Auditory perception following hair cell regeneration in European starling (*Sturnus vulgaris*): Frequency and temporal resolution[a]

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Behavioral detection thresholds, auditory filter widths, and temporal modulation transfer functions were obtained from four starlings before, during, and after 11 days of subcutaneous injections of kanamycin, an aminoglycoside antibiotic. Birds were operantly conditioned to respond to pure tones and amplitude modulated noises ranging in frequency from 0.25 to 7 kHz using adaptive staircase procedures and were tested daily for 92 days after the first injection of aminoglycoside. All birds had threshold shifts of at least -60 dB at frequencies above 4 kHz. Lower frequencies were affected in some birds, although none of the birds had hearing loss below 3 kHz. All four birds had wider auditory filters at 5 kHz immediately after the aminoglycoside series. Any changes in frequency resolution at frequencies below 5 kHz were slight, transitory, and rarely observed. Two of the four birds had permanently wider auditory filters at 5 kHz. Temporal modulation transfer functions were briefly affected in two birds during the time of greatest threshold shift. Recovery of detection thresholds began soon after the injections ceased and continued for approximately 60 days. Recovery in frequency resolution lagged behind auditory threshold by about 10 days. Normal temporal resolution was observed in the context of impaired intensity and frequency resolution. Changes in auditory threshold and frequency resolution were closely associated for all birds at 5 kHz, but were correlated with statistical significance in only two birds. Scanning electron microscopy was performed on all four birds after 90 days of recovery and confirmed that the extent of initial damage was consistent with the pattern of observed hearing loss. © *1998 Acoustical Society of America.* [S0001-4966(98)02706-4]

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INTRODUCTION

Noise or drug induced damage to hair cells in the basilar papilla of birds results in a deterioration in behavioral detection threshold and an increase in the critical ratio (Hashino and Sokabe, 1989; Marean *et al.*, 1993; Linzenbold *et al.*, 1993). The damage pattern observed by scanning electron microscopy in these studies is a good predictor of changes in the shape of the audibility curve, and visa versa. After trauma, hair cells and associated structures in the avian basilar papilla regenerate (Ryals and Rubel, 1988; Corwin and Cotanche, 1988; Lippe *et al.*, 1991). Hair cell regeneration appears to lead to partial, and, in some cases, full behavioral recovery (Hashino and Sokabe, 1989; Marean *et al.*, 1993; Linzenbold *et al.*, 1993; Saunders *et al.*, 1995).

Little is known about auditory perception in birds following hair cell regeneration beyond detection thresholds. Critical ratio measurements in budgerigars (Hashino and Sokabe, 1989) suggest that critical bands broaden substantially following aminoglycoside ototoxicity and that thresholds and critical bandwidths partially recover in a linear relationship to each other. The bandwidths calculated from critical ratios in these birds are wide enough, however, to beg the question as to whether or not budgerigars have any "useful" degree of frequency resolution remaining after hair cell regeneration. Moreover, changes in efficiency in extracting signal from noise which might occur with hearing loss and hair cell regeneration could not be assessed with the critical ratio method.

The only behavioral study to examine temporal resolution is Saunders *et al.* (1995). They exposed adult chickens to a 120-dB pure tone at 525 Hz for 48 h which led to a relatively flat hearing loss from 500 Hz to 5 kHz. The slope of the temporal integration function became quite shallow following the damage, but gradually recovered over 25 days as thresholds returned to normal.

The species used, the type of damage, and the degree of damage obtained were quite different in these two studies. It would be useful to obtain a set of data from the same birds on a variety of perceptual measures in order to provide a better qualitative and quantitative description of audition following hair cell trauma and regeneration. Moreover, obtaining measures of frequency and temporal resolution before, during, and after hair cell regeneration is a way of investigating relationships among auditory functions in normal and impaired vertebrate hearing.

The two experiments reported here measured frequency and temporal resolution before, during, and after aminoglycoside ototoxicity in starlings, a passerine songbird. We chose to use notched-noise masking to calculate auditory filter shapes (Patterson, 1976), and temporal modulation transfer functions (''TMTFs,'' Viemeister, 1979) to measure temporal resolution.

A previous study in our lab (Marean *et al.*, 1993) found that a 10-day dose of 200-mg/kg/day dose kanamycin results in both temporary and permanent threshold shifts, depending on frequency. Below 3 kHz, auditory threshold remained normal. We chose, therefore, to measure the auditory filter (shape, bandwidth, and efficiency), and TMTF at 1, 3, and 5 kHz, because these frequencies should be associated with differing degrees of threshold shift. Measurements obtained at 1 kHz should reflect cochlear function of presumably normal, undamaged hair cells. At 3 kHz, auditory processing should reflect both the contribution of normal, damaged, and regenerated epithelium. Finally, at 5 kHz, the response should be primarily from regions of regenerated hair cells (Marean *et al.*, 1995, 1993).

I. METHODS

A. Subjects

Four adult European starlings served as subjects. The birds were caught in the wild in West Seattle in the Spring of 1993. All birds were in juvenile plumage at the time and were thus approximately 1 3/4 years of age when training began.

Birds were acclimated to individual home cages in our aviary and given an unlimited supply of dog food kibble (*ad lib* feeding). After a month on this diet the birds were weighed. From this point on birds were weighed daily and rationed to maintain a weight equal to 90% of their weights, when allowed to feed ad libitum.

B. Stimulus and apparatus: Frequency resolution study

Noise bands were created by multiplying a low-pass filtered noise by pure tones. The noise was generated by a Tucker-Davis WG2 function generator.[1] The noise source was fed to a Frequency Devices 901 low-pass filter set to 300 Hz. The output of the FD901 was fed through a Tucker-Davis programmable filter (PF1) set to low pass at 300 Hz using a twelfth-order 32BIQUAD configuration. Double filtering increased the slope of the low-pass filter attenuation from 70 dB per octave to >120 dB per octave prior to multiplication. The output of the PF1 was then split, with each being fed into a signal multiplier (Tucker-Davis MT3). Two Tucker-Davis function generators (WG1s) were used to generate pure tones. The output of each WG1 was fed into the signal multipliers. One WG1 determined the center frequency of the high-side noise band, and the other, the low-side noise band. The noise bands created by the multiplication process were 600 Hz wide with extremely sharp skirts (>400 dB per octave).

The pure-tone probes were generated digitally at a 44-kHz sampling rate and stored on a NEC 386SX PC. During presentation, they were played through a Data Translation DT2821 AD/DA board and fed to a Tucker-Davis digital programmable attenuator (PA4). The pure tones were 500 ms in duration and ramped on and off with 20-ms rise/fall times.

The outputs of the multipliers and the attenuator were sent to a Gemini PMX-1000 preamp/mixer console and fed to a Technics SA303 amplifier. A Phillips D2223 speaker was mounted to a wire mesh assembly and hung on the side of a special test cage at 6 in. from the response key (to the bird's right). The speaker face was protected by a material which did not influence the acoustic output of the speaker below 10 kHz, and the entire cage, including portions of the response key panel, was lined with acoustic foam.

The test cage was placed in an IAC single-walled acoustic sound chamber during testing. Calibration of test signals were made weekly with a Hewlett-Packard signal analyzer through a General Radio microphone to ensure that the noise bands were flat and that the drop-off of the notches was >400 dB per octave. The combination of the IAC sound chamber and specially designed test cage provided for a low noise floor: Notch depth was greater than 50 dB SPL in the 50-dB spectrum level (dBN0) conditions.

Figure 1 (panel A) is a schematic of how the stimuli were generated and a representative power spectrum measured at the approximate location of a bird's right ear in the test chamber. In this example, the notch width is 40% of center frequency.

C. Stimuli and apparatus: Temporal resolution study

Broadband noise was generated by a Tucker-Davis WG-3 noise generator. The noise was fed to a signal multiplier (Tucker-Davis MT3) and multiplied by a dc offset (4 V) pure tone from a Tucker-Davis WG-1 waveform generator. The output was fed to a digital attenuator (Tucker-Davis PA-4) where an attenuation correction factor was applied for the increase in intensity of the modulated conditions. The degree of attenuation was determined by the formula:

$$10*\mathrm{Log}(1+m^2/2),$$

where the modulation index (m) = the amplitude of the pure-tone modulator divided by the dc offset.
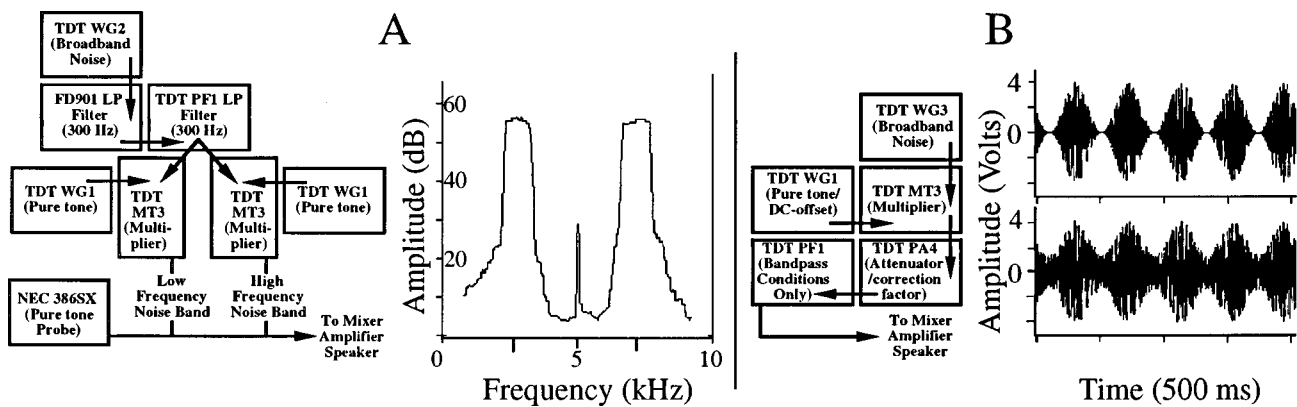
FIG. 1. Stimuli used in the current experiment. A. Schematic of how stimuli were produced and the acoustic power spectrum of a 5-kHz pure tone in a 50-dBNo notched-noise masker. Notch width is 2 kHz or 40% of center frequency. B. Schematic for temporal resolution study and amplitude modulated broadband noise at 100% modulation (upper panel) and 50% modulation (lower panel).

