Perception of complex sounds in budgerigars (*Melopsittacus undulatus*) with temporary hearing loss

Robert J. Dooling[a)]
Department of Psychology, University of Maryland, College Park, Maryland 20742

Brenda M. Ryals
Department of Communication Sciences and Disorders, James Madison University, Harrisonburg, Virginia 22807

Micheal L. Dent[b)] and Tracy L. Reid
Department of Psychology, University of Maryland, College Park, Maryland 20742

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Songbirds and parrots deafened as nestlings fail to develop normal vocalizations, while birds deafened as adults show a gradual deterioration in the quality and precision of vocal production. Beyond this, little is known about the effect of hearing loss on the perception of vocalizations. Here, we induced temporary hearing loss in budgerigars with kanamycin and tested several aspects of the hearing, including the perception of complex, species-specific vocalizations. The ability of these birds to discriminate among acoustically distinct vocalizations was not impaired but the ability to make fine-grain discriminations among acoustically similar vocalizations was affected, even weeks after the basilar papilla had been repopulated with new hair cells. Interestingly, these birds were initially unable to recognize previously familiar contact calls in a classification task—suggesting that previously familiar vocalizations sounded unfamiliar with new hair cells. Eventually, in spite of slightly elevated absolute thresholds, the performance of birds on discrimination and perceptual recognition of vocalizations tasks returned to original levels. Thus, even though vocalizations may initially sound different with new hair cells, there are only minimal long-term effects of temporary hearing loss on auditory perception, recognition of species-specific vocalizations, or other aspects of acoustic communication in these birds.
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I. INTRODUCTION

It is well known that songbirds and parrots rely on hearing to develop and maintain a normal vocal repertoire (see review in Kroodsma and Miller, 1996). Budgerigars (*Melopsittacus undulatus*) are particularly interesting because they learn new vocalizations throughout life, especially in response to changes in their social milieu (see review in Dooling, 1986; Dooling *et al.*, 1987; Farabaugh *et al.*, 1994; Farabaugh and Dooling, 1996; Brittan-Powell *et al.*, 1997; Hile *et al.*, 2000). In the most extreme case of experimental auditory manipulation in budgerigars, permanent deafening by cochlear removal during development (Dooling *et al.*, 1987; Heaton and Brauth, 1999) or in adulthood (Heaton *et al.*, 1999) results in a dramatically impoverished vocal repertoire. In a more subtle auditory manipulation, recent experiments show that the role of auditory feedback in vocal production can also be more immediate. Budgerigars trained to produce particular vocalizations under controlled experimental conditions exhibit the Lombard effect—an increase in vocal intensity in response to an increase in ambient noise. This is suggestive of a sensitive, real-time monitoring of vocal output as occurs in humans (Manabe *et al.*, 1998).

The importance of hearing for the maintenance of a normal adult vocal repertoire in these birds raises the question of the effects of hearing loss (defined by an increase in absolute thresholds) on the discrimination and perception of vocalizations. To this end, we examine the effect of severe, but temporary, hearing loss on the discrimination and recognition of species-specific vocalizations in budgerigars. Numerous reports have shown that following auditory trauma, birds can regenerate hair cells which lead to varying degrees of physiological and behavioral recovery of hearing (e.g., Corwin and Cotanche, 1988; Ryals and Rubel, 1988; Hashino and Sokabe, 1989; Tucci and Rubel, 1990; Hashino *et al.*, 1991; Lippe *et al.*, 1991; Hashino *et al.*, 1992; Marean *et al.*, 1993; Niemiec *et al.*, 1994; Saunders *et al.*, 1995, 1996; Dooling *et al.*, 1997; Marean *et al.*, 1998; Ryals *et al.*, 1999). Recent reports by Rubel and his colleagues show that the Bengalese Finch, an age-dependent vocal learner, demonstrates both auditory and vocal recovery before hair cells have fully regenerated (Woolley and Rubel, 1999; Woolley *et al.*, 2001; Bermingham-McDonogh and Rubel, 2003). Except for a brief and preliminary earlier summary (Dooling *et al.*, 1997), there are no detailed reports on the effect of temporary hearing loss on the discrimination or recognition of vocalizations in birds that exhibit vocal learning throughout adulthood.

[a)]Corresponding author. Electronic mail: dooling@psyc.umd.edu
[b)]Current address: Department of Psychology, University at Buffalo SUNY, Buffalo, NY 14260.

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In the following experiments, we tested whether hearing, auditory discrimination, and the perception and recognition of complex vocalizations were affected by temporary hearing loss. In our tests we measured absolute thresholds, intensity difference limens (IDLs), frequency difference limens (FDLs), the discrimination between natural and synthetic contact calls, and the identification of contact calls in budgerigars before and after injections of kanamycin. Histological evaluations were also carried out to verify the time course of the loss and regeneration of hair cells. Because budgerigars learn new vocalizations throughout life and rely on hearing to maintain a normal adult vocal repertoire, they may provide an important animal model for some aspects of human hearing and speech. It is well known that hearing impairment in humans, for instance, can have a profound effect on speech perception, and the quality of speech production and acoustic communication.

II. GENERAL METHODS

A. Subjects

Fifteen budgerigars were used in the behavioral experiments. These, as well as an additional 14 birds, were also used for histology. Nonbehavioral birds were sacrificed at intervals sufficient to determine the extent of hair cell loss and recovery over time after cessation of drug injection (at 1 day postkanamycin, $n=3$; at 9–14 days postkanamycin, $n=4$; at 30 days postkanamycin, $n=3$; and control birds, $n=4$). The birds were individually housed in an aviary at the University of Maryland and kept on a normal photoperiod correlated with the season. During behavioral testing, the birds were food deprived to 85–90% of their free-feeding weights. Animal care and housing met all standards of the Animal Care and Use Committee at the University of Maryland.

