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Vocalization-related stapedius muscle activity in different age chickens (*Gallus gallus*), and its role in vocal development

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The stapedius muscle activity associated with vocalization was analyzed in young and adult roosters. Our results show that remarkable differences in the behavior of vocalization-related stapedius muscle activity exist between these two ages. Unlike young roosters, electrical stimulation in the midbrain of adult cocks yields vocalizations associated with stapedius muscle EMG responses that always show a higher threshold and a longer latency than those of the vocalization induced. Moreover, the maximal amplitude of the stapedius muscle EMG response is consistently lower than that detected in young roosters, despite the fact that the maximal vocalization amplitude of the adult birds is much higher. On the whole our results demonstrate that vocalization-related stapedius muscle activity is strongly reduced in adulthood. The possibility that stapedius muscle may play a role during the vocal development was verified by comparing the crow of normal roosters with that of cocks from which the stapedius muscle had been removed shortly after hatching. Strong differences exist in the amplitude/frequency distribution of the crowing of normal and stapedectomized roosters, suggesting that the stapedius muscle exerts an important role in auditory feedback modulation, and that this feedback is used for normal vocal development.

INTRODUCTION

It is known that, unlike mammals, chickens (as other avian species) do not show an acoustic stapedial reflex^{1,4,16}, while the stapedius muscle is activated during the animal's own vocalization, causing a change of the acoustic impedance of the ear^{1–6}.

Stapedius activity associated with vocalization in chickens occurs in response to a central stimulus that reaches the mesencephalic stapedius controlling neurons and is produced by neurons involved in vocalization^{8,9}.

Despite some hypotheses, the physiological significance of vocalization-related stapedius muscle activity in chickens has not been fully clarified. It has been proposed that it may: (1) insure normal 'auditory vigilance' during vocalization; and (2) 'protect' the inner ear receptor cells against overstimulation by the bird's own voice^{1,4,5}.

Since the variables affecting vocal development in birds have not been fully described, a possible involvement of stapedius muscle activity in this development seems possible.

Comparative studies of different species show that the course of vocal development is inflexible in some birds and plastic in others^{11,12}. The domestic chicken is considered to belong to the former group, because the

basic pattern of its calls and crowing as they appear on audiospectrograms is not affected by early deafening¹⁰.

By contrast, it has been suggested that songbirds can develop a normal vocalization only by hearing themselves vocalize, using acoustic feedback control of voice^{11–15}.

Our personal unpublished observation that stapedius muscle size is reduced in adult roosters, compared with that of young ones, induced us to begin the present study aimed at verifying whether stapedius muscle, activated only by the cock's own voice, might play a role during vocal development. This work was carried out first by analyzing the vocalization-related stapedius muscle activity at different stages of development and then by comparing the crow (considered the most elaborate vocalization) of normal roosters with that of cocks from which the stapedius muscle had been removed shortly after hatching.

MATERIALS AND METHODS

Vocalization-related stapedius muscle activity in chickens of different age

The experiments were performed on 20 male Leghorn chickens (*Gallus gallus*) divided into two groups: one made up of 10 young (40–60 days old) roosters (group A) and the other of 10 adult (130–150 days old) ones (group B).

Group A roosters, weighed 1.5–1.8 kg and were characterized by juvenile secondary sexual characters (cockscomb and wattles) and

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by an immature vocalization. Group B roosters, weighing 3.0–3.5 kg showed mature secondary sexual characters and crowed.

In both young and adult cocks, a stapedius muscle electromyogram (EMG) was recorded under urethane anesthesia (1 g/kg b. wt., i.v.) during vocalization elicited by electrical stimulation of the mesencephalic 'calling area' according to Grassi et al.⁷⁻⁹.

Stapedius muscle response alone, elicited by stimulating the mesencephalic stapedius controlling area, was also analyzed. The sites stimulated were tested by increasing stimulus intensities (from 0 to 100 μA), square pulses 0.5 ms duration delivered at a rate of 100 pulses/s¹⁷.