In this experiment, dc offset was held constant, and the voltage of the WG-1 was varied to change modulation depth. For temporal modulation transfer function (TMTF) conditions with bandpass carriers of center frequencies 1 kHz, 3 kHz and 5 kHz, the signals were sent to a Tucker-Davis PF-1 programmable filter. Filter settings were calibrated so that bandwidth in absolute Hertz remained constant at @ 2200 Hz for each frequency at the half-power point (-3 dB). The skirts of the noise bands were @70 dB/oct.

The output of the attenuator was sent to the same mixing and amplification apparatus previously described. The same speaker assembly, test cage, and response panel was used as well. Stimuli were calibrated using the same system used in the auditory filter study, plus the addition of a Hewlett-Packard digital storage oscilloscope. The stimuli were calibrated to be 30 dBNo and 50 dBNo.

Figure 1 (panel B) shows several periods of a broadband noise modulated at 10 Hz. In the upper trace, the modulation depth is nearly 100%; in the lower trace, the modulation depth has been reduced by 50%.

D. Training and testing procedure: Frequency resolution[2]

Birds were trained to peck an ''observation key'' until they detected a pure tone. During an 1100-ms interval following the tone, the computer waited for the bird to hit the response key. If the bird responded during the interval, a ''hopper'' containing dog food kibble was extended for 1000 ms. Failure to respond caused the lights in the test chamber to turn off for 500 ms.

In masking conditions, the masker was turned on at the beginning of the session and remained on throughout. On a certain percentage of the trials, the computer presented no tone but waited for a response. If the subject responded during the trial interval (a false alarm), the lights in the test chamber were turned off for 8000 ms. Correct rejections were neither rewarded or punished; the panel lights flashed to indicate that the trial was over and a new observation interval was begun.

Prior to beginning a threshold run, subjects were put through a ''warm-up period.'' During this time (a minimum of 10 trials), the probability of a signal trial was 0.5, and all

signal trials were at least 60 dB (70 dB in some conditions), but varied in level up to 85 dB. When the subject attained a percent correct of at least 90%, the threshold run was begun. Masker properties and signal frequency were set at the beginning of the warm-up period to be the same as during the adaptive procedure. Masker intensity was either 30 dBNo or 50 dBNo in the final experiment. For the high intensity condition, a higher level masker would have been preferable, but birds would not tolerate noise intensities greater than 50 dBNo. In humans, the difference between 30 dBNo and 50 dBNo maskers would not be expected to lead to significant changes in auditory filter shape (Patterson, 1976; Rosen and Baker, 1994).

The adaptive procedure used a two-down, one-up rule to estimate the 70% point on the psychometric function (Levitt, 1970). The probability of a signal trial was 0.64; the probability of a no-signal trial was 0.2, and the probability of a probe trial was 0.16. Probe trials were constrained to be no lower than the lowest values used during the warm-up period. Only signal trials were used in the stepping procedure. False alarm rate and probe trial hit rate were monitored.

After each reversal, a set of rules similar to PEST (Taylor and Creelman, 1967) was used to decrease or increase step size. The adaptive staircase began at a randomly selected intensity between 60 dB and 70 dB; initial step size was 20 dB. Minimum step size was 1 dB. A minimum of six reversals was required for a complete threshold run, with the final four being averaged to obtain threshold. If the standard deviation of the last 4 reversals was greater than 10 dB, the run was continued, for up to a maximum of 16 reversals, until the standard deviation was below 10 dB.

Sessions were discounted if the false alarm rate was above 0.39, if the probe hit rate was below 0.9, or if any of the reversals were above the probe level. Sessions with an uneven number of reversals were also not used in the final sample. These exclusion criteria were selected based upon several years of observing starling psychoacoustic performance and receiver operating characteristic analysis (Green and Swets, 1966).

Thresholds in quiet were obtained at 0.5, 1, 2, 3, 4, 5, and 7 kHz. Thresholds in notched-noise were obtained at 1, 3, and 5 kHz. Quiet thresholds were used to ensure that each

bird had normal pre-exposure audiograms across the frequency range. They also served as a check on performance at the widest notch conditions, which should approximate performance in quiet. For thresholds in quiet, the order of testing frequency was randomly determined.

When noise bands were placed symmetrically about center frequency, total notch width in Hertz is described as a percentage of center frequency. For example, a total notch width of 10% at 5 kHz is a 500-Hz-wide notch, and a 10% total notch width at 1 kHz is a 100-Hz-wide notch. When noise bands were placed asymmetrically about center frequency, the distance in Hertz from probe frequency to the lower and upper noise bands are described as a proportion of probe frequency. Thus a notch configuration of 0.15–0.3 at 5 kHz describes an asymmetrical notched-noise masker with a lower noise band 750 Hz below 5 kHz and an upper noise band 1500 Hz above 5 kHz (total notch width: 45%).

At 1 and 3 kHz, birds were tested at total notch widths of 0%, 20%, 40%, 60%, and 80% of center frequency. Due to the relatively narrower filter at 5 kHz, total notch widths of 0%, 10%, 30%, 40%, and 60% were used. When birds were tested on notched-noise, they were tested on one frequency per day. Block order of different notch configurations was randomly determined.

To test for the symmetry of the auditory filter, two to four blocks were included with the upper and lower noise bands asymmetrically placed about center frequency (Stone *et al.*, 1992). At 1 kHz and 3 kHz, the asymmetrical notches were placed at 0.2–0.4 and 0.4–0.2. At 5 kHz, the asymmetrical notch conditions were 0.15–0.3 and 0.3–0.15. After dosing, more blocks were added in 5-kHz conditions. The birds were tested at total notch widths of 20% and 80%, as well as the asymmetrical notch conditions of 0.2–0.4 and 0.4–0.2. This was done so that changes in the auditory filter expected to occur at 5 kHz could be tracked more accurately.

Filter shapes were fitted to the data from a program obtained from Dr. Richard Baker (see Rosen and Baker, 1994). The procedure approximates filter shape in a manner similar to the ROEX (p,r) filter originally described by Patterson *et al.* (1982):

$$W(g) = (1-r)(1+pg)\exp(-pg) + r,$$

where g is the normalized deviation from the center of the filter, p is a parameter determining the slope of the skirts, and r is a parameter which flattens the filter at frequencies remote from the center frequency.

The Rosen and Baker (1994) polynomial fitting (Poly-Fit) procedure replaces each parameter with a linear function which changes with masker level. Filter shapes were also fitted to the data from a program obtained from Drs. B. C. J. Moore and B. R. Glasberg (see Glasberg and Moore, 1990). The filter shapes obtained by both methods were similar, but the results obtained from the Rosen and Baker (1994) procedure are reported here. This was done primarily because of extensions in the PolyFit procedure to the ROEX p,r model which allowed us to estimate r separately for the high- and low-frequency side, as well as taking into account absolute threshold (which we expected to change dramatically after dosing) in the fitting procedure. The PolyFit procedure al-

lowed us to find the values of p (upper and lower) and r (upper and lower) that best fit the data. The best fitting values define the filter shape.

Data from incomplete sessions were not included in the final analysis because the sum-squared deviations of the fit from the data were routinely higher than for complete sets despite there being fewer residuals. For sessions using at least seven notch conditions, the sum-squared deviations for a fixed masker level were below 100 in all of the sessions reported here (mean=48, ±1 s.e.=18). Thus the average deviation between data and fit was @ 2.7 dB.

Bandwidth estimates from fitted filters represent the passband at the −3 dB down points. Equivalent rectangular bandwidth estimates were also obtained. The −3 dB passbands are less sensitive to changes in dynamic range than equivalent rectangular bandwidth (ERB) estimates, since the ERB of an auditory filters is an area measure. On average, the −3 dB passbands were 5%–15% narrower than ERB estimates of the same auditory filters.

E. Training and testing procedure: Temporal resolution

The basic training and testing procedure did not differ between the frequency and temporal resolution studies. At the beginning of each session, the noise carrier was turned on. It remained on for the duration of the session. On some trials, the computer presented no modulation of the background noise but waited for a response. If the bird responded during this time (a false alarm), the lights in the test chamber were turned off for 8000 ms. Correct rejections were neither rewarded or punished; the panel lights flashed to indicate that the trial was over and a new observation interval was begun.

