Role of Mammalian Auditory Cortex in the Perception of Elementary Sound Properties

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Received 12 June 2000; accepted in final form 8 March 2001

Talwar, Sanjiv K., Pawel G. Musial, and George L. Gerstein. Role of mammalian auditory cortex in the perception of elementary sound properties. J Neurophysiol 85: 2350–2358, 2001. Studies in several mammalian species have demonstrated that bilateral ablations of the auditory cortex have little effect on simple sound intensity and frequency-based behaviors. In the rat, for example, early experiments have shown that auditory ablations result in virtually no effect on the rat’s ability to either detect tones or discriminate frequencies. Such lesion experiments, however, typically examine an animal’s performance some time after recovery from ablation surgery. As such, they demonstrate that the cortex is not essential for simple auditory behaviors in the long run. Our study further explores the role of cortex in basic auditory perception by examining whether the cortex is normally involved in these behaviors. In these experiments we reversibly inactivated the rat primary auditory cortex (AI) using the GABA agonist muscimol, while the animals performed a simple auditory task. At the same time we monitored the rat’s auditory activity by recording auditory evoked potentials (AEP) from the cortical surface. In contrast to lesion studies, the rapid time course of these experimental conditions preclude reorganization of the auditory system that might otherwise compensate for the loss of cortical processing. Soon after bilateral muscimol application to their AI region, our rats exhibited an acute and profound inability to detect tones. After a few hours this state was followed by a gradual recovery of normal hearing, first of tone detection and, much later, of the ability to discriminate frequencies. Surface muscimol application, at the same time, drastically altered the normal rat AEP. Some of the normal AEP components vanished nearly instantaneously to unveil an underlying waveform, whose size was related to the severity of accompanying behavioral deficits. These results strongly suggest that the cortex is directly involved in basic acoustic processing. Along with observations from accompanying multunit experiments that related the AEP to AI neuronal activity, our results suggest that a critical amount of activity in the auditory cortex is necessary for normal hearing. It is likely that the involvement of the cortex in simple auditory perceptions has hitherto not been clearly understood because of underlying recovery processes that, in the long-term, safeguard fundamental auditory abilities after cortical injury.

INTRODUCTION

The mammalian auditory cortex, situated bilaterally in the temporal regions of the brain, is a complicated structure made up of a cluster of separate, acoustically responsive, territories. At the center of these territories lies what is commonly known as the primary auditory (or AI) cortex. The AI field receives its dominant input from the main auditory pathway, which begins at the cochlea and passes through successive, interlinked, brain stem and thalamic nuclei on its way to the cortex. Individual neurons in AI, like those of lower nuclei in the pathway, are selective for a narrow frequency band and are organized by their frequency tuning properties to form a complete spatial representation of the frequencies to which an animal is sensitive (for a review, see de Ribaupierre 1997). Homologues of AI have been mapped in several species; in the rat, for example, the AI field closely approximates cortical areas 41 and Te1 (Sally and Kelly 1988). Surrounding this core region are fields that make up the secondary or belt auditory areas (Roger and Arnault 1989).

Despite the progress in mapping the acoustic response properties of the auditory cortex, its role in the perception of sound remains unclear. Over the last five decades the role of the auditory cortex in dealing with basic auditory sensation has been explored in a large number of lesion studies that have looked for specific behavioral deficits after cortical ablations (for a comprehensive review see Pickles 1982; others: Buser and Imbert 1992; Clarey et al. 1992; Heffner and Heffner 1990). In general, these studies have shown that simple pitch discriminations are not affected by bilateral auditory ablations, while effects on sound detection range from virtually none in many species to moderate in primates and humans. In the rat, for example, ablation of all known auditory areas has resulted in little or no change in either frequency discrimination thresholds or sound sensitivity (Kelly 1970). In addition, the rat’s ability to localize sound also does not appear to be affected by cortical lesions (Kelly and Glazier 1978), although this finding may be species and task dependent (Jenkins and Merzenich 1984).

The evidence from lesion studies indicates therefore that an intact neocortex is not necessary for basic auditory functions such as detection of tones and simple discrimination of sound frequencies. Nevertheless, such a conclusion does not speak clearly to the nature of cortical involvement in normal hearing. For example, it is possible that reorganization of the central auditory system (a property of the CNS that is being increasingly recognized) may cause recovery of basic auditory functions after cortical ablation, in ways perhaps similar to that underlying the recovery from cerebrovascular accidents in humans. The transient behavioral deficits that are often seen...
after surgical ablations may well reflect underlying reorganization; these, however, are difficult to study systematically and to isolate from nonspecific deficits associated with the general trauma of surgery. Although some lesion studies have attempted to chart the time course of behavioral recovery (Heffner and Heffner 1986; Kavanagh and Kelly 1988), their focus has also been to define behaviors for which the auditory cortex is essential. Naturally, the possibility of auditory reorganization after cortical ablation complicates the interpretation of lesion studies.

