Physiological Plasticity of Single Neurons in Auditory Cortex of the Cat During Acquisition of the Pupillary Conditioned Response: I. Primary Field (AI)

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The effects of conditioning on the discharges of single neurons in primary auditory cortex (AI) were determined during acquisition of the pupillary conditioned response in chronically prepared cats. Acoustic stimuli (1-s white noise or tone) were presented with electrodermal stimulation unpaired during a sensitization control phase followed by pairing during a subsequent conditioning phase. Stimulus constancy at the periphery was ensured by the use of neuromuscular blockade. Discharge plasticity developed rapidly for both evoked and background activity, the former attaining criterion faster than the latter. The pupillary dilation conditioned response was acquired at the same rate as were changes in evoked activity (i.e., 10–15 trials) and faster than background activity (i.e., 20–25 trials). Increases in background activity were correlated with increasing level of tonic arousal, as indexed by pretrial size of the pupil.

Behavioral adaptation requires accurate information about the environment. That sensory systems provide such information has never been in serious question. However, the mechanisms involved have not yet been elucidated fully. This problem appears to be further complicated by the fact that electrophysiological responses to environmental stimuli within sensory systems are modified by learning. Numerous studies of classical and instrumental conditioning in animals have demonstrated that responses to conditioned and discriminative stimuli in sensory systems are altered systematically by associative processes (for reviews, see John, 1961; Sokolov, 1977; Thompson, Patterson, & Teyle, 1972). Such response plasticity is particularly evident in sensory cortex; it has been documented most extensively in auditory cortex (e.g., Buchwald, Halas, & Schramm, 1966; Cassady, Cole, Thompson, & Weinberger, 1973; Galambos, Sheatz, & Vernier, 1955; Oleson, Ashe, & Weinberger, 1975) and has been reported as well in olfactory (Freeman, 1980), somatosensory (e.g., Voronin, Gerstein, Kudryashov, & Ioffe, 1975), and visual (e.g., Shinkman, Bruce, & Pfingst, 1974) cortices. Thus, sensory responses are affected by two types of variables: (a) the physical parameters of stimuli and (b) the meaning or cue value of stimuli. This situation suggests a paradox because the requirement for veridical responses to the physical parameters of stimuli appears to be compromised by the effects of stimulus meaning.

In recent years, this issue has been investigated most thoroughly within the auditory system, and there now appears to be a partial resolution of the paradox. Anatomical and physiological studies have revealed that the auditory system contains both lemniscal and non-lemniscal or "lem-
niscal adjunct" ascending pathways (Graybiel, 1972; Herkenham, 1980; Ryugo & Killackey, 1974; J. Winer, Diamond, & Raczkowski, 1977). At the level of the thalamic auditory system, these subsystems engage different subdivisions of the medial geniculate body (Morest, 1964, 1965). The lemniscal line projects to the ventral medial geniculate nucleus (MGv), the neurons of which are tonotopically organized, with narrow tuning functions (Aitkin & Webster, 1972), whereas the nonlemniscal line projects to the magnocellular medial geniculate nucleus (MGm), the neurons of which are not tonotopically organized and have very broad tuning functions (Aitkin, 1973). Analysis of the physical parameters of sound and the meaning of sound is also compartmentalized at this level of the auditory system. Neurons in the lemniscal MGv respond to the physical parameters of the acoustic environment, but these responses are unaffected by learning. In contrast, neurons in the nonlemniscal MGm are not particularly sensitive to changes in the physical parameters of sound, but they develop discharge plasticity rapidly during learning. These findings have been obtained in cat during classical defensive conditioning (Ryugo & Weinberger, 1976, 1978), rabbit during instrumental avoidance conditioning (Gabriel, Miller, & Saltwick, 1976), and rat during hybrid classical-instrumental appetitive conditioning (Birt, Nienhuis, & Olds, 1979; Birt & Olds, 1981).

At the level of auditory cortex, the situation has not yet been clarified, but it is probably more complex than at the thalamus. Auditory cortex consists of several fields, which have either lemniscal or nonlemniscal characteristics. The primary auditory cortex (AI) and the anterior (AAF) and posterior (P) fields are tonotopically organized; in contrast, the secondary auditory cortex (AII) and insular (I) and temporal (T) fields are not so organized and thus seem to be nonlemniscal in nature (e.g., Reale & Imig, 1980). Almost all previous studies of learning in the auditory cortex have investigated AI, and, as pointed out above, there is abundant documentation that evoked potentials and multiple-unit activity in this cortical field are modified by associative processes. Insofar as both the ventral and the magnocellular medial geniculate nuclei project upon primary auditory cortex, we have suggested that the former, being nonplastic, is not a source of associative effects on AI whereas the latter might be involved in learning-induced cortical discharge plasticity (Weinberger, in press). Additional analysis of this and many related issues requires investigation of the effects of learning on the discharge properties of single neurons in auditory cortex, because of the inherent limitations of evoked potentials and multiple-unit data. Single-unit studies during the acquisition of behavioral conditioned responses are technically difficult, requiring that adequate recordings be obtained continuously during a prolonged period of training, and they yield data from only one cell per training session. Nonetheless, such studies are important because they provide a link between the fields of single-unit auditory physiology and learning and they can reveal the changes underlying neurophysiological plasticity as indexed by evoked potentials and particularly by multiple-unit recordings (Kraus & Disterhoft, 1982).