In adult cocks a stapedius muscle electromyogram (EMG) was also recorded during vocalization elicited by electrical stimulation of the occipitomesencephalic tract rostral to the midbrain¹⁷. Vocalization was recorded using a microphone placed at a distance of about 7 cm from the cock's beak (thus delaying the response by about 0.2 ms) and stapedius muscle EMG response was recorded by means of a bipolar electrode consisting of two 0.1 mm copper wires, insulated except at the tip. Electrical stapedius muscle response (EMG, filtered between 100 and 10 000 Hz) and vocalization (300–3000 Hz) were recorded on a digital storage oscilloscope, transferred to a computer and stored on diskettes.

The amplitude of both EMG and vocalization responses was evaluated by measuring the area (integration) of the full-wave rectified EMG and of vocalization activity. The localization of the stimulating electrode in the midbrain, was subsequently verified after appropriate histological processing of the brain. For further technical details see Grassi et al.^{8,9}.

Stapedius muscle and vocalization development

In order to verify whether the stapedius muscle was involved in vocal development, 15 male Leghorn chickens were used, all born on the same day, on the same chicken farm. Five chicks underwent bilateral stapedius muscle removal at 10 days of age. Five chicks underwent a sham operation and the others served as controls. Experimental animals were anesthetized with ether by inhalation, and the stapedius muscle was exposed and completely detached from its origin along the temporal and occipital bones, and cut at the level of its entrance into the middle ear cavity. During this procedure the tympanic cavity was not at all opened or damaged. For sham operations, all surgical procedure, except the muscle removal, was performed. Both experimental and control chicks were handled equally, and maintained in identical social conditions for equal lengths of time.

Testing of vocal behavior was performed in operated and control cocks every 20 days from birth until the appearance of crowing at about 100–120 days of age. Crows were recorded until their pattern became stable. Sound recordings (at 9.5 cm/s speed) were low-pass filtered (5000 Hz) and digitalized at 10 000 Hz through an IBM-XT computer equipped with Computerscope ISC-16 System (R.C. Electronics, Inc.) at 0.1 ms sampling rate.

The frequency response of the tape recorder was ± 3 dB in the range 200–10 000 Hz. Each crow, lasting 1.8–2.2 s, was analyzed by a program (I.L.S., Signal Technology, Inc.) which computed the average power spectrum based on Fast Fourier Transform (FFT) of 256 points time series (25.6 ms). The amplitude value for each spectral peak was expressed as percentage of the total energy of that spectrum. The amplitudes of crow components of normal and operated roosters were statistically compared by using the Mann-Whitney test.

RESULTS

Vocalization-related stapedius muscle activity in young and adult roosters

As previously reported^{8,9} electrical stimulation of the mesencephalic 'calling area' of *Gallus gallus* consistently

elicited stapedius muscle activity, which appeared to be associated with vocalization both in young and adult roosters. In young roosters (group A), in full agreement with previous results⁹, stapedius muscle EMG response showed the same or a slightly higher threshold than that of vocalization, depending on the exact locus stimulated within the mesencephalic 'calling area' and its latency changed according to a direct or secondary activation of the mesencephalic stapedius controlling neurons⁹. The amplitude of both responses increased with the stimulus intensity until a maximal response was elicited (Fig. 1A).

In adult roosters (group B) stimulation of any point within the mesencephalic 'calling area', elicited stapedius muscle EMG at a threshold much higher than that of vocalization (Fig. 1B). In fact stapedius muscle EMG response appeared only at stimulus intensities evoking loud vocalizations and its latency was constantly longer than that of vocalization, independently of stimulus intensities. Moreover in group B roosters, the amplitude of vocalization response strongly increased with increasing stimulus strength, but the EMG response amplitude rose little and the maximal EMG response was always much lower than that observed in group A cocks. This effect is illustrated in the histogram in Fig. 2, showing a reduction of the vocalization-evoked stapedius muscle activity in adult roosters compared to young ones.

Stapedius muscle EMG response alone, elicited by stimulating the mesencephalic stapedius controlling area, was also analyzed in both groups. In group A roosters the threshold for stapedius muscle EMG response was lower ($12 \pm 1.3 \mu\text{A}$) than that detected in adult ones ($72 \pm 3.7 \mu\text{A}$). Also in this case, the maximal response recorded in adult roosters was always lower than that elicited in young ones (Fig. 3). This result was followed by the finding, in group B roosters, of a reduction in the size of the mesencephalic locus, from which stimulation elicited stapedius muscle response independent of vocalization (Fig. 4). This area, located extensively around the mesencephalic 'calling area' in young roosters⁹, was confined to a narrow mesencephalic locus in adult animals, where, on the contrary, an enlargement of the 'calling area' could be detected (Fig. 4).