In this study we revisit the role of auditory cortex in elementary acoustic behaviors by applying an acute and reversible chemical blockade of it using the GABA-A agonist muscimol, a potent neuronal inhibitor. In the bat, a species with specialized cortical areas for complicated parametric analysis of the echo, experiments using muscimol on particular areas have shown selective behavioral impairments corresponding to their known functions (Riquimaroux et al. 1991). However, analogous work on the auditory cortex of any of the vast majority of animals that lack ethologically related specializations seems not to have been done. Our experiments applied muscimol to the AI cortex of rats while they were engaged in a simple auditory task that required the ability both to detect pure tones and to discriminate frequencies. Simultaneously, we monitored auditory evoked potentials (AEP) from the AI cortex. Separate muscimol experiments then related the AEP to multiunit activity (MUA) within the cortex.

**METHODS**

We performed both chronic and acute experiments over the course of this study, which used a total of 17 subjects, all of whom were female white rats, 300–350 gm (Charles River). Depending on their aim, these experiments were classified as behavioral experiments, AEP and MUA experiments, or initial control experiments.

**Behavioral experiments**

Five female white rats were first trained in a task that involved both tone detection and frequency discrimination. The details of the procedure, methods, and duration of training, sound apparatus and calibration, and the analysis of behavior have been previously detailed (Talwar and Gerstein 1998).

Behavioral tests were conducted in a sound-transparent operant cage (12 × 10 × 10 in.) made of slim metal bars (½ in. diam) with a ½-in. wire mesh (½-in. wire) floor and ceiling, and suspended from the ceiling of an anechoic room (IAC, Bronx, NY). A speaker (Realistic no. 40-1377) was attached to the top of the front wall of the cage, making an angle of 60° with the cage floor. It delivered pure tones and had a near flat frequency response between 5 and 40 kHz. Sound calibration showed that the space within the cage approximated to tones and had a near flat frequency response between 5 and 40 kHz.

A correct detection, or hit, was defined as a nosing response within 2 s of target tone onset. Responses outside this time window counted as false alarms and were not rewarded. Frequency discrimination performance was evaluated by defining hit rates and false alarm rates (guessing rate). In the context of our behavioral task, these quantities, in turn, depended on the notion of a trial. A single trial was considered as any 2-s time window during which a possibility of reward existed. A target trial therefore was the 2-s time window from target tone onset; responses during these trials led to reward. A nontarget (standard) trial, similarly, corresponded to any 2-s time interval outside a legitimate hit window; responses during these trials were not rewarded (but could potentially have lead to a reward, as far as the rat was concerned). Thus hit rates were defined as number of hits/number of target trials. Since two discrimination task levels, coarse and fine, existed within a session, each was associated with its individual hit rate. By contrast, a single false alarm rate was defined for the session: number of false alarms/total number of nontarget trials.

In any given test session frequency discrimination performance at each task level was measured by the signal detection index A’ (Green and Swets 1966; Talwar and Gerstein 1998, 1999). This index, which is a function of hit and false alarm rates, has the computational formula (Grier 1971)

\[ A' = \frac{1}{2} + \frac{1}{2} \left[ \left( h_i - fa_i \right) \left( 1 + h_i - fa_i \right) \right]^{1/2} \]

where \( A' \) is the discrimination performance at task level \( i \) = coarse or fine; \( h_i \) = hit rate, at the corresponding task level; \( fa_i \) = false alarm rate. Values of \( A' \) from 0.5 to 1 indicate chance to perfect discrimination performance. Normal subjects always scored more than 0.88 on coarse discriminations and more than 0.83 for fine ones (these were the worst performances recorded). Values lower than these minima were taken to indicate corresponding deficits in frequency discrimination.

In contrast to the measurement of frequency discrimination ability, the measurement of tone detection ability was built naturally into the behavioral task. In the operant cage subjects were first observed for 1–3 min before the tones began. In the normal test situation, the onset of tones was accompanied by an easily identified pattern of behavior on the part of the rats. This pattern included one or all of the following responses to tone onset: a pricking of the pinna and a sudden cessation of ongoing movement, a movement toward the nosing hole, and a false-alarm operant response within 3 s. The occurrence of any of these qualified as tone detection. In training and test sessions, all normal subjects exhibited a very clear detection response for tone intensities greater or equal to ~20 dB SPL at 8 kHz. Thus the behavioral task did not require an explicit operant response for tone detection (as against discrimination). [Our study was actually piloted in a rat for which an additional operant response for tone detection was also required; this task, similar to that used in a previous study (Talwar and Gerstein 1999), gave results identical to what we report here. Note also that, in the task we used here, performance was independent of location of the sound source; during training the location of the sound source was varied to confirm this.]