The present experiment is concerned with the discharges of single cells in primary auditory cortex (AI) during the acquisition of a behavioral conditioned response. A companion report is concerned with the effects of classical conditioning on the secondary auditory cortical field (AI1) which is prototypical of nonlemniscal auditory cortex (D. Diamond & Weinberger, 1984). Such comparative information may be important for understanding the functional role of multiple sensory cortical fields (I. Diamond, 1979; Merzenich & Kass, 1980) as well as bearing more directly on issues regarding the neuronal bases of learning. Further, it is hoped that the data so obtained will promote the rapprochement of the fields of sensory neurobiology and learning, as both areas are critically concerned with how the brain acquires information about the environment.

A preliminary report of some of these results has been presented (Hopkins & Weinberger, 1980).
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Method

Surgical Preparation

The subjects were 8 adult cats, 3.2-5.0 kg, in good health. The animals were anesthetized with sodium pentobarbital (Nembutal, 40 mg/kg, ip) and placed in a stereotaxic frame, with care taken to preserve the integrity of the external auditory meatus and tympanic membrane. The scalp was incised and reflected, and the calvarium was cleared of connective tissue. A pedestal containing metal fixtures was built with dental acrylic and secured to the skull with stainless steel screws. The pedestal allowed for immobilization of the head during subsequent training sessions without direct pressure on the animal. Body temperature was maintained by a thermostatically controlled warm water pad during the surgery and recording sessions. Ophthalmic ointment (Terramycin) was applied to prevent corneal drying. Antibiotics (Panalog and Bicillin) were administered for 2-3 days following the surgery. Training began after a recovery period of 1-2 weeks.

Experimental Design and Procedures

At the beginning of a training session, the animals underwent neuromuscular blockade induced by gallamine triethiodide (Flaxedil, 10 mg/kg, ip). The trachea was intubated with a pediatric catheter, coated with a local anesthetic (Xylocaine), under laryngoscopic control, and the animal was artificially respired with a Harvard respirator. Following immobilization, the animal was positioned in a modified stereotaxic frame, and the animal was artificially respired under laryngoscopic control, and the animal was artificially respired with a Harvard respirator. Following immobilization, the animal was positioned in a modified stereotaxic frame fitted with bars which attached to the pedestal. All procedures were carried out with the animals enclosed in an acoustically isolated chamber (IAC 1202). Neuromuscular blockade was maintained with intravenous infusion of Flaxedil (20 mg/hr, iv). Expired CO₂ levels were not monitored because we had found in previous experiments that pupillary size and motility in response to sensory stimulation are more sensitive indexes of the animal's condition. At the end of a training session, the animals were recovered with the assistance of Tensilon (0.6 ml im).

Acoustic stimulation was delivered to the ear contralateral to the recording electrode by a Beyer earphone which was attached to plastic tubing that fitted into a mold of the external auditory meatus. The mold was sealed with plasticine to provide a closed acoustic system. Acoustic stimulation consisted of 1-3 kHz tones and were controlled from outside the acoustic chamber and advanced through the dural slit until the discharges of single neurons were encountered (see below). Discharges were amplified with a conventional differential preamplifier (bandwidth 80 Hz-10 kHz) and led to an active high-pass filter (0.4-6 kHz), the output of which was displayed on a storage oscilloscope and recorded on a direct channel of a Hewlett-Packard 3964A tape recorder. A single unit was identified by visual inspection of the spike waveform. Neuronal data were considered acceptable if the record consisted of a clearly distinguishable unit whose waveform exhibited no notches or other signs of injury, with a signal-to-noise ratio of at least 3:1. Data reported here are only for units meeting these criteria.

A training session consisted of two parts, a sensitization phase and a conditioning phase. During sensitization, 15 CSs and USs each were presented in an unpaired fashion, at pseudorandom intervals at an average density of two per minute with the restriction that the stimuli not occur within 10 s of each other. During conditioning, the CS and US were always paired, the US being presented at CS offset. Stimulus density was maintained at two per minute, the average intertrial interval was 60 s (range, 30-90 s). The sensitization phase served as a control for nonassociative factors. This phase used unpaired rather than randomized presentation of the CS and US because conditioned inhibition does not develop during the presentation of small numbers of unpaired stimuli (Furedy, 1971; Furedy, Poulos, & Schiffman, 1975). Backward pairing was not used because it can produce conditioned inhibition to the CS due to its cue value as a “safety” signal of the termination of the US (e.g.,...
Segundo, Galeano, Sommer-Smith, & Roig, 1961). Conditioning trials were initiated without break after the last sensitization trial and were continued for up to 60 trials or until acceptable recordings from a single cell could not be obtained. Animals received only one training session on a single day, and at least 7 days intervened between successive training sessions.