Stimulation at a more anterior level (A 10.00, L 3.00, H 5.00)⁶, corresponding to tractus occipitomesencephalicus, was also performed in adult roosters to rule out the possibility that a higher stapedius muscle activity could be associated with the activation of supramesencephalic loci. In full agreement with previous results stimulation of these higher loci evoked vocalization responses like those evoked by stimulating the mesencephalic 'calling area'; these vocal responses were associated with a low stapedius muscle EMG response perfectly superimposable on

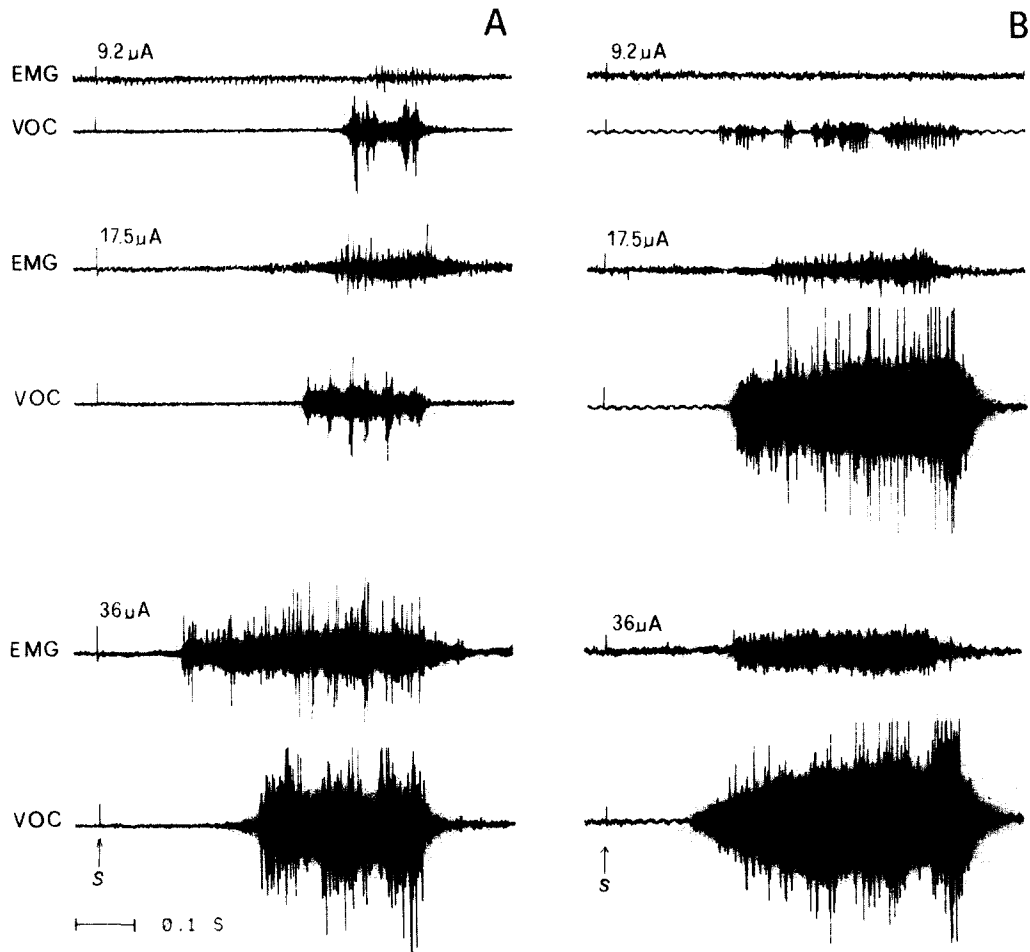


Fig. 1. Stapedius muscle EMG response (EMG) and vocalization (VOC) elicited by stimulating the low threshold mesencephalic vocalization loci in young (A) and adult (B) roosters. Stimulus intensities (μA) are indicated on the left of each recording and the beginning of the stimulation by S. Time calibration applies to all recordings.

that elicited by stimulating the mesencephalic 'calling area' (Figs. 5 and 6).

On the whole these results suggest that vocalization-related stapedius muscle activity is strongly reduced in adulthood.

