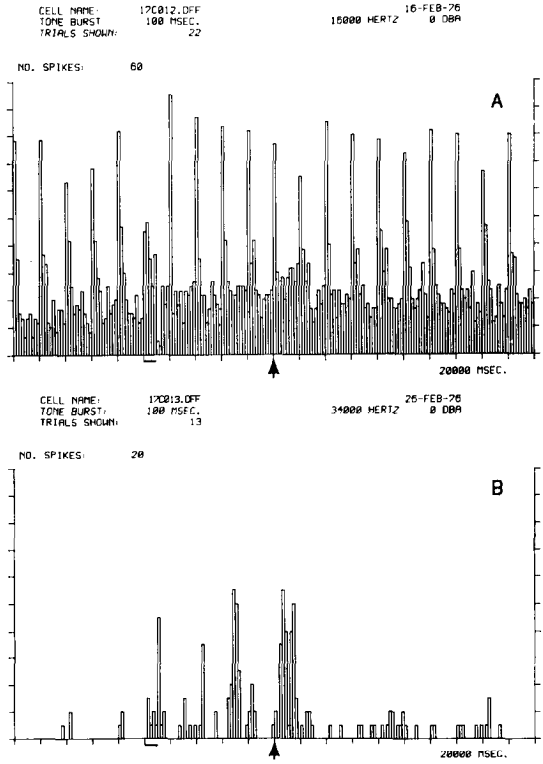


FIG. 5. Augmented spontaneous activity. A—unit 17C012 illustrates the case of increased spontaneous activity without significantly altered evoked activity. Probe tones: 16.0 kHz delivered at 60 dB SPL. B—augmented spontaneous discharge rate of unit 17C013 during and after 13 conditioning trials. This unit would not respond to tonal stimuli. Increased activity was not a consequence of probe tones but of behavioral trials. Probe tones: 34.0 kHz delivered at 36 db SPL.

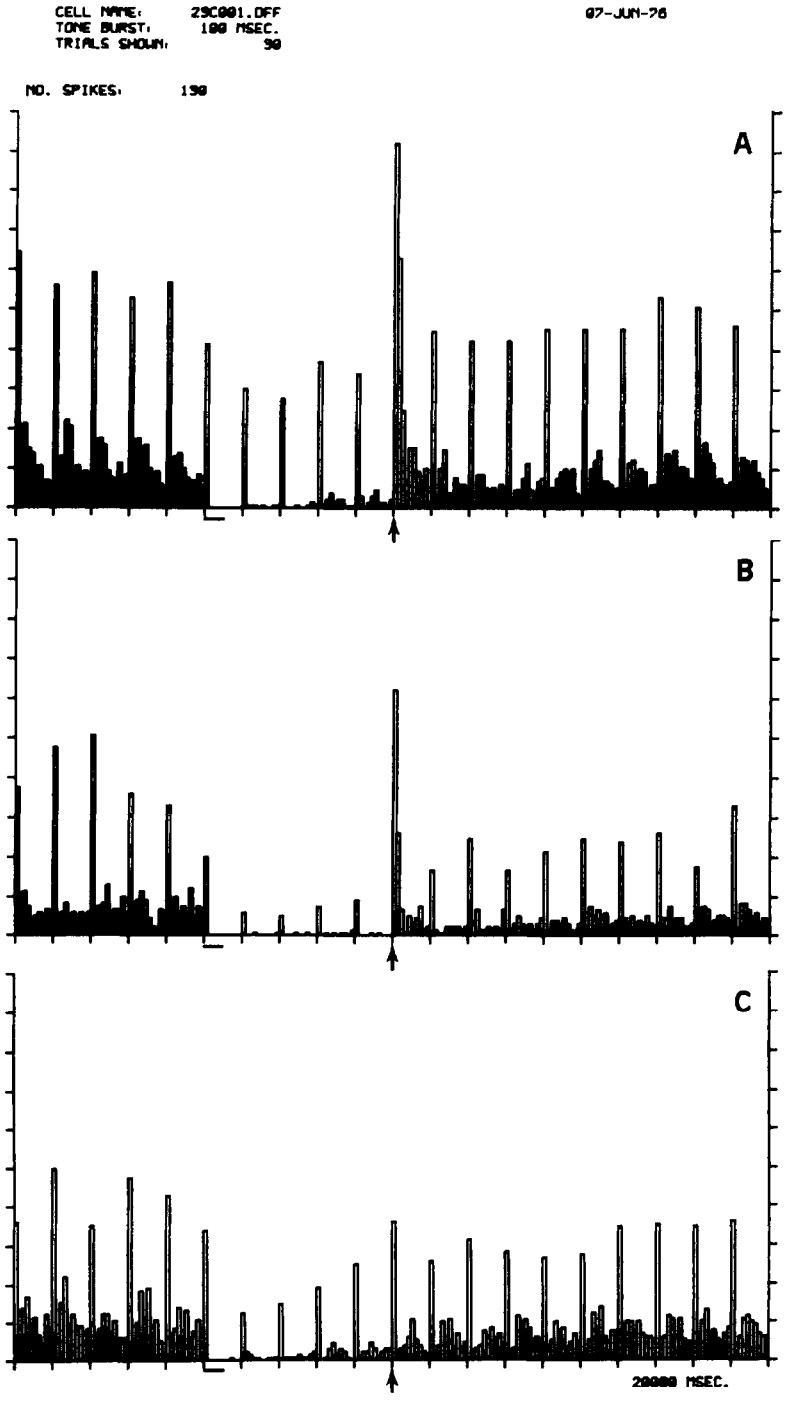
the responses occurring during the 100-ms tones were greater than those occurring in the pre-CS period. Spontaneous activity was, on the other hand, significantly ($P < 0.05$) increased during the CS–US period and remained significantly ($P < 0.05$) above the control level for at least the duration of the post-US period shown in Fig. 5A.