B. Drug injections

The birds were injected with kanamycin for 8–10 days. The single daily intramuscular injections were 100 mg/kg the first day and 200 mg/kg each day after that. The injections were in the pectoris muscle, and the injection site changed from day to day. The birds were weighed daily and injected at approximately the same time every day using a Hamilton (microliter No. 710) 0.1 ml glass syringe with a 30G1/2 needle. Psychophysical tests were carried out before, in some cases during, and after the administration of kanamycin. Long-term psychophysical tests were conducted at approximately biweekly intervals for the next 24 weeks following the end of injections.

C. Behavioral testing apparatus

The testing apparatus has been described in detail previously (Okanoya and Dooling, 1987; Dooling and Okanoya, 1995). A custom-made operant chamber with a food hopper and two response keys consisting of light-emitting diodes and microswitches was used. A standard pigeon grain hopper delivered food with response keys mounted just above the hopper opening. The stimuli were delivered from a loudspeaker mounted above the test cage (Realistic Soft Dome midrange speaker, Model 40-1281A). The entire operant apparatus was suspended in a small animal sound isolation chamber (Industrial Acoustics, Model IAC-3). A microcomputer controlled all experimental events including stimulus delivery and reinforcement contingencies. The behavior of the animals during test sessions was monitored by a video camera.

D. Auditory stimuli and stimulus calibration

Pure tone stimuli ranging from 500 Hz to 5700 Hz (300 ms duration, 5 ms rise/fall times) were used in Experiments 1 and 2. Species-specific contact calls were used in Experiments 2 and 3. These contact calls were recorded from five budgerigars using a Simul-Sync 4-track Teac tape recorder (Model A-3440). To elicit contact calls, birds were isolated individually in small sound-isolation chambers (Industrial Acoustics Company, Model AC1), each fitted with an omnidirectional Realistic Electret Microphone (Model 33-1063) connected to separate tracks of the tape recorder. After at least 30 min of isolation, the doors to the chambers were opened slightly so the birds could hear the faint calls of the other birds. The computer software package SIGNAL (Beeman, 1992) was used to create synthetic versions of the recorded contact calls. The calls were analyzed using overlapping, serial fast Fourier transformations and then synthesized by reconstructing the peak frequency trace and amplitude contour throughout the call (see, for example, Dooling and Okanoya, 1995).

Stimulus calibration was performed using a General Radio Model 1982 sound-level meter with octave band filters. Sound pressure levels (SPLs) in the test chamber were measured by placing the microphone in front of the keys in the position normally occupied by the bird's head during testing. The intensities of test tones and calls were measured several times during the testing phase to ensure they remained constant.

E. Training and testing procedures

The training and testing procedures have been described in detail previously (Okanoya and Dooling, 1991; Dooling and Okanoya, 1995). Briefly, the birds were trained by a standard operant autoshaping program to peck at the left microswitch key (observation key) until a tone was presented. Then, a peck to the right microswitch key (report key) within 2 s of stimulus presentation produced food reinforcement. A failure to peck the report key was recorded as a miss, and a new trial was started. 30% of all trials were sham trials in which no tone was presented. A peck to the report key during a sham trial was recorded as a false alarm. Sessions with a false alarm rate of 16% or higher were discarded.

The birds were tested in two daily sessions typically consisting of about 100 trials each. Stimuli were presented according to the method of constant stimuli and the targets were chosen so that the lowest target tested was below the bird's suspected threshold while the highest targets were well

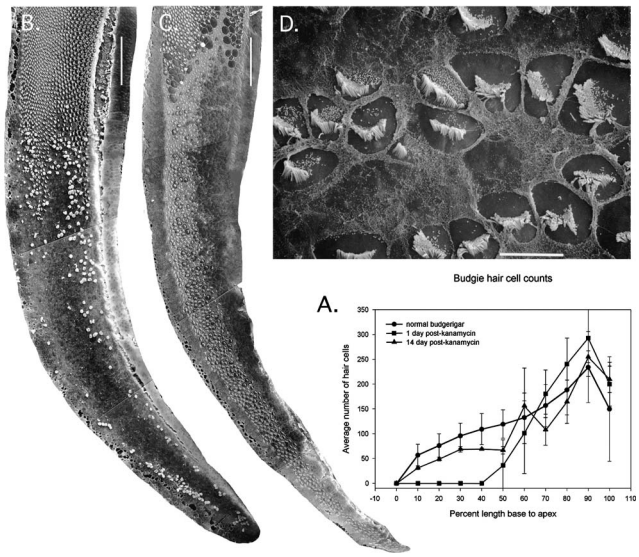

FIG. 1. (A) Average number of hair cells counted from 700× SEM montages at regular intervals from base to apex. Hair cells are completely absent from the basal 40% of the papilla one day after the cessation of kanamycin injections in all birds examined ($n=3$). Hair cell number has increased to nearly normal levels within 14 days of kanamycin cessation ($n=4$). Error bars represent standard deviations. (B) SEM photomicrograph of the basal half of the basilar papilla in a bird one day after the cessation of eight days of kanamycin injections (bar=100μ). Swollen hair cells, ejected from the epithelia are seen as large white blebs on the surface of the papilla. (C) SEM photomicrograph of the basal half of the basilar papilla in a bird 14 days after cessation of 8 days of kanamycin injections (bar=100μ). (D) Higher magnification (bar=10μ) of area on the basilar papilla of a bird 14 days after kanamycin injections. Areas with small microvilli are the swollen surfaces of supporting cells.

above threshold. Threshold was defined as the target level that the bird detected 50% of the time (Dooling and Okanoya, 1995).