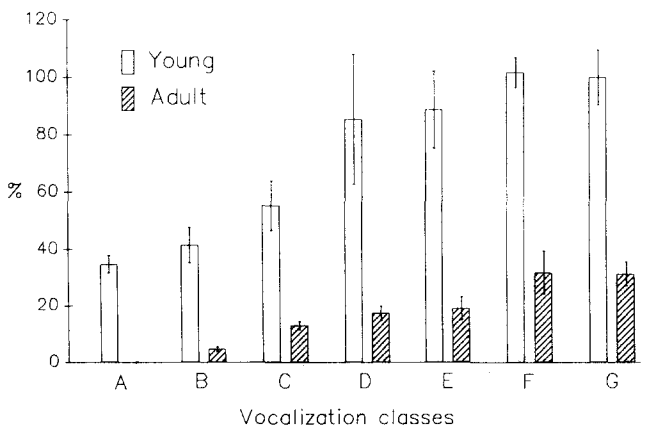


Fig. 2. EMG amplitude as a function of vocalization amplitude, expressed as classes of percentage of the maximal vocalization amplitude obtained in young and adult roosters (A, 0-10%; B, 10-20%; C, 20-30%; D, 30-40%; E, 40-50%; F, 50-60%; and G, 60-100%). The amplitude values are the area of full-wave rectified EMG and of vocalization activity. Mean of the recordings obtained in 10 fowl \pm S.E.M.

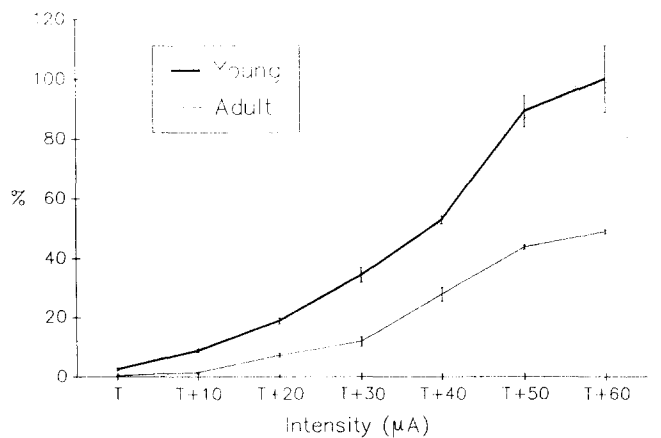


Fig. 3. Plot of the amplitude of EMG elicited without vocalization (percentage of the maximum, ordinate) as a function of stimulus intensity above threshold (T abscissa) in young and adult roosters. The EMG amplitude values are the area of full-wave rectified EMG activity. Each point is the mean of the responses obtained in 10 fowl \pm S.E.M.

Acoustic structure of calls in normal and stapedectomized adult roosters

Both operated, sham operated and normal roosters began to crow at about 100–120 days, after a progressive change of their vocalization, characterized by a shifting of vocal acoustic energy from higher frequencies (2000–4000 Hz), typical of the chick, to lower ones (400–2000 Hz), typical of the adult cocks.

The removal of the stapedius muscle did not affect the volume of crowing and its temporal pattern. In fact, the total energy value of each crow and the crow duration (1.8–2.2 s) were similar in both normal, sham operated and operated roosters. Furthermore, both roosters crowed with the same repetition rate during the day.

On the contrary, significant differences in the energy content of various crow components existed between normal and stapedectomized roosters. Although slight differences could be already detected in vocalization at about 60–80 days of life, they were particularly evident in the crowing, and progressively increased from the time the crow appeared until its pattern became stable (Fig. 7).

In fact, the power spectrum of crow showed the acoustic energy distributed in two main peaks, at 633 ± 31 Hz and 1300 ± 81 Hz in the normal and sham operated roosters, and at 700 ± 17 Hz and 1400 ± 56 Hz in the operated ones (Fig. 7). The differences in the peak frequencies between normal and operated roosters were not statistically significant ($P > 0.05$). However, the energy content of each peak was clearly different between operated and unoperated roosters.