Data Analysis

The effects of training on pupillary behavior were assessed as described previously (Ashe, Cassady, & Weinberger, 1976; Oleson et al., 1975; Oleson, Vododnick, & Weinberger, 1973; Oleson, Westenberg, & Weinberger, 1972; Ryugo & Weinberger, 1978). Briefly, the pupillometer write-out was measured immediately preceding the presentation of the CS throughout training and preceding the US for sensitization trials on which EDS was given alone. The peak amplitude of dilations to these stimuli was measured and the pretrial level was subtracted, which yielded a difference score hereafter referred to as a pupillary response. The scores and baseline levels were determined for every trial throughout training. They were normalized for comparison across animals by expressing each value as a percentage change from the average of the last five-trial block of the sensitization phase. The number of trials to criterion for pupillary conditioning was defined as five consecutive trials during the conditioning phase all of which had responses greater than the average of the last five trials of the sensitization phase. The probability of this occurring by chance is .03 (Feller, 1968).

Neuronal discharges were analyzed with the assistance of an LSI/11 computer. Single-unit discharges were passed through a voltage detector or window that produced a single pulse for each discharge detected. The output of the trigger source was led to a pulse generator which recorded the occurrence of spikes during 0.5-4 s immediately preceding a trial and during the trial. Spike counts were stored in consecutive bins of 2 or 3 ms. The average number of counts per bin was determined for every pretrial period and every during-trial period. The pretrial average was subtracted from the during-trial average for each trial; this yielded a difference score. The pretrial scores are hereafter referred to as background activity, and the difference scores as evoked activity. Each background and each evoked score were normalized by expressing it as a percentage change from the average pretrial and evoked scores, respectively, for the last five-trial block of the sensitization period. The number of trials to criterion of discharge plasticity was defined as five consecutive trials during the conditioning phase all of which had greater or smaller values than the average of the last five trials during the sensitization phase \( p = .03 \). All data were evaluated by parametric (B. Winer, 1971) or nonparametric (Siegel, 1956) statistics.

Histology

Following a training session, a small electrolytic lesion was produced by passing anodal current through the recording electrode. After the final session, the animal was given an overdose of Nembutal and perfused through the carotid arteries with 0.9% saline followed by 10% formalin; the brain was removed and stored in formalin. Frozen serial sections (50 \( \mu m \)) were taken throughout auditory cortex and stained with cresyl violet. Recording sites were reconstructed according to the cytoarchitectural distinctions of the various subfields of auditory cortex described by Rose (1949) and Sousa-Pinto (1973).

Results

Pupillary Behavior and Conditioning

Pupillary dilation responses were recorded for all experimental sessions. At the beginning of sensitization, the acoustic stimulus generally elicited brief, low-amplitude dilations. By the end of the sensitization phase, dilation responses were reduced in both amplitude and duration. The EDS (US) produced consistently large dilation responses throughout the experimental session. During conditioning, acoustically evoked dilations increased rapidly over trials, as has been reported previously (Ashe et al., 1976; Oleson et al., 1972, 1973, 1975; Ryugo & Weinberger, 1978; Weinberger, Oleson, & Haste, 1973). The average conditioned dilations typically exceeded the largest acoustically elicited responses of the preceding sensitization period during Trials 6-10, attained asymptote by Trials 21-25, and maintained high values thereafter (Figure 1). The pupillary dilation conditioned response attained criterion in 16 of 21 sessions. For these sessions, the mean trials to criterion was 11.81 \( (SD = 5.76, \text{range}, 6-27 \text{trials}) \).

Of the 8 animals, 7 received more than one, and some as many as four, training sessions at weekly intervals. In order to assess the cumulative effect of earlier training sessions, each animal was assigned a savings score based on a sequential comparison of values for the trials-to-criterion measure from session to session. Thus, a subject would receive a net positive savings score if the number of all possible session comparisons for which there was a reduction in the trials-to-criterion measure was greater than the number of comparisons that yielded an increase in this measure. In this manner it was determined that 4 of 7
animals had net positive savings scores, 1 had a zero savings score, and 2 had negative savings scores.

**Location of Neurons**

Data were obtained from 21 neurons with recording sites verified histologically in primary auditory cortex. The laminar sites of recording could be determined in 15 cases. Thirteen sites were in infragranular layers V and VI, and two sites were in layer IV. This distribution is insufficient for correlating anatomical lamina with neurophysiological data. The results presented here should be regarded as representing mainly the deep lamina of primary auditory cortex.

**Neuronal Data—Interpretation of Negative Findings**

The establishment of behavioral conditioned responses provides a framework within which to interpret neuronal activity during conditioning procedures. As explained previously (Weinberger, 1982a, 1982b), we do not seek the neural circuit underlying the pupillary dilation conditioned response. However, this index of behavioral learning is useful as a sign that the subject is "adequate," in order to permit unambiguous interpretation of "negative" neuronal findings. The failure of a neuron to develop a systematic change in its discharges during conditioning might be due to its presumptive membership in a group of "nonplastic" neurons, at least nonplastic for the circumstances of a given experiment. Although this is the usual interpretation of negative findings, an alternative explanation is that the subject was "inadequate," that is, a "substandard" preparation. Thus, a neuron in question might have the capacity to develop plasticity but be unable to express it because of the inadequacy of the subject. In short, if the subject is unable to acquire a conditioned response and a neuron in that animal also fails to express discharge plasticity, no conclusions can be drawn about the functional plasticity of the cell. Accordingly, the data from such neurons should be set aside. In contrast, a cell that does not develop discharge plasticity in an animal that does develop a behavioral conditioned response can be said to be functionally nonplastic for that situation because the alternative explanation of an inadequate preparation can be rejected.