III. ANATOMICAL EXPERIMENTS

A. Experiment 1—Histology

1. Methods

Basilar papillae were fixed (3.5% glutaraldehyde followed by 1% osmium tetroxide) and dehydrated to 70% ethanol. Then, the tectorial membrane was removed, dehydrated to 100% and critical point dried in CO_2. Papillae were mounted on aluminum stubs, sputter coated with gold, and viewed at 15 kV using either an Amray 1820 or a Joel 820 scanning electron microscope (SEM). In order to quantify the degree and location of hair cell loss, hair cell counts were made from SEM montages (700×). Montages were divided into ten equidistant intervals (approximately 10% increments of length) from basal to apical tip. Each interval was 60 μm wide (along the basal to apical dimension) and extended from neural to abneural edge; hair cells were counted in each of these ten intervals. This method for hair cell counts from SEM montages is similar to that used by other investigators (see, for example, Gleich and Manley, 1988).

2. Results

Figure 1 shows examples of papillae one day [Fig. 1(B)] and 14 days [Fig. 1(C)] after an 8-day course of kanamycin injections. Hair cells are completely absent in the basal 40% of the papilla immediately after kanamycin injections cease, but return to nearly normal numbers within 14 days [Fig. 1(A)]. While there are no published place-frequency maps for the budgerigar, estimates from other avian species predict that this hair cell loss likely corresponds to hearing for frequencies above 500–1000 Hz (Gleich and Manley, 2000). Even though the hair cell number has nearly returned to normal within 14 days, swelling of support cells continues and small regenerating hair cells continue to be present [see Fig. 1(D)]. Our histological results are comparable to those reported by Hashino *et al.* (1992) and Hashino and Sokabe (1989). The hair cell number continues to increase, but at a slower rate, over the next several weeks. Figure 2(A) shows the number of hair cells present in several of the birds tested behaviorally in the present experiments and sacrificed at either 2–3 months postkanamycin ($n=6$) or 7 months postkanamycin ($n=4$). At 2–3 months postkanamycin treatment, even though hair cell number is within one standard deviation of normal, some hair cell abnormalities (multiple and/or abnormal stereovilli bundles, and abnormal stereovilli bundle orientation) and regenerating hair cells are present [see Fig. 2(B)]. After 7 month survival, no immature hair cells were seen, but stereovilli bundle orientation remained irregular in the area where hair cells had been completely lost [see Fig. 2(C)]. These abnormalities are similar to those reported in other avian hair cell stereovilli after ototoxic drug administration (Cotanche, 1999) and remain in the basal portion of the papilla, even after 7 months.

IV. PSYCHOPHYSICAL EXPERIMENTS

A. Experiment 1 - Absolute thresholds

1. Methods

To track hearing loss, three birds were tested on the detection of pure tones in quiet. Within a ten-trial block, the test tone was presented at seven different predetermined levels using a step size of 3 dB. In addition, three sham trials were included in each block of ten trials. The levels were preselected so that only the quietest one or two tones could not be heard by the birds, whereas the loudest tones were always above threshold. In all, thresholds were measured at six tone frequencies (at 0.5, 1.0, 2.0, 2.86, 4.0, and 5.7 kHz) before and after kanamycin injections. Each bird was run on at least 100 trials weekly at each of the six frequencies following the injections for up to 24 weeks total.

2. Results

As is typical of birds (Marean *et al.*, 1998; Woolley *et al.*, 2001), and as summarized earlier for budgerigars (Dooling *et al.*, 1997), absolute thresholds were elevated at all frequencies following kanamycin injections, but the hearing loss was especially severe at high frequencies as shown in Fig. 3. Within a few weeks of kanamycin treatment, threshold shift was greatest (50–60 dB) at frequencies above 2 kHz and less (10–30 dB) at frequencies below 2 kHz. By 20 weeks following injections, absolute thresholds improved to asymptotic levels of within 10–15 dB of normal at low frequencies at about 20–30 dB at higher frequencies. Recov-