The average energy content of the low frequency

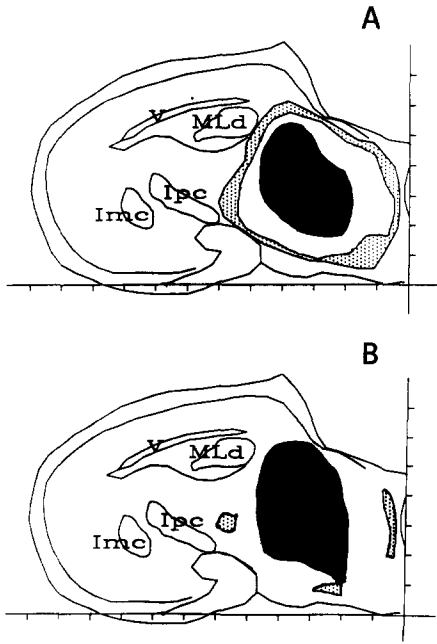


Fig. 4. Mesencephalic areas from which vocalization response (black area) and stapedius muscle activity alone (dotted area) could be elicited in young (A) and adult (B) roosters are plotted on a schematic section of the chicken brain stereotaxic atlas⁷. On both axes, each division represents 1 mm. Imc, nucleus isthmi, pars magnocellularis; Ipc, nucleus isthmi, pars parvocellularis; MLd, nucleus mesencephalicus lateralis, pars dorsalis; V, ventriculus.

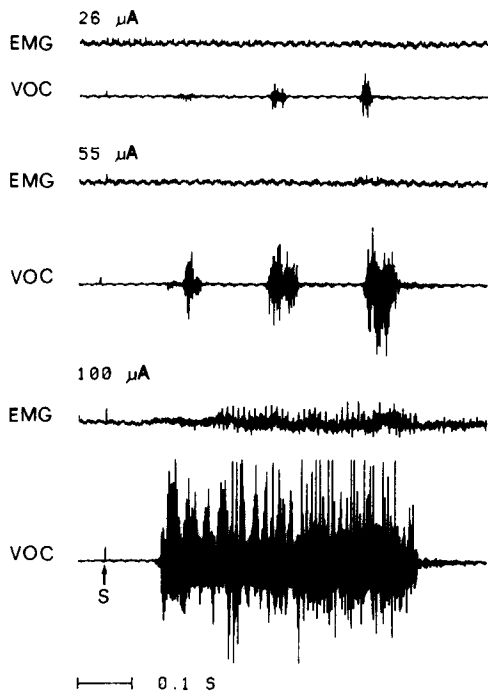


Fig. 5. Stapedius muscle EMG response (EMG) and vocalization (VOC) elicited in adult roosters by stimulating tractus occipitomesencephalicus at supramesencephalic level. Stimulus intensities (μA) are indicated on the left of each recording and the beginning of the stimulation by S. Time calibration applies to all recordings.

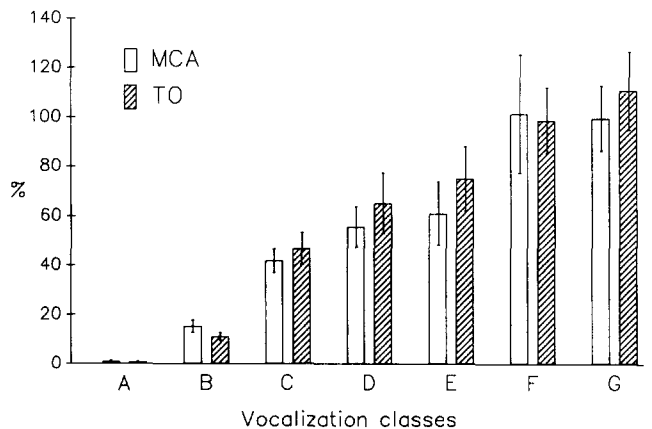


Fig. 6. EMG amplitude as a function of vocalization amplitude, expressed as classes of percentage of the maximal vocalization amplitude (A, 0–10%; B, 10–20%; C, 20–30%; D, 30–40%; E, 40–50%; F, 50–60%; and G, 60–100%), evoked in adult roosters by stimulating mesencephalic ‘calling area’ (MCA) and tractus occipitomesencephalicus (TO). The amplitude values are the area of full-wave rectified EMG and of vocalization activity. Mean of the recordings obtained in 10 fowl \pm S.E.M.