When searching for responsive units with the probe stimuli, occasionally the presence of a cell would be indicated only by its spontaneous discharge rate as the cell failed to respond reliability to any tonal stimulus at our command. When the responsiveness of such cells was tested within the context of a set of behavioral trials, the activity of some remained unaltered while the activity of other cells increased significantly.

This type of unit behavior is illustrated by unit 17C013 (Fig. 5B). Its presence was evident only in a very low spontaneous discharge rate, indicated in Fig. 5B by the six discharges that occurred during the pre-CS periods of 13 trials. There was a minimal number of discharges during the latter 400 ms of WN and an indication of an off response after the termination of the broad band stimulus. During the CS–US period, 50 discharges occurred. There was, however, no systematic indication that the discharges were related in time to the presentation of the probe tones. Fifty discharges occurred during the 1-s periods in which shock was presented. Relative to the pre-CS period, a significantly increased discharge rate is apparent in the post-US period shown in the histogram. These results indicate that the augmented activity of unit 17C013 was determined principally by the concomitants of the behavioral task rather than by the probe tones.

Suppression of both tone-evoked and spontaneous activity was commonly observed during and after classical conditioning trials (Table 1). The three panels of Fig. 6 each show the activity of unit 23C001 during 30 classical conditioning trials. Mean tone-evoked activity was reduced from 75 discharges in the pre-CS period to 42 discharges in the CS–US period, a 45% suppression of evoked activity (A). Spontaneous activity was reduced from an average of 48 discharges to seven discharges in the CS–US period. During the post-US period the levels of spontaneous and evoked activity were significantly ($P < 0.05$) augmented above the mean CS–US levels but remained significantly below the mean pre-CS discharge rates for at least 10 s after shock.

After unit data at the best frequency and most effective intensity were collected, additional data were sometimes collected with nonoptimal probe stimuli, i.e., sets of behavioral trials were presented in which the frequency or intensity of the probe stimulus was changed. Due to the time required to complete each set of conditioning trials, only a few points within the response area of any one cortical neuron were examined. These data,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however, uniformly suggest that the type of activity change may be independent of the frequency and intensity of the probe tone. Unit 23C001 exemplifies this observation. The modulatory pattern apparent in Fig. 6A occurred also when the level of the 25.5-kHz probe tone was reduced to 21 dB SPL (Fig. 6B) and also when the probe tone was changed to a 20-kHz signal presented at 60 dB SPL (Fig. 6C). Mean tone-evoked and spontaneous activity occurring during both the CS–US and post-US periods were significantly ($P < 0.05$) below the respective activity in the pre-CS periods.

