Physiological Plasticity of Single Neurons in Auditory Cortex of the Cat During Acquisition of the Pupillary Conditioned Response: II. Secondary Field (AII)

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The discharges of 22 single neurons were recorded in the secondary auditory cortical field (AII) during acquisition of the pupillary dilation conditioned defensive response in chronically prepared cats. All 22 neurons developed discharge plasticity in background activity, and 21/22 cells developed plasticity in their responses to the acoustic conditioned stimulus (CS). Nonassociative factors were ruled out by the use of a sensitization phase (CS and US [unconditioned stimulus] unpaired) preceding the conditioning phase and by ensuring stimulus constancy at the periphery by neuromuscular paralysis. Changes in background neuronal activity were related to measures of behavioral learning or to changes in the level of arousal. Specifically, decreases in background activity (17/22 cells) developed at the time that subjects began to display conditioned responses. Increases in background activity (5/22) developed in animals that became more tonically aroused during conditioning. However, both increases (11/22) and decreases (10/22) in evoked activity developed independently of the rate of pupillary learning, tonic arousal level, or changes in background activity. These findings indicate that changes in background activity are closely related to behavioral processes of learning and arousal whereas stimulus-evoked discharge plasticity develops solely as a consequence of stimulus pairing. A comparative analysis of the effects of conditioning on secondary and primary (AI) auditory cortex indicates that both regions develop neuronal discharge plasticity early in the conditioning phase and that increases in background activity in primary auditory cortex are also associated with elevated levels of tonic arousal. In addition, the overall incidence of single neurons developing learning-related discharge plasticity is significantly greater in AII than in AI. The relevance of these findings is discussed in terms of parallel processing in sensory systems and multiple sensory cortical fields.

This article forms the second part of an investigation of single-unit discharges in auditory cortex of the cat during learning. The first part described the effects of classical conditioning on the primary auditory cortical field (AI; Weinberger, Hopkins, & Diamond, 1984). The present study is concerned with the secondary auditory field (AII). The two fields differ in their physiological organization, response properties to acoustic stimulation, thalamocortical connections, and cytoarchitectonics. AI is tonotopically organized, and its neurons have narrow tuning curves. Its major thalamic source of input is the ventral medial geniculate nucleus (MGv), which is also tonotopically organized and comprises part of the lemniscal auditory system (Aitkin &...
Functional aspects of parallel thalamocortical auditory pathways have been revealed at the thalamic level in learning tasks in which acoustic stimuli serve as cues predicting reward or punishment. Neurons in the lemniscal MGv respond in an unchanging fashion to acoustic stimuli during learning irrespective of their behavioral relevance. In contrast, neurons in the nonlemniscal MGm develop discharge plasticity in a rapid and pronounced manner during learning. These results have been found in three orders of mammals in three different tasks: in the cat during classical defensive (pupillary) conditioning for both multiple-unit (Ryugo & Weinberger, 1976, 1978) and single-unit activity (Weinberger, 1982), in the rabbit during instrumental avoidance conditioning (Gabriel, Miller, & Saltwick, 1976), and in the rat during a hybrid instrumental–classical appetitive task (Birt, Nienhuis, & Olds, 1979; Birt & Olds, 1981). The discharge plasticity in the magnocellular medial geniculate develops in the absence of proprioceptive feedback or changes in the effective stimulus intensity at the tympanic membrane (Ashe, Cassady, & Weinberger, 1976). Thus, at the level of the thalamus, there is a fundamental dichotomy with respect to the ability of a region of the medial geniculate nucleus to convey either an accurate representation of the acoustic environment (MGv) or respond differentially to sounds that acquire greater salience as a function of learning (MGm).

At the cortical level, the accompanying investigation of primary auditory cortex (Weinberger et al., 1984) demonstrated that single neurons developed learning-related discharge plasticity rapidly, for both background and evoked discharges. In that report, we suggested that such plasticity may result, in part, from the regulatory influence of the MGm to layer I of the primary auditory cortex. The present study of the secondary auditory cortex provides both a characterization of single neurons in MG during learning and the first comparative data of neuronal activity in different sensory cortical subfields during learning.

A preliminary report of some of these results has been presented (Diamond & Weinberger, 1982).

Method

The surgical and training procedures were identical to those described in the companion investigation of the primary auditory cortex (Weinberger et al., 1984), with one exception as noted below. Briefly, the subjects were 12 adult cats (3–5 kg) in good health. Pedestals were affixed to the skull under general anesthesia (Nembutal, 40 mg/kg, ip) to provide foratraumatic fixation of the head during subsequent recording sessions. Subjects recovered from the anesthesia in an incubator, and recordings began 1–2 weeks later. On the day of recording, paralysis was induced with gallamine triethiodide (10 mg/kg, ip). The trachea was intubated, and the animal was artificially respired. The corneas were protected from drying with an application of ophthalmic ointment. Pupillary diameter was monitored with an infrared pupillometer. Epoxylite-coated tungsten electrodes were lowered through a burr hole into All in a ventromedial direction at an angle of 25°–30° to the vertical. Histological verification of the recording sites indicated that the electrode typically entered the brain near the AI–All border and then passed at least 3 mm into All. Consequently, the final position of the electrode was in the infragranular layers (V and VI) during all recordings. Single-unit activity was recorded on magnetic tape and fed to an LSI 11/03 computer for on- and off-line analysis.

The conditioned stimuli (CS) were white noise or tones (70–80 dB) 1 s in duration, presented to the ear contralateral to the recording site. The unconditioned stimulus (US) was electrodermal stimulation (EDS; 375 ms) presented to the forelimb contralateral to the recording site. Training consisted of 15 trials of sensitization (unpaired CS and US, 15 each) followed by conditioning (CS paired with US at CS offset) up to 60 trials.

The only differences in procedure between this study and the companion investigation of primary auditory cortex was that several sessions at the start of this study involved the extensive presentation of...
white noise or tones as search stimuli preceding training.

At the conclusion of the experiment, the animal was deeply anesthetized and perfused through the carotids with normal saline and 10% formalin. Recording sites were verified on the basis of cytoarchitectonic distinctions (Rose, 1949).

Results

General Response Characteristics of Neurons in AII

Data were obtained from 22 cells in 21 recording sessions. The recording sites were histologically verified in layers V and VI of auditory field AII. The best stimuli for evoking activity were complex sounds such as keys jangling, squeaky sounds, and the experimenter's voice. White noise was more effective than tones in evoking responses. Consequently, white noise was used as the CS in 18 sessions and a 2-kHz tone in the other three sessions. The background firing rates of 21 of the 22 cells were less than 7 spikes/s, averaging 2.5/s ($SE = 0.56$). One cell was unique in that its background activity was very high (26 sp/s), and it was the only cell to exhibit a sustained excitatory response to acoustic stimuli.