**Surgery and recordings**

After subjects had mastered the behavioral task, they underwent surgery for later bilateral muscimol application to the AI cortex and AEP recordings. Anesthesia was induced by intra-peritoneal injection of a mixture of ketamine (70 mg/kg) and xylazine (8 mg/kg). Smaller supplemental doses (1⁄3 of induction) were occasionally given to main-
tain surgical depths of anesthesia. After clearing the temporal regions of the rat skull, bilateral craniotomies, 2 mm diam, were made over the center of each AI field. Identification of AI center was made stereotactically; it corresponded to the center of region Te1 as defined by Paxinos and Watson (1986). Vascular markings in this area of the rat cortex provided additional easy reference to AI location (Sally and Kelly 1988). Note that the rat AI surface area is roughly around 3.5 mm².

Small stainless steel well cylinders, 3.5 mm OD, 0.3 mm wall, and 4 mm height, were fixed to bone surrounding each craniotomy with acrylic. The well cylinders doubled as future receptacles into which muscimol was injected and from which it then diffused into the AI cortex, and as surface electrodes through which the AEP was recorded. The dura that was exposed by the craniotomies was deliberately left intact; initial experiments demonstrated that it was highly permeable to muscimol. In two subjects, electrical connections were made from the steel wells to a connector that was also cemented to the skull top. (A separate lead, embedded in the nasal bone, acted as signal reference for the AEP.) The well cylinders were placed so that the recording surface overlaying the cranialium sat inside the estimated surface margins of AI, and not over the secondary auditory areas, Te2 and Te3 (Paxinos and Watson 1986; Roger and Arnault 1989). To achieve this correctly, the wells, instead of being centered on the craniotomies were placed with lateral eccentricity.

After surgery the dura was cleaned daily with fine cotton sponges. Despite this precaution, the growth of granulation tissue over the dura, starting first from the edges of the craniotomy, presented a growing barrier to muscimol with time. Our experience indicates that the muscimol effect we report here is not reliable if carried out more than 10–12 days after craniotomies are performed, at least if an intact dura is an experimental concern. Nevertheless, one advantage of initial granulation at the edges of the craniotomy was its role as a sealant, preventing leakage of muscimol around AI (the granulation also tended to limit the cortical surface access to muscimol to an actual area <2 mm²). Placing gel-foam over the local AI dura in several experimental runs also guarded against the possibility of muscimol leakage into the cortical areas surrounding AI.

During behavioral test sessions a suitable adapter was plugged into the skull-top connector of the two subjects in which electrical connections were made to the implanted steel wells. This allowed easy and continuous recording of bilateral AI surface potentials in response to ongoing acoustic stimuli. Signals were buffered by head-stage amplifiers and, after being led through the ceiling of the operant cage, were appropriately filtered (2–300 Hz), amplified, digitized, and stored. The time and frequency of each tone presented to the rat as part of its ongoing behavioral test were integrated into these records. The behavioral AEP was defined as the average AI surface potential evoked by a given set of standard 8-kHz tones.

AEP and MUA experiments

These experiments aimed to record the normal AEP in the rat, both from the AI surface and its depth, and to study how the normal AEP changed as muscimol, when applied to the AI surface, began to diffuse into the underlying cortex. At the same time MUA within the AI cortex, and as surface electrodes through which the AEP was recorded. The dura that was exposed by the craniotomies was deliberately left intact; initial experiments demonstrated that it was highly permeable to muscimol. In two subjects, electrical connections were made from the steel wells to a connector that was also cemented to the skull top. (A separate lead, embedded in the nasal bone, acted as signal reference for the AEP.) The well cylinders were placed so that the recording surface overlaying the cranialium sat inside the estimated surface margins of AI, and not over the secondary auditory areas, Te2 and Te3 (Paxinos and Watson 1986; Roger and Arnault 1989). To achieve this correctly, the wells, instead of being centered on the craniotomies were placed with lateral eccentricity.

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Appropriate muscimol dosage for complete AI inactivation was determined in separate preliminary experiments, both acute (n = 4) and chronic (n = 4), in which microelectrodes were tangentially placed in deep AI cortical tissue below overlying craniotomies that were identical to those used later in the behaving animals. In the awake animal we generally found that a 20-μg topical muscimol application over the dura reliably inactivated the click-evoked response of the deep layers of AI cortex (all muscimol dosages used in this study were 1 μg/μl saline concentration). Amounts much smaller than 20 μg, such as those used for studies in the bat (Riquimaroux et al. 1991; Zhang and Suga 1997) proved to be insufficient (the desired volume of inactivation in our study was much larger). Gradual withdrawal of electrodes in acute preparations showed that the area of deactivation extended to ~1 mm around the craniotomy margins, a result consistent with the dynamics of muscimol diffusion (Martin 1991). Cells at the periphery of the inactivated zone tended to fire in bursts with periods of quiet between them; beyond this region cells were often spontaneously active. The limited craniotomies (2 mm) we made were therefore designed to contain the zone of inactivation within the AI region (~3.5 mm²). Even so, some diffusion effect on the cortical areas in the immediate neighborhood of AI cannot be completely ruled out, just as the possibility of individual subject variation of AI location cannot be. Still, the influence of both these factors on the results is expected to be relatively small, given the relatively large extent of the secondary auditory fields (see Paxinos and Watson 1986).