Prior to beginning a threshold run, birds were run through a warm-up period. During this time (a minimum of ten trials), the probability of a signal trial was 0.5, and all signal trials were near 100% modulation. When the bird attained a percent correct of at least 90%, the threshold run was begun. Modulation and carrier frequency were set at the beginning of the warm-up period to be the same as during the adaptive procedure.

At each carrier band, the birds were tested at five modulation rates: 10, 50, 100, 500, and 1000 Hz. The carriers were presented at 30 dBNo; the 5-kHz carrier and the broadband noise carrier were also presented at 50 dBNo.

The adaptive procedure was the same as in the frequency resolution study. The adaptive staircase began at a modulation depth at or close to 100%. The initial step size was 0.5 V; minimum step size was 0.1 V. Probe trials were near 100% modulation depth. A minimum of six reversals was required for a complete threshold run, with the final four being averaged to obtain threshold. If the standard deviation of the last 4 reversals was greater than $m=10$, the run was continued until the standard deviation was below 10 for up to a maximum of 16 reversals. All other exclusion criteria were the same.

F. Kanamycin injections, testing schedule, and scanning electron microscopy

Once baseline performance had been determined for all conditions (absolute threshold, frequency, and temporal resolution), each bird was given subcutaneous injections of 100 mg/kg/day for 2 days and 200-mg/kg/day kanamycin for 9 days. An 11-day dosing period was used instead of the standard 10-day dosing period so that the first day's dose could be spread over 2 days to reduce the shock of these high dosages of aminoglycoside.

Testing was continued during this dosing period: Birds were tested several hours after their daily injection. Food rations were provided immediately after behavioral testing.

One bird, 95–134 (identified in the results as Bird IV), was given an extra two days of injections because its threshold shift lagged behind the other three birds (see results).

After all post-injection data were collected (92 days after the first dose), the inner ears of each bird were examined by scanning electron microscopy (SEM). For SEM, birds were decapitated following administration of an overdose of pentobarbital sodium. Both cochleas from each bird were perfused via the round window with 2.5% gluteraldehyde and 2% paraformaldehyde in 0.1 molar phosphate buffered saline (PBS). The temporal bones were removed and the basilar papilla exposed using standard microdissection techniques. The specimens were then placed in 1% osmium tetroxide for 1 h, followed by several washes in PBS. Specimens were then dehydrated in a graded ethanol series, followed by final dissection. The tectorial membrane was removed under 70% ethanol. After critical point drying, specimens were mounted on aluminum stubs and coated with gold palladium prior to examination with the JEOL 63005 electron microscope.

Our previous work suggested that the extent of damage from the dosing regimen could be ascertainable from orientation patterns of stereociliary bundles even after long survival times (@90 days in the current study). We also expected that the linear extent of disorientation (measured from the base of each papillae) would be predictable from the pattern of behavioral threshold shift observed (and vice versa). However, our goal in the present study was not to provide detailed correlations between structural and functional properties as a function of frequency and place. Thus other than measuring the area of presumed damage (disoriented hair cell bundles) along the superior edge of each papilla, detailed quantitative analyses of hair cell parameters such as number, size, and bundle orientation were not undertaken.

II. RESULTS

A. Predose thresholds in quiet

Average threshold data across birds at seven frequencies agree with previously published data from our laboratory (Marean *et al.*, 1993) and other labs using similar methods (e.g., Dooling *et al.*, 1986).
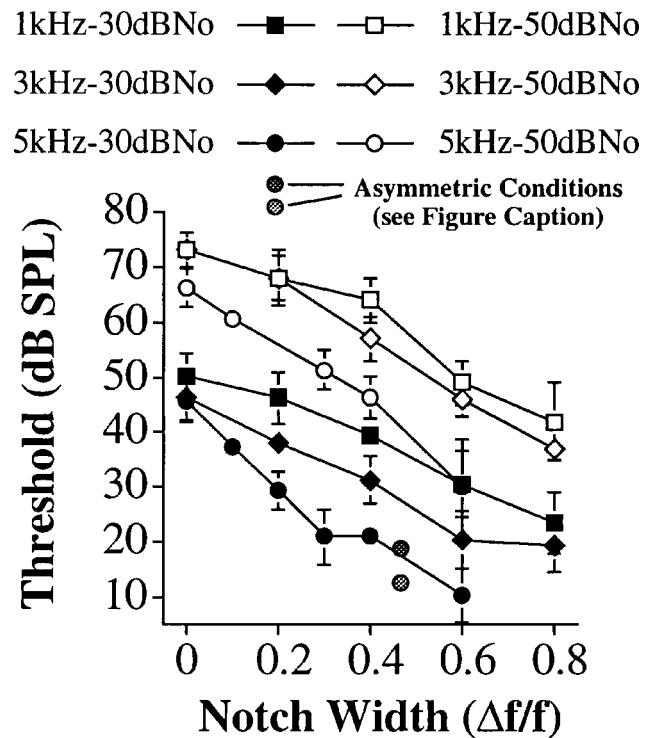
FIG. 2. Pre-dose average masked thresholds in 30-dBNo and 50-dBNo notched-noise conditions. Error bars are ± 1 standard deviation of all the points used to make the figure. Dark stimpled filled circle shows data from an asymmetric notch condition at 5 kHz (0.15–0.3: Total notch width $= 45\%$). Light stimpled filled circle shows data from an asymmetric notch condition at 5 kHz (0.3–0.15: Total notch width $=45\%$). Slope values for 30 dBNo: 1 kHz$= -0.34$; 3 kHz$= -0.37$; 5 kHz$= -0.57$. Slope values for 50 dBNo: 1 kHz$= -0.4$; 3 kHz$= -0.47$; 5 kHz$= -0.59$.

B. Auditory filters[3]

Figure 2 shows the average raw data for birds in all symmetrical notch conditions; the filled symbols are data at a masker level of 30 dBNo. The open symbols show data from 50-dBNo masker level conditions. Asymmetrical notch conditions for the 30-dBNo maskers at 5 kHz are also shown.

The data show that relative width of the auditory filter of starlings narrows as a function of frequency since the slope of the 5-kHz data is steeper than 3 kHz, which in turn is steeper than 1 kHz (see figure caption). These data also show that thresholds at the widest notch widths are comparable to thresholds obtained in quiet. Finally, since performance at 5 and 3 kHz at notch widths of 0% are better than performance at 1 kHz, these data also illustrate that efficiency improved with increasing frequency. Efficiency, the signal to noise ratio at threshold independent of filter bandwidth, was estimated from both Glasberg and Moore's (1990) and Rosen and Baker's (1994) procedures and was determined to be 10–15 dB better at 5 kHz than 1 kHz.

Masker level influenced performance in a predictable manner in that thresholds were 20 dB higher in the 50-dBNo conditions than in the 30-dBNo conditions. Unfortunately, birds found the noise level of 50 dBNo to be aversive at 1 and 3 kHz, and they were frequently unwilling to complete these sessions. Thus for both masker intensities, the predose average raw data at 5 kHz is comprised of several complete sessions from each bird, but at both 1 and 3 kHz, the predose
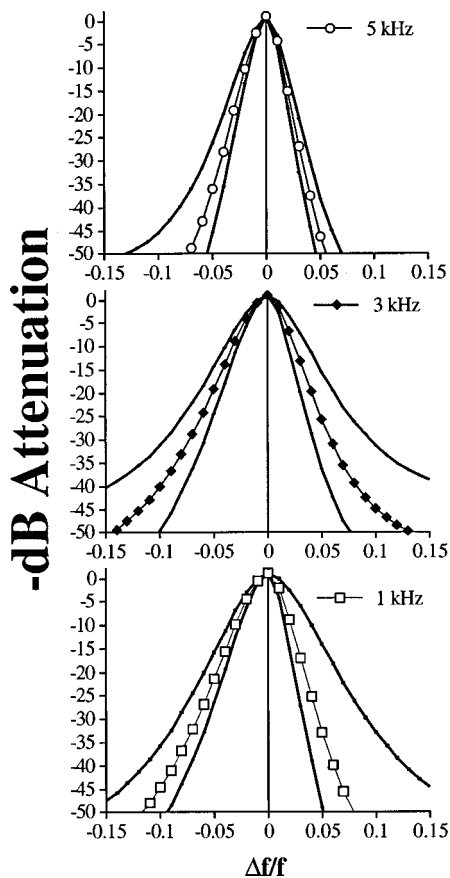
FIG. 3. Average predose auditory filters at three frequencies. Average band-width at 5 kHz=480.1 Hz (s.d. 19.87); 3 kHz=382.1 Hz (s.d. 44.84); 1 kHz=135.5 Hz (s.d. 21.07). Unmarked lines are filters representing ±2 standard errors of predose slopes and dynamic ranges obtained from all birds predose.

average raw data for the 50-dBNo masker intensities may be based upon only a single estimate per bird. Despite fewer data points, the pattern of response remained the same at all frequencies.