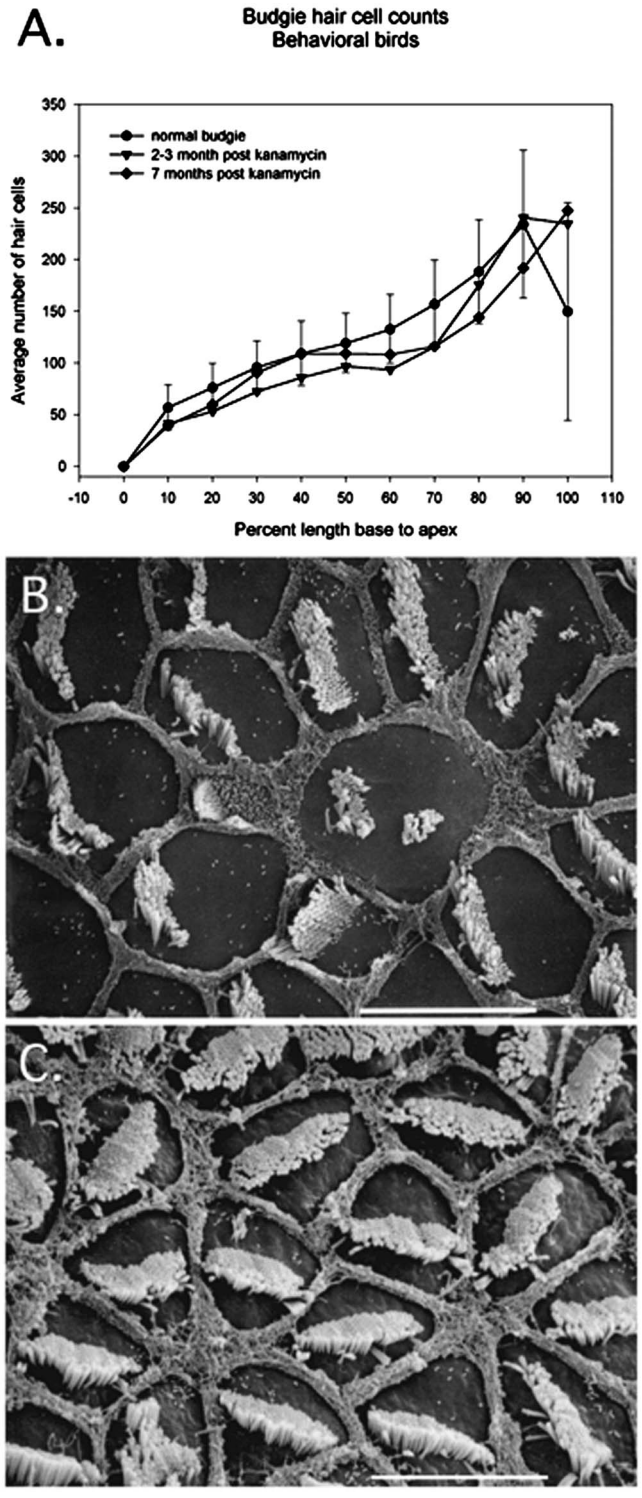

FIG. 2. (A) Average hair cell counts in birds tested behaviorally and allowed to survive either 2–3 months ($n=6$) or 7 months ($n=4$) after kanamycin injections. Hair cell number is within one standard deviation of normal for each group. (B) SEM photomicrograph of hair cell abnormalities (cell with two stereovilli bundles) and regenerating hair cell in a bird sacrificed 2–3 months after kanamycin injections (bar=10μ). (C) SEM photomicrograph of hair cells with abnormal stereovilli bundle polarity 7 months after kanamycin injections. These disordered stereovilli orientation patterns are similar to those previously reported in other avian species (Cotanche, 1999).

ery proceeded most quickly at low frequencies and slower at high frequencies but a permanent threshold shift was evident at all frequencies.

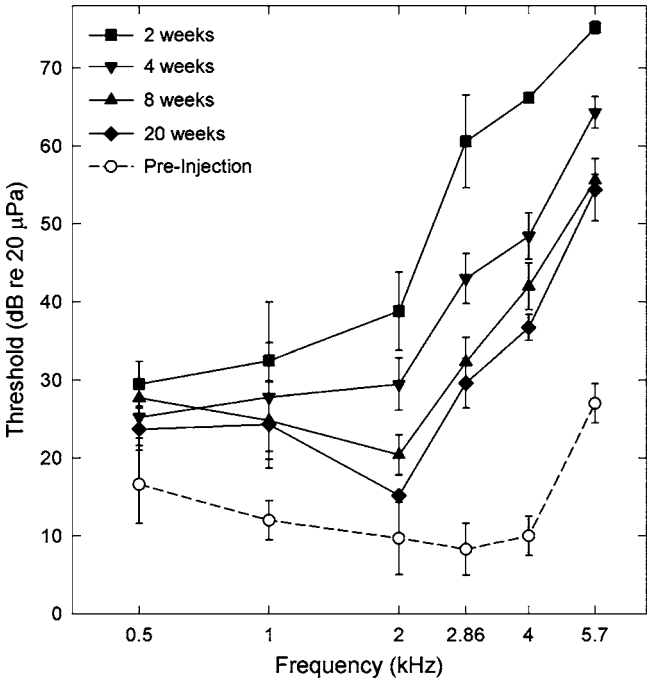

FIG. 3. Hearing loss audiograms for three budgerigars prior to injections (open circles) and 2 weeks (closed squares), 4 weeks (closed upside-down triangles), 8 weeks (closed triangles), and 20 weeks (closed diamonds) following kamanycin treatment. Error bars represent between subject standard errors.

B. Experiment 2—Discrimination tests: Intensity and frequency difference limens and the discrimination of contact calls

1. Methods

For these experiments, three different birds were each tested on three different discrimination tasks. These tasks included IDLs, FDLs, and the discrimination of species-specific contact calls. For all tests, the birds were required to discriminate a change in a repeating background of sound. For the measurement of difference limens, the repeating background consisted of either 1.0 or 2.86 kHz pure tones played at a rate of 2/s at a level of 65 dB SPL [measured as root-mean square (rms) on the fast scale of the sound level meter]. For the IDLs, test tones were presented at seven different predetermined levels increasing in step sizes of 3 dB (three sham trials were included in each block) within a ten-trial block. For the FDLs, the test tones were presented at seven different predetermined frequencies increasing in step sizes of 5 Hz (three sham trials were also included in each block of ten trials). For each bird, a minimum of a 100 trials were collected on IDLs and FDLs immediately before kanamycin injections and approximately every three weeks following injections for a total of 19 weeks.