RESULTS

Effect of muscimol on auditory behavior

Three days after well-implantation surgery the subjects were reacclimatized to the behavioral task. On the fourth day they were tested before and after 20 μg of muscimol was injected bilaterally, into each well. Typically, baseline sessions were first recorded ½ h before muscimol application. Immediately afterward, subjects were again tested. Thereafter, several tests were conducted over a prolonged time period, sometimes lasting more than 24 h. The time intervals of the tests were often dictated by our ongoing observations.

Figure 1, A–E, depicts auditory performance, both tone detection and frequency discrimination, in all five subjects at various times relative to AI muscimol application. The sound intensities at which subjects were tested, and the corresponding occurrence/non-occurrence of tone detection, are shown in the top half of the panels. The bottom half depicts frequency discrimination performance (measured by the index A’) when detection occurred, at both coarse and fine discrimination task levels.

Behavioral testing began at 50 dB SPL. If a clear detection
response was not apparent, sessions were terminated. New sessions were then begun, in which tone intensity was increased in steps of 20 dB, to a maximum of 90 dB SPL. When, and if, a clear detection response was observed, frequency discrimination performance was evaluated.

The effect of AI-applied muscimol on the rat’s auditory performance was striking and consistent in all five subjects. As Fig. 1 demonstrates, pre-muscimol baseline sessions always showed both normal detection and normal frequency discrimination ability at 50 dB SPL. At 15–30 min postmuscimol, their behavior continued to be near normal; only the ability to discriminate fine frequencies was affected in three subjects, with the index $A_9$ falling to $0.8$.

Behavioral tests that were conducted at 2–3 h post-muscimol, however, showed a profound, and surprising, effect on auditory performance. During this period there was no evidence of tone detection in any subject, even at maximum 90 dB SPL. Otherwise, the rats appeared healthy; their motor functions, for example, were normal. Although the usual exploratory behavior typical of rats in the operant cage appeared to be decreased, their grooming behavior was unchanged. In about 50% of the experiments, we observed that the acoustic startle response to a loud hand clap near the rat appeared to be present, although somewhat diminished. This may indicate that the reflex mediating this response, which involves the lower auditory nuclei, was not much affected (Koch and Schnitzler 1997).

Tone detection remained completely absent at all intensities for a further 2–3 h. Thereafter, some recovery became evident with detection occurring at high intensities. During this recovery period, however, there was no corresponding indication of frequency discrimination ability in any animal. Subjects responded indiscriminately, with a very high rate of false alarms, while tones were being presented (i.e., stimulus control by frequency was lost). Subsequently, the ability to detect tones made a complete recovery. However, the recovery of frequency discrimination ability lagged considerably, taking a much slower time course. Even when normal detection ability had returned for 50 dB SPL tones (within 6–7 h), frequency discrimination performance remained close to chance level. Discrimination ability only gradually returned over an 8- to 15-h period. In all subjects, the ability to make coarse discriminations recovered much more quickly than the ability to make fine ones.

The above experimental sequence was repeated a minimum of three times in each subject, at intervals of at least 24 h of complete recovery. In all, the experiment was replicated in 20 separate muscimol applications, over all 5 subjects. At several points during this period, we substituted saline for muscimol as a simple control, with no discernable effect on either tone discrimination performance.

**FIG. 1.** The effect of muscimol-induced inactivation on auditory performance in 5 rats (A–E). The x-axis shows the times of testing relative to muscimol application at 0 h. The 2nd time point was 15–30 min after muscimol application. The y-axis shows tone intensities in dB SPL and frequency discrimination performance ($A'$) when sound detection occurred; $\times$, tone intensities that were detected; $\circ$, no detection occurred. For frequency discrimination performance: $\bullet$, easy discriminations; $+$, fine discrimination.
detection or frequency discrimination performance. Finally, the possibility of water satiation of subjects, at any time, was ruled out, in the light of results from previous behavioral studies that used identical behavioral protocols (Talwar and Gerstein 1998). For example, false alarm rates, which are good indicators of subject motivation and response bias, remained high in many sessions that otherwise showed diminished auditory performance, indicating that subjects remained vigilant.