The raw data from the asymmetric conditions suggested a slight skewing of the auditory filter toward the low-frequency side, since thresholds were lower in the asymmetric conditions where the low-frequency masker was further from the probe frequency than the high-frequency masker (total notch width was the same). The same effect was seen at both masker intensities for all frequencies, although only the 30-dBNo data from 5 kHz are shown. The asymmetric notch conditions figure prominently in filter slope estimates; since only two asymmetric conditions are plotted in Fig. 2, only qualitative comparisons can be made between Fig. 2 and subsequent filter estimates.

Auditory filters were fitted to raw data using the PolyFit procedure of Rosen and Baker (1994). The output provided estimates of the high- and low-frequency slopes, the dynamic range, efficiency in extracting signal from noise (in dB), and the bandwidth −3 dB (in Hertz).

In Fig. 3 and all subsequent figures showing fitted auditory filters, symbols have been added to the fits for identification purposes. In the case of group data, additional filters have been plotted using slopes and dynamic ranges ±2 standard errors from the mean plotted in the figure. Group filter
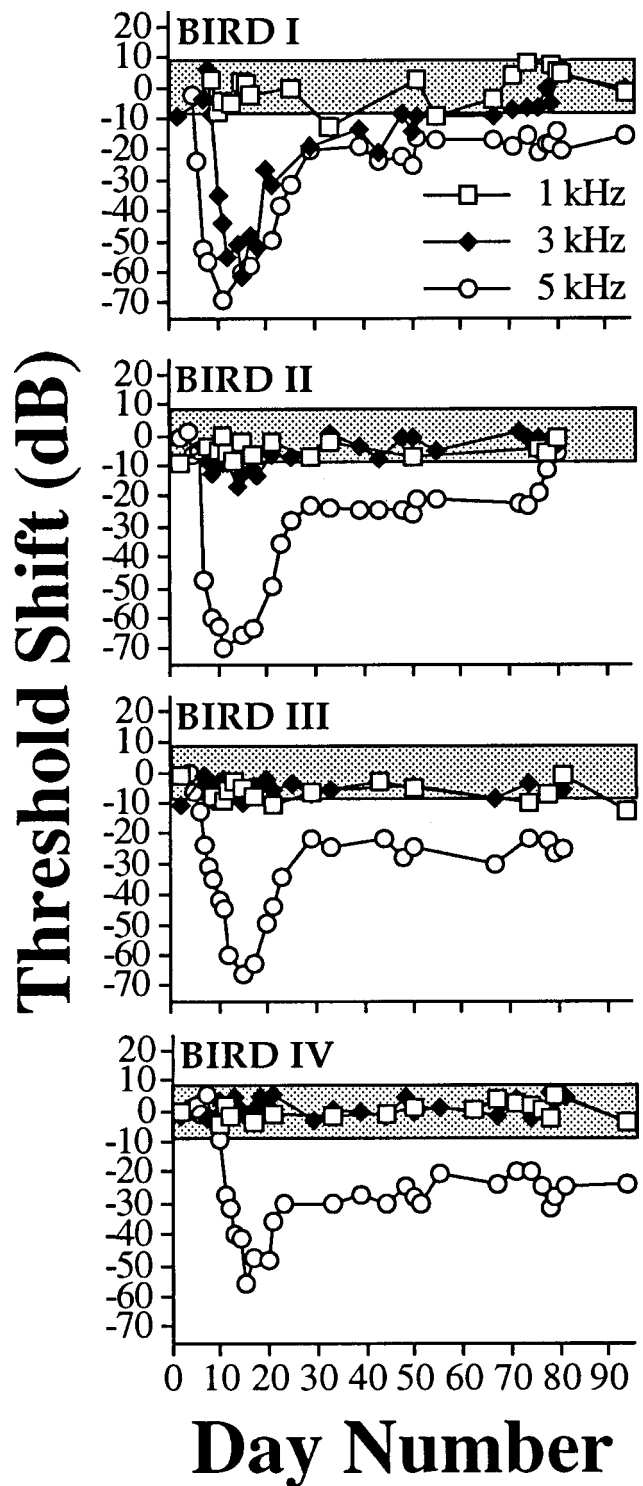

FIG. 4. Threshold shifts throughout the experiment for individual birds. Day numbers refers to the number of days from the first kanamycin injection. Shaded area represents ±2 standard errors of the mean for all pre-dose thresholds.

estimates are calculated from average individual filter estimates, and not from group means of thresholds at different notch widths (plotted in Fig. 2).

Predose group data at three frequencies are shown in Fig. 3. The −3 dB bandwidth for each frequency is given in the figure caption, and shows that especially for 1 and 5 kHz, bandwidth estimates are remarkably stable within and across

TABLE I. Bandwidths for individual birds at four time periods during the experiment. Bandwidths in boldface are outside the range of predose measures. The symbol (^) appears after data showing bandwidths narrower than obtained predose.

BIRD	Freq.	Week	BW	Freq.	Week	BW	Freq.	Week	BW
	5 kHz	Pre	454.33	3 kHz	Pre	406.5	1 kHz	Pre	163.36
Bird I		0–2	**891.4**		0–2	332.67^		0–2	**278.92**
		3–6	**677.07**		3–6	**507.66**		3–6	**218.3**
		7–10	**694.38**		7–10	425.92		7–10	108.65^
	5 kHz	Pre	499.89	3 kHz	Pre	380.21	1 kHz	Pre	127.79
Bird II		0–2	**1,821.22**		0–2	426.92		0–2	130.02
		3–6	**581.44**		3–6	**469.53**		3–6	158.56
		7–10	429.41^		7–10	352.9		7–10	110.83
	5 kHz	Pre	490.6	3 kHz	Pre	421.75	1 kHz	Pre	137.65
Bird III		0–2	**641.32**		0–2	392.53		0–2	128.84
		3–6	**552.64**		3–6	370.56		3–6	109.34
		7–10	**547.41**		7–10	371.24		7–10	125.77
	5 kHz	Pre	475.75	3 kHz	Pre	319.94	1 kHz	Pre	113.34
Bird IV		0–2	**659.44**		0–2	295.47		0–2	103.4
		3–6	**689.9**		3–6	282.95		3–6	106.29
		7–10	476.4		7–10	303.49		7–10	**141.4**

birds. Individual differences in filter shape were primarily differences in skew and did not result in highly variable filter bandwidths.

Filter shapes and bandwidths were most variable at 3 kHz, and least variable at 5 kHz. The fitted data show the slight low-side skewing of the auditory filter suggested by the raw data reviewed above and represent the first published data of auditory filter shapes in birds.

C. Aminoglycoside-induced changes in detection threshold

Figure 4 shows individual threshold shifts during and after the aminoglycoside treatment at the three primary frequencies: 1, 3, and 5 kHz. Not shown are threshold shifts at 7, 4, and 2 kHz, and frequencies below 1 kHz. In all birds, threshold shift at 7 kHz preceded threshold shift at 5 kHz by two days. Thresholds at 4 kHz shifted to the same degree as 5 kHz, but lagged behind 5 kHz by one day. No threshold shift was observed at 2 kHz or below in any of the four starlings. The pattern of results replicated our earlier findings (Marean *et al.*, 1993) in that threshold shift occurred first at higher frequencies, and proceeded toward lower frequencies.

These data were used to order subject numbers so that each subject's number would describe their relative degree of hearing loss. Thus Bird I had the greatest degree of hearing loss (severe threshold shift at all frequencies >2 kHz). Bird II had only a mild shift at 3 kHz, and severe shifts at all frequencies >3 kHz. Bird III had no hearing loss at frequencies below 4 kHz, and severe threshold shift at 4 kHz and above. Finally, Bird IV had significant hearing loss only at frequencies 5 kHz and higher.

The course of recovery in auditory threshold at 3, 4, and 5 kHz proceeded rapidly over a period of 20 days after the last dose. Thresholds recovered from a loss of approximately −70 dB to approximately −30 dB at 5 kHz. An additional 10–20 dB improvement sometimes occurred during the next 20-day period, except for at 3 kHz where the threshold returned to normal by 20 days after the aminoglycoside treat-

ment. No additional recovery occurred after 60 days following the last dose in birds I, III, and IV, consistent with our earlier findings. Bird II had further improvement at 5 kHz beginning on day 70, but not at 4 kHz or 7 kHz.

D. Post-dose changes in auditory filter bandwidth

Changes in filter bandwidth were considered significant if they exceeded ±2 standard deviations of predose estimates for individual data, and ±2 standard errors for group data. Table I shows bandwidth data from auditory filters averaged for individual birds within four discrete time periods of the experiment. The first time interval was pre-dose; the second was during the first two weeks following the dose; the third was during the period of three to six weeks following the dose, and the final time interval was the average of auditory filter bandwidths seven to ten weeks after kanamycin injections. Filter bandwidths were significantly wider for all birds at 5 kHz in the first two weeks following the dosing period. Birds I and II also had wider bandwidths at 3 kHz, although the effect was not observed until at least three weeks following the dose. Finally, the bird with the broadest range of hearing loss also had a broadening of the auditory filter at 1 kHz.

In all but three cases, bandwidth recovered to within normal limits by the end of the experiment. Bird I and Bird III had permanently wider filter bandwidths at 5 kHz. Bird IV had a wider bandwidth at 1 kHz at the end of the experiment; however, given the pattern of results observed in this and other birds, this is likely due to an outlier filter estimate.

