Published in final edited form as:

J Comp Physiol A Neuroethol Sens Neural Behav Physiol. 2009 February ; 195(2): 193–202. doi:10.1007/s00359-008-0398-z.

Psychophysical Evidence of Damaged Active Processing Mechanisms in Belgian Waterslager Canaries

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Abstract

Belgian Waterslager canaries (BWC), bred for a distinct low-pitched song, have an inherited high-frequency hearing loss associated with hair cell abnormalities. Hair cells near the abneural edge of the papilla, which receive primarily efferent innervation in normal birds, are among the most severely affected. These cells are thought to support nonlinear active processing in the avian ear, though the mechanisms are poorly understood. Here we present psychophysical evidence that suggests degraded active processing in BWC compared to normal-hearing non-BWC. Critical ratios, psychophysical masking patterns and phase effects on masking by harmonic complexes were measured in BWC and non-BWC using operant conditioning procedures. Critical ratios were much larger in BWC than in non-BWC at high frequencies. Psychophysical tuning curves derived from the masking patterns for BWC were broadened at high frequencies. BWC also showed severely reduced phase effects on masking by harmonic complexes compared to non-BWC. As has been hypothesized previously for hearing-impaired humans, these results are consistent with a loss of active processing mechanisms in BWC.

Keywords

psychophysical tuning curve; harmonic complex; active processing; masking; bird hearing

Introduction

Belgian Waterslager canaries (BWC) have long been bred for a distinctive low-pitched song, and have a permanent high frequency hereditary hearing loss (Güttinger 1985; Okanoya and Dooling 1985, 1987a; Okanoya et al. 1990; Wright et al. 2004). This hearing loss develops before adulthood and is associated with damaged and missing hair cells and irregular stereociliary bundles along the basilar papilla (Gleich et al. 1994; Weisleder and Park 1994; Gleich et al. 1995; Weisleder et al. 1996; Brittan-Powell et al. 2002). Hair cells near the abneural edge appear to be the most severely affected in BWC (Gleich et al. 1994; Weisleder and Park 1994; Weisleder et al. 1996). These cells receive primarily efferent auditory nerve contacts in normal-hearing species of birds (reviewed in Gleich and Manley 2000). The BWC basilar papilla also shows missing supporting cells, a smaller tectorial membrane, and cuticular plate abnormalities (Weisleder et al. 1996). Despite normal absolute thresholds at lower

frequencies and elevated high frequency thresholds, hair cell abnormalities are found throughout the length of the papilla, and there is a greater degree of damage in apical regions of the basilar papilla than in basal regions (Gleich et al. 1994; Weisleder and Park 1994; Weisleder et al. 1996). These basilar papilla abnormalities are probably associated with irregularities in the mechanical and electrical properties of the BWC basilar papilla, although the physiological functioning of the BWC basilar papilla has not been tested directly.

Damage to the pigeon (*Columbia livia*) basilar papilla has been shown to disrupt basilar membrane motion, resulting in reduced sensitivity and frequency selectivity, increased phase lag, and altered tonotopic mapping (Smolders et al. 1986; Gummer et al. 1987). These functional changes may be partly due to the loss of active processing mechanisms in damaged papillae. Though the active processing mechanism in the songbird basilar papilla is poorly understood, it is thought to operate through stereociliar bundle movements that provide energy to the radial movement of the tectorial membrane (Gleich and Manley 2000; Fettiplace 2006; Hudspeth 2008). Based on this theory, BWC would be expected to show reductions in active processing for two reasons. The loss of efferently innervated hair cells could decrease the overall amount of energy that is transmitted to the tectorial membrane via active stereocilia movements. Alternatively, damaged or missing stereocilia could compromise the coupling between the tectorial membrane and hair cells, thereby further reducing the amount of energy provided to the tectorial membrane.

Loss of active processing in the mammalian cochlea has been associated with a suite of perceptual effects (reviewed in Moore 1995). In the following experiments, we use three psychophysical tests that have been implicated in reduced active processing in humans to evaluate auditory processing in BWC. First, critical ratios were measured as an indirect estimate of auditory filter bandwidth. We also measured tone-on-tone masking patterns and psychophysical tuning curves (PTC) derived from the masking patterns to further characterize the effects of hair cell abnormalities in BWC on cochlear processing. Third, masking by harmonic complexes with identical frequency spectra but different phase spectra were used to estimate the phase response of the basilar papilla in BWC. On all three of these measures, BWC were expected to show evidence of masking deficits related to loss of active processing.