**AEP in the behaving animal**

In 2 subjects, we examined AEPs during 10 separate muscimol applications. This provided a picture of ongoing AI neuronal activity while the rats were being tested. We found that changes in auditory performance were consistently mirrored by changes in the AEP (both AI cortices showed identical changes). Figures 2 (A–H) and 3 (A–H) depict sequential snapshots of the AEP in both subjects. Each AEP waveform was averaged over 200 successive tone presentations of the 8-kHz base frequency at 70 dB SPL. The observations that follow are described in terms of Fig. 2 (the data from both subjects were very similar).

The normal AEP seen before muscimol application is shown in Fig. 2A. The peaks of the waveform are labeled sequentially according to polarity. The first four, middle latency, peaks of

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**FIG. 2.** Auditory evoked potentials (AEP) relative to the time of muscimol application. Data were from the right cortex in one subject (m, minutes; h, hours). The behavioral observations that accompanied each recording are inscribed above the respective AEPs. Eight-kilohertz tones were either detected (Det ✔) or not detected (Det ☐). When detection occurred, discrimination performance (dsc) was described as 100% (normal performance at both fine and coarse frequency differences), 50% (fine frequency discrimination affected), or 0% (no frequency discrimination ability).

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**FIG. 3.** The AEP and accompanying behavior relative to the time of muscimol application in a 2nd subject. The presentation of the data is identical to that in Fig. 2.
the waveform (P1 to N2) are similar to the AI surface-evoked response, which has previously been described for the awake rat by Hall and Borbely (1970). In our study, a long-latency P3 component (a 5th peak) was also seen. Note that, in comparison to the anesthetized rat, the AEP in the awake behaving rat has a well-developed N2 wave (it is largely missing in the anesthetized preparation; compare Fig. 2A and Fig. 4A). The mean latencies of the AEP peaks in our behaving subjects, were (in ms) as follows: P1 = 13; N1 = 20; P2 = 36; N2 = 50; P3 = 150.

Figure 2B is the AEP recorded 1 min after muscimol application. The figure catches both the N1 and P2 peaks of the waveform in the process of vanishing. Invariably, the N1 and P2 components vanished completely within 2–3 min, and the normal AEP changed into a single, large-amplitude positive-negative waveform. Figure 2C shows the new AEP waveform, recorded in test sessions 10–15 min after muscimol application. The waveform is labeled as P14 N50, indicating the polarity and approximate latency of its main peaks. The algebraic difference between the normal premuscimol AEP and the P14 N50 wave is also shown in Fig. 2C, superimposed as a dashed wave. This “difference” waveform is labeled as N20 P50, and it represents the AI activity that has vanished. The distinct shapes, both time course and amplitude, of the P14 N50 and the N20 P50 waveforms indicate that mainly different underlying processes generate them. Note that during these AEP changes, there was little corresponding effect on basic auditory performance (the ability to make difficult discriminations was affected). The P3 component of the AEP, or equivalently the P150, was still evident during these changes, although it was somewhat reduced.

After ~15 min post-muscimol the energy in the newly emerged P14 N50 wave (energy was measured as the absolute area under the waveform) began to decrease, with the amplitudes of its peaks P14, N50, and P150, diminishing together. The P150 vanished first, in <30 min. Within 2 h the P14 N50 wave had reached its minimum, and the AEP was reduced to a small P14 peak in an otherwise nearly flat record, shown in Fig. 1D. The rat’s corresponding auditory behavior was a complete loss of tone detection even at the maximum, 90 dB SPL, tone intensity. A P14N50 energy reduction of more than 72% of its value recorded at 15 min post-muscimol application was invariably accompanied by the loss of detection. The rat’s returning ability to detect sound, but not as yet to discriminate frequencies, was heralded by an increase in P14 wave amplitude and the appearance of the N50 wave (Fig. 2E). The slower, more gradual, improvement in discrimination ability appeared to be linked to further increases in the energy of the P14 N50 wave (Fig. 2F).

The transition of the P14 N50 waveform back to the normal AEP involved the gradual reappearance first of P150 and then of the N20 and P50 components (Fig. 2G). These components, in due course, completely overlaid the P14 N50 wave to give the normal surface AEP of the rat (Fig. 2H). Two additional observations on the P14 N50 wave may be worth noting here. 1) At any stage of its existence, the energy in N50 relative to the rest of the AEP appeared to be the best predictor of frequency discrimination ability. For example, note that the discrimination performance accompanying the AEP of Fig. 2C is better than that accompanying the AEP of Fig. 2F. While the energies of the P14 component in both figures are roughly the same, it is the extra energy in the N50 component of Fig. 2C that is better correlated with frequency discrimination performance. 2) The relationship of auditory performance to total P14 N50 energy seemed to take the functional form of a hysteresis: during P14 N50 decay, deficits in auditory performance generally appeared at much lower energy levels than the levels associated with their recovery.