Virtually all cells (21/22) responded to acoustic stimuli with an increased rate of activity over background, but this proportion may not be representative of the AII population as a whole because inhibition was difficult to detect due to the extremely low background firing rates. The typical evoked pattern was an onset response consisting of single or multiple discharges. The mean latency to onset was 147 ms, the median was 80 ms, and the range was from 22 to 500 ms (Figure 1). Long-latency responses might have resulted from an initial period of inhibition followed by rebound excitation, but the low background rates precluded an analysis of this possibility.

Pupillary Behavior and Conditioning

Pupillary data were recorded during 20 of the 21 recording sessions. Early in the sensitization phase, the acoustic stimulus (CS) generally elicited a small amplitude dilation. By the end of the sensitization phase, dilation responses were reduced in both amplitude and duration. Electrophysiological stimulation (US) produced consistently large dilation responses throughout the experimental session. Acoustically evoked dilations increased in amplitude during the conditioning phase, as has been reported (Ashe et al., 1976; Oleson, Vodocknick, & Weinberger, 1973; Oleson, Westenberg, & Weinberger, 1972; Ryugo & Weinberger, 1978; Weinberger, Oleson, & Haste, 1973).

The rate of acquisition of the pupillary dilation conditioned response was measured relative to the averaged evoked dilations.

1 Two cells were recorded from one electrode during one session.
tions on the last 5 trials of the sensitization phase. The criterion of learning was five consecutive dilation responses to the CS greater than the sensitization reference level; the fifth consecutive trial was noted as the trial(s) to criterion. This criterion was met in 17 of the 19 sessions in which acoustically evoked dilations were recorded. During the 20th session, the pupil dilated to a maximum level early in the conditioning phase and remained there. Consequently, the development of conditioned responses (CRs) could not be measured for this session. For the 19 sessions in which conditioned responses were recorded, the mean trials to criterion was 26.6. However, it became evident that the trials to criterion were distributed bimodally; pupillary conditioned responses developed either rapidly (in less than 20 trials, \( n = 10 \)) or slowly (greater than 20 trials, \( n = 9 \)). The rapid learners attained criterion in an average of 11.9 trials, and slow learners required 42.5 trials. The difference between the two groups is significant (\( t \) test, \( p < .05 \); Figure 2). It is likely that the disparity in learning rate is due to latent inhibition as a result of the extensive use of tones and white noise as search stimuli in the earlier recording sessions (see Method). In later recording sessions, we did not use these stimuli to locate cells. Squeaky sounds and jangling keys served as effective stimuli for evoking neuronal activity, and the retardation of learning during these sessions was not evident. To examine the latent inhibition hypothesis, we compared the rate of pupillary learning in sessions that involved the extensive use of tones and white noise as search stimuli with the rate in sessions in which complex ambient sounds were used. For the sessions that involved the extensive use of tonal search stimuli, the criterion was met in an average of 42.0 trials. Subjects conditioned in sessions without such search stimuli learned significantly more rapidly, in a mean of 15.1 trials (\( p < .005, \ U \) test). Therefore the rate of conditioning appears to have been influenced by the amount of prior exposure to tones and white noise.

Of the 12 animals, 10 were trained more than once: 5 received three conditioning sessions and 5 received two sessions, at intervals of 7–30 days. In order to assess the cumulative effect of earlier training sessions on later ones, each animal was assigned a “savings score” based on whether conditioned responses developed more rapidly in the later sessions than they did in the first session. Thus an animal would be assigned a 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 such comparisons in the first session. Consequently, they were included in the slow-learning category, with their total number of conditioning trials used as their trials to criterion (34 and 50).

Figure 2  Pupillary learning curves. (Each point represents the mean (±1 SE. Values are computed as percentage change in the acoustically evoked pupillary dilation from the average response for each of the last 5 trials of sensitization. The animals are divided into two groups on the basis of the rate of acquisition of conditioned responses. For the slow learners, the magnitude of the dilation response remains at the sensitization level for the first 20 trials of conditioning. Rapid learners display conditioned responses within the first 10 trials of the conditioning phase and reach asymptote by the 20th trial. The rate of acquisition is illustrated in finer detail for the first 5 trials of conditioning. The \( N \) totals 19 because the evoked pupillary dilations were not monitored during 2 of the 21 recording sessions. In some cases, recording was terminated after the sixth block of conditioning (Trial 30) due to deterioration of isolation of discharges from single units; the numbers of subjects for Blocks 7–9 were 12, 8, and 7.)

Two subjects in the slow category failed to exhibit five consecutive CRs. However, they developed significantly greater CS-evoked pupillary dilation responses late in conditioning compared with the sensitization phase (\( U \) test, \( p < .05 \)). Consequently, they were included in the slow-learning category, with their total number of conditioning trials used as their trials to criterion (34 and 50).
than the number of comparisons that yielded an increase in this measure. In this manner, it was then determined that 4 of 10 animals had a positive savings score and 6 had negative savings. Hence there was little evidence of savings when the subjects were trained more than once.

**Neuronal Data**

To facilitate comparison of pupillary and neuronal data, we used a criterion to classify neuronal plasticity in the same manner as pupillary learning was classified. However, to accommodate decreases as well as increases in neuronal activity, the criterion for neuronal plasticity was five consecutive trials, all of which had either greater or fewer discharges than the mean of the last five trials of the sensitization phase. We would have excluded any neurons that, although meeting the criterion, were simply continuing a trend existent during sensitization (Weinberger et al., 1984); however, no such trends were found.

**Background Activity**

Background activity was measured for the 1.5 s immediately preceding the initiation of a trial. All 22 units satisfied the criterion of plasticity for background activity (Table 1). A significant majority of the neurons (17/22) developed a decrease in background activity during conditioning (binomial test, p < .02). The mean number of trials to criterion between increases and decreases did not differ significantly (increases, n = 5, M = 14.0; decreases, n = 17, M = 18.9; p < .05 (Figure 3A). Poststimulus histograms of the development of decreases in background activity during single sessions are presented in Figure 4 and of increases in Figure 5.

The fact that animals learned at different rates led to an analysis of the relation between behavioral learning and the development of neuronal plasticity. The most salient finding was that the development of decreases in background activity was correlated with the acquisition of pupillary conditioned responses. Subjects classified as rapid learners displayed a rapid decrease in background activity during stimulus pairing. Slow learners did not reach the behavioral criterion until considerably later in the conditioning session, and the development of changes in background activity was also retarded. The relation of decline in background activity to acquisition of the pupillary conditioned response is presented in Figure 6. The difference in background activity between the respective groups is

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Background Activity and Pupillary Conditioned Responses</th>
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<td>SD</td>
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*a Pupillary behavior was not monitored during these sessions. b Cells II-2B1 and II-2B1' were recorded simultaneously from the same electrode during one session; therefore, this value applies once to both cells.
Evoked Activity

Evoked activity was computed as the difference between background and evoked...
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SENSITIZATION

CONDITIONING

Figure 5. Peristimulus histograms of examples of single-unit activity in AII during individual conditioning sessions. (A: Cell II-1C developed an increase in both background and evoked activity. B: Cell II-12A developed an increase in background activity and a decrease in evoked activity. The bar from 0 to 1,000 ms indicates the duration of the conditioned stimulus, and the second bar [only present during the conditioning phase] indicates the duration of the unconditioned stimulus [US], which was 375 ms. Neuronal activity during the US is not presented for cell II-1C.)

discharges. This method eliminated apparent changes in evoked activity that merely reflected a general change in the background excitability of a cell, rather than a specific effect that occurred during acoustic stimulation. Of the 22 neurons, 21 developed stimulus-evoked plasticity during conditioning (Table 2). Eleven developed increases \((M = 21.7\) trials to criterion), and 10 developed decreases \((M = 12.5\) trials to criterion; Figure 3B). Decreases in evoked activity were significantly more rapid than increases \((U\) test, \(p < .01)\). Poststimulus histograms of the development of increases in evoked activity during single sessions are presented in Figures 4B and 5A; decreases are presented in Figures 4A and 5B. There were no significant relations between the development of changes in evoked activity and pupillary learning.