Aside from the lack of suppression of evoked responses after WN (which may be due to the small sample of modulated cells observed in the habituated animals) and the relatively low probability of significant alterations of responsiveness, the kinds of modulation that were observed in the control animals did not differ from those seen in the conditioned animals.

Correspondence of Evoked and Spontaneous Discharge Modulation. It is not possible to determine from the present data whether auditory cortex was itself the source or the target of the modulatory processes or whether the cortical cells were faithfully reflecting altered input arriving from subcortical auditory structures. Nevertheless, additional information about the modulatory process is available from an analysis of the direction in which spontaneous and tone-evoked activity changed during the behavioral task. If evoked and spontaneous activity changed in a dependent manner one could postulate a common source of modulation affecting the general excitability of the cortical cells.

This analysis was carried out on those units recorded in the trained and control animals for which statistical analyses were available for both evoked and spontaneous activity and for which at least one kind of activity was significantly altered after WN presentation. Of the 12 units recorded in the control animals meeting these conditions, the two kinds of activity changed in the same direction in two cases, and changed in the opposite direction in one case, and in nine cases only one kind of activity was significantly altered. Thus, in the control animals there was no predictable relationship between the modulation of tone-evoked and spontaneous activity. In the trained animals, on the other hand, the spontaneous and tone-evoked activity of eight of the 14 available cells changed in the same

FIG. 6. Modulation of spontaneous discharge rate and responses of unit 23C001 evoked by probe tones of 25.5 kHz delivered at 51 dB SPL (A), 25.5 kHz delivered at 21 dB SPL (B), and at 20.0 kHz delivered at 60 dB SPL (C). Activity of 30 conditioning trials in each panel. Maximal value of ordinate in each panel is 130 discharges. Duration of each histogram is 20 s. Modulation pattern similar with each probe stimulus.

TABLE 2

Magnitude of Modulation of Auditory Cortex Responsiveness[a]

	Trained	Control
Spontaneous activity		
Number of units tested[b]	16	10
Mean ± SE pre-CS period	0.98 ± 0.26	0.91 ± 0.29
Mean ± SE CS–US period	1.33 ± 0.35	0.88 ± 0.25
Mean ± SE change	0.35 ± 0.11[c]	−0.03 ± 0.17
Expected mean change[d]	0.25	0.01
Mean absolute change	0.47 ± 0.08[c]	0.34 ± 0.11[c]
Expected absolute change[d]	0.34	0.09
Evoked activity		
Number of units tested	17	7
Mean ± SE pre-CS period	3.97 ± 0.86	3.08 ± 1.21
Mean ± SE CS–US period	4.27 ± 1.09	4.21 ± 1.66
Mean ± SE change	0.30 ± 0.40	1.13 ± 0.51
Expected mean change[d]	0.19	0.14
Mean absolute change	1.25 ± 0.25[c]	1.13 ± 0.51
Expected absolute change[d]	0.79	0.14

[a] Discharge rate per 100 ms.

[b] Number of units whose activity was modified significantly (Table 1).

[c] Differs significantly ($P < 0.05$) from zero (t-test).

[d] Average change multiplied by the percentage of units that changed (Table 1).

direction, and changed in the opposite direction in two cases, and one class of activity did not change significantly in four cases. These frequencies indicate an increased tendency for spontaneous and tone-evoked activity alterations to covary in the trained animals. Even for these animals, however, the tone-evoked and spontaneous activity of nearly half the units did not vary together.