It proved hard to make more quantitative observations between the AEP and performance, given that continuous behavioral testing over long time periods is difficult and that behavioral measurements, inherently, have insufficient resolution.

**AEP in relation to MUA**

As noted in METHODS, separate acute experiments (n = 4) focused on understanding the above AEP findings in relation to neuronal activity within AI. A single microelectrode was lowered into the cortex while recording the AEP to click stimuli both from the surface and, locally, from within AI. Both local AEP and MUA in the cortical depth were recorded.

The normal AEP of the anesthetized rat has been exhaustively studied by Barth and colleagues (e.g., Barth and Di 1990; Sukov and Barth 1998). Here we found that the polarity

![Figure 4](image-url)
and timing of the normal AEP waveform changed with increasing depth in a manner consistent with what these authors have reported. The P1 peak began to invert in polarity within 0.4 mm, while N1 began to invert at depths of ~1 mm. The AEP recorded from the white matter immediately beneath AI, at 2 mm, was a mirror image of the surface AEP. These observations indicate that all components of the normal surface AEP waveform have their origins within AI.

Electrodes were positioned in AI, at cortical depths between 0.8 and 1 mm, and auditory clicks delivered continuously (1 per s). After 100–120 clicks, muscimol was applied to the cortex. (Muscimol dose varied from 5 to 40 μg in individual experiments.) Results are summarized in Fig. 4 (A–C). Figure 4A shows the normal AEP recorded simultaneously at the surface and in the depth, along with the peristimulus time histogram (PSTH) of local neuronal activity (AEP and PSTH averaged over 100 stimuli). Figure 4B depicts identical recordings made 10 min after muscimol was applied to the dura. At the time Fig. 4B was captured the N1 and P2 peaks of the normal surface AEP had long vanished, and the unmasked P14N50 wave was in the process of diminishing. These changes are similar to those seen in the chronic behaving subject, as detailed above.

As Fig. 4B shows, the surface and depth P14N50 (and including P150) are near mirror images indicating that the P14N50 is generated from within the AI cortex, within cortical tissue <1 mm deep. Although the P150 wave is clearly seen in this example, it was, more often than not, less well defined in the anesthetized rats as compared with the awake ones. The PSTH accompanying the AEP shows that MU discharge pattern in the depth accurately reflects the shape of the P14N50 wave both in the latencies and amplitudes of its peaks. Figure 4C plots P14 amplitude and accompanying maximum MUA at different time delays during the decay of the P14N50 wave. As can be seen, peak MUA is strongly correlated with P14 amplitude (P < 0.0001 in all recordings, by calculation of correlation coefficients). This strong correlation was independent of the rate of decay of P14. Thus the amplitude of P14 is a very accurate indicator of the initial short-latency response of AI cells to appropriate stimuli.

In general our findings clearly indicated that the rate of muscimol-induced decay of the P14N50 is much faster in the anesthetized animal than in the awake. (In 2 additional experiments, recording from the same subjects in the anesthetized versus awake condition, we directly confirmed this comparison.) This observation, however, is unlikely to be physiologically significant. In the general experimental context the rate of P14N50 decay as muscimol diffused into the cortex can be assumed to depend on certain variables: 1) the initial dose of topically applied muscimol; 2) the state of the dura (thickening of the dura in the chronic animal would slow diffusion time); and 3) the initial baseline MU response (the much higher firing rates, and sensitivities, of neurons in the awake animal implies that the P14N50 waveform would take longer to die out).

**DISCUSSION**

In this study we applied muscimol, a GABA-A agonist and strong neuronal inhibitor, to the surface of the auditory cortex of rats while they were being tested in a simple auditory task that required them to detect tones and discriminate frequencies. At the same time, we monitored activity within the auditory cortex by recording auditory evoked potentials. In separate experiments, we related the AEP to multunit spike activity (MUA) with the auditory cortex during muscimol application. Within the auditory cortex, itself, we focused on experimentally inactivating the primary auditory cortex, or AI field, to the exclusion of surrounding areas. As noted in METHODS, the nature of the experiment makes it difficult to completely rule out accompanying small inactivations of the secondary auditory areas immediately surrounding AI; however, these are expected to be insignificant.

