male was silenced or the subordinate males were deafened, the results were very similar.

A dominant male that was audible to his subordinates devoted less time to fighting and more to courting and, consequently, had a higher rate of copulation. Our study, as well as more detailed studies of female behavior (*5*), indicates that male chirping does not affect female behavior directly. We conclude that acoustic signals influence male mating success because inaudible males are interrupted by other males more often during courtship than are audible males. The structural similarity in chirps produced during courtship and during aggression may reflect a convergence of function: no matter what the context, male chirps signal an aggressive warning to other males.

The chirps of many species of crickets have a different structure for each of several contexts (*13*). Most species of field crickets are solitary, and the males produce loud species-specific "calling" songs that attract females from a distance and possibly serve a role in the territorial spacing of neighboring males (*3*). The less intense "courtship" chirps of solitary males are audible to females in the immediate vicinity, but may not be detectable by other males (*5*); female field crickets discriminate against males that do not chirp during courtship (*4*). "Aggressive" chirps usually have yet another structure (*13*). A gregarious cricket such as *Amphiacusta maya* need not produce a calling song but is very likely to be interrupted during courtship. Thus a male in a gregarious species, at risk of constant interruptions and fights, produces "war propaganda" whenever he chirps.

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6. Another gregarious Phalangopsine cricket, *Phaeophilacris spectrum*, communicates with infrasound rather than with audible signals. The same signal is produced during courtship and aggression, but a second signal is added in high-intensity aggressive interactions [M. von Dambach and L. Lichtenstein, *Z. Tierpsychol.* **46**, 14 (1978)].
7. Three marked males were housed in each cage. One female was put in immediately before observations began. Most aggressive interactions occurred in the vicinity of the female, when a male was interrupted during courtship. The interactions varied in intensity from one male antennating the other and displacing him, to fights in which both males stood on their hind legs, hit each other with their front legs, tried to bite, and chirped continuously. All aggressive interactions [described in (*5*)] and all attempts to copulate were recorded for 0.5 hour. If the female had mated during this period, she was removed; otherwise, she was left in the cage for another 0.5 hour. The ranks of males were assigned on the basis of the number of aggressive interactions that each male won during each observation session. A cage was observed only once per day.
8. Fifty cages each containing three males were observed for one to four sessions each (*7*). If mating in this species occurs by chance alone, then each male should have achieved approximately 0.33 of the copulations in his cage. To test this hypothesis, one day on which just one copulation occurred was chosen at random for each cage, and the number of times that the dominant or a subordinate male copulated was compared with an expected value of the dominant male copulating in 0.33 of the cases and the subordinates in 0.67. Dominant males copulated in 39 cases and subordinates in 11 ($\chi^2 = 45$, d.f. = 1, $P << .001$).
9. Males were silenced by first anesthetizing them with CO_2 or N_2 or restraining them, then waxing the stridulatory file on the underside of the right wing. This allowed them to move their wings normally but they were unable to produce sound. Control males were anesthetized or restrained similarly and in the second experiment a drop of wax was put on their pronota.
10. Seven of 40 copulations in 57 observations were made by silent males.
11. Fisher test, not significant, 14 experimental and 9 control cages.
12. Subordinate males were deafened by tearing the tympana on their prothoracic legs. The dominant males were handled in similar fashion except that the analogous sites on their metathoracic legs were scratched rather than their prothoracic tympana. All of the males walked normally after this operation.
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Intracellular Recordings from Cochlear Outer Hair Cells

Abstract. *Intracellular recordings were made from outer hair cells in the third turn of the guinea pig cochlea, and the electrical characteristics of the cells were compared to those of inner hair cells, supporting cells, and extracellular spaces from the same recording region. Outer hair cells have higher membrane potentials than do inner hair cells, but they produce smaller a-c receptor potentials. The frequency response characteristics of both types of hair cells are probably not significantly different. In the frequency region where tuning is optimal, both cell types produce depolarizing d-c receptor potentials, but outer hair cells also generate hyperpolarizing responses at low frequencies.*

The most advanced auditory organs have two morphologically distinct sensory receptors. Electrical characteristics of individual mammalian receptors have been described only for one type, the inner hair cell (*1*). We now describe results of a 4-year study of cochlear outer hair cells (*2*). Information from single outer hair cells may explain some central questions of cochlear physiology, such as the role of this receptor cell in hearing and the types of interaction, if any, that occur between outer and inner hair cells.