As noted above, data were obtained from 21 neurons in primary auditory cortex. The data from 2 neurons were eliminated from the total sample because the animals failed to acquire the pupillary dilation conditioned response and the neurons developed neither background nor evoked discharge plasticity. In all other cases in which discharge plasticity did not develop, there was independent evidence of the adequacy of the preparation.

**Evoked Activity**

For the 19 cells in the analysis set, 14 attained the criterion of discharge plasticity. However, we excluded any neurons that, although meeting this criterion, were simply continuing a trend extant during sensitization. This was accomplished by computing the slopes of functions for sensitization and conditioning, on a trial-by-
Table 1
Effects of Conditioning on Evoked Discharges

<table>
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<th>Decrease</th>
<th>No change</th>
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<td>6C</td>
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<td>7</td>
</tr>
<tr>
<td>7E</td>
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</tr>
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<th>SD</th>
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<th>M</th>
<th>SD</th>
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<td>10.25</td>
<td>3.20</td>
<td>6</td>
<td>11.83</td>
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<td>14.50</td>
<td>11.83</td>
</tr>
</tbody>
</table>

* Deleted from analysis because neither pupillary learning nor neuronal plasticity developed.

trial basis. Cells that had the same slope during conditioning as during sensitization were considered not to be plastic. Two neurons failed the slope test and are hereafter classed as nonplastic with respect to evoked activity. Thus, 12 cells (63%) were classified as plastic.

Changes in evoked discharges developed rapidly and were evident during the first block of 5 trials. Statistical criterion was attained on the average in 13.17 trials. Six neurons developed increased responses ($M = 11.83$), and six developed decreased responses ($M = 14.50$; Table 1). Functions for evoked activity are presented in Figure 2. These functions are significantly different from each other (Mann-Whitney U tests: increases vs. decreases, $p < .001$; increases vs. no change, $p < .001$; decreases vs. no change, $p < .001$). Examples of increases in evoked discharges are given in Figures 3B and 4 (cell 18D) and decreases in Figures 3A and 4 (cell 6C).

Kraus and Disterhoft (1982) reported that some portions of the evoked discharge of neurons in auditory association cortex of the rabbit are affected differently than others, during conditioning of the nictitating membrane response. Therefore, we analyzed separately various portions of evoked activity, in particular discharges having a latency less than 50 ms and longer latency

![Figure 2](image-url)
PLASTICITY OF SINGLE AI NEURONS DURING LEARNING

Figure 3. Representative poststimulus histograms for two neurons that developed discharge plasticity during conditioning. (In this, and Figures 4 and 7, the horizontal bars below the abscissa of each graph denote presentation of the acoustic conditioned stimulus. A: Cell I-3E—each histogram is the sum of discharges for 5 consecutive trials, as designated. Background discharges were unaffected by conditioning, whereas discharges evoked by the conditioned stimulus decreased. B: Cell 1-6—each histogram is the sum of 10 consecutive trials, as indicated. Background discharges decreased during conditioning, whereas evoked discharges did not develop a statistically significant change relative to background activity.)

activity. We were unable to find consistent effects of conditioning on various portions of the evoked response.

The probability of developing discharge plasticity was unrelated to whether the cell was recorded during the first training session for an animal or a later session, \( \chi^2(1, N = 18) = 0.078, p > .05. \)

Comparisons of the pupillary and neuronal evoked data indicated that there was no significant difference in trials to criterion for the entire group (pupil \( M = 11.81; \) neuronal \( M = 13.17 \), \( t(24) = 0.89, p > .05. \)). Within subjects, the pupil reached criterion (if at all) more rapidly than did changes in neural activity (if at all) in 9/19 cases, more slowly in 8/19, and at the same time in 3/19 cases. There was no significant difference between the rates of change for this group (pupil \( M = 12.25; \) evoked \( M = 15.75, \) Mann-Whitney \( U \) test, \( p < .05 \)). For those 12 cells attaining criterion, the pupil also attained criterion in 8 cases. There was no significant difference in trials to criterion for this group (pupil = 12.25; evoked = 15.75, Mann-Whitney \( U \) test, \( p > .05 \)). Also, within subjects, the pupil attained criterion more rapidly than evoked activity in 4/12, more slowly in 7/12, and at the same time in 1/12 cases. Overall, then, there was no statistically significant difference between the rate of pupillary learning and the rate at which evoked discharge plasticity developed.
Figure 4  Representative poststimulus histograms for three neurons that developed discharge plasticity during conditioning. (Each histogram is the sum of 15 consecutive trials, as indicated. Left: Cell I-8B—background discharges increased over the course of conditioning, whereas evoked activity was not significantly altered. Middle: Cell I-6C—background activity was not altered by conditioned, whereas evoked discharges decreased. Right: Cell I-18D—background discharges were not changed, whereas evoked activity increased during conditioning.)
Background Activity

Twelve neurons attained the criterion for discharge plasticity. Data from one cell were discarded because the slopes of its changes in discharges from sensitization and conditioning were equal. The 11 cells remaining attained criterion on the average in 22.91 trials. Seven neurons developed increased background activity ($M = 23.00$ trials; e.g., Figure 4, cell 8B). Four neurons developed decreased discharges ($M = 21.25$ trials; e.g., Figure 3B). Changes in background activity typically were initiated during the first block of five conditioning trials. The cells that were classified as unchanged ($n = 8$) tended toward increases in background discharges, but these were not maintained consistently at a level sufficient to attain the statistical criterion. Those cells that developed decreases had a small (20%-50%) but highly consistent change. Functions for background activity are presented in Figure 5. These functions are significantly different from each other (Mann-Whitney $U$ tests), increases vs. decreases, $p < .001$; increases vs. no change, $p < .01$; decreases vs. no change, $p < .001$).