This is in contrast to the direct relation found for decreases in background activity and the acquisition of pupillary conditioned responses, discussed above. However, one consistent aspect of evoked plasticity was that decreases always developed rapidly, even when pupillary learning occurred much later in the conditioning session. Decreases in evoked activity satisfied the plasticity criterion in 10.8 conditioning trials.

Figure 6 Illustration of the relation between the development of decreases in background activity and the rate of pupillary conditioning. (Each point is the mean value for a block of five trials and is expressed as percentage change from the mean value of the last five trials of sensitization. Fast learners [open triangles] displayed conditioned responses within the first five trials of conditioning, whereas slow learners [solid triangles] did not begin to display conditioned responses until after the 20th trial of conditioning. Rapid learners developed decreases in background activity [open circles] within the first five trials of conditioning. Background activity for the slow learners [solid circles] did not decrease until the fifth block of conditioning trials, at which time the slow learners began to exhibit conditioned responses and the background activity abruptly decreased. The \(n\) for fast pupillary learners equals 8, and the neural data contains 9 cells because two units were recorded from one electrode during one session. In Figure 3, the \(n\) for decreases in background activity equals 17 because it represents the activity of all cells that developed changes in activity during conditioning. One of the cells could not be included in this analysis \((n = 16)\) because pupillary behavior was not monitored during a session in which a decrease in background activity was recorded.)
Table 2  
Evoked Activity and Pupillary Conditioned Responses

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Total  

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Note Cell II-2B' did not develop evoked plasticity.  
*Pupillary behavior was not monitored during these sessions.

for slow learners and 14.3 trials for fast learners. The difference was not significant (U test, p > .1). In contrast, increases in evoked plasticity were just as likely to occur before as after the development of pupillary conditioned responses.

Relation of Evoked to Background Activity

The rates of change for background and evoked discharges were not significantly different; the overall average trials to criterion for background and evoked discharges were 17.9 and 17.3, respectively. However, the directions of change (increase or decrease) within a training session were independent, $\chi^2(1, N = 21) = 0.153, p > .05$; for example, increases in background activity were accompanied by the development of increases or decreases in evoked activity. These findings are summarized in Table 3.

Relation Between Tonic Arousal and Neuronal Discharge Plasticity

The pupillomotor system provides sensitive indexes of the general state of excitability or arousal of a subject, in addition to yielding prime data on the acquisition of an associative relation between a CS and a US, that is, a conditioned response. As an index of tonic arousal, we measured the baseline level of pupillary dilation throughout each recording session. Pupillary baseline was measured immediately preceding presentation of each acoustic stimulus. The average of the last 5 CS trials of sensitization served as the reference level, and changes in the baseline of pupillary dilation were measured as a percentage change from this reference value. The tonic level of dilation increased by about 30% early in the sensitization phase, reaching asymptote between the 5th and 10th sensitization trials (see Figures 7A and 7B). Decreases in neuronal background activity ($n = 17$) always occurred when the tonic level of dilation remained at about the same relative level during the conditioning phase as it was during the last 10 trials of sensitization. In contrast, the 5 sessions in which neuronal background activity increased were unique in that the pupillary baseline also increased.

Table 3  
Effects of Conditioning on Background and Evoked Activity

<table>
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<th>Evoked discharges</th>
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Note $\chi^2 (1, N = 22) = 0.153, p > .05$.  

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The pupillomotor system provides sensitive indexes of the general state of excitability or arousal of a subject, in addition to yielding prime data on the acquisition of an associative relation between a CS and a US, that is, a conditioned response. As an index of tonic arousal, we measured the baseline level of pupillary dilation throughout each recording session. Pupillary baseline was measured immediately preceding presentation of each acoustic stimulus. The average of the last 5 CS trials of sensitization served as the reference level, and changes in the baseline of pupillary dilation were measured as a percentage change from this reference value. The tonic level of dilation increased by about 30% early in the sensitization phase, reaching asymptote between the 5th and 10th sensitization trials (see Figures 7A and 7B). Decreases in neuronal background activity ($n = 17$) always occurred when the tonic level of dilation remained at about the same relative level during the conditioning phase as it was during the last 10 trials of sensitization. In contrast, the 5 sessions in which neuronal background activity increased were unique in that the pupillary baseline also increased.

Table 3  
Effects of Conditioning on Background and Evoked Activity

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<tr>
<td>No change</td>
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Note $\chi^2 (1, N = 22) = 0.153, p > .05$.
changes significantly during the conditioning phase ($U$ test, $p < .001$). This indicates that increases in neuronal background activity were accompanied by increases in tonic arousal during conditioning but that decreases in background activity were not accompanied by decreases in tonic arousal (Figure 7A).

In a related issue, recall that decreases in background activity developed early in conditioning for rapid learners and after the 20th trial for slow learners. The relation between arousal and background activity led to an analysis of the possibility that the differential rate of decline may have resulted from a difference in generalized arousal between the two groups. In order to investigate this possibility, the baseline levels of the pupil during the first 20 trials of conditioning were compared for the rapid and slow learners. The tonic levels of dilation of rapid and slow learners were not different during the first 20 trials of conditioning (Mann-Whitney $U$ test, $p > .1$). Therefore, the differential rate of decrease in background activity during conditioning for the rapid and slow learners was not due to a difference in their levels of tonic arousal.

In contrast to background activity, plasticity of evoked activity to the CS did not bear any relation to the baseline level of the pupil (Mann-Whitney $U$ test, $p > .1$; Figure 7B).

**Relation Between Phasic Arousal and Neuronal Discharges in AII**

Phasic increases in pupillary dilation (2–5 s) were reliably evoked by electrodermal stimulation. This unconditioned response may be considered a reliable index of phasic increases in general behavioral excitability or arousal. In order to examine the possible effects of phasic arousal on cellular activity, neuronal discharge rate was measured for the 1.5 s immediately preceding and immediately following each EDS during the sensitization phase. For each animal, the numbers of discharges during the pre- and post-EDS periods for each trial were evaluated across the 15 sensitization EDS trials by the Mann-Whitney $U$ test (Siegel, 1956). Of the 22 neurons, 17 exhibited significant responses to EDS ($p < .05$); the rates of discharges were increased for 6 cells and decreased for 11 neurons.