Anesthetized guinea pigs were maintained at a constant core temperature and their heart rates were monitored; in later experiments, exhaled CO_2 was also measured. To assess the normalcy of the ear, a wire electrode was placed in the scala tympani of the first cochlear turn, and tone-burst–generated compound action potentials were recorded. In preparing for the recording of hair cell potentials a fenestra (~ 0.3 by 0.5 mm) was made in the bone over the stria vascularis in the third turn of the cochlea. Microelectrodes were introduced through the stria and aimed at the organ of Corti. The cochlea was back-lighted with a fiber optics illuminator, so that the shadow of the organ of Corti could be seen through the fenestra. Attempts were made to insert the electrodes so that they would travel parallel with the reticular lamina (*3*) (Fig. 1A). Electrodes were driven by a motorized microdrive in increments of multiples of 2 μm (*4*). Only responses obtained with tone-burst stimuli are presented.

The continuous recording of electrode position and a characteristic sequence of d-c potential changes (Fig. 1B) help identify the location of the electrode tip within the organ of Corti. Cell types may be identified by a combination of recording depth, membrane potential, and response magnitude. In all supporting cell types the membrane potential is high (steady-state values range up to −100 mV) and electrical responses are smaller, at any frequency, than those mea-

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sured in scala media or in the extracellular spaces in the organ of Corti. Inner hair cells (N = 19 stable recordings) are characterized by low steady-state membrane potentials (median, −20 mV; highest, −47 mV) and large receptor potentials (up to 25 mV, peak to peak). At the best frequency of the cell, these responses can be up to a hundred times larger than the corresponding extracellular response. These findings are in harmony with the observations of Russell and Sellick on first-turn inner hair cells (*1*). Outer hair cells (N = 22) can be recognized from their large steady-state membrane potentials (median, −71 mV; highest, −94 mV) and large receptor potentials (up to 15 mV, peak to peak) (*5*). The cell's a-c response is between 3 and 15 times greater than the extracellular potential. Response patterns at various electrode locations are shown in Fig. 1C.

We compared observations made among a-c response magnitudes recorded at various locations in the same organ of Corti (Fig. 2A). All functions, except the scala media plot (*6*), depict bandpass filter characteristics. Around the best frequency there is a relatively sharply tuned tip segment with a high frequency slope of more than 40 dB per octave. The low frequency slope flattens out in a tail section which begins about 10 dB below the maximum at the tip. Direct comparison between the inner and outer hair cells in the same cochlea show that the tuning characteristics are comparable, even though the tip-to-tail ratio tends to be smaller in outer hair cells. This ratio, however, is highly variable among animals, ranging from 0 to 20 dB. Higher ratios probably correspond to better preparations.

When a-c and d-c receptor potentials recorded from an outer hair cell are compared, several striking observations can be made. First, outer hair cells produce both depolarizing and hyperpolarizing d-c responses, whereas the d-c output of inner hair cells is always positive (*1*). Second, the d-c response of outer hair cells appears to be much more sharply tuned than the a-c component. Third, around the best frequency of the cell, the a-c and d-c response magnitudes are commensurate. The behavior of the d-c receptor potential is similar to the patterns described for the gross summating potential (*7*), which also undergoes a polarity reversal along the frequency axis. In addition, we have shown that the summating potential is much better tuned than the gross cochlear microphonic (*8*), further suggesting a similarity between intracellular responses of outer hair cells and the gross cochlear potentials. Both a-c and d-c intracellular responses are largest at the same frequency when measured at low sound levels. With an increase in the magnitude of the stimulus both types of responses saturate, and the a-c response peak shifts to lower frequencies. Thus, for example, at 70 dB (Fig. 2B) the a-c maximum occurs at 600 Hz even though at low sound levels it is at 800 Hz, the same frequency at which the d-c maximum is maintained over the whole range of intensities.