Overall, the direction of change during conditioning was opposite to that during sensitization. Thus, neurons that developed increased background discharges during CS-US pairing exhibited decreased background activity during the preceding sensitization control period, and vice versa for neurons that developed decreased background activity.

The pupil reached criterion more rapidly than did changes in background activity in 13/20 cases, more slowly in 4/20 cases, with 3 ties. This outcome is statistically significant ($p < .05$, two-tailed binomial test). In 8 cases, both pupil and background attained criterion, the former being significantly faster than the latter (pupil $M = 11.39$, background $= 22.38$, Mann-Whitney $U$ test, $p < .001$). In fact, the pupil reached criterion more rapidly than did background activity in all 8 cases ($p < .002$, binomial test). Thus, across-subjects and within-subjects comparisons indicated that the development of the pupillary conditioned response preceded the development of changes in background activity.

Relation Between Background and Evoked Activity

Of the 19 cells in the primary auditory field, 15 (79%) developed discharge plasticity during conditioning, either for background or for evoked activity, or for both. Inspection of the data suggested that for any given neuron, the type of activity (background or evoked) and the effect of training (increase, decrease, no change) were independent (Table 2). This was validated statistically, $\chi^2(1, N = 19) = 0.88, p < .05$. However, for the 8 cells that attained criterion for both background and evoked activity, the direction of change was different for the two aspects of neuronal activity.
Table 2
Effect of Conditioning on Background Activity

<table>
<thead>
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<th>Cell no.</th>
<th>Pupil</th>
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<th>Cell no.</th>
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* Deleted from analysis because neither pupillary learning nor neuronal plasticity developed.

in all cases (Table 2), which is statistically significant $\chi^2(1, N = 8) = 4.50, p < .05$.

The rates of change for evoked and background activity were found to be significantly different: Evoked activity attained criterion in fewer trials than did background activity, $t(21) = 2.27, p < .05$.

Relation Between Arousal Level and Neuronal Discharge Plasticity

The measurement of pupillary diameter during conditioning provides an opportunity to investigate the relation between arousal level and neuronal discharge plasticity. The relation between pupillary diameter and arousal level is well established: increased diameter, increased arousal level, and vice versa (e.g., Nunnally, Knott, Duchnowski, & Parker, 1967). It is useful to distinguish between transient or phasic arousal and enduring or tonic arousal (Sokolov, 1963). In the present study and its companion study on secondary auditory cortex (D. Diamond & Weinberger, 1984), unconditioned phasic arousal is operationally defined as the dilation that is evoked by the unconditioned stimulus and attains peak within 1.5 s of its onset. Tonic arousal is defined as the baseline (pretrial) level of the pupil.

To assess the effects of phasic arousal on neurons in AI, we compared cellular discharges for the 1.5 s immediately preceding the EDS with discharges for the 1.5 s immediately following this stimulus for every US trial during the sensitization phase. This yielded 15 pairs of values which were then evaluated by the Mann-Whitney $U$ test for each cell separately. EDS produced pupillary dilation indicative of phasic arousal on every trial. Only 6 of 19 cells were responsive to EDS (Mann-Whitney $U$ tests, $p < .05$ or less); 5 cells responded with increased firing, and 1 cell with decreased discharges. There were no significant relations between the effects of phasic arousal and the effects of training, for either background or evoked activity.

Tonic arousal was indexed by the size of the pupil immediately preceding the onset of each acoustic stimulus, hereafter referred to as pupillary baseline. These data were averaged for consecutive blocks of five trials and are expressed as percentage change from the mean value of the last five CS trials of the sensitization period for each case. In order to determine the relation of pupillary baseline to background or evoked activity, the data were assigned to groups on the basis of the effects of training on background and evoked activity, and averaged. Thus, the average pupillary baseline data were computed for six groups: in-
creases, decreases, and no change for background discharges and for evoked discharges, respectively. These findings are presented in Figure 6.

Two sets of findings emerged from this analysis. First, with respect to background discharges, those neurons that developed increased background activity during CS–US pairing were in subjects that developed increased tonic arousal during pairing (Mann-Whitney U test, p < .001). Those cells that developed decreased background activity or failed to change in background activity were in subjects that tended toward lower, but not significant, levels of tonic arousal (Figure 6A).

Second, with respect to evoked activity, those neurons that failed to develop discharge plasticity were in subjects in which tonic arousal increased during CS–US pairing (Mann-Whitney U test, p < .02). There was no statistically significant relation between pupillary baseline and neurons that developed either increases or decreases in evoked discharges (Mann-Whitney U test, p > .05; Figure 6B).