This outcome is in contrast with evidence that increases in tonic arousal were always accompanied by increases in background activity, as discussed above. Moreover, of the five cells that developed increases in background activity along with elevated levels of tonic arousal, four exhibited a suppression of ongoing activity during phasic increases in arousal.

As further evidence that tonic and phasic changes in arousal level have different effects on neurons in AII, we may consider
data from cells II-3B and II-2A, illustrated in Figure 8. Subject II-3B developed an increase in pupillary baseline during the conditioning phase, and its neuronal background activity also increased. However, EDS caused suppression of its discharges (Figure 8). On the other hand, subject II-2A was more typical in that it did not develop an increase in pupillary baseline during conditioning and its background activity decreased during the conditioning phase. Nonetheless, increases in phasic arousal elicited an increase in firing rate (Figure 8).

Comparison of Primary (AI) and Secondary (AII) Cortical Auditory Fields

At this juncture, it is possible to compare the effects of training on the discharges of single neurons in AII with those reported in the companion article for AI (Weinberger et al., 1984).

The probabilities of the development of discharge plasticity during conditioning were compared in $2 \times 2$ contingency tables. These analyses indicated that neurons in AII had a significantly higher proportion of discharge plasticity than did neurons in AI, both for evoked, $\chi^2(1, N = 41) = 4.87, p < .05$ (Table 4), and background, $\chi^2(1, N = 41) = 8.98, p < .01$, activity (Table 5). The direction of change for background and evoked activity was evaluated in the same manner. There was no significant difference between AI and AII for evoked activity, $\chi^2(1, N = 33) = 0.05, p > .05$. For background activity, AII developed predominant decreases in contrast to AI, but the differences did not reach statistical significance, $\chi^2(1, N = 33) = 3.68, p < .10$.

The effects of phasic arousal on single-unit discharges were also evaluated. It should be recalled that the effects of phasic arousal were measured as the responses, or lack thereof, to EDS during presentation of the US in the sensitization phase of the experiment. Only 6/19 neurons in AI showed a significant response to EDS, in contrast to 17/22 cells in AII. This difference was statistically significant, $\chi^2(1, N = 41) = 6.89, p < .01$.

Comparison of the rates of development of discharge plasticity was complicated by the fact that in the present study of AII, acquisition was retarded in several cases due to latent inhibition that was caused by the extensive use of acoustic search stimuli
Table 5
Comparison of Effects of Conditioning on Background Discharges of Primary (AI) and Secondary (AII) Cortical Auditory Fields

<table>
<thead>
<tr>
<th>Training outcome</th>
<th>AI</th>
<th>AII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plastic</td>
<td>11</td>
<td>22</td>
</tr>
<tr>
<td>Nonplastic</td>
<td>8</td>
<td>0</td>
</tr>
</tbody>
</table>

Note. $x^2 (1, N = 41) = 8.98, p < .01.$

preceding training, as discussed above. Therefore, this comparison was restricted to those neurons in AII that were recorded during sessions that had training conditions identical to those used in the study of AI, that is, no extensive use of acoustic search stimuli.

In the case of evoked activity, this comparison yielded data from 10 neurons from AII, compared with 12 cells in AI in which conditioning resulted in significant changes in evoked activity. There was no significant difference between the rates of change for AI and AII (AI $M = 13.17$ trials to criterion; AII $M = 14.50$; Mann-Whitney $U$ test, $p > .05$). In the case of background activity, there were 11 neurons in AII and also 11 in AI that attained the criterion of change. The AI neurons had a mean of 22.91 trials to criterion, and the mean for AII was 14.63. This difference approached but did not attain statistical significance (Mann-Whitney $U$ test, $p < .10$).

Summary of Results

All 22 cells recorded in AII developed physiological plasticity in either background or evoked activity during the conditioning phase, and 21 of the 22 cells were plastic in both measures. Furthermore, changes in background activity were related to behavioral measures of arousal and learning rate. Increases in background activity occurred in animals that became more tonically aroused during conditioning than in the sensitization phase. Decreases in background activity developed in subjects that remained at the same relative tonic level of arousal during the conditioning and sensitization phases. The decrease in background activity developed along with the acquisition of pupillary conditioned responses. Increases in phasic arousal also affected the activity of most neurons, but the effects of phasic and tonic arousal were not necessarily concordant.

The rates at which neurons in AI and AII developed discharge plasticity for evoked and background activity were not significantly different. However, the proportion of cells that developed discharge plasticity was significantly greater in AII than AI both for evoked and for background activity. Finally, phasic arousal had greater effects on cells in AII than in AI.

Discussion

Changes in sensory system activity during learning may reflect mechanisms that underlie alterations in the processing of information. However, central auditory activity can be affected by changes in the effective intensity of a stimulus at the receptors during postural adjustments and middle ear muscle contractions (Imig & Weinberger, 1970; Marsh, Worden, & Hicks, 1962; Starr, 1964; Wiener, Pfeiffer, & Backus, 1966). In this investigation, the subjects developed pupillary conditioned responses while they were maintained under neuromuscular blockade. Consequently, the changes recorded in secondary auditory cortex during learning cannot be attributed to proprioceptive feedback or to a change in the physical parameters of the stimulus at the tympanic membrane.

Given that the stimulus remained constant throughout the conditioning session, it is extraordinary that every cell recorded in AII developed physiological plasticity during learning. The only comparable degree of learning-related changes, of which we are aware, is that of the magnocellular medial geniculate nucleus (MGm) of the thalamus. It has been reported that all neurons in this nucleus exhibit discharge plasticity for either or both background and evoked activity during pupillary conditioning (Weinberger, 1980, 1982). The possible role of MGm plasticity in that of AII is considered in a later section.

In addition to the detection of neuronal plasticity during conditioning, changes in AII single-unit activity covaried with be-
behavioral measures of learning rate and level of arousal. However, before further discussing relations between neuronal activity and behavioral measures, it will be helpful to provide a general description of the discharge characteristics of the cells that were encountered in this region.

**General Characteristics of Neuronal Activity in AII**

The cells recorded in this study typically responded best to squeaky sounds and other complex acoustic stimuli. Onset latencies ranged from 22 to 500 ms; the average was 147 ms, and the median was 80 ms. These values are similar to previous recordings of single cells in AII in anesthetized preparations. Katsuki, Watanabe, and Maruyama (1959) noted that most cells in AII responded with latencies greater than 100 ms whereas AI units had considerably shorter onset latencies. Others extended their analysis of AI to note the broadness of tuning curves in AII and its lack of tonotopic organization (Merzenich, Knight, & Roth, 1975; Middlebrooks & Zook, 1983; Reale & Imig, 1980).