There were three instances where auditory filter bandwidths were narrower than those obtained predose: The 5-kHz auditory filters of Bird II at the end of the experiment, the 3-kHz auditory filter for Bird I immediately following the injection series, and the final auditory filters obtained for Bird I at 1 kHz were all narrower than seen prior to dosing.
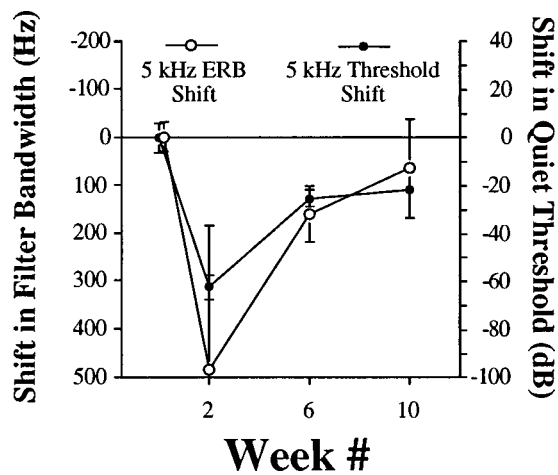
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FIG. 5. Group average shift in filter bandwidth (left ordinate) plotted with shift in quiet threshold (right ordinate) for 5 kHz. ±1 standard deviation is shown.

FIG. 6. Average auditory filters during four time periods of the experiment at 5 kHz.

E. The relationship between threshold shift and filter bandwidth

A correlation matrix of threshold shift and bandwidth shift was constructed for all birds at all frequencies. Out of this matrix, only two correlations were significant: Auditory threshold at 5 kHz was significantly correlated to filter bandwidth at 5 kHz for Bird I [$r=0.831$, $p<0.05$]* and Bird II [$r=0.912$, $p<0.05$]*. One other correlation approached significance: For Bird I, filter bandwidth at 5 kHz correlated with auditory threshold at 3 kHz [$r=0.808$, $p<0.06$].

Although not statistically significant, the group data at 5 kHz illustrate the close association between changes in frequency resolution and detection threshold (see Fig. 5). The open circles are estimated bandwidth shifts (left ordinate) and the filled symbols are detection threshold shifts (right ordinate). The lack of a significant correlation was likely due to the high variability in filter bandwidth immediately following the dosing period and at the very end of the experiment. Normal frequency resolution was observed in two of
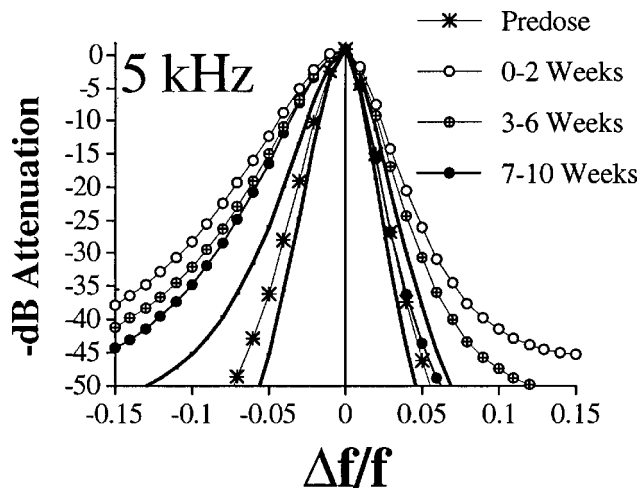
the four birds in the context of abnormal detection thresholds.

F. Post-dose changes in filter shape: Group Data

High- and low-frequency slopes and dynamic ranges were averaged for the four time periods displayed in Table I in order to examine general trends in the response of the auditory filter to ototoxic damage and hair cell regeneration. The resulting average filters at 5 kHz are shown in Fig. 6. Predose slope estimates of ±2 standard errors are also shown in the figure. Average filters from three other time periods are shown and correspond to the same intervals used to average individual data in Table I. Filters broadened on both sides, with nearly complete recovery on the high-frequency side by the end of the experiment. Permanently shallower slopes were observed on the low-frequency side of the filter. No significant bandwidth changes were evident in the group data at lower frequencies.

Changes to the 5-kHz filters shown in Fig. 6 do not look as austere as the ERB bandwidth changes shown in Fig. 5
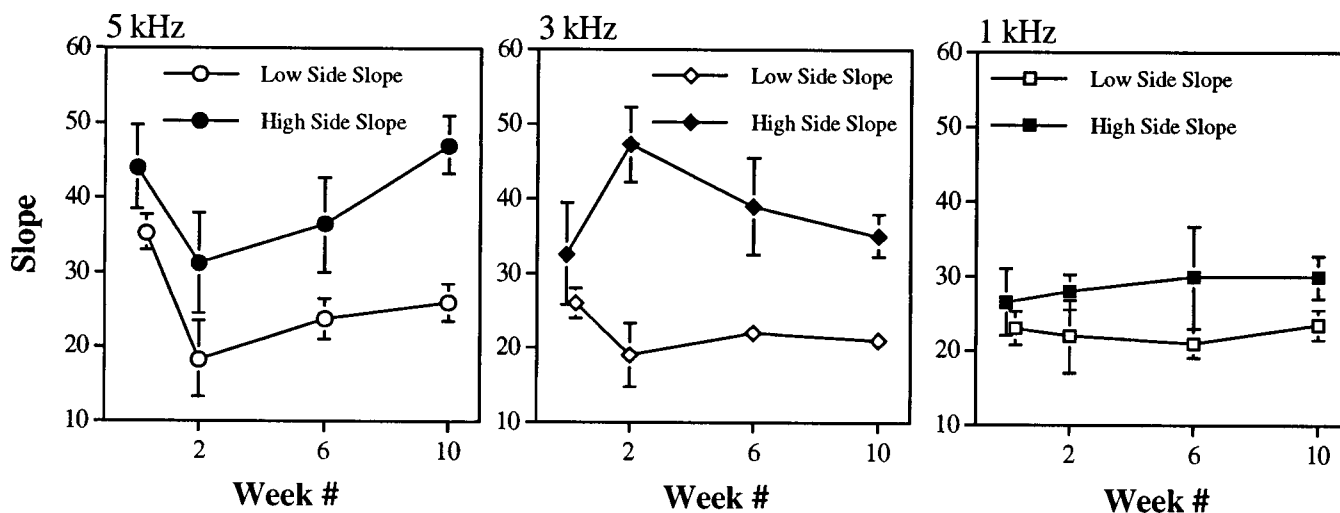

FIG. 7. Average slopes for both the high- and low-frequency side of the auditory filter during four time periods of the experiment. Error bars show ±2 standard errors.
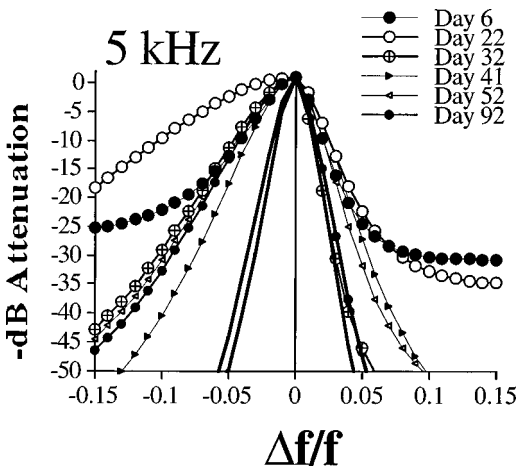
FIG. 8. Auditory filter estimates for Bird I at 5 kHz during the experiment. In this and the following three figures, thin symbol-less lines show the extent of variation predose in high- and low-frequency slopes; each estimate is independent.

FIG. 9. Same as Fig. 5 but for Bird II at 3 kHz.

because Fig. 6 shows frequency as a proportion of center frequency, whereas Fig. 5 shows frequency in absolute Hertz.

Permanent changes in slope were found at both 5 and 3 kHz. Figure 7 shows the average slope values (± 2 s.e.) at four time periods during the experiment at each frequency. At 5 kHz, both the high-frequency and low-frequency slopes become shallower after the dose period. Recovery is greater on the high-frequency side than the low-frequency side with a permanent significant change in slope occurring on the low-frequency side.

At 3 kHz, where no significant changes in bandwidth for the group occurred, the high side slope became steeper and the low side shallower, indicating that the skew of the filter was significantly shifted toward lower frequencies. The high side recovers to within normal limits, but with a higher average slope. Combined with a permanently significant shift on the low-frequency side, the filter appears to be permanently shifted in skew while retaining its normal bandwidth.

At 1 kHz, none of the changes in slope are significant for the group, although the trend mimics that of the 3-kHz filters. Although individual birds did have significant changes in slope at 1 kHz (see below), changes in skew were transitory when observed.