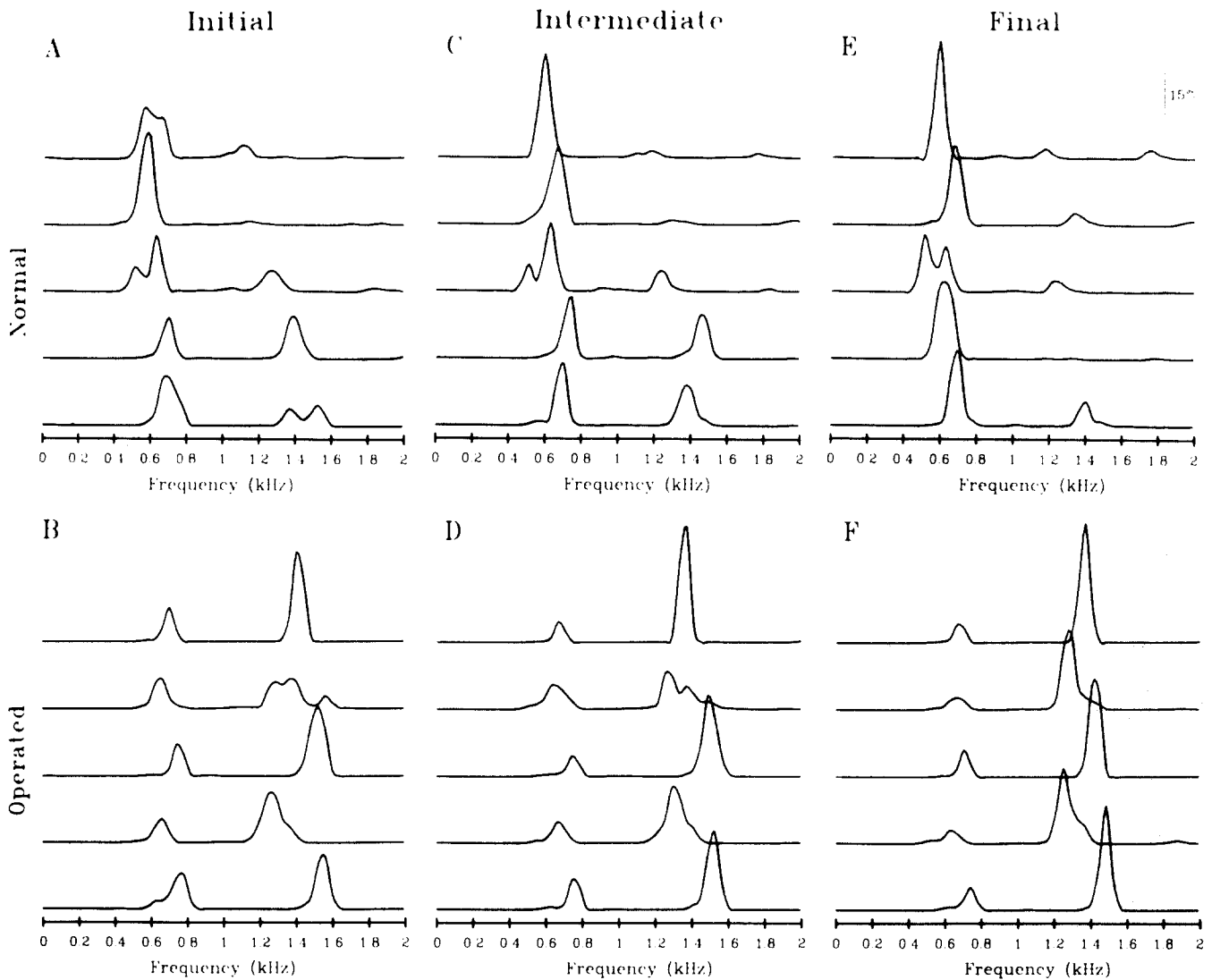


Fig. 7. Power spectra of crow of 5 normal (A, C, E) and 5 stapedectomized (B, D, F) roosters. In each rooster, the crow was analyzed at the time of its appearance (initial), some days later (intermediate) and when its pattern became stable (final).

component (633 Hz) in normal and sham operated roosters was $30.3 \pm 3.67\%$ of the total energy when the crowing became stable, while that of the higher frequency component (1300 Hz) was $4.35 \pm 1.4\%$. On the contrary, in operated roosters, when the crowing was stabilized, the low frequency component (700 Hz) showed an average energy content of $7.04 \pm 1.05\%$, whereas the average energy content of the higher frequency component (1400 Hz) was $35.02 \pm 3.1\%$.