Thus, increasing tonic arousal during conditioning was compatible with the development of increased background discharges but was incompatible with the development of plasticity of evoked discharges.

Single-Unit Data and Multiple-Unit Data

One reason that the present data for single neurons were obtained is that multiple-unit data, that is, unsorted discharges recorded simultaneously from more than one neuron by a single electrode, may be insensitive to different types of discharge plasticity; for example, such data may mask divergent changes in various neurons. Although this experiment did not involve the recording of multiple-unit data, it was feasible to construct composite “multiple-unit” histograms by combining the records of all individual neurons. Because this had to be done laboriously by hand on a bin-by-bin basis, we undertook this process only for selected blocks of trials, specifically for the last 5 CS trials of sensitization and Trials 16–20 during conditioning. The latter were selected because most discharge plasticity was present during this part of training. These compilations yielded the “multiple-unit” histograms shown in Figure 7. They reveal an effect of conditioning.
Note that there is an increase in evoked discharges following onset of the CS during Trials 16–20 of conditioning relative to the last 5 trials of sensitization. Decreases that are evident in single unit data are masked, as are the changes in background activity of single neurons.

Discussion

Acoustically evoked pupillary responses developed progressive increases during the conditioning phase of training. Such changes never occurred during sensitization during which the CS and US were not paired. Further, increments in the pupillary response developed rapidly, reaching criterion in an average of 12 trials. In the present case, a discrimination paradigm, to demonstrate stimulus-specific pupillary conditioning, was not employed because it would have prolonged the duration of training and thus greatly reduced the probability of obtaining continuous recordings from single cells during the development of conditioned responses. We have reported that pupillary conditioning exhibits all of the major characteristics of Pavlovian conditioned responses: systematic increase in magnitude due to stimulus pairing, discrimination both within and between modalities, discrimination reversal, conditioned inhibition, and inhibition of delay (Oleson et al., 1972, 1973, 1975; Ryugo & Weinberger, 1978; Weinberger et al., 1973). Therefore, the pupillary dilation conditioned response can serve as a framework for the analysis of the effects of associative processes on the discharges of neurons in auditory cortex. Further, as discussed in Results, it provides a basis for the interpretation of “negative” neuronal data, that is, instances in which discharge plasticity for background or evoked activity did not develop during the pairing of the conditioned and unconditioned stimuli.

A major finding is that discharge plasticity is prevalent in AI neurons during classical conditioning. Of 19 cells, 15 developed statistically significant changes in either evoked or background activity, or both. These effects are attributable to associative processes, for several reasons. The effects of CS–US pairing were assessed relative to a sensitization control period. Stimulus constancy was assured by neuromuscular blockade, which eliminates movement of the head or pinna with respect to the sound source (Marsh, Worden, & Hicks, 1962; Wiener, Pfeiffer, & Backus, 1966), contraction of the middle ear muscles (Starr, 1964), and movement-induced masking noise (Imig & Weinberger, 1970). Finally, neuronal changes due to alleged sensory feedback from conditioned responses, that is, the pupillary dilation conditioned response, can be ruled out because the pupillary musculature does not contain proprioceptors (Lowenstein & Loewenfeld, 1969).

Although these effects are associative,
Kitzes, Farley, and Starr (1978) argued that such discharge plasticity in sensory systems may be unrelated to the cue value of the conditioned stimulus. These experimenters presented brief tones continuously throughout defensive Pavlovian training in which the CS was white noise. They reported changes in AI single-unit activity to the tones and periods of silence present during CS–US intervals relative to intertrial intervals. They concluded that training causes merely a general change in cortical excitability so that evoked discharge plasticity is not directly related to the significance of the conditioned stimulus. However, this conclusion rests on the assumption that the tone pips were devoid of significance during the CS–US interval, but Kitzes et al. failed to provide independent evidence to support this assumption. Quite the contrary, these stimuli may have had CS properties because of the particular training regimen employed. The subjects were trained initially with a 5-s white noise CS, followed by electrodermal stimulation. After establishment of the pupillary conditioned response, they were shifted from a delay to a trace paradigm, which resulted in a 0.5-s CS (white noise) followed by 4.5 s of silence during which “neutral” tone pips could be presented. But the original training caused conditioning to 5 s of acoustic stimulation, and the pips filling the CS–US interval could have acquired CS value due to within-modality stimulus generalization, that is, the effective CS was actually white noise followed by tone pips. A more severe problem is that the control group received only tones, not tones unpaired with EDS, hence the effects were not demonstrably associative. Finally, the relevance of these findings to the acquisition of pupillary conditioned responses (CRs) and evoked discharge plasticity are rapid and do not differ from each other and (b) development of pupillary CRs and evoked discharge plasticity were not closely related, for example, conditioned responses developed during some sessions in which neuronal plasticity did not appear, and vice versa. These findings suggest that the neuronal changes are not causal to the pupillary CRs. That both develop at the same rapid rate during associative learning suggests that they are related to a common process which has not yet been delineated.