Magnitude of the Modulation. Estimates were derived of the degree of modulation exhibited by the population of cortical units influenced by state-dependent variables. Estimates were obtained by analyzing the activity of each of the units recorded in both the trained and control animals that showed significantly altered evoked or spontaneous discharge rates after WN. The number of tone-evoked and spontaneous discharges occurring in the pre-CS and CS–US periods were divided by both the number of trials the individual cell was studied and by the number of 100-ms bins occupied by the particular activity. The latter manipulations were necessary to analyze across cells the tone-evoked and spontaneous activity of different durations and to relate the magnitude of the change of spontaneous activity to the altered evoked rate. Averages of these values represent, therefore,

the mean number of discharges evoked by a single presentation of a probe stimulus and the average accompanying spontaneous activity.

The average evoked and spontaneous activity and the standard errors of the means are given in Table 2. In the pre-CS period neither evoked nor spontaneous activities recorded in the trained animals differed significantly from the respective activity recorded in the control animals. Analyses of mean differences between the pre-CS and CS–US data (t-test for correlated means) showed that only the spontaneous activity of cells recorded from trained animals was significantly augmented ($P < 0.05$). In the control animals the increment of evoked activity was significantly ($P < 0.05$) greater than that of spontaneous activity. This result is due to the lack of suppression of evoked responses after WN and the mixed changes of spontaneous activity (Table 1).

The actual extent of the modulation is, however, obscured by analyses of mean differences between the pre-CS and CS–US values because augmented and suppressed responses of different units tend to cancel each other in calculations of average differences. Average absolute differences between the pre-CS and CS–US periods, independent of the direction of the change, are more descriptive of altered single-unit activity as suppression and augmentation are given equal significance as modes of modulation.

Analyses of absolute change demonstrated that the tone-evoked and spontaneous activity of units recorded in the trained animals and the spontaneous activity of units recorded in the control animals were significantly altered. Only the tone-evoked activity of the control units was not significantly changed. Neither tone-evoked nor spontaneous activity recorded in the trained animals was modified significantly more than was the respective activity recorded in the control animals. The degree of modulation of evoked and spontaneous activity did not significantly differ in the control animals, whereas in the trained animals tone-evoked activity was altered to a significantly ($P < 0.05$) greater extent than was spontaneous activity. When the mean absolute change is weighted by the probability of change (Table 1), i.e., the percentage of units that changed, it is evident that, as a group, the activity of the modifiable neurons was affected by the behavioral task and that, in contrast to the preceding analysis of average differences, tone-evoked responses were altered to a much greater extent than was spontaneous activity.

Location of Cells. The vertical orientation of the recording chamber restricted the orientation of the electrode penetrations to an oblique course through auditory cortex. As the result of repeated penetration and manipulation, the dura became progressively more difficult to penetrate, precluding the large number of penetrations required to map fully the auditory cortex and to determine exactly that the units studied were all within the primary

auditory field. Merzenich *et al.* (12) showed that topographical landmarks such as the anterior and posterior ectosylvian sulci are not totally reliable indicators of the location of AI.

It is likely, nevertheless, that most or all of the units encountered in this study were situated within the primary auditory field because, for each animal, higher best frequency units were always found in the anterior portion of the recording region and lower best frequency units in the posterior region. Second, the extent of the penetrations was apparently insufficient to reach AII as the frequency reversal to be expected upon entering the second auditory field was not encountered. Last, each penetration did enter auditory cortex at the commonly defined locus of AI, between the anterior and posterior ectosylvian sulci near the center or dorsal half of the ectosylvian gyrus.

DISCUSSION

The purposes of this experiment were (i) to determine if the discharge properties of auditory cortex neurons are influenced by the behavioral context in which acoustic stimuli occur and (ii), if so, to characterize the observed modulation. The context of acoustic stimulation was determined experimentally by eliciting a previously acquired behavioral response at specified times during the study of spontaneous and tone-evoked activity. As the physical parameters of the acoustic stimuli were constant during a set of behavioral trials presented in the study of a cortical neuron, any systematic changes in response to the stimuli can be interpreted as being related to the behavioral context in which the signals occurred.