Our experiments yielded two main results, related in the context of the overall experiment: 1) the profound and reversible effect of muscimol-induced inactivation of auditory cortex on the rats auditory performance—both tone detection and frequency discrimination; 2) the curious transformation of the normal AEP when muscimol is topically applied to the auditory cortex. The behavioral and electrophysiological findings, taken together, show that sudden and large auditory inactivation is sufficient to cause a complete loss in a rat’s ability to detect tones. Partial deactivation, on the other hand, causes varying deficits in the ability to discriminate frequencies. Here, soon (>30 min) after muscimol application, rats were unable to detect tones as loud as 90 dB SPL at 8 kHz, more than 70 dB greater than the rat’s normal threshold (Fay 1988; Kelly and Masterton 1977). Normal detection ability only returned with increasing activity within the auditory cortex (>5–8 h). Normal frequency discrimination ability, on the other hand, took much longer to recover (>10 h), with the ability to make coarse discrimination returning hours earlier than the ability to make fine discriminations.

What the rats actually perceived while exhibiting diminished auditory performance, of course, is difficult to know. For example, it is possible that altered perception and/or cognition, may have contributed to diminished performance. Note, however, that our rats were set a very simple behavioral task. In addition, under conditions of auditory deactivation, when either tone detection or frequency discrimination was affected, behavioral control along the relevant stimulus dimension was often exercised by stronger stimuli: higher intensities and larger frequency differences. This conjunction suggests that the cortex may be directly concerned with auditory sensation rather than with, or in addition to, the cognitive components of the present task. For the same reason, altered sound perception is unlikely to have significantly contributed to decreased performance. The overall behavioral deficits we observed therefore probably represent an acute and total deafness soon after muscimol application and, later, during partial recovery, a true increase in frequency discrimination thresholds. In any event, whatever the exact nature of the behavioral deficits, our findings show that the rat cortex is intimately involved in the perception of sound intensity and frequency, the two essential parameters of sound.

It seems likely that the findings of this study will also hold for other mammals. The literature supports a loose relationship between a species’ auditory performance (especially frequency discrimination) and the degree of its immunity to cortical lesions (Buser and Imbert 1992; Clarey et al. 1992; Heffner and Heffner 1990; Pickles 1982). The rat’s auditory abilities, for example, are among the least acute in mammals (Fay 1988; Talwar and Gerstein 1998); its auditory abilities, correspond-
A clear distinction should be made between cortical necessity of the auditory cortices of many mammalian species (see view seems to have been influenced by the lack of clear tasks that involve elementary sound attributes like intensity and concerned with the abstract and cognitive aspects of behavioral traditional textbook notion that the neocortex may only be acoustic computation.

1991), a study that first showed that the mammalian neocortex recently been shown to mediate fine frequency discriminations of a type essential to its natural behavior (Riquimaroux et al. 1991), a study that first showed that the mammalian neocortex may in fact be concerned with some types of elementary acoustic computation.

The findings of this study stand in some contrast to the traditional textbook notion that the neocortex may only be concerned with the abstract and cognitive aspects of behavioral tasks that involve elementary sound attributes like intensity and frequency (e.g., Buser and Imbert 1992; Pickles 1982). This view seems to have been influenced by the lack of clear behavioral deficits in simple auditory tasks following ablations of the auditory cortices of many mammalian species (see introduction). Such lesion experiments typically examine behavior some time after recovery from surgery. When chronic deficits have been observed after cortical ablation, they have often been linked with task complexity, differences in species, the biological relevance of stimuli, and sometimes, nonspecific consequences of ablation surgery. These variables may have clouded the issue. The fact that the cortex is not necessary for elementary sound perception may only be true for the long term; in the short term, by our results, the cortex appears to be both involved in and necessary for any sound perception. Thus a clear distinction should be made between cortical necessity and its involvement for normal auditory behaviors.

The involvement of the cortex in normal hearing thus far may have been obscured by the ability of the mammalian auditory system to reorganize itself after lesioning. The contrasting affect on the rat’s auditory performance seen here after acute cortical inactivation and that recorded by Kelly (1970) after cortical ablation suggests that the auditory system has evolved a mechanism that ensures the recovery of sound intensity and frequency based behaviors after cortical injury. The time course of underlying reorganization and consequent behavioral recovery of simple auditory tasks after cortical lesions may well vary among species. It may be rapid in species like the rat, measurable in days and making it difficult to observe, and much longer in animals like the macaque monkey, in whom it may be relatively easy to catalog (Heffner and Heffner 1986).

The locations of auditory sites that may underlie the above recovery process after cortical injury seem at present difficult to pinpoint. Putative sites include tonotopic and nontonotopic auditory regions, both cortical and subcortical. Two natural candidate sites would seem to be the secondary auditory regions of the cortex and the medial geniculate body (MGB) of the thalamus, the ventral division of which provides the main thalamic projections to AI (Roger and Arnault 1989). However, lesion experiments have often ablated extensive areas of the neocortex, and neurons in the MGB would be expected to undergo retrograde regeneration following cortical ablation (Lashley 1941). In the rat, for example, Kelly widely ablated cortical regions including all known auditory areas prior to testing auditory performance; simultaneously, Kelly also reported widespread degeneration within the main divisions of the MGB. The foregoing observations imply that auditory regions that offer substitute computations after cortical ablation might be found in the MGB outside of its main divisions or in sub-thalamic auditory nuclei like the inferior colliculus (IC).