Our results reveal some potentially significant similarities, as well as differences, between the electrical characteristics of inner and outer hair cells. The a-c potential produced by either the inner or outer hair cells peaks at the same frequency which is determined by the cell's location along the cochlear spiral. In other words, there does not appear to be a systematic tuning disparity between the two types of receptors located at the same place. The sharpness of tuning

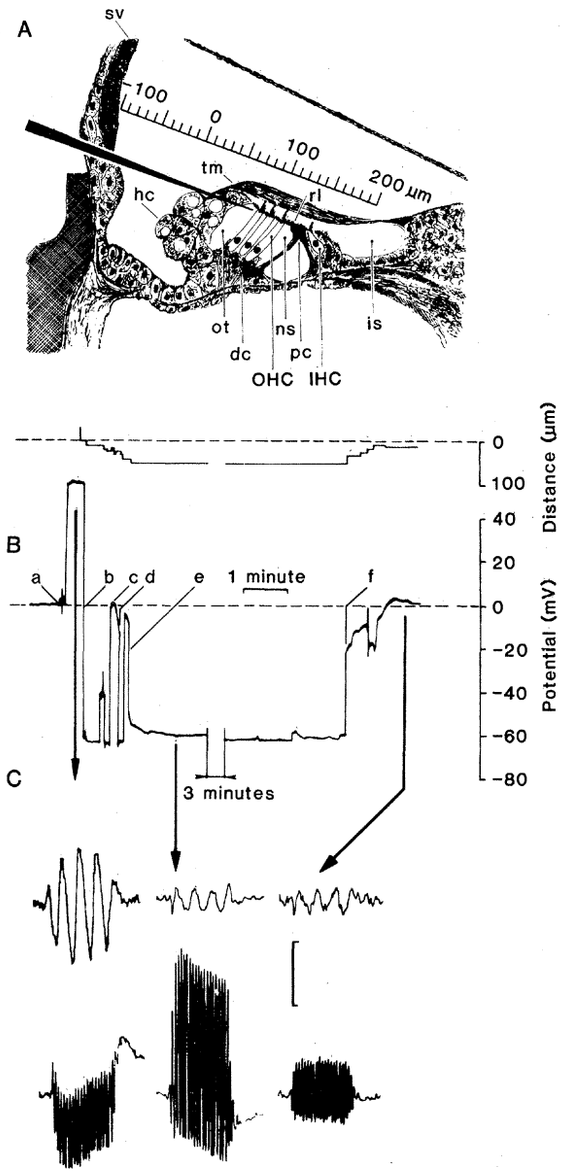

Fig. 1. (A) Cross-section of the third turn of a guinea pig cochlea. The recording electrode was inserted through the stria vascularis by a fenestra in the cochlear bone. The optimal electrode path is parallel to the reticular lamina and situated just below it. *OHC*, outer hair cell; *IHC*, inner hair cell; *sv*, stria vascularis; *hc*, Hensen's cell; *tm*, tectorial membrane; *rl*, reticular lamina; *ot*, outer tunnel; *dc*, Deiters' cell; *ns*, Nuel space; *pc*, pilar cell; and *is*, inner sulcus. (B) The d-c potentials measured during a typical electrode track through the cochlear scala. The appearance of a potential can be correlated with the location of the electrode tip with the aid of the distance scale provided. Electrode tip potentials are balanced to zero on the outer surface of the tissue lining the cochlear duct. At point *a* the electrode penetrates the stria vascularis and enters the endolymphatic space. This is signaled by the positive endocochlear potential (EP = 57 mV). After encountering the EP, the electrode travels within the endolymph from 80 to 180 µm, depending on its position and orientation, before reaching the organ of Corti. Hensen's cells are contacted first, and their penetration is clearly marked by a d-c shift from positive to negative of up to 180 mV (point *b*). A further advance of the electrode traverses the Hensen's cell layer and locates the tip within the outer tunnel of Corti (point *c*). The d-c potential in the outer tunnel or in any fluid space (*12*) within the spiral organ is zero or slightly negative (less than −10 mV). At electrode location *d* a cell is registered; it is, however, quickly lost. This is probably a third-row outer hair cell. At a depth of 50 µm another cell is penetrated (point *e*) and held with a stable membrane potential. (The range of holding times for outer hair cells is from 1.2 to 33 minutes; the many cells that were lost before a minute are not considered.) This is another outer hair cell. At marker *f* contact is lost with the hair cell in which the electrode dwelled for 7.5 minutes. A 