The differences in the energy content of both crow components between normal and operated roosters were statistically significant (Mann-Whitney, $P < 0.05$). The finding that no consistent difference in the acoustic structure of the crow, existed between normal and sham operated roosters, ruled out completely the possibility that the changes observed in stapedectomized roosters were due to the surgical procedure.

DISCUSSION

Our results show remarkable differences in the stapedius muscle activity associated with electrically induced vocalization in young and adult roosters.

Unlike young roosters, the stapedius muscle EMG response associated with vocalization in adult cocks always shows a higher threshold and a longer latency than that of vocalization. Moreover, its maximal amplitude is constantly lower than that detected in young roosters, despite the fact that the amplitude of the vocalization response is much higher.

More evidence was obtained by stimulating the occipitomesencephalic tract in adult roosters, the major projection from the Archistriatum to mesencephalic 'calling area'¹⁷. A vocalization-related stapedius muscle EMG response showing the same characteristics as that

elicited by stimulating the mesencephalic 'calling area' was observed. This finding rules out the possibility that, because of the enlargement of the mesencephalic 'calling area' in adulthood, the low vocalization-related stapedius muscle activity was due to the inability of electrical stimulation to activate a sufficient number of vocalization neurons to drive a higher stapedius muscle response. Furthermore, stapedius muscle activity alone, elicited in adult roosters⁹, was also reduced, and this finding was associated with a reduction in the size of mesencephalic loci, from which electrical stimulation could elicit the stapedius response. This observation suggests that the decrease in adult stapedius muscle activity could be due to a loss of mesencephalic stapedius controlling neurons, which could result from neuronal death. The shrinking of the stapedius controlling area can also account for the threshold and latency increase of the stapedius muscle response in adulthood. In fact in young roosters, when the direct activation of stapedius controlling neurons occurs, the latency of the stapedius muscle EMG response decreases, becoming shorter than that of the vocalization^{8,9}. In adult cocks, as a consequence of the presumed reduction in the number of stapedial neurons, only a secondary activation can probably take place at the stimulus intensities we used.

Our findings suggest that vocalization-related stapedius muscle activity is acutely reduced in adult roosters. These results, obtained during electrically induced vocalization, have to be validated by recording from awake birds, to confirm that the changes in stapedius muscle activity, detected in our experimental conditions, persist in awake spontaneously vocalizing birds. However, this seems to be likely considering that there is a datum in literature⁴ showing that stapedius muscle activity associated with spontaneous vocalization in young chickens is like that electrically induced.

Our findings question the hypothesis that the stapedius muscle only acts to protect the inner ear receptor cell during vocalization, as its activity appears markedly reduced just when the animal produces its loudest

vocalizations. Hence, our findings suggest that stapedius muscle activity occurs during vocalization mostly in young fowl and may be involved in vocal development. A possible mechanism could be the modulation of the auditory feedback during such development, so that feedback occurs at a specified frequency and amplitude.

It has been suggested that song birds learn to sing only by hearing themselves vocalize and use the auditory feedback to match their voice with the intended pattern¹¹⁻¹⁵. This mechanism has been ruled out for the development of crowing in chicken¹⁰ which is thought to be completely independent of auditory input.

Our results demonstrate that strong differences exist in the crowing of normal and stapedectomized roosters, and so we suppose that auditory feedback can play a role in the development of adult vocalization also in this avian species. The modulatory action exerted by the stapedius muscle on auditory feedback, probably filters the acoustic input generated by the cock's own voice. When this filtering is not possible then perhaps sound production is modified until it generates the expected feedback that would have occurred with filtering. Recent work in an other avian species has shown that the energy of various harmonics of a same sound can be willfully controlled and imitated¹⁸ and this same control is apparently available to young roosters. As vocal development ends, so too ends most of the stapedial attenuation of self-generated vocal input.

It is conceivable that in adults the persistent low stapedius muscle activity is aimed at protecting the inner ear receptor cells against overstimulation by the bird's own voice as suggested^{1,4,5}.

Work aimed at identifying the exact mechanism by which the stapedius muscle exerts its modulatory role during the different stages of the vocal development is in progress.

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