G. Post-dose changes in the auditory filter: Individual data

1. 5 kHz

Figure 8 shows individual auditory filters from Bird I at 5 kHz during and after kanamycin injections. The broadest and the narrowest high- and low-frequency slopes obtained prior to dosing for that bird have been drawn on the figure with lines without plot symbols. Data outside the range in these figures also fall outside ± 2 standard deviations of pre-dose estimates for each individual bird. By day 6, both the high- and low-frequency slopes decreased, with substantially greater decreases on the low-frequency side of the filter. The dynamic range of the filter was reduced. The threshold shift at this time was -30 dB.
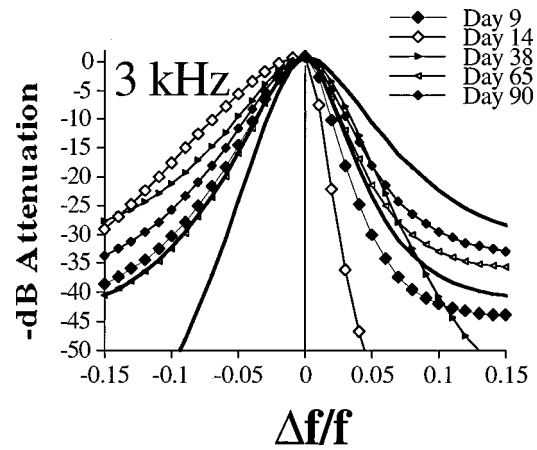
By day 22, the filter broadened further on the low-frequency side, and was only slightly broader on the high-frequency side. This pattern was observed in all birds, although filter widening was more drastic in some birds. For example, the change in filter bandwidth for Bird I (shown in Fig. 8) was from 9% predose to 27% on day 22; for bird II, the change was from 9% to 66%.

By day 32, the low- and high-frequency sides of the auditory filter recovered and stabilized. The low-frequency slope was permanently shallower; the high-frequency slope returned to normal. The overall effect in this bird was a permanently wider auditory filter at 5 kHz accompanied by a change in skew toward the low frequencies. Two of the birds had complete recovery of auditory filter bandwidth at 5 kHz, but all four birds showed the same pattern of permanent changes in skew.

Day 41 and day 52 have been plotted with smaller and different identification symbol shapes. Although there is an orderly pattern of broadening and partial recovery of the auditory filter, these data illustrate the variability in performance observed post-dose compared to pre-dose.

2. 3 kHz

Figure 9 shows individual auditory filters for Bird II at 3 kHz during kanamycin dosing (day 9), and at day 14, immediately following kanamycin dosing. Also shown is the last filter estimate from data obtained at day 90. As shown in Fig.
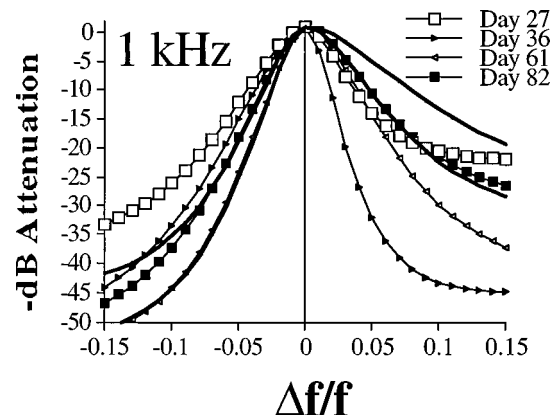

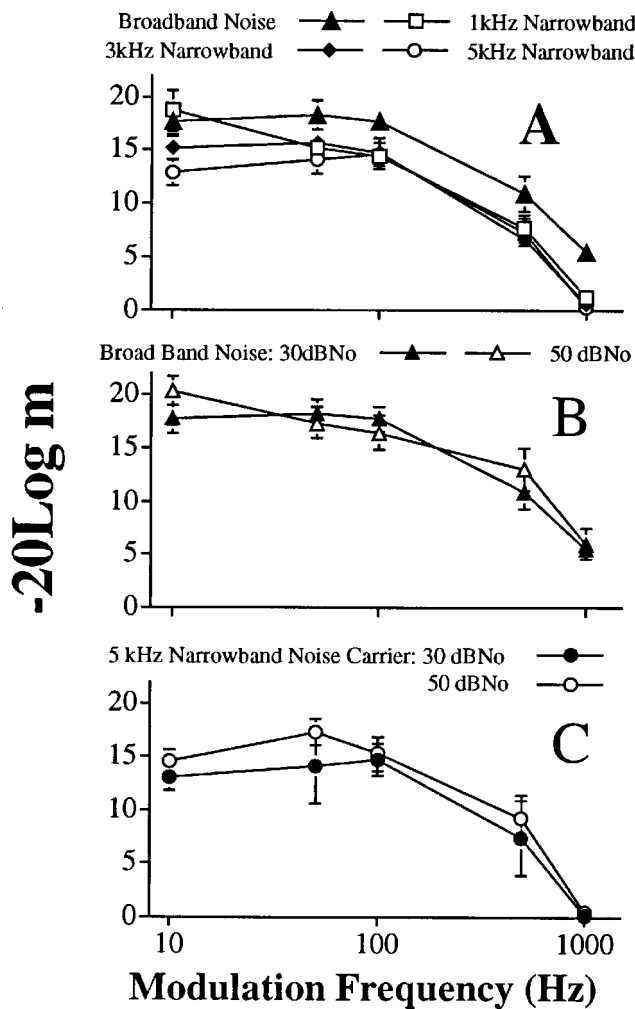FIG. 10. Same as Fig. 5 but for Bird III at 1 kHz.

FIG. 11. A. Pre-dose average TMTFs in all conditions with 30-dBNo carriers. B. For broadband noise carriers at 30 dBNo and 50 dBNo. C. For 5-kHz narrow-band carriers at 30 dBNo and 50 dBNo. ±1 standard error of the means is shown.

4, this bird had a mild threshold shift at 3 kHz by day 9. The bandwidth of the auditory filter was not significantly broader, but the filter noticeably changed in skew toward lower frequencies. By day 14, the filter was 5.3% broader, which was a significant change in bandwidth: pre-dose filters for this bird at 3 kHz did not vary by more than 2%. Bird I also had hearing loss at 3 kHz and also had a broadening of the auditory filter. Birds III and IV had no change in threshold or filter width at 3 kHz, but did show the same change in skew toward lower frequencies.

The change in skew was permanent for this bird, but not the change in bandwidth. The permanent decrease in the low-frequency slope of the filter did not result in a wider auditory filter, since the high-frequency slope increased proportionally.

3. 1 kHz

Bird III's post-injection auditory filters are shown in Fig. 10. Prior to dosing, its filters had an unusual tendency to be skewed toward high frequencies at 3 kHz and 1 kHz. The filter bandwidths for this bird at 1 kHz did not change at any time during the experiment, and yet its filters underwent
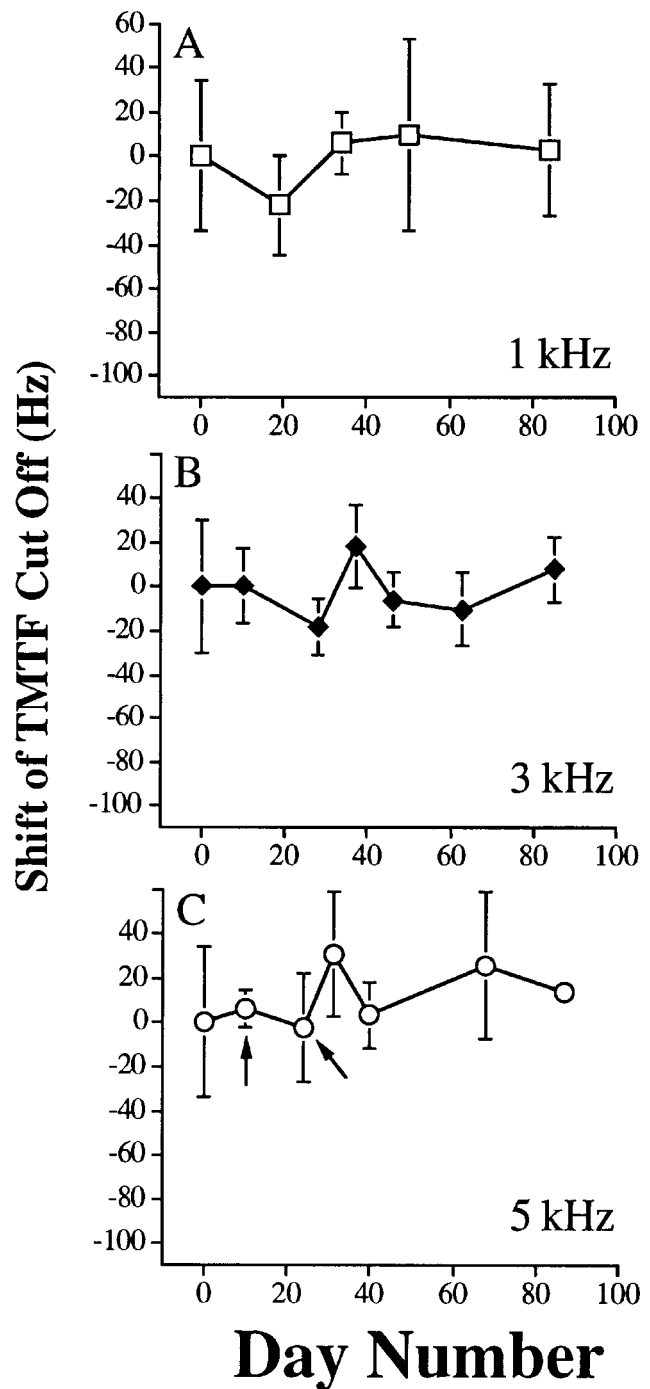

FIG. 12. A. Average shift in upper frequency cutoff (−3 dB) of TMTFs at 1 kHz; calculations were made from exponential fits to raw data. B. Same at 3 kHz. C. Same at 5 kHz. Two data points are marked with arrows to show when averages were determined from less than four birds. Representative raw data from excluded birds are shown in Fig. 13.

changes in skew toward lower frequencies during the time of threshold loss and recovery at higher frequencies. By the end of the experiment, the skew returned to normal. Birds II and IV also had no change in filter bandwidth. Their auditory filters skewed toward lower frequencies as well, although the changes in slope were not significant, and the skew changes were also not permanent.