Background activity for most cells was very low, rarely exceeding 5 spikes/s. It was common to find cells that fired only 5-10 spikes/min. An example of this class of unit is presented in Figure 4A. Its evoked response consisted of one or two discharges at a latency of about 30–50 ms after stimulus onset, followed infrequently by further firing. This particular unit had a background firing rate of 8 spikes/min during the sensitization phase, which declined to an almost complete cessation of background activity during conditioning. This essentially led to a circumstance such that the stimulus-evoked discharges were the only times the cell fired for periods as long as 10–15 min.

One cell was unique in that its background firing rate was considerably higher than the rest of our sample (27 spikes/s), and it was the only unit to exhibit a sustained excitatory response to acoustic stimulation. The discharge characteristics of this cell were similar to a class of cells recorded by De Ribaupierre, Goldstein, and Yeni-Komshian (1972) in AI of the cat. (See also Mountcastle, Talbot, Sakata, and Hyvarinen, 1969, for similar data in somatosensory cortex.) Others have also detected a low incidence of auditory cortical units that have high background firing rates (Goldstein & Abeles, 1975). The major features that this unit had in common with the AI cells in De Ribaupierre's study were its high rate of background activity and sustained excitatory response to acoustic stimulation. It is unlikely that the high-rate units of De Ribaupierre were axons because extensive intracellular recordings were obtained from many cells in this category. In the present study, the unique behavior of this cell cannot be attributed to peculiarities in the recording site or a result of an instability in the preparation because a second cell was recorded from the same electrode and it responded in a more typical fashion, that is, it had a very low rate of background activity (5 spikes/min) and rarely discharged more than one action potential to the acoustic stimulus.

Mountcastle et al. (1969) and De Ribaupierre et al. (1972) postulated that cells that received predominantly excitatory input and lacked inhibitory postsynaptic potentials at the soma were stellate cells whereas units with lower background rates and transient evoked responses were pyramidal cells. These points are noteworthy because the high-rate cell in this study was the only one that failed to develop evoked plasticity during conditioning. Although the sample size is too small to speculate extensively on the nature of this finding, it is suggestive of a fundamental difference between plastic changes in cortical projection cells and interneurons during learning.

**Relation Between AII Neuronal Activity and Learning**

AII unit activity was profoundly influenced by the conditioning procedure. All 22 units displayed plasticity in their background activity, and 21 of the 22 developed evoked plasticity. The probability of obtaining increases and decreases in evoked activity was approximately equal; 11 units increased and 10 decreased evoked activity
during conditioning relative to the sensitization phase. Decreases in background activity predominated; 17 units decreased, and only 5 developed increases. The direction and rate of change in background activity were not related in any obvious manner to evoked changes.

Given that there was such a large degree of plasticity, we investigated the relation between alterations in firing rates as a function of the rate of learning. A serendipitous finding aided such an investigation. The subjects acquired the pupillary conditioned response either rapidly (range, 6–16 trials) or slowly (range, 25–59 trials). It is likely that this was the result of two different procedures that were employed while searching for cells. The low background rates encountered in All made it difficult to locate units in the absence of acoustic stimulation. In earlier recordings, tones and white noise stimuli were extensively used as search stimuli. However, the development of conditioned responses was delayed in these sessions. In later recording sessions, it was discovered that the best stimuli for evoking All activity were complex sounds such as squeaks and shaking keys. By using these stimuli, we were able to obviate the problem of locating cells with low background firing rates without inducing a latent inhibitory effect on conditioning. The subjects that learned slowly allowed for the investigation of the temporal relation between the development of neuronal plasticity and the acquisition of conditioned responses.

The rate at which evoked plasticity developed was not related to the rate of pupillary conditioning during individual recording sessions. In contrast, changes in background activity were correlated with learning rate. Specifically, for units that developed decreases in background activity during conditioning, the rate of decrement was directly related to the rate of development of the pupillary conditioned response. Rapid learners displayed a rapid decrease in background activity, whereas the decrement was not evident in slower learners until they began to associate the acoustic stimulus with paw shock (see Figure 6). Disterhoft and Stuart (1976) also reported that decreases in auditory cortical background activity predominated during acquisition of a tone-signaled, appetitive classically conditioned response in rats. As in the present study, the decreases began with the first evidence of conditioned responses. Our findings are also in agreement with the aforementioned study with regard to the differential nature of evoked and background changes. In rat auditory cortex, evoked plasticity developed later than did background changes and the early signs of behavioral learning. The fact that we found changes in evoked activity both before and after learning is likely to be a function of the differences in the sampling of neuronal populations. Disterhoft and Stuart recorded from more than one cell simultaneously, whereas the present study characterized the activity of single neurons.

Changes in evoked and background activity are likely to involve different mechanisms for the following reasons. Evoked plasticity developed gradually and reached asymptote after 20–35 conditioning trials (see Figure 4A). Further, the development of changes in evoked activity did not coincide with the acquisition of pupillary conditioned responses. Thus, stimulus-evoked plasticity develops solely as a consequence of stimulus pairing and maintains a gradual increase in plasticity beginning early in the conditioning phase. In contrast, decreases in background activity developed abruptly, along with the first signs of associative learning (see Figure 6). When the subjects developed pupillary conditioned responses rapidly (fewer than 10 trials), the decrease occurred abruptly, within the first 5 trials of conditioning, and stabilized at depressed levels of activity for the duration of the recording session. The subjects that exhibited slower learning began to develop conditioned responses after the 20th trial. Notice in Figure 6 that background activity of cells in the slower learners remained at the sensitization level for the first 20 trials of conditioning and then abruptly decreased and stabilized.

The relation between learning rate and neuronal plasticity held for decreases in background activity only. The five units that showed increases in background activ-
ity during conditioning did so at a rate that was independent of the rate of learning. However, increases in background activity were related to other measures of behavior, and these are discussed in the next section.

Relation Between Neural Activity and Tonic Arousal

In addition to providing an index of learning, pupillary dynamics serve as an indicator of the behavioral state of the animal. In humans, the relation between arousal level and pupillary dilation is well documented (Nunnally, Knott, Duchnowski, & Parker, 1967). By using the relative levels of pupillary dilation during a recording session as an indicator of the state of the subject, the relation between neuronal activity and arousal level could be quantified. The analyses focused on the tonic or baseline level across the entire recording session and phasic increases in arousal following EDS. In this section, we take up the former; in the next section, the latter.

The tonic level of arousal in awake animals has not been investigated widely in previous neurophysiological studies of learning. Generally, it has been assumed that whereas distinctions among states of waking, drowsiness, and sleep are important, finer distinctions need not be addressed. Perhaps this is so because animals are clearly awake during conditioning training and sensitive measures of tonic arousal are not generally employed. However, we were able to analyze the tonic level of arousal in awake animals because we recorded the diameter of the pupil during the intertrial intervals as well as during the presentation of the conditioning stimuli.