Another experiment was conducted to determine the relation between the detection and discrimination of standard pure tone stimuli and the discrimination of vocalizations that these birds use in communication. A set of vocalizations were prepared consisting of five different natural species-specific contact calls and their synthetic analogs as shown in Fig. 4(A) (see Dooling and Okanoya, 1995). The birds were tested on a pair-wise discrimination of all possible combina-

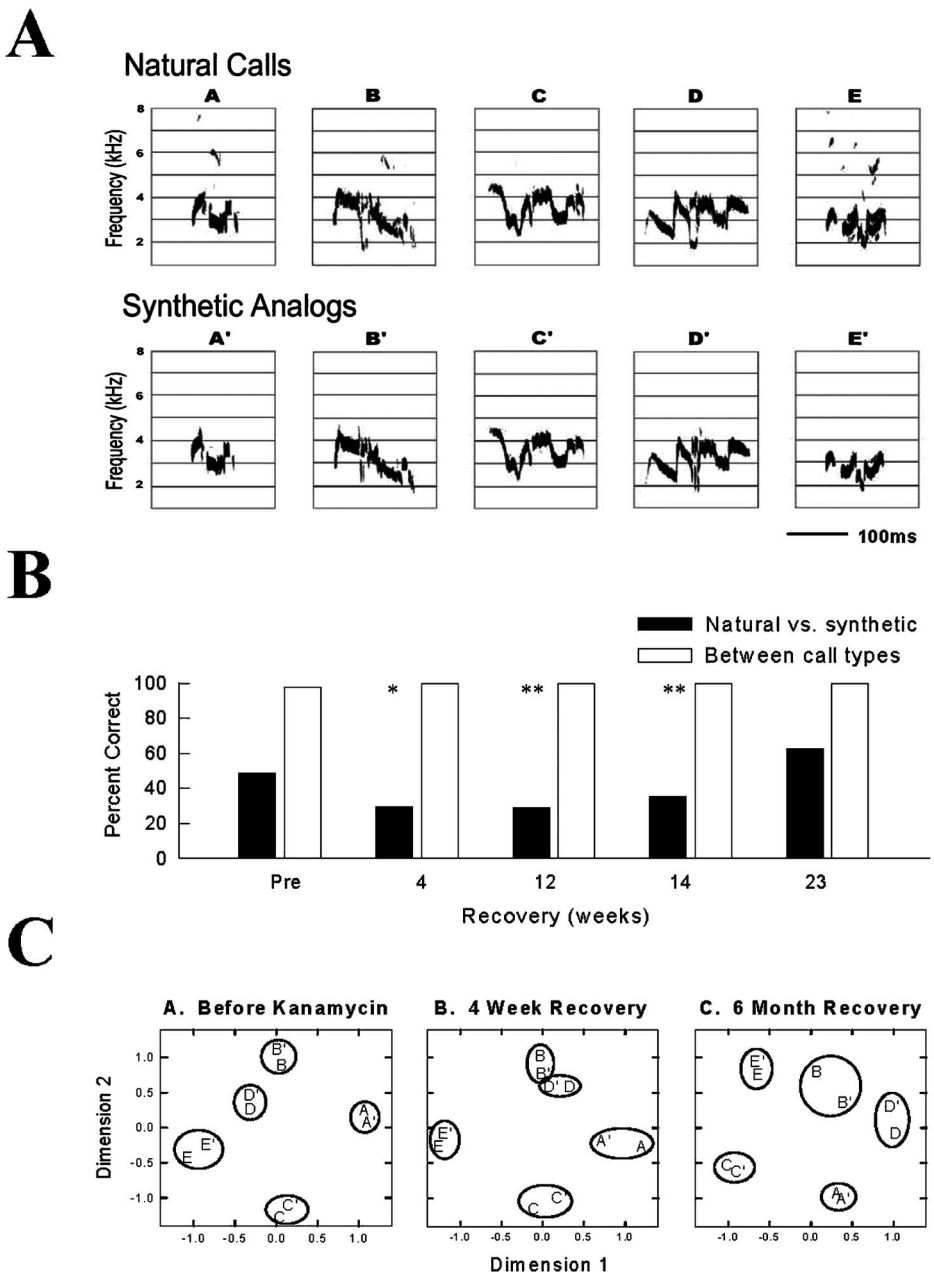

FIG. 4. (A) Sonograms of contact calls from five different birds and their synthetic analogs plotted as frequency by time (taken from Dooling and Okanoya, 1995). (B) The average percent correct for three budgerigars discriminating among the five natural contact calls and their synthetic analogs before treatment with kanamycin and at 4, 12, 14, and 23 weeks after injections. The stars represent significantly different results from the preinjection condition (single star: $p=.05$, double star: $p<.05$). (C) Two-dimensional perceptual maps by MDS from three budgerigars tested before kanamycin treatment (a), after 4 weeks of recovery (b), and after 6 months of recovery (c).

tions of the ten calls shown in Fig. 4(A). This set of calls was designed to allow for both fairly easy discriminations (e.g., between call types as between A and B) and more difficult discriminations (e.g., as between A and A′)—a discrimination that humans find impossible and birds find very difficult (Dooling *et al.*, 1997). During testing, the calls were played out at a rms level of 65 dB SPL measured at the birds' ears. Sessions consisted of 100 trials with one call selected as the repeating background and the other calls selected as targets. This procedure continued until all possible combinations of calls were tested in random order. Each bird was tested on the entire ten-call set both before and approximately every 4 weeks following injections up to about 24 weeks following cessation of kanamycin injections.

2. Results

Though kanamycin treated birds have a substantial hearing loss remaining at 4 weeks following kanamycin injections (as shown in Experiment 1, Fig. 3), FDLs and IDLs are not significantly different from prekanamycin thresholds (see Fig. 5). For all three budgerigars in Experiment 2, FDLs appeared to be only slightly elevated at both frequencies at 4–6 weeks following the cessation of injections. A repeated measures analysis of variance (ANOVA) for each frequency before and at three time periods following kanamycin injections showed that FDLs were not significantly different at any of the subsequent time periods up to 19 weeks at 1.0 kHz $[F(2,6)=2.15, p>0.05]$ or 2.86 kHz $[F(2,6)=0.27, p>0.05]$, IDLs were also only slightly elevated at both frequencies at 4–6 weeks after injections but a repeated measures ANOVA for each frequency showed that IDLs were not significantly different at any of the time periods either at 1.0 kHz $[F(2,6)=1.47, p>0.05]$ or 2.86 kHz $[F(2,6)=1.00, p>0.05]$.