Materials and methods

Subjects

Adult canaries (*Serinus canaria*) of the BWC and non-BWC strains (3 unspecific strain, 1 Fife, 1 Red Factor; none were bred for particular song characteristics) were used as subjects. All subjects did not participate in all experiments. Birds were housed in an avian vivarium at the University of Maryland. All birds were maintained at approximately 85-90% of their free-feeding weight, and had free access to water and grit. The Animal Care and Use Committee of the University of Maryland, College Park approved the care and use of animals in this study (A3270). Birds were screened for hearing loss by measuring absolute thresholds for several tones prior to testing in the masking experiments. Thresholds for individual subjects are shown in Table 1.

Apparatus

All experiments were conducted in a wire test cage (23×25×16 cm²) mounted in a sound-attenuated chamber (Industrial Acoustics Company, IAC-3) lined with acoustic foam. The custom-designed test cage consisted of a perch, an automatic feeder (food hopper), and two response keys made of red and green 8mm light-emitting diodes (LEDs) attached to two microswitches. The left key (red LED) was designated as the observation key, and the right

key (green LED) was designated as the report key. The speaker was mounted from the roof of the sound-attenuated chamber at a 45-degree angle aimed toward the front of the test cage, approximately 25 cm from the bird's head. The animals were monitored at all times by an overhead video camera system.

The experiments were controlled by an IBM Pentium III microcomputer operating Tucker-Davis Technologies (TDT, Gainesville, FL) System 2 modules. Stimuli were generated prior to the beginning of the experiment, stored digitally, and output via a timing generator (TDT, Model TG6) to a 4-channel D/A converter (TDT, Model DA3-4). Each signal was then output from the D/A converter to a digital attenuator (TDT, Model PA4) and amplifier (TDT, Model HB6) to a loudspeaker (KEF Model 80C, England) in the sound-attenuated chamber. Stimulus calibration was performed periodically using a Larson-Davis sound level meter (Model 825, Provo, UT) with a 20 ft. extension cable attached to a ½ in. microphone positioned in the place normally occupied by the birds' head during testing. All test sessions were automated using a custom-designed computer program. Data were stored digitally and analyzed using commercially available statistics software and a custom designed analysis program.

General training and testing procedures

Birds were trained to peck two keys for food reward using an operant auto-shaping program. The basic training and testing procedures have been described in detail elsewhere (Dooling and Okanoya 1995). For all experiments, birds were required to peck the observation key for a random interval of 2 to 6 s during a repeating background sound. A peck to the report key during this time resulted in a time-out period during which all of the room lights were extinguished. The trial was restarted after the time-out period. After the random observation interval, the background sound was alternated with a target sound. The bird was required to peck the report key within 2 s of this target/background alternation to receive a food reward. A report key peck during this response period was recorded as a hit. If the bird failed to peck the report key within 2 s of the target/background alternation, a miss was recorded. Thirty percent of all trials were sham trials, during which there was no target/background alternation. Pecks to the report key during the response period on these sham trials were recorded as false alarms and punished with time-out periods. Sessions with false alarm rates of 18% or higher and sessions with hit rates lower than 80% for the two targets that were easiest to detect were not used for analysis.

Experimental sessions consisted of approximately 50 to 100 trials, and birds were usually tested twice a day, 5 days a week. Trials were run in blocks of ten, presented in a random order with 3 sham trials and 7 target sounds presented within one block. Targets were presented at 7 different levels bracketing the expected threshold within a block of trials. Each bird ran a minimum of 300 trials on each experimental condition until threshold changed less than 1/3 of the target stimulus step size across sessions. The last 200 trials were used for analysis. Threshold was defined as the level of the tone that was detected 50% of the time corrected for the false alarm rate [$Pc^* = (Pc - FA) / (1 - FA)$] (Gescheider 1985; Dooling and Okanoya 1995).

Masking by broadband noise and critical ratio measurement procedures

Stimuli were 400 ms (total duration) pure tones with frequencies of 1000, 2000, 4000, and 5700 Hz. The tones had rise/fall $\cos^2$ ramps of 20 ms, and were generated with a sampling rate of 40 kHz. The background was continuous broadband noise with a relatively flat frequency spectrum (± 5 dB) between 500 Hz and 8500 Hz. The noise was played at an overall level of 65 and 75 dB SPL.

Thresholds for tones were measured in quiet and in noise. Masked thresholds for tones were measured in continuous broadband noise with a spectrum level of 26 dB (overall level of 65 dB SPL). Thresholds for BWC were also measured in noise that was 10 dB higher (spectrum level 36 dB) because the lower noise level did not produce sufficient masking at 4000 and 5700 Hz. An earlier study that measured CRs in BWC and non-BWC at 2000 Hz showed no change in CR with increasing noise level (Okanoya and Dooling 1985). The average false alarm rate was 2.79% for non-BWC and 1.46% for BWC. None of the data from non-BWC or BWC were discarded due to high false alarm rates or low hit rates, an indication of the ease of the task for these animals.