As noted above, increases in background activity were unrelated to the rate of pupillary conditioning. However, they proved to be related to tonic arousal; animals that became more tonically aroused also developed increases in background activity (Figure 8). This relation holds for the 5 neurons that developed increased background activity but not for the 17 neurons that exhibited decreased background discharges. Thus, decreases in background activity, although related to the rate of learning, were not associated with the tonic level of arousal; decreases in background activity occurred in training sessions that involved no significant changes in tonic arousal level.

With further respect to changes in background activity, we previously noted that decreases in background activity during conditioning develop at a rate that is correlated with the rate of learning; rapid learners develop decreases rapidly, and slow learners develop decreases more slowly. Perhaps the reason why there was a differential rate of decline of background activity was a difference in the tonic level of arousal between rapid and slow learners; for example, if the slow learners were temporarily in a state of increased tonic arousal early in conditioning, their background activity could have been elevated and thus retarded the rate of decrease of background activity. If this was the case, then one would expect the pupillary baseline of the slower learners to be greater than that of the rapid learners in the early stage of conditioning. However, an analysis of this possibility revealed that the baseline level of pupillary dilation did not differ between the two groups early in the conditioning session. Therefore, the differential development of the decrement in background activity is not a function of differences in generalized arousal. Rather, it appears to reflect associative processes, the exact nature of which remains to be determined.

It is important to point out that animals can still learn rapidly even with increases in pupillary baseline level and background neuronal activity. Therefore, the decrease in background activity related to behavioral learning should be considered to be a component of acquisition during a relatively stable state of tonic arousal, but it is not a necessary component of learning per se.

The overall picture presented with respect to background activity, tonic arousal, and conditioning is as follows. Decreases in background activity are correlated with the rate of acquisition of the conditioned response; further, they are themselves associative in nature. This is the usual state of affairs, as revealed to the experimenter when the level of tonic arousal does not
change much during training; in the present experiment, such effects occurred in 17/22 cases. However, when, for reasons unknown, subjects develop increases in their level of tonic arousal, background activity increases in a correlated manner, and such increases override or "mask" processes that generate the usual decrease in background activity. The tonic level of arousal need not change in order for associative behavioral and neuronal events to develop, as such events are obtained in the absence of changes in tonic arousal in most cases. Thus, changes in tonic arousal should be viewed as performance rather than as associative factors. They are nonetheless important because they can mask or prevent the appearance of neuronal changes that are associative (i.e., decreases in background activity) and are neurophysiological correlates of acquired responses (e.g., conditioned pupillary dilation responses). One implication of these findings is that caution should be exercised in the interpretation of the absence of a neurophysiological correlate of learning in a given experiment unless there has been a concurrent analysis of performance variables that could obscure a brain–behavior relation, such as the level of tonic arousal.

Relation Between Neuronal Activity and Unconditioned Phasic Arousal

Phasic (transient) arousal is evidenced by pupillary dilations that are evoked by sensory stimulation and have a relatively brief duration (e.g., 2–5 s). Strictly speaking, conditioned dilations to acoustic stimulation are instances of phasic arousal. However, for purposes of discourse, we distinguish between these learned effects, which have been discussed previously, and unconditioned arousal, such as that which occurs in response to presentation of the unconditioned stimulus. To assess the possible relations between phasic arousal and neuronal discharges, we analyzed the effects of EDS during the sensitization phase. As noted in Results, 17 of the 22 cells responded to EDS: 6 exhibited increases, and 11 exhibited decreases in activity, relative to pretrial discharge activity. It is important to note that for a given neuron, the effects of phasic arousal were not necessarily the same as the effects of tonic arousal. Of the 5 neurons that had increases in background activity, along with increasing tonic levels of arousal, 4 displayed a decrease in activity during phasic arousal. Hence, increases in tonic arousal were always accompanied by increases in background cellular discharge activity, whereas increasing phasic arousal caused either increases or decreases in neuronal activity. Such evidence indicates that tonic and phasic changes in arousal level represent distinct physiological processes: the latter is apparently not merely a brief episode of the former.

The present findings on arousal-related processes in sensory cortex are somewhat similar to the effects of stimulation of the reticular formation on cortical cellular function, which also may be either facilitatory or inhibitory (Feeney & Orem, 1972; Inubishi, Kobayashi, Oshima, & Torii, 1978; Singer, Tretter, & Cynader, 1976; Spehlmann & Downes, 1974). However, it is not yet possible to draw conclusions regarding the extent to which the normative function of the reticular formation is responsible for the increases and decreases in cellular discharge reported here for tonic and phasic arousal, for several reasons. First, the phasic arousal effects herein reported were elicited by sensory stimulation, and it has not been determined whether a given cortical cell responds in the same manner for peripherally and centrally induced arousal. Second, the previous studies did not measure the changes in pupillary diameter that undoubtedly occur following reticular stimulation (Loewy, Arajo, & Kerr, 1973), so that one cannot yet infer that the two sources of arousal yield the same behavioral results. Third, there is as yet insufficient understanding of the presumptive different mechanisms underlying phasic and tonic arousal (Jasper, 1960). However, at least the first two issues may be approached directly by concurrent use of reticular and sensory stimulation while records are obtained from single cortical neurons. Such studies should also shed light on the extent to which phasic arousal
induced by EDS is similar to phasic conditioned arousal induced by a conditioned acoustic stimulus.

**Arousal, Attention, and Learning**

Miller, Pfingst, and Ryan (1982) reviewed studies of single-unit activity in the auditory system of primates during the performance of various tasks and concluded that changes in neuronal activity are largely a consequence of shifts in arousal and attention, rather than learning. Although this line of research has been fruitful in identifying neuronal correlates of attention, the designs employed do not allow one to distinguish between the effects of learning and the effects of arousal or attention. The subjects had been trained in complex instrumental tasks for many months before recordings were initiated. Miller and associates found that auditory system neuronal activity was significantly altered during trials when the subject was attending to acoustic cues, compared with nonattending trials. The fact that neuronal activity in a sensory system is different while a subject is attending to environmental stimuli is consistent with the view that sensory cortex plays an active role in the processing of information, rather than only providing an accurate representation of the physical parameters of the stimuli. However, the findings in this study and in the investigation of Al (Weinberger et al., 1984) indicate that nonspecific processes of arousal and attention can be separated from associative processes for the following reasons. First, recordings were obtained during a sensitization as well as a conditioning phase. During sensitization, the subjects were aroused by the EDS, yet there was little evidence of changes in acoustically evoked activity (see Figure 3). Second, during the conditioning phase, evoked plasticity developed at a rate that was independent of the rate of pupillary learning, which is a sensitive behavioral index of arousal. Third, previous neural recordings in the ventral division of the medial geniculate nucleus indicated that the activity of these cells changes as a function of the arousal level of the animal (Humphrey & Orman, 1977; Imig, Weinberger, & Westenberg, 1972; Orman & Humphrey, 1981), but learning studies have consistently failed to obtain discharge plasticity in this nucleus (Birt et al., 1979; Gabriel et al., 1976; Ryugo & Weinberger, 1976, 1978). Hence, arousal and learning effects are clearly separable. These data indicate that processes of attention and arousal may affect widespread regions of the brain but that the capacity to develop discharge plasticity as a function of associative learning may involve more restricted populations of cells. In order to determine such a capacity, recordings must be obtained during the acquisition phase of learning rather than during the performance of an overlearned task.