In discriminating among the natural and synthetic contact calls before and after kanamycin injections, all birds

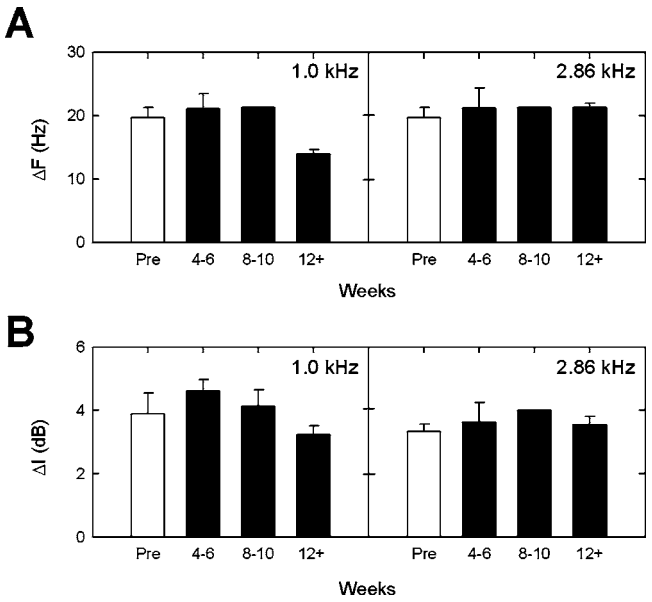

FIG. 5. (A) FDLs and (B) IDLs for 1.0 and 2.86 kHz pure tones before, and at three time periods following injections. There is no negative effect of kanamycin KM treatment on these difference limens and there is even a suggestion of improvement at both frequency and intensity difference limens at 1.0 kHz after 3 months. Error bars represent standard errors.

showed similar patterns. The more difficult acoustic discriminations in the test set as between natural calls and their synthetic analogues were significantly affected for up to 14 weeks. Only at 23 weeks did discrimination return to pre-kanamycin levels [Fig. 4(B)]. Easy disciminations, as between contact calls from different birds, were discriminated at nearly 100% before kanamycin injections and were hardly affected at four weeks following kanamycin treatment. Paired *t*-tests were used to compare each week of testing to pretesting discrimination performance. Discrimination performance at 4 weeks $[t(2)=4.99, p=0.05]$, 8 weeks $[t(2)=4.99, p<0.05]$, and 12 weeks $[t(2)=10.99, p<0.05]$ following kanamycin injections were found to be significantly different from preinjection levels.

These data were also analyzed using a three-way multidimensional scaling (MDS) algorithm (SYSTAT, v7.01, 1997). A matrix of response latencies was constructed where each call in the set was related to every other call in the test set by the response latency. Previous work has shown that a matrix of such response latencies for birds has properties of a similarity matrix in which longer latencies are correlated with greater stimulus similarity (see Dooling and Okanoya, 1995; Dooling *et al.*, 1990). MDS places calls in a multidimensional space in such a way that perceptual similarity is represented by spatial proximity (Dooling and Okanoya, 1995; Shepard, 1980). Two-dimensional MDS solutions are shown in Fig. 4(C) for the combined analysis of the three birds tested on the call set before kanamycin injections, and at postinjection periods of 4 weeks and 6 months. These MDS solutions accounted for 73%, 73%, and 72% of the variance in the birds' response latencies, respectively, before kanamycin treatment, at 4 weeks and at 6 months postinjection.

Before kanamycin treatment, the calls were arranged in perceptual space as pairs, with each natural call close in proximity to its synthetic analog. In other words, the birds discriminated more quickly on trials involving two different contact call types (i.e., calls from different birds such as A versus B) than they did on trials involving a natural contact call and its synthetic analog (i.e., such as A and A′). At 4 weeks following kanamycin treatment, the perceptual space was still significantly distorted by this analysis since two pairs of calls that were previously separated before kanamycin treatment, were now clustered together. At 6 months following kanamycin, however, the birds' perceptual space, in terms of separation of call types, more closely approximated the pattern seen before kanamycin treatment. These results show that frequency and intensity difference limens, as well as relatively easy discriminations between complex sounds such as species-specific vocalizations (contact calls), are relatively unaffected. Even so, a more refined analysis of response latencies which can be used to define perceptual categories show minor perturbations in perceptual space still apparent at 16 weeks.

C. Experiment 3—Recognition tests: Identification of previously familiar contact calls

From the previous experiments, we know that discrimination of frequency and intensity differences in pure tones is relatively unaffected after only four weeks of recovery and discrimination between similar contact call types (as measured by percent correct) also returns to pretreatment levels by that time. However, the analyses of response latencies in discrimination tests with contact calls using MDS also show that the birds' perceptual space for contact calls at this recovery time point is still distorted in that two contact calls that were perceptually distinct prior to kanamycin treatment, sound similar to birds at four weeks following kanamycin.