With regard to the first question, this experiment indicates that in the primary auditory field the level of spontaneous activity and responses to behaviorally irrelevant tonal stimuli may be significantly modulated during the performance of a behavioral task. The difference between the proportions of units that were modulated in the trained and control groups of animals indicates that the altered unit activity recorded in the trained animals could only partly be due to intramodality interactions between WN and the probe tones, independent of the conditioning paradigm. Rather, the differences between the two groups must be related to differences between the behavioral contexts of acoustic stimulation. Thus, the behavioral context in which acoustic signals occur is an important determinant of both the evoked and spontaneous activities of a large proportion of auditory cortex neurons.

Perhaps the most interesting observation of this experiment is the variety of changes occurring in the responses of different cortical cells and the nonuniform modulation of tone-evoked responses of individual neurons. All components of an evoked response were often not equally modified.

Furthermore, spontaneous activity may decrease or increase with altered evoked responses or may change significantly although tone-evoked responses remain unaltered. Spontaneous activity may even increase in the absence of accompanying evoked responses. It is, therefore, unlikely that the modulation of cortical unit activity arises from a simple gain control mechanism that diffusely alters sensory system activity. Rather, these observations suggest quite specific influences that dynamically bias the activity of auditory system neurons.

The time course of the altered responsiveness often extended several seconds beyond presentation of WN. Such extended changes of spontaneous activity and responses to acoustic signals could not be predicted from the results of the multiple-unit (4, 5, 9, 11, 15, 16) or single-unit experiments (1, 13), cited earlier. The prolonged nature of the modulation suggests that in the unanesthetized condition the excitability of auditory cortex may be continuously modulated by state-dependent variables.

The tone-evoked responses of six of the 17 modulated units recorded in the trained animals were significantly reduced during the classical conditioning task while evoked activity after WN was augmented in all seven instances of modulated responses observed in the control animals (Table 1). Although the sample of modulated units recorded in the control animals is too small to allow definite conclusions, the lack of suppression in those cells and the frequency of suppression in the trained animals suggest that response suppression may be a mode of modulation evoked only within the context of particular behavioral states.

It is unclear how the dependence of auditory cortex activity on state-dependent variables relates to the sensory processes of coding physical parameters of acoustic signals. It is clear, however, that auditory cortex activity reflects the influences of both auditory and nonauditory processes.

Several aspects of the classical conditioning task acting alone or together may have contributed to the observed activity changes. Although the pupillary dilation responses began prior to the first probe tones of the CS–US period and may, therefore, have been a contributing factor, the behavioral responses were themselves not sufficient for the cortical modulation as they accompanied both modulated and unaltered unit activity. Tonic changes of arousal level cannot account for the modulation as the activity preceding each conditioning trial comprised the control data, i.e., the pre-CS activity.

Statistical comparisons of spontaneous and tone-evoked pre-CS activity demonstrated no significant differences between the respective activities in the two groups of animals. The occurrence of tail shock in a conditioning trial, therefore, did not significantly influence probe tone responses and spontaneous activity preceding the subsequent behavioral trial. It is never-

theless possible that tail shock could have had a sensitizing influence upon the response to the subsequent white noise stimulus, resulting in altered CS–US activity in that trial. Determination of the relative contribution of these and other aspects of the conditioning trials to the modulation of cortical responsiveness, such as the modality of the conditioned stimulus, will be analyzed in complementary behavioral paradigms.

Relevance to Learning Studies. Although the present study was not intended to fulfill the requirements of a learning experiment, the results are nevertheless pertinent to that area of research. Behavioral paradigms often used in studies concerned with the role of sensory system activity during behavior typically use a relatively prolonged conditioned stimulus. The observation in the present study that spontaneous activity is often altered during conditioning trials raises the question whether augmented discharge rates commonly observed in the presence of conditioned stimuli derive solely from enhanced responses to those signals or in part from enhanced background activity that would have occurred during those time periods even in the absence of the on-going conditioned stimuli. The query applies, of course, similarly to neuronal responses to differentiated stimuli often included in learning studies and generally suggests caution in interpreting the involvement of sensory systems in learning.

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