**Relation to Previous Studies of Acquisition of Conditioned Responses**

There are two previous studies that investigated neural recordings in nonprimary auditory cortical fields during learning. Kraus and Disterhoft (1982) reported on single units in auditory association cortex of the rabbit during acquisition of the nictitating membrane response, and Gabriel, Orona, Foster, and Lambert (1982) reported on multiple-unit activity in a secondary auditory cortical field of rabbits during active avoidance conditioning. The results of our study contrast with those reported in the two investigations of neuronal plasticity in terms of the incidence of plasticity and the time course of such changes. Kraus and Disterhoft detected significant changes in background and evoked activity in only 50% of their recordings, and the changes that did occur developed late in conditioning. Gabriel et al. (1982) did not find any changes in neuronal activity related to learning. An assessment of the anatomical, procedural, and methodological differences between the paradigms

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3 Kraus and Disterhoft reported using the same method of processing neurophysiological data as that used by Olds (1973). They stated that “cells with similar waveforms were sometimes combined by this technique and were not further discriminated” (p. 206). Therefore, the extent to which their data actually reflect the discharges of single neurons is unclear.
is necessary in order to properly evaluate the disparity in experimental findings.

The anatomy of the auditory system has been most thoroughly investigated in the cat. The feline auditory cortex is comprised of at least four tonotopically organized subfields surrounding AI, which is not tonotopic (Merzenich & Kaas, 1980; Merzenich et al., 1975; Reale & Imig, 1980). Kraus and Disterhoft (1982) recorded in an auditory cortical field they described as "auditory association cortex." However, it is likely that this area is homologous to one of the tonotopically organized fields that have been described in the cat because their cells had distinct best frequencies. Recent electrophysiological evidence indicates that the rabbit contains at least two tonotopically organized auditory cortical fields (Mullen & Glaser, 1982). The location of these fields coincides with the recording sites in the Kraus and Disterhoft study and supports the view that AI of the cat is not the homologue of auditory associational cortex of the rabbit.

Although the two fields are not homologous, there is still the issue of substantial differences in the time course of the development of neuronal plasticity between the present study and that of Kraus and Disterhoft. Changes in activity in AI developed soon after stimulus pairing began and tended to stabilize by the 20th conditioning trial for animals that did not exhibit latent inhibition. Kraus and Disterhoft found that plasticity developed about 100 trials after stimulus pairing began, which was after the somatic conditioned response appeared. However, their training sessions did not include a sensitization phase, which could serve as a reference for which to compare changes in activity during conditioning. Animals were either sensitized or conditioned. They reasoned that because somatic conditioned responses were not evident early in the conditioning phase, these trials could serve as a reference point for learning-related plasticity. However, we have shown in this study and in the investigation of AI (Weinberger et al., 1984) that auditory cortex is in a dynamic rather than static state early in the conditioning phase. Therefore, even though somatic conditioned responses were not evident early in their conditioning sessions, rapid associative neuronal changes may have still developed but were not detected.

It has been argued that classical defensive conditioning is a two-stage process (Weinberger, 1982). The initial stage consists of a rapidly conditioned process in which the conditioned stimulus elicits conditioned arousal or conditioned fear. This is followed by a slowly acquired process involving the acquisition of a conditioned somatic response which is specific to the nature of the US. The present study and that of Kraus and Disterhoft (1982) indicate that both stages involve changes in the discharge characteristics of auditory cortical neurons. However, we detected changes in neuronal activity involving both background and evoked activity. Kraus and Disterhoft noted that all of their changes involved stimulus-evoked activity whereas background activity remained relatively constant. A lack of change in background activity is also a common finding during the performance of tasks involving animals that are highly overtrained (Beaton & Miller, 1975; Benson & Hienz, 1978; Goldstein, Benson, & Hienz, 1982; Pfingst, O'Connor, & Miller, 1977). Perhaps the initial recognition of stimulus contingencies involves a widespread damping of ongoing neuronal activity and an accentuation of the activity of neurons that are specifically involved in the processing of salient cues. Once such a process takes place and contextual cues remain constant, all further changes may proceed on a relatively constant level of background activity. The elaboration of conditioned somatic responses as well as the performance of overlearned tasks may involve a fine tuning of neural processing that is more likely to be expressed during stimulus-evoked activity than during interstimulus periods of background activity. Resolution of this issue will have to await studies in which continuous recordings are maintained from single neurons throughout the course of acquisition of conditioned fear and conditioned specific somatic responses.

Gabriel et al. (1982) recorded neural activity in AI of the rabbit during condi-
tioned avoidance. As noted above, they failed to find learning-related changes. However, owing to the lack of data on the comparative anatomy between the rabbit and cat, it is not known if their recording sites were in a cortical field that is homologous to AII of the cat. In addition to the issue of homologous recording sites, there was a crucial difference in the manner in which neuronal activity was recorded and analyzed. In our study, single neurons were monitored during an entire conditioning session. Gabriel et al. recorded neural activity which was composed of an indeterminate number of neurons over the course of many days. All of the multiple-unit recordings from different animals were pooled into one cumulative record, which was found to be nonplastic. This technique is not likely to be fruitful when attempting to detect learning-related changes in neocortical activity for a number of reasons. Single-unit recordings in many different cortical fields indicate that most cells have very low background firing rates and respond transiently to sensory stimuli. A small population of cortical cells has high rates of neuronal activity and respond in a sustained manner to sensory stimulation. Recall that one of the cells recorded in this study had firing characteristics that suggested that it belonged within this class of cells. This was also the only cell that did not develop evoked plasticity during learning. Such activity, if present in multiple-unit recordings, would mask the plasticity of other types of neurons. Evidence of masking in multiple-unit recordings was presented in Weinberger et al.'s (1984) article on primary auditory cortex. Further, the present study has shown that changes in evoked activity are equally likely to be increases and decreases. These differences would tend to average to no change in a multiple-unit record containing both types of data. This very effect has been demonstrated by multiple-unit recordings (Ryugo & Weinberger, 1978) and single-unit recordings (Weinberger, 1982) in the magnocellular division of the medial geniculate nucleus. Thus, negative findings obtained from multiple-unit recordings are subject to incorrect conclusions.

Sources of Plasticity in Secondary Auditory Cortex

AII receives input from two auditory regions that display learning-related changes in neuronal activity, AI and the magnocellular division of the medial geniculate (MGm). Of these two regions, the cells in MGm exhibit a greater probability of plasticity than those in AI. It is possible that the plasticity that develops in AII is dependent in part upon the plasticity that develops in AI or MGm. However, the most prominent AII afferent is the dorsocaudal division of the medial geniculate (MGdc; Anderson, Knight, & Merzenich, 1980). Although there are no published studies of single-unit recordings in MGdc in behaving animals, Calford and Webster (1981) characterized the activity of cells in this region in anesthetized cats. The majority of the units were broadly tuned, responded to acoustic stimuli at long latencies (greater than 40 ms), and habituated rapidly to repetitive stimulation. These findings suggest that MGdc may play a role in the processing of acoustic information with respect to its behavioral relevance. In addition, the long onset latencies recorded in this study may have resulted, in part, from input from MGdc. The extent to which the plasticity found in AII is conferred upon it by MGdc remains to be investigated.