This suggests a more subtle and complicated effect of hearing loss on the perception of complex sounds in these birds. Taking speech as another example, there are a number of ways to assess the role of hearing loss in speech perception. We know from a variety of perceptual tests that it is one thing to hear speech, or even to discriminate among speech sounds, but it is quite another thing to understand what is being said (e.g., Newby, 1964). The next experiment was designed to explore the phenomenon of complex sound perception during hearing loss from a slightly different perspective. To address this last point, we assessed auditory perception during and after severe, but temporary, threshold shifts using a recognition task involving contact calls. In other words, birds were trained to recognize and classify two different contact calls and then were treated with kanamycin and retested on the recognition of those calls.

1. Methods

In this experiment, six new experimental birds were tested with a different behavioral procedure. This procedure tested the birds on a Go/NoGo task which assessed their ability to classify contact calls before and after kanamycin injections. This task was more difficult than the discrimination task described above in that the birds had to remember

from trial to trial which call was the "Go" call and which call was the "NoGo" call. The natural contact calls used in this test were produced by birds in another flock that the experimental subjects had never heard before. During testing these calls were presented at a rms level of 65 dB SPL.

Testing occurred in the same apparatus as in the detection and discrimination experiments. However, here the budgerigars were required to peck the observation key just once to hear one presentation of one of two contact calls—either a Go call or a NoGo call. A correct response when presented with the Go call was to peck the report key within 2 s to receive food. A correct response when presented with the NoGo call was to withhold responding on the report key for an interval of 5 s. Correct responses on NoGo trials were not rewarded but incorrect responses were punished with a blackout. If the birds pecked the report key within 5 s of the presentation of a NoGo call, the lights in the test chamber were extinguished for 10 s during which another trial could not be initiated.

During initial training, the birds always pecked the report key when presented with either call resulting in an overall performance level of 50% correct (100% correct on the Go trials and 0% correct on the NoGo trials). With continued testing, however, the birds learned to withhold responding when the NoGo contact call was presented. The birds were run in 100 trial sessions until they had achieved a criterion level of at least 85% correct for three successive 100-trial sessions.

Once trained, the six birds were tested daily for five days before and then the ten days during injections. Following the cessation of injections, they were tested again for one session at day 24 and again at 38 days (4 weeks following the end of injections). Daily testing also resumed at day 38 and the number of sessions it took for these birds to reach criterion performance was measured. Four birds were trained and tested on the same pair of contact calls and two birds were tested on a different pair of contact calls.

Prior to being tested, four of the birds were trained to criterion and then given a 2 week pause in testing and then retested on the same task involving the same pair of vocalizations. Two of these birds and two of the others were also retrained to criterion and then given a 4-week pause in testing, and then retested on the same vocalizations. These control tests measuring the birds' performance after a two or four week pause in testing served as a control for the absence of testing during recovery from kanamycin. Past experiments (Park and Dooling, 1985) have shown that birds trained and tested under these conditions maintain much of their performance even after long periods of no testing. An additional group of birds ($n=3$) was trained to criterion on the original pair of contact calls, then switched to a new pair of contact calls, and tested daily until criterion performance was reached on the new vocalizations. This condition also provides important control information for interpreting the effects of hearing loss by kanamycin treatment on vocal recognition by establishing the time it takes to learn to classify a new pair of contact calls.

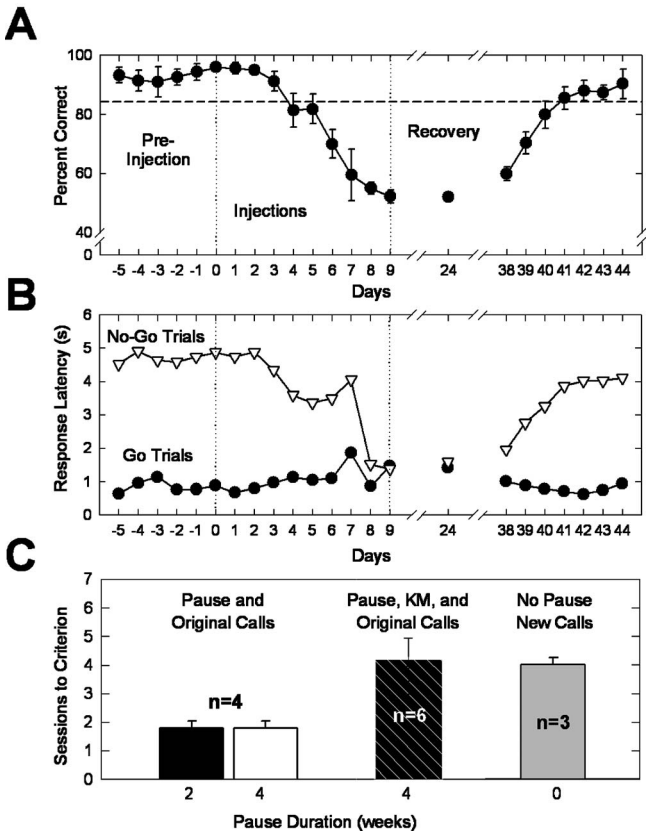

FIG. 6. (A) Average percent correct responses of six budgerigars on a Go/NoGo recognition task involving two contact calls before, during, and after treatment with kanamycin. 4 weeks following the end of kanamycin treatment, classification performance returns to preinjection levels after 4 days of testing. Error bars represent standard errors. (B) The average response latencies to both Go and NoGo stimuli for the six budgerigars before, during, and after kanamycin treatment. (C) Summary of the test sessions required to reach criterion on original calls or new calls with or without antibiotics and a pause in testing. Error bars represent standard errors.

2. Results

Figure 6(A) shows the average percent correct for the six budgerigars on the classification task before, during and after ten days of kanamycin injections. Prior to injections of kanamycin, the birds' performance was well above the 85% criterion level. However, performance falls to chance levels (50%) after several days of kanamycin injections and remains there when assessed in a single 100-trial test session 24 and 38 days later. At 38 days (4 weeks following cessation of kanamycin injections), the birds were retrained and tested daily on the same task in 100-trial sessions. The birds returned to preinjection performance levels in four days.