3-minute segment of recording is omitted. (C) Averaged responses (N = 32) to tone bursts from various electrode locations. Two stimulus frequencies were used (100 and 600 Hz), and all presentations are at the same sound level (70 dB with respect to 20 µPa). The vertical bar represents 1 mV, except for the two, top right-hand traces, for which it is 0.2 mV. The recording electronics is a-c coupled with a low frequency cutoff of 1 Hz. Recording electrodes typically possess a bandwidth extending to 1700 Hz (upon optimal capacitance compensation).

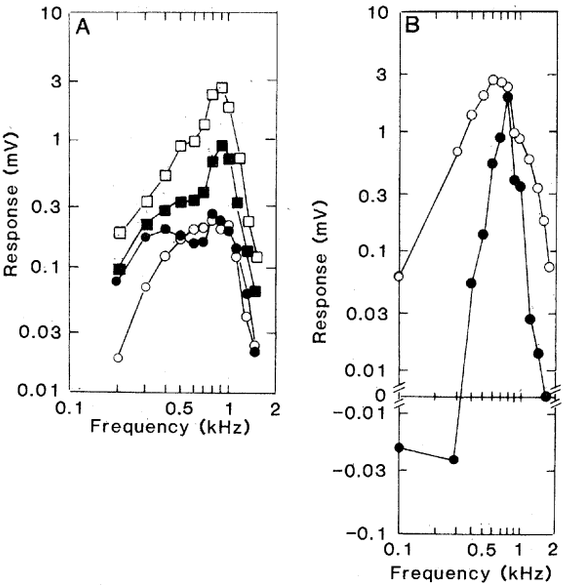

Fig. 2. (A) Peak-to-peak a-c response magnitude as a function of stimulus frequency, recorded from various locations within the same organ of Corti. Sound pressure is constant at 40 dB (with respect to 20 μPa); at this level the responses are linear. Inner hair cells (□), outer hair cells (■), scala media (●), and organ of Corti fluid space (○). (B) Comparison of the frequency dependence of the a-c and d-c receptor potential components from one outer hair cell. Data are obtained from averaged responses with tone-burst stimuli having a constant sound level of 70 dB (with respect to 20 μPa); a-c responses are peak-to-peak magnitude, and d-c responses are measured between prestimulus baseline and half the excursion of the a-c waveform; a-c (○) and d-c (●).

appears to be similar in both types of cells. Although some evidence suggests that inner hair cells can be better tuned, tuning appears to vary more from animal to animal than from cell to cell in a particular animal. This variation most likely reflects the nonuniformity of physiological conditions among our subjects. We have compared equal intensity response curves with single auditory nerve fiber rate functions obtained at the same sound level from fibers (recorded from chinchillas in our laboratory) for which the best frequency matched those of our hair cells. The range of tuning for these single fibers encompassed our sample, but our best tuned hair cell is not as sharply tuned as the best nerve fiber; it is much better tuned, however, than the poorest one. Since single fiber tuning itself is relatively shallow in the frequency range of interest (600 to 1000 Hz), quantitative comparisons between fiber and hair cell tuning are not particularly revealing. Our data therefore support the suggestion that the sharpness of tuning seen in single fiber discharge patterns is already established at the hair cell level, and the degree of sharpness is probably determined by the mechanical tuning of the basilar membrane-organ of Corti complex (*9*).