Bird I, the most seriously affected bird, had a nonpermanent broadening of the auditory filter at 1 kHz. The primary change was to the high-frequency slope (measured on

BIRD I
- ● Predose TMTF at 5 kHz
- ⊕ 24 Days after 1st Dose
- ○ 68 Days after 1st Dose

BIRD II
- ● Predose TMTF at 5 kHz
- ⊕ 24 Days after 1st Dose
- ○ 31 Days after 1st Dose

-20 Log m

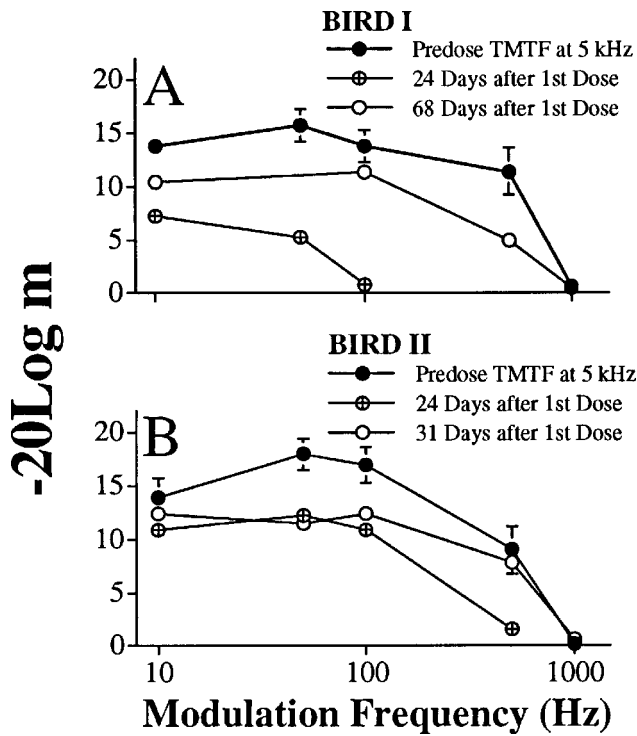**Modulation Frequency (Hz)**

FIG. 13. A. TMTFs from bird I at 5-kHz pre-dose and at two different days after the dose. B. Same for bird II.

days 17 and 36), a pattern not observed at other frequencies. By day 61, the filter had returned to its normal shape.

H. TMTFs: Pre-dose group data[4]

Figure 11A shows group average data for TMTFs with a broadband noise carrier and narrow-band carriers at 30 dBNo. There was no difference in the TMTF as a function of level for broadband carriers or the 5-kHz narrow-band carrier (Fig. 11B and C). The broadband data are consistent with previously published data from starlings (Klump and Okanoya, 1991).

- ▲ Broad Band Noise TMTF 30 dBNo
- ○ 5 kHz Quiet Threshold
- ▽ 7 kHz Quiet Threshold

Shift of TMTF Cut Off (Hz)

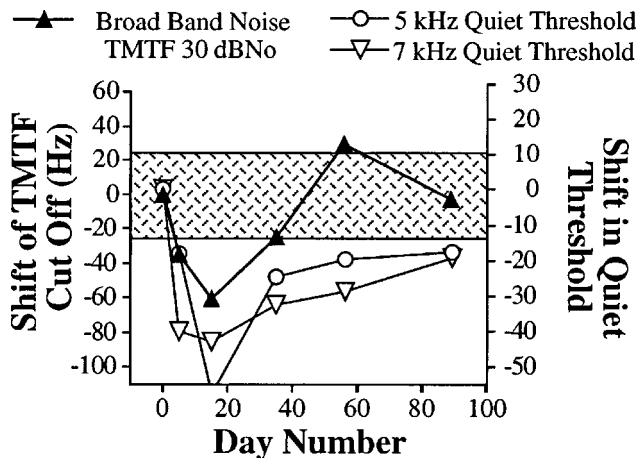**Shift in Quiet Threshold**

Day Number

FIG. 14. Average change in the upper frequency cutoff of the TMTF with a broadband 30-dBNo carrier (left ordinate) plotted along with average shift in auditory threshold on the same days at 5 kHz and 7 kHz. The darkened area shows ±2 standard error of the mean of predose TMTFs in this condition.

TMTF bandwidth was determined by the 1/2 power points of group and individual functions, which were calculated from exponential curves fitted to raw data. Group average TMTF bandwidth was 49 Hz for the narrow-band carriers; performance in a broadband noise was about 100 Hz better (149 Hz, with individual averages ranging from 115 to 155 Hz). Although generally less sensitive than humans to low-frequency amplitude modulation, the upper cutoff of the TMTF bandwidth is higher for starlings (see also Dooling and Searcy, 1981; Klump and Okanoya, 1991).

I. Post-dose changes in the TMTF

No change in TMTF bandwidth cutoff occurred at 1 and 3 kHz for any bird (see Fig. 12A–B). No apparent change occurred in the TMTF at 5 kHz in terms of the group average bandwidth, but Bird I and Bird II had functions which showed a severe loss in sensitivity soon after the dosing period. When sensitivity was reduced to a point where data could not be obtained from at least four modulation frequencies, exponential fits could not be obtained. Thus the second and third data points in Fig. 12C (indicated by arrows) do not reflect the performance of all four birds.

Examples of some of the less sensitive functions are shown in Fig. 13A–B. The obvious changes which occurred for Birds I and II immediately following the dose period coincided with the period of maximum hearing loss at 5 kHz. Energy from the carrier band may have been available to less impaired regions of the basilar papilla in Birds III and IV which is why the performance of these birds did not change. The TMTF bandwidth quickly returned to normal in Birds I and II as soon as the stimuli became audible within the carrier band.

Figure 14 shows the shift of the upper frequency cutoff averaged for all birds for broadband TMTFs at 30 dBNo (left ordinate). Also shown are high-frequency absolute thresholds measured on the same day. The cutoff is lower when the audible portion of the stimulus is restricted to lower frequencies. As soon as threshold recovered to a point where frequencies above 4 kHz were audible at this stimulus level, TMTF bandwidth returned to normal. No significant change in TMTF bandwidth occurred in the 50-dBNo condition, indicating that sensation level was sufficiently compensated for at the higher intensity.

J. Scanning EM

The basilar papillae of all four birds were examined with SEM. Measurements were made along the superior edge from the base toward the apex to measure the extent of previous damage due to the aminoglycoside ototoxicity. This was identifiable from regions of hair cell disorientation which was limited to regenerated portions of the epithelium (Marean et al., 1993, 1995). The proportion of regenerated epithelium was compared to a frequency place code map available from Manley (1990). As in our previous work, the spread of threshold shift to lower frequencies is predicted (and vice versa) by comparing previously damaged regions to the map. We used these observations simply to confirm that the association between damage patterns and threshold

shifts seen in our earlier work was true for the current group of animals. The reader is referred to Marean *et al.* (1993) for representative scanning electron micrographs.

III. DISCUSSION

A. Normal hearing in starlings

The pre-dose performance of starlings in the current study replicates and confirms recent published reports from other laboratories. In the case of frequency resolution, Langemann *et al.* (1995) measured critical bands and critical ratios in starlings and found that the critical band grew from 135 Hz at 1 kHz (13.5% of center frequency) to 233 Hz at 2 kHz (11.6% of CF), to 345 Hz at 4 kHz (8.6% of CF). In the current study, -3 dB filter bandwidth increased from 135 Hz at 1 kHz (13.5% of CF) to 382 Hz at 3 kHz (12.7% of CF) to 480 Hz at 5 kHz (9.6% of CF). Equivalent rectangular bandwidth (ERB) estimates (Glasberg and Moore, 1990) of our data are slightly wider at each frequency (16.87% at 1 kHz, 13.47% at 3 kHz, and 11% at 5 kHz). ERBs are probably a more appropriate comparison measure, since the ERB of an auditory filter is meant to estimate critical bandwidth from notched-noise data. The frequencies above 1 kHz were not the same in the two studies, but curves fitted to show the growth of bandwidth with frequency in each study show a parallel increase, with our estimates being slightly wider. Both studies show the widening of the auditory filter in absolute Hertz, but a relative narrowing of the filter as a proportion of center frequency, characteristic of most vertebrates.

The only notable difference between Langemann *et al.* (1995) and the current study was the behavior of the critical ratio. In that study, the critical ratio estimated the critical band without a correction factor. This result was also apparent in our data, but only at 1 kHz. At 3 and 5 kHz, thresholds in notch widths of 0%[5] showed that threshold improved with increasing frequency, which incorrectly indicates that the auditory filter narrowed in absolute Hertz. Our auditory filter data revealed, however, that the filter was widening in absolute Hertz with increasing frequency, as expected. The auditory filter data also revealed that birds became increasing efficient in extracting signals from noise at higher frequencies (at least up to 5 kHz). When our critical ratio data were corrected for changes in efficiency, they accurately predicted our ERB estimates. This result demonstrates the importance of using a measure which can estimate efficiency, since there are evidently differences between populations of birds in efficiency as a function of frequency.