Comparison of AI and AII

Previous analyses of AI and AII distinguished these cortical fields on the basis of cytoarchitecture (Rose, 1949), thalamocortical connections (Anderson et al., 1980), and sensory response characteristics (Katsuki et al., 1959; Merzenich et al., 1975; Phillips & Irvine, 1981). AI is tonotopically organized, with narrowly tuned cells. It is the major projection site of the primary lemniscal pathway of the auditory system. Subcortical nuclei that comprise this pathway are tonotopically organized, with narrowly tuned cells. In contrast, AII does not contain a tonotopic organization and has broadly tuned cells. It is the cortical projection field of the so-called "diffuse" or "lemniscal-adjunct" auditory pathway entailing
PLASTICITY OF SINGLE AI NEURONS DURING LEARNING

subcortical nuclei that are not tonotopically organized (Aitkin, 1973; Aitkin, Webster, Veale, & Crosby, 1975; Graybiel, 1972). Cells in these regions are more broadly tuned than those in the lemniscal pathway. By recording neuronal activity in AI and AII during learning, we are attempting to uncover evidence of general principles governing the functional organization of primary and secondary cortical fields and gain a greater understanding of the nature of parallel pathways in sensory systems.

The cells that were recorded in these two regions were in layers V and VI. Therefore, this analysis is concerned with the discharge characteristics of neurons that convey information from each field toward other cortical and subcortical regions (Beyerl, 1978; Imig & Brugge, 1978; Kelly & Wong, 1981).

Three aspects of differences in neuronal activity of AI and AII are discussed next: the rate of development of discharge plasticity during the course of the conditioning phase, the relation between changes in neuronal activity and arousal level, and the relative incidence of single cells developing learning-related discharge plasticity.

In both cortical fields, evoked and background neuronal activity changed rapidly once the acoustic stimulus was paired with EDS (see Figures 2 and 5 of Weinberger et al., 1984, and Figure 3 of the present study). As the changes developed at about the same rate in both regions, it is unlikely that the plasticity developed first in one of the fields which then conferred its plasticity upon the other field. Further, as the magnocellular division of the medial geniculate (MGm) projects to the upper layers of both fields (Niimi & Naito, 1974; Wilson & Cragg, 1969), and it also develops discharge plasticity early in conditioning (Weinberger, 1982), aspects of the plasticity may have resulted, in part, from the modulatory influence of MGm in concert with processes intrinsic to each field.

In terms of the relation between discharge activity and arousal level, one interesting finding was that both fields were similar with respect to changes in background neuronal activity and the tonic level of behavioral arousal. In AI and AII, increases in background activity developed in subjects that became more tonically aroused during the conditioning phase than during sensitization. In addition, the increase in cellular excitability was not expressed as an increase in evoked discharge rate. In fact, the direction of change of evoked activity in AII was completely unrelated to the tonic level of arousal, that is, the incidence of increases or decreases in evoked plasticity was not a correlate of the behavioral state of the subject. The only correlation between evoked activity in AI and arousal level was that cells failed to develop evoked plasticity when the subjects were in a state of elevated tonic arousal (see Figure 6 of Weinberger et al., 1984). In contrast, cells in AII developed evoked plasticity even when subjects were in an increased state of arousal during conditioning. Perhaps the information that is processed in this field is qualitatively less specific in nature than that in AI and may be less susceptible to interference by elevated states of arousal during learning.

In another analysis of the relation between arousal and discharge activity, cells in AII were more likely to display altered firing rates following EDS than were cells in AI (77% in AII vs. 32% in AI). EDS consistently evoked large transient pupillary dilations lasting from 2 to 5 s. The observation that more cells in AII were responsive to the EDS alone may reflect a greater involvement of this region with nonspecific processes governing rapid changes in arousal level. It is also possible that the cells were responding to somatosensory input which may reach AII via the posterior nucleus of the thalamus (J. Wiener, Diamond, & Raczkowski, 1977). In any case, the capacity of cells in AII to respond to the US as well as the CS may have increased the likelihood that discharge plasticity developed during conditioning. Related findings were reported by Cohen, Gibbs, Siegelman, Gamlin, and Broyles in the lateral geniculate of the pigeon (1982), Yoshii and Ogura in the reticular formation (1960), and O'Brien and Fox in motor cortex (1969).

Although there is the potential for a sampling bias, the difference in the incidence
of cells that developed learning-related plasticity in AI and AII provides evidence for a functional distinction. All 22 cells in AII were plastic in either background or evoked activity, and 95% (21/22) were plastic in both measures. In AI, 79% (15/19) were plastic in either measure, and only 42% (8/19) developed changes in both background and evoked activity during conditioning. Constraints on the degree of discharge plasticity that develops in AI may result from the necessity of this region to also perform aspects of an analysis of the physical properties of sound. AI may be necessary for such complex processes as integrating the acoustic environment into a coherent framework (see Whitfield, 1979). The fact that AI contains binaural response bands (Imig & Adrian, 1977; Middlebrooks & Zook, 1983) and that lesions within this field lead to sound localization deficits (Jenkins & Merzenich, 1981; Neff, Diamond, & Cassady, 1975) supports the notion that an analysis of the sensory properties of acoustic stimuli takes place at the cortical level. Such a requirement may restrict the modifiability of a subpopulation of cells in AI.

Because plasticity was so pervasive in AII, this may indicate that information in this region is processed with a greater emphasis on the psychological than on the physical properties of sound. However, the specific nature of such changes in terms of information processing remains unknown. Perhaps learning-related changes in the discharge activity of AI and AII neurons represent dynamic changes in certain aspects of their receptive field characteristics. Studies now in progress are investigating this and related issues.

Summary

Single-unit activity was recorded in AII of the cat during acquisition of the pupillary dilation conditioned response. All 22 cells displayed physiological plasticity during conditioning relative to a sensitization phase. Background activity for all cells changed, and 21 of 22 cells displayed stimulus-evoked plasticity. In addition to detecting changes in cell activity, the direction of change of activity could also be correlated with behavioral measures. Decreases in background activity occurred in conjunction with the development of pupillary conditioned responses. Increases in background activity were recorded when subjects became more tonically aroused during conditioning. In contrast, CS-evoked plasticity was not correlated with either the rate of pupillary learning or the tonic level of arousal during the conditioning phase. Thus, single-unit activity in secondary auditory cortex reflects components of behavioral processes, namely, changes in arousal level and associative learning.

References


(Eds.), Handbook of sensory physiology (Vol. 5, Pt. 2, pp. 307-400) New York: Springer Verlag.


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