Given that subcortical auditory nuclei appear to possess, or perhaps evolve, the ability to subserve basic auditory behaviors in the absence of the cortex, a possible mechanism for the acute loss of hearing ability after suppression of cortical activity by muscimol might involve simultaneous associated effects on lower auditory nuclei through descending feedback pathways from the cortex. In the rat, anatomical evidence for descending pathways from the cortex to lower levels of the auditory system is plentiful (e.g., Faye-Lund 1985; Herbert et al. 1991; Weedman and Ryugo 1996; Winer et al. 1999). These cortico-fugal pathways have been shown to mediate positive feedback and to strongly modulate activity in both MGB and IC in the rat (Zhang and Suga 1997). Initial studies in our laboratory suggest that a similar auditory feedback system operates in the rat. Acute suppression of AI activity would then likely have a considerable acute effect on the function of the lower nuclei. If this is so, we could infer that the cortex is normally involved in basic acoustic processing at least to the extent that it selectively gates and modulates subcortical sensory signals for its own computational needs. Nevertheless, other experimental approaches are needed to help clarify the significance of some of the foregoing speculations. One such approach may involve maintaining a continuous muscimol inactivation of the cortex while simultaneously monitoring behavior for signs of recovery.

The electrophysiological approach we employed in this study (simultaneous monitoring of the AEP) may help point to key elements of cortical sound processing. The AEP can be understood as a measure of summed postsynaptic potentials generated within AI (Mitzdorf 1985). The sequential AEPs that we constructed over the course of an experiment reflect the gradual diffusion of muscimol through dura to successively deeper AI layers. We found that the normal AEP is completely transformed as a result of surface muscimol application. A set of AEP components vanishes almost instantaneously to unmask an underlying waveform, the P14N50 wave. This waveform, whose size parallels the severity of behavioral deficits, gradually diminishes with a time course of hours. Assuming linear summation, the AEP components that vanish instantaneously were identified as forming the N20P50 wave. The foregoing observations indicate that the normal surface AEP can be considered to be the sum of at least two distinct AI processes. The first process underlies the P14N50 wave (and P150 as well); this wave contributes to the P1, N2, and P3 components of the normal AEP of the awake rat. The second process can be assumed to underlie the N20P50 wave, which contributes mainly to the N1 and P2 components of the normal surface AEP.
The $N_2P_{50}$ wave presumably disappears as a result of muscimol action in the most superficial cortical layers. Its disappearance indicates a break in the normal sequence of cortical processing of acoustic stimuli. Surprisingly, this does not appear to have major consequences for simple auditory behaviors. The neuronal events associated with the $N_2P_{50}$ wave, or the N1 and P2 components of the normal AEP may only be involved in fine frequency discrimination. By contrast, the AI processes underlying the $P_{14}N_{50}$ wave or, equivalently, those underlying the P1 and N2 peaks of the normal AEP, are likely to be essential for elementary sound perception. A minimum amount of energy in $P_{14}N_{50}$ appears to be critical for the detection of sound; above this critical amount the total energy in $P_{14}N_{50}$ provides a rough guide to frequency discrimination ability. More specifically, the noticeable presence of the $N_{50}$ component in the AEP appears to be a consistent predictor of near normal detection and discrimination behavior. As a measure of postsynaptic activity, the $P_{14}N_{50}$ waveform is sufficient to explain the classical AI neuronal response to best frequency tones (Sally and Kelly 1988): a strong short-latency response ($P_{14}$), with a subsequent period of inhibition/suppression ($N_{50}$), and often followed by an afterdischarge ($P_{150}$). The amplitude of $P_{14}$ therefore is a strong predictor of tone-detection ability in the rat and an accurate index of the short-latency AI response to sound, summed over the AI active cell population.

The finding that surface muscimol application cleaves the AEP into two cleanly identifiable waveforms, for the first time, provides direct physiological confirmation of theoretical conclusions drawn from several current source density studies (e.g., Barth and Di 1990; Mitzdorf 1985; Sukov and Barth 1998). These studies suggest that different cell assemblies, each of which generates a specific AEP waveform, underlie the normal AEP. The peculiar effects of muscimol on the surface AEP, when studied in conjunction with systematic depth recordings of both local AEPs and local MUA within the cortical depth, may help to provide new insights into the microcircuitry of the cortex.

We thank Dr. Mike Kisley for invaluable assistance during this study. This work was supported by National Institutes of Health Grants DC-01249 and MH-46428.

REFERENCES