In the case of temporal resolution, the current findings concur with previously reported TMTFs in both parakeets (Dooling and Searcy, 1981) and Starlings (Klump and Okanoya, 1991). These studies show the transfer function for modulation detection to be low pass or band pass, with the function ending at about 1.2 kHz modulation rate.

B. Effects of hearing loss on frequency and temporal resolution

In humans, hearing loss is associated with an increase in the critical ratio and a broadening of the auditory filter (see Moore, 1995 for a review). Hearing impairment in birds clearly shows the same effects (see also Hashino and Sokabe, 1989).

Tyler *et al.* (1984) measured auditory filters in hearing impaired human listeners at 2 kHz. Their results showed that similar patterns of hearing loss can lead to very different behavior of the auditory filter (assuming that the pre-hearing loss auditory filters of listeners were similar). Some listeners had an decrease in slope to the high-frequency side of the filter, while others showed the same effect on the low-frequency side. In the current study, most of the variability in the behavior of auditory filters occurred at frequencies without hearing loss. At 5 kHz, where all birds had significant threshold shifts, the auditory filter broadened on both the high- and low-frequency sides, although the low-frequency slope decreased on average more than the high-frequency slope. The only marked variability was the degree of filter broadening. One bird's filter broadened by 2800 Hz, while another broadened by only 400 Hz. At 3 and 1 kHz, on the other hand, a variety of outcomes were observed. These changes were typically changes in the skew of the filter rather than the bandwidth, although bandwidth changes did occur at 1 kHz for one bird.

Individual differences to filter shape in humans have been attributed to selective impairments to either inner hair cells or outer hair cells (see Liberman *et al.*, 1986). In the case of birds, the initial effects of ototoxicity are not selective to presumably analogous tall hair cells or short hair cells (Marean *et al.*, 1993, 1995), although selective differences in their regenerative capacity could play a role in the recovery of function.

There has been considerable debate in the human psychophysics literature regarding hearing impairment and impairments to temporal resolution. Several studies in humans have shown that when hearing impaired subjects and normal hearing subjects are tested at equal sensation levels, they can perform equivalently (Florentine and Buus, 1984; Grose *et al.*, 1989; Bacon and Viemeister, 1985; Bacon and Gleitman, 1992; Turner *et al.*, 1995). Our data were consistent with the assertion that changes in threshold have little to no effect on temporal resolution, but we did not hold sensation level constant for the 5-kHz carrier in the current study. Two of the birds could either not perform the task at all, or could only respond to low-frequency amplitude modulation during the period of greatest threshold shift at 4, 5, and 7 kHz.

In the case of the broadband noise carriers, the data were consistent with a reduced internal bandwidth of the stimulus as a result of high-frequency hearing loss. Shailer and Moore (1983) and Formby and Muir (1988) have shown that performance on temporal tasks improves with increasing bandwidth of the stimulus. The restricted range of frequencies due to hearing loss in the current study would have reduced the effective bandwidth of the broadband carrier. However, as soon as the 30-dBNo carriers became audible in the region of impaired hearing, the effective bandwidth increased and performance returned to normal. For broadband carriers, 50 dBNo sufficiently maintained effective internal bandwidth, and thus no changes in temporal resolution were observed.

C. Recovery of function

Hair cell regeneration led to functional recovery of threshold, frequency resolution, and temporal resolution. The degree of functional recovery varied based on several factors, some known and some unknown. One factor which was clearly related to functional recovery was degree of impairment. Mild to moderate impairments were not permanent, but severe to profound hearing loss resulted in some degree of permanent threshold shift. However, in both Marean *et al.* (1993) and the current study, one out of four birds had complete recovery of threshold at 5 kHz, although complete recovery of threshold was not observed at 4 or 7 kHz in both of these birds.

A similar statement can be made in the case of frequency resolution. Increases in filter bandwidth of less than a 10% were not permanent. Increases in filter bandwidth of greater than 10% may or may not be permanent. Interestingly, the bird with the greatest increase in filter bandwidth at 5 kHz had normal frequency resolution at 5 kHz by the end of the experiment. Neither resolution at CF (or adjacent frequencies) nor threshold shift predicted which would occur.

All birds show malformed and disoriented stereociliary bundles throughout the region of ototoxic damage, but some birds appear to hear normally despite these structural changes. Permanent changes in hearing may not be solely due, however, to structural changes within the basilar papilla. Temporary hearing loss may have caused changes to the central auditory system in some birds, but not in others. There are, however, a variety of changes which might occur in the avian cochlea to account for individual variation in the recovery of function (see Cotanche *et al.*, 1994, 1991).

Auditory filters at both high and low frequencies typically change their skew toward low frequencies in the regenerated cochlea. Any number of factors may contribute to this behavior, including the loss of off-frequency, high-frequency suppression regions. This explanation does not account for changes in skew without concomitant changes in bandwidth. Salvi *et al.* (1994) showed that damage to high-frequency regions in the chicken cochlea influenced the tuned response of low-frequency regions of the auditory nerve. The skew and occasional bandwidth changes seen at low frequencies in our data with high-frequency hair cell damage is consistent with their findings.

D. Relation of auditory functions

There was no way to predict changes in frequency resolution directly from auditory threshold. There were instances where frequency resolution was normal in the context of permanent threshold shifts (and in one case, Bird II, vice versa). Auditory threshold at adjacent frequencies did not correlate with frequency resolution either. On average, filter bandwidth at 5 kHz was associated with threshold shift at 5 kHz, but there was no way to predict from threshold data which auditory filters would recover completely, and which would not.

One aspect of the relationship between recovery of threshold and recovery of frequency resolution which stands out is that frequency resolution continued to improve after auditory thresholds had stabilized. This was evident in the 5-kHz data from Birds I, III, and IV. Bird II also showed improvement in frequency resolution between week 7 and week 10, but threshold at 5 kHz also improved during this time. By the time thresholds stabilize, the permanent changes in the low-frequency slope of the auditory filter were fixed, and additional improvement tended to be the high-frequency slope.

Changes in frequency resolution appeared to have little or no effect on temporal processing as assessed by the TMTF. Changes to the TMTF bandwidth were limited to time periods when the carrier bands were at extremely low sensation levels. This made obtaining high modulation rate thresholds more difficult for Birds I and II, presumably because even the low-frequency edge of the 5-kHz carrier band was barely audible at 50 dBNo. It is probable that had we increased the intensity of the carrier band during this time, TMTFs might have appeared normal.

It is also *not* the case that wider auditory filters led to *improved* temporal resolution, an idea sometimes discussed in the literature as being hypothetically possible due to changes in the impulse response of wide filters versus narrow ones (e.g., Evans, 1985). Evidently, other than affecting stimulus audibility, regenerated hair cells did not alter the auditory system's ability to follow the temporal properties of a stimulus. Apparently, normal orientation of hair cell bundles was not functionally required for this task.

Data from the current study suggest that more complex auditory behavior could be preserved after hair cell regeneration in birds. The temporal aspects of birdsong, for example, should be well preserved in the regenerated basilar papilla based on TMTFs. Only minor changes to the spectral representation of song would be expected. In humans, frequency resolution is highly correlated to the perception of speech in noise, but not in quiet (Festen and Plomp, 1983). Thus even birds with permanent changes in frequency resolution may be able to perceive song in a controlled setting. The effects of temporary hearing loss on the neural control centers for song behavior are unknown. Thus we cannot conclude at this time that hair cell regeneration results in the restoration of all complex auditory functions, especially those involved in acoustic communication.

[1] The noise bands used in this study were correlated since they were generated by the same source. There is no evidence that having the noise bands generated by a common source resulted in any release from masking, which can occur when tones are presented in the presence of co-modulated noise bands.

[2] In the present experiment we fixed masker level and varied probe level to find threshold. A preferable method would have been to vary masker level and fix probe level in order to keep the overall stimulus power in the auditory filter constant across different notch width conditions. We chose not to use this method for the sake of simplicity in the trial structure which facilitated animal training. See Rosen and Baker (1994) for discussion and comparison of results obtained by these two methods.

[3] Exclusions for quiet thresholds and auditory filter study: Prior to dosing the four birds with kanamycin, a total of 157 sessions were run to obtain thresholds in quiet and in notched-noise, resulting in 835 individual threshold runs. Of these, 22 runs were incomplete, 61 had reversal values above the probe level, 100 had high false alarm rates, and 4 were excluded due to experimenter error. Each bird had approximately 162 usable threshold runs, or roughly five thresholds per condition.

[4] Exclusions for TMTF study: Prior to dosing, 48 sessions resulted in 224 threshold runs. Of these, 30 runs were incomplete, 15 had reversals above the probe levels, and 17 were excluded due to high false alarm rates. There were thus 158 usable threshold runs, a yield rate of 70%. Most of the excluded sessions were from carrier intensities of 50 dBNo. Individual birds in some cases had but one completed function at this higher level.

[5] Broadband noise data are not reported because they did not differ from data obtained at 0% notch widths.

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