Response latencies for the Go stimuli remained below 1 s and were relatively unchanged throughout the experiment, attesting to the birds' excellent health, attentiveness, and behavioral responsiveness [Fig. 6(B)]. Response latencies to the NoGo stimuli averaged near 5 s (the duration of the response interval) before and during the first few days of treatment with kanamycin. As testing continued, the birds began to respond by pecking the report key to both Go and NoGo stimuli. Response latencies to the NoGo stimuli approached the levels recorded to the Go stimuli, indicating that the birds were unable to recognize the NoGo stimuli and performance fell to chance responding.

The six kanamycin-treated birds tested after four weeks following kanamycin treatment required an average of over four 100-trial sessions to reach criterion instead of the average of less than two 100-trial sessions to reach criterion following a 4 week pause in testing but with no kanamycin treatment. These results are summarized in Fig. 6(C). The first two control conditions are for the four noninjected birds that were given either a 2-week or a 4-week pause in testing. Because these calls were familiar, the time to criterion was much less than the time required to learn the new classification. Birds performing above criterion on one pair of contact calls (original calls) and switched to another pair (new calls) also required slightly over four 100-trial sessions to reach criterion. These results suggest that previously familiar contact calls do not sound the same to budgerigars who have been treated with kanamycin even though they have undoubtedly regained their full complement of hair cells. Instead, the birds behave as if these calls sound unfamiliar as indicated by the time required to relearn the classification. While the exact cause of this phenomenon is unclear, we know even at 2–3 months postkanamycin treatment, some hair cell abnormalities remain in the basal portion of the papilla including multiple and/or abnormal stereovilli bundles, and abnormal stereovilli bundle orientation, some immature hair cells, and an irregular pattern of hair cells as has been reported in other avian species after ototoxic drug administration (e.g. Cotanche, 1999).

V. DISCUSSION

An earlier study demonstrated that budgerigars show changes in vocalizations produced under operant control around the time when hair cell loss from aminoglycoside administration was greatest (Dooling *et al.*, 1997). Here, we address the effect of a severe but temporary threshold shift on the hearing and auditory perception of vocalizations in budgerigars in more detail. Absolute thresholds in budgerigars recover considerably by 4–8 weeks following aminoglycoside administration. These results are generally similar to those found previously both behaviorally or physiologically in budgerigars as well as several other species of birds including starlings, chicks, and Bengalese finches (Dooling *et al.*, 1997; Hashino and Sokabe, 1989; Marean *et al.* 1993; 1998; Tucci and Rubel, 1990). The time course of hearing recovery and the amount of permanent threshold shift in budgerigars suggests that they may be more sensitive to damage from ototoxic antibiotics than are starlings or Bengalese finches (Marean *et al.* 1993; 1998; Woolley *et al.*, 2001). The present findings also show that frequency and intensity difference limens 4 weeks after kanamycin treatment in budgerigars are already at near normal levels and remain so for the duration of testing out to as long as four months. We could infer from these data using relatively simple sounds that the recovery of auditory function with regeneration of hair cells is nearly complete, and there are only relatively small residual effects after kanamycin-induced trauma.

The present experiments addressed how temporary hearing loss affects the discrimination and the perception of more complex sounds in birds. We expected that the effects of hearing loss on the perception of species-specific vocalizations are more relevant and more complicated than the effects on the detection and discrimination of simple sounds. Previous work has shown that, as in humans, hearing loss in birds is accompanied by an increase in critical ratios (Hashino and Sokabe, 1989) and a broadening of auditory filters and changes in temporal resolution (Marean *et al.*, 1998). As hair cells regenerated, both of these deficits eventually improved to near pre-treatment levels, suggesting that complex sound perception may also return to near normal as new hair cells develop.

In our experiments, discrimination among contact calls of different birds as measured by percent correct performance in an operant task was near 100% before kanamycin treatment and at 4 weeks after kanamycin treatment and remained at high levels out to 6 months. Difficult discriminations, such as between a natural contact call and its synthetic analog (a task that human listeners find challenging) were affected for up to twenty weeks following kanamycin treatment. A more refined analysis using MDS of the response latencies of birds working in this task showed that the perceptual map of contact calls at 4 weeks of recovery still showed overlapping categories of contact calls that were perceptually distinct prior to kanamycin administration. As far as we know, these are the first experiments to examine how perceptual categories of species-specific vocalizations are affected following hair cell loss and regeneration. In budgerigars, at least, the results show a large recovery within a few weeks but some residual perceptual problems that persist for up to 14–16 weeks.

A common refrain of hearing impaired humans is that speech can be heard as well as before the hearing loss occurred but cannot be as easily understood (Newby, 1964). This is an intriguing phenomenon and one that is even more interesting in an organism that has the capability of auditory hair cell regeneration. When trained to recognize two different contact calls, budgerigars completely lose the ability to correctly label these calls when their hair cells have been destroyed by injections with kanamycin. Four weeks into recovery, these birds quickly relearn the classification of previously familiar contact calls to a high level but they do so with a time course that suggests that these previously familiar calls now sound unfamiliar. In other words, although the ability to detect, discriminate, and classify complex acoustic sounds approaches pretreatment levels 4 weeks into recovery, the perceptual world of vocalizations is not the same as before hair cells were lost. These findings have relevance for hearing restoration efforts in humans since they suggest an enduring change in an organism's auditory world of vocalizations in spite of a new set of hair cells and relatively normal hearing otherwise.

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