Masking patterns and derived psychophysical tuning curves procedures

Psychophysical tuning curve stimuli consisted of 20-ms (total duration) 1000, 2000, and 2860 Hz pure tone signals with $\cos^2$ rise/fall times of 5 ms and 300-ms tone maskers with $\cos^2$ rise/fall times of 20 ms. Maskers had frequencies of 700, 800, 900, 950, 1050, 1100, 1200, 1400, 1600, or 1800 Hz for the 1000 Hz signal; 1400, 1600, 1800, 1900, 1950, 2050, 2100, 2200, 2400, 2600, or 2700 Hz for the 2000 Hz signal; and 2060, 2260, 2460, 2660, 2760, 2810, 2910, 2960, 3060, 3260, 3460, or 3660 Hz for the 2860 Hz signal.

Prior to measuring masked thresholds, absolute thresholds in quiet for the 20-ms pure tone signals of 1000, 2000, and 2860 Hz were measured as described above. PTCs are traditionally measured by determining the level of a masker that just masks a signal frequency fixed at a constant level (usually about 10 dB SL). This type of task is difficult for the birds to learn, because they are trained to respond to any *change* from the background stimulus. On a given trial, the bird might respond to either of two events: 1) the presence of the signal tone, or 2) a change in the masker level. From this, it would be impossible to determine an accurate masked threshold. To circumvent this issue, tone-on-tone forward-masking patterns were measured for tones at 1000, 2000, and 2860 Hz and PTCs were estimated from these masking patterns measured in BWC and non-BWC. This procedure significantly reduced training and testing time, which was already extensive due to the number of conditions tested. A similar procedure was used to measure tone-on-tone masking patterns in chickens and budgerigars (Dooling and Searcy 1980, 1985; Saunders and Salvi 1995). A nonsimultaneous masking paradigm was used in which the signal tone immediately followed the masker tone to avoid two-tone suppression effects that may occur, such that the basilar papilla response to one tone may be suppressed by the presence of another tone (Sachs and Kiang 1968; Sachs et al. 1974).

For each block of trials, the masker level was held constant and the signal was presented at a range of levels bracketing the estimated threshold. This procedure was repeated for each masker frequency at three masker levels to generate masking functions. The levels of the signal tones were changed in 5 dB steps according to the Method of Constant Stimuli and varied randomly within a set range from trial to trial. Maskers for the 1000 Hz signal were presented at 40, 50, and 60 dB SPL for non-BWC and at 60, 70, and 80 dB SPL for BWC. Maskers for the 2000 Hz signal were presented at 30, 40, and 50 dB SPL for non-BWC and 60, 70, and 80 dB SPL for BWC. Maskers for the 2860 Hz signal were presented at 30, 40, and 50 dB SPL for non-BWC and 70, 80, and 85 dB SPL for BWC. These masker levels were chosen to ensure that at least 10 dB of masking was produced by six masker frequencies centered around each signal frequency. The average false alarm rate was 2.33% for non-BWC and 3.09% for BWC. In all, 10% of all non-BWC data and 4% of all BWC data were discarded due to high false alarm rates or low hit rates for the easiest two targets.

Phase effects on masking procedures

Stimuli were either harmonic complex maskers alone, or maskers with an embedded signal tone. Maskers were constructed by summing equal-amplitude pure tones from 200 to 5000 Hz, with a fundamental frequency of 100 Hz. Component starting phases of the harmonics were selected according to a modification of the Schroeder (1970) algorithm:

$$\theta_n = C\pi n(n+1)/N$$

where θ_n represents the starting phase of the n^{th} harmonic, N is the total number of harmonics, and C is a scalar (Lentz and Leek 2001). This systematic assignment of component phase produces maskers with frequencies gliding monotonically within each period of the waveforms. The rate of within-period frequency change depends on the fundamental frequency and the scalar used to select component phases.

Maskers were generated for scalars (C) ranging between -1.0 and $+1.0$. When $C=0.0$, a highly modulated cosine-phase waveform is produced, characterized by very peaky envelopes with long low-energy portions within each period. In contrast, C values of -1.0 and $+1.0$ produce waveforms with very flat envelopes and very short low-energy portions. The different scalars generate maskers on a continuum of relative proportion of low versus high energy within each period. Negative scalar values produce waveforms with increasing instantaneous frequency within the masker period, and positive scalar values produce waveforms with decreasing instantaneous frequency within each period. Changing the scalar alters the rate of the frequency change within each period, so that scalars closer to zero produce more rapid frequency changes than those close to ± 1.0 . Time waveforms for several of the harmonic complexes are shown below the x-axis in Figure 4 (see also Lauer et al 2006).