Although neuronal changes did not precede acquisition of the pupillary dilation conditioned reflex, it is likely that they precede the acquisition of several other conditioned responses because the rate of pupillary conditioning is among the fastest of any response system (Weinberger, 1982a, 1982b). During defensive conditioning, responses that are not specific to the nature of the unconditioned stimulus develop conditioned responses more rapidly than do those systems in which the conditioned response is determined by the specific characteristics of the unconditioned response (Schneiderman, 1972). Among the former are pupil, cardiovascular, respiration, and general skeletal movement. Responses that are specific to the nature of the US include limb flexion, eye blink, and extension of the nictitating membrane. The present findings indicate that discharge plasticity for single neurons in primary auditory cortex develops as rapidly as does pupillary conditioning. Such rapidly developing behavioral and neuronal plasticity may comprise part of the first stage of a two-stage (Konorski, 1967) or three-stage (Thompson et al., in press) conditioning process in which the second stage is the elaboration of a somatic conditioned response which is specific to the nature of the unconditioned stimulus (Weinberger, 1982a, 1983).

The functional role of associatively induced discharge plasticity in primary auditory cortex, and for that matter in other sensory systems, is unknown. Evoked plasticity may reflect changes in the threshold functions or in the receptive field properties of sensory neurons, but appropriate exper-
iments have not yet been reported. It is known that ablations of auditory cortex produce deficits in the discrimination of complex acoustic stimuli (Neff, Diamond, & Cassady, 1975), and evidence suggests that auditory cortex is essential for the appreciation of stimulus constancy or equivalence (Whitfield, 1979). The fact that discharge plasticity develops rapidly in auditory cortex during Pavlovian conditioning suggests that even apparently simple learning situations may be involved in such complex processes.

Relation to Previous Studies

The present report appears to be the first in which the discharges of single neurons were recorded in the primary auditory cortex during the acquisition of a behavioral conditioned response. Issues relating to auditory cortical unit discharges during the performance of previously acquired responses are considered in a companion article on auditory cortical field AI (D. Diamond & Weinberger). However, there are several previous reports in which multiple-unit activity was recorded during conditioning.

With respect to evoked discharges, there is consistent evidence that responses in auditory cortex to an acoustic conditioned stimulus are augmented during conditioning. This has been found in the cat during Pavlovian defensive conditioning both in freely moving and in muscle-blocked subjects (Buchwald et al., 1966; Halas et al., 1970; Oleson et al., 1975) and during instrumental conditioning (Halas et al., 1970), in the rat during a hybrid classical–instrumental appetitive task (Disterhoft & Olds, 1972; Disterhoft & Stuart, 1976; Olds, Disterhoff, Segal, Kornblith, & Hirsh, 1972) and in the rabbit during instrumental avoidance learning (Foster, Orona, Lambert, & Gabriel, 1980). This concordance is noteworthy, given the variations in subjects, tasks, and conditions of training, plus the fact that the data reported for the rat include activity from cortical sites beyond the limits of auditory cortex. The present results for single neurons reveal that decreases in evoked discharges also develop and that they do so at the same rapid rate as do increases in evoked discharges. Thus it appears likely that multiple-unit recordings mask decreases in discharge plasticity during learning. This conclusion is underscored by the analysis in which we constructed “multiple-unit” histograms by combining the data from single neurons (Figure 7).

In regard to background multiple-unit activity, there is less agreement. Disterhoft and co-workers reported the development of decreases in background activity (Disterhoft & Olds, 1972; Disterhoft & Stuart, 1976), whereas we found a pronounced increase in background discharges during differential conditioning and reversal of differential conditioning (Oleson et al., 1975). A further difference is that those experimenters reported that background activity changed before evoked activity whereas we found the opposite relation. The present study extends this finding to single units: Evoked activity attained criterion significantly earlier than did background discharges. It is difficult to reconcile the contrary findings, particularly because the concordance regarding evoked activity in studies of multiple-unit activity suggests that differences in subjects and training conditions may still yield similar findings for evoked activity. It may be that changes in background activity are more tightly linked to the cytoarchitectonically delimited region of primary auditory cortex than are changes in evoked activity. In any event, it is now evident that previous studies have reported either increases or decreases in background activity whereas the present study of single neurons has revealed both increases and decreases under the same conditions of training and acquisition of a behavioral conditioned response. Studies, such as the current investigation, that undertake finer grain analyses than previous experiments are likely to find complexities heretofore not discovered. Determination of the functional role indexed by such findings remains a challenge.

Single-Unit and Multiple-Unit Data

Kraus and Disterhoft (1982) argued for the importance of obtaining discharge data
from single neurons because of the limitations of multiple-unit recordings. We are in complete agreement with this view, and the present findings offer empirical support for the position that multiple-unit records are not adequate representations of the changes that develop for single cells; for example, as pointed out above, multiple-unit histograms indicative of response plasticity indicate an increase in evoked discharges during learning. In this study, we constructed "multiple-unit" histograms by adding the histograms of single neurons. These histograms are not identical to standard multiple-unit records because they were obtained from separate experimental sessions and various loci and their composition is known. Nonetheless, they also provided a picture of an increase in evoked response to the CS during conditioning (Figure 7). Yet the histograms of the individual neurons are heterogeneous, including decreases and no changes as well as increases during CS-US pairing. Thus, multiple-unit records are not necessarily comprised of the discharges of a homogeneous population of neurons, all or most of which develop an increase in response. Also, the "masking" of unit heterogeneity holds for background as well as evoked activity (Figure 7). Therefore, it would be incorrect to conclude that the general excitability of sensory cortex simply increases during conditioning.