In agreement with work (*1*) on inner hair cells, we find that the magnitude of a-c and d-c receptor potentials is remarkably large. The responses of inner hair cells appear to be about three times greater than the ones recorded from outer hair cells. There also does not appear to be a systematic difference in sensitivity between the two types of hair cells. Thus schemes that assign different operating regions of sound intensity for outer and inner hair cells are not supported by these data. At their best frequency the operation of both inner and outer hair cells becomes markedly nonlinear above approximately 50 dB. The nonlinearity is manifested by saturation and production of harmonics and of a d-c component. Although it has been suggested that outer and inner hair cells operate in phase opposition (*10*), we find no evidence to support such a contention. At the best frequency the a-c receptor potentials produced by the two sensory cell types are approximately in phase, and, as was mentioned above, the d-c receptor potentials are depolarizing. Thus the intrinsic electrical behavior of the two hair cell types is similar around their best frequency.

The most striking difference between the electrical characteristics of outer and inner hair cells is in their resting membrane potentials: the inner hair cells appear to operate at about half the membrane potential of the outer hair cells, and thus resemble supporting cells in this respect. There is no readily evident explanation for this difference in resting potentials. Although the input resistance of our receptor cells is highly variable, the two cell types show overlapping distributions ranging between 10 and 34 megohms (*11*).

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3. Initially electrodes filled with aqueous solutions of Niagara sky blue, Procion yellow, or Lucifer yellow were used to mark the cells. Dye injection followed recording and was accomplished by passing a 1 to 5 nA negative current through the cell. After the experiments, the cochleas were perfused with 4 percent glutaraldehyde buffered to *p*H 7, removed from the animal, and stored in the perfusing solution. Subsequently the ears were dehydrated, embedded in Epon or Spurr, and sectioned in 10-μm increments. Dye-filled cells were identified under epifluorescent illumination, and 4 inner hair cells, 13 outer hair cells, 28 Deiters' cells, 41 pillar cells, 19 Hensen's cells, and a small assortment of other supporting cell types were recovered. Once the electrical characteristics of various cell types had been clarified, only 3*M* KCl-filled pipettes (tip, 0.1 μm in diameter with 120 to 200-megohm resistance) were used for recording.
4. There may be interference with cochlear partition movement when an electrode is introduced into the organ of Corti. It may be questioned if our approach (with an electrode track roughly parallel to the basilar membrane instead of perpendicular to it) compounds the problem. To study this issue we recorded gross receptor potentials from a third turn scala vestibuli electrode during various stages of microelectrode penetration. In most cases no changes were observed in the gross potential, either in its waveform or its spectral content.
5. All organ of Corti cells, including outer hair cells, had negative membrane potentials. This is in contrast to the report of Y. Tanaka, A. Asanuma, and K. Yanagisawa [*Hearing Res.* **2**, 431 (1980)].
6. The observation that in some cases (for example, see Fig. 1C at 100 Hz) the scala media potential can be larger than the intracellular outer hair cell response appears disturbing, since gross potentials are largely produced by outer hair cells [P. Dallos and M. A. Cheatham, *J. Acoust. Soc. Am.* **60**, 510 (1976)]. We have shown that the low frequency lobe of the scala media response function does not represent a locally produced electrical activity. Instead, these responses are conducted from more apical regions of the cochlea which are tuned to the low frequencies and produce much larger potentials. When the fourth turn of the cochlea is amputated, the large, low frequency response disappears, and the scala media function assumes the form of the organ of Corti gross response, without a change in its magnitude at the best frequency.
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11. Input impedance of cells was measured with a single-electrode bridge-balance technique.
12. In contrast to the fluid spaces within the organ of Corti proper, in the inner spiral sulcus and in the subtectorial space the resting potential is identical to the positive endocochlear potential.
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