Previously, we reported similar results for discharge plasticity in the magnocellular medial geniculate nucleus (MGm). Poststimulus histograms of multiple-unit activity exhibit the same general pattern of activity and the same sort of increase in evoked activity during conditioning as reported for the primary auditory cortex (Ryugo & Weinberger, 1976, 1978). However, changes in the discharges of individual neurons in the MGm also are heterogeneous for both background and evoked activity (Weinberger, 1982a). Thus, multiple-unit activity overshadows certain aspects of single-unit discharge plasticity in the thalamus as well as in the cortex. There is no reason to doubt that the same problem may exist for multiple-unit recordings from other parts of the brain, as well. Therefore, appropriate caution should be exercised in the interpretation of multiple-unit records.

Discharge Plasticity, Conditioning, and Tonic Level of Arousal

Plasticity of background and evoked discharges was a common event in this study. Given the caveat that any microelectrode study may be limited by sampling bias and that classes of neurons not yet described may exist in primary auditory cortex, these findings are in essential agreement with the observations of Kraus and Disterhoft (1982), who emphasized the heterogeneity of discharge plasticity in the auditory association cortex of the rabbit. This can be seen in the highly variable shapes of post-stimulus histograms and in the fact that conditioning is accompanied by both increases and decreases in discharges as well as lack of change.

However, one facet of this heterogeneity may be explicable. With the prettrial size of the pupil used as an index of the level of tonic arousal, it was found that increased tonic arousal was accompanied by the failure of neurons to develop discharge plasticity in evoked activity. Although no causal roles can be assigned yet, it does seem that increased tonic arousal level is not conducive to evoked discharge plasticity. This seems to be a neurophysiological expression of the well-known Yerkes-Dodson law that high levels of arousal impair learning and performance (e.g., for review, see Eysenck, 1982).

Possible Mechanisms of Discharge Plasticity in Primary Auditory Cortex

The discharge properties of neurons in AI during conditioning may be, in part, a function of the combined influences of their thalamic sources of input, that is, the ventral and magnocellular medial geniculate nuclei. As the ventral medial geniculate does not develop discharge plasticity during conditioning whereas the magnocellular does (see introduction), the plastic properties of AI neurons might relate to input from the latter region. In addition, there may be mechanisms of plasticity intrinsic
to the cortex and other extrinsic sources as well. However, there is a striking similarity between the effects of conditioning on MGm and the effects on AI neurons. Using an identical preparation and training regimen, we reported that evoked discharge plasticity developed at the same rate as for AI cells, that is, 10-20 trials.

Although the present picture is very incomplete, it is now possible to propose a preliminary hypothesis of how associative evoked discharge plasticity may develop in the primary auditory cortex. The lemniscal, tonotopic projection from the ventral medial geniculate body (MGv) terminates densely in the middle layers of AI (I. Diamond, 1979; Herkenham, 1980; Jones & Rockel, 1971). However, the discharges of the MGv are not altered by learning, so that the predominant input to the middle layers of AI probably reflects only the physical parameters of stimuli. It should be noted that the rates of discharges of individual neurons in MGv can vary widely in response to a physically constant stimulus when background activity changes in the unanesthetized cat (Humphrey & Orman, 1977; Imig & Weinberger, 1973). However, the pattern of response is conserved despite fluctuations in the actual number of discharges (Imig & Weinberger, 1973). Therefore, it is not necessary to hold that primary auditory cortex always receives the same number of discharges to the same acoustic stimulus when background activity changes in the unanesthetized cat (Humphrey & Orman, 1977; Imig & Weinberger, 1973). However, the pattern of response is conserved despite fluctuations in the actual number of discharges (Imig & Weinberger, 1973). Therefore, it is not necessary to hold that primary auditory cortex always receives the same number of discharges to the same acoustic stimulus via the MGv but only that there is some constant relation between the physical parameters of an acoustic stimulus and the input from the MGv to primary auditory cortex. In contrast, the magnocellular medial geniculate body projects to layer I of AI (Burton & Jones, 1976; Herkenham, 1980; Jones & Rockel, 1971; Ryugo & Kilacky, 1974). This arrangement suggests that the MGm regulates the responses to acoustic information of neurons whose soma are in lower layers, perhaps by effects on apical dendrites that ascend to the upper lamina.

The failure to develop evoked discharge plasticity in primary auditory cortex has been linked in the present study to high levels of tonic arousal, as discussed above. The possible mechanisms cannot be considered yet because there are essentially no pertinent data on this point. Rather, it would seem that future inquiry into the mechanisms of response plasticity in auditory cortex, and perhaps in many other areas as well, also will have to attempt to account for such failures to develop plasticity.

Finally, the present findings were obtained from single neurons located mainly in the infragranular lamina (V and VI) which are the major sources of subcortical projections from AI (I. Diamond, 1979; Kelly & Wong, 1981). It is therefore noteworthy that these "output" neurons are altered by associative processes. The consequences of associative discharge plasticity in these neurons, and the intracortical mechanisms that may lead to these changes, will require extensive detailed study, including laminar analyses during learning.

References


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