Birds were tested using 13 different maskers. The maskers were 260 ms in duration with 20-ms raised-cosine rise/fall times and presented at an overall level of 80 dB SPL (63 dB SPL per harmonic component). The signals were 2800 Hz tones added in phase to the corresponding masker component. The duration of the signal was the same as the masker, including the rise and fall times.

The procedures were identical to those described by Lauer et al. (2006). Birds were trained to detect the presence of a 2800 Hz tone embedded in a harmonic complex masker. Maskers were background sounds and the target sounds were the masker plus the tone. Tones of different levels relative to the level of the masker component at the signal frequency were presented using the Method of Constant Stimuli at 7 different levels within a block of trials. Step sizes were either 1 or 2 dB, depending on the bird's behavior. Birds were tested with different maskers in a random order. The average false alarm rate was 2.56%. Two percent of all sessions were discarded.

Results

Masking by broadband noise and critical ratios

Average thresholds and standard deviations for tones masked by broadband noise are shown in Table 2. Masked thresholds for all frequencies were elevated by about 20-30 dB compared to quiet thresholds in non-BWC. Masked thresholds in BWC were elevated by about 7-20 dB compared to quiet thresholds below 4000 Hz for the 65 dB SPL noise level. There was little to no masking at 4000 and 5700 Hz for BWC by the 65 dB SPL noise. Even a noise level of 75 dB SPL did not produce masking at 5700 Hz in BWC. The noise levels used approach threshold levels at these higher frequencies, and may even be below threshold for some

individual birds. Higher noise levels were not tested due to the risk of incurring more hair cell damage.

CRs, signal-to-noise ratio at masked threshold, were calculated from the masked thresholds in non-BWC and BWC ($CR = \text{masked threshold} - \text{noise spectrum level}$). CRs measured in 65 dB SPL noise for BWC and non-BWC are shown in Figure 1. No CR could be calculated for BWC at 5700 Hz because the level of the noise needed to mask the tone was beyond the system hardware's capabilities (75 dB SPL) and could potentially have resulted in more hearing loss in the birds. Average CRs increased by about 20 dB between 1000 and 4000 Hz in BWC.

A mixed factor (frequency x strain) ANOVA using only 1000, 2000, and 4000 Hz showed a significant effect of frequency ($p=0.001$) and strain ($p<0.001$) and a significant interaction of frequency and strain ($p=0.001$). Post Hoc analysis revealed that CRs were not significantly different between BWC and non-BWC at 1000 Hz and 2000 Hz. At 4000 Hz, CRs for BWC were much larger than those of non-BWC ($p<0.05$).

Masking patterns and derived psychophysical tuning curves procedures

The amount of masking of each signal frequency was calculated as the difference in threshold of a signal tone in the presence of a masker and in quiet for each masker-signal combination at each level tested. Average tone-on-tone masking patterns (the amount of masking as a function of masker frequency) are shown in Figure 2 for signal frequencies of 1000 Hz, 2000 Hz, and 2860 Hz for non-BWC and BWC.

The masker level at each masker frequency required to produce 10 dB of masking of the signal frequency was used to derive a PTC. These masker levels were plotted as a function of masker frequency, as demonstrated in the insert of Figure 2A. The dotted horizontal line in that panel shows the 10-dB levels for these average functions. For some masking patterns, the masker level was too high to produce 10 dB of masking at the signal frequency, which would correspond to the tip of the tuning curve. In those cases, the tip was estimated by calculating the difference in amount of masking between the data point just below or above the signal frequency and the masking at the signal frequency. The tip of the derived tuning curve was estimated to be that dB amount less than the masker level for the point just below or above the signal frequency on the PTC, as shown in the inset in Figure 2A. The masker levels in dB SPL for each bird and frequency were averaged within subject groups to produce derived PTCs at each frequency for the two groups. These PTCs are shown in Figure 3.

A measure of the sharpness of tuning, the quality factor (Q) was calculated for each PTC for the averaged data. Q is calculated as the signal frequency divided by the bandwidth of the PTC at some level above the tip. Larger Q values indicate sharper tuning, or increased frequency selectivity. Q values were calculated at 10 dB above the tip level (Q_{10}) and at 20 dB above the tip (Q_{20}). Q values for non-BWC and BWC canaries are shown in Figure 3 for 10 dB and 20 dB bandwidths.

Both Q_{10} and Q_{20} values increased with increasing frequency in non-BWC, indicating increased frequency selectivity at higher frequencies compared to lower frequencies. Q_{20} values were smaller than Q_{10} values in non-BWC, reflecting the widening of the skirts of the PTC as the masker frequency moves away from the center frequency. In BWC, Q_{10} values were similar for 1000 Hz, 2000 Hz, and 2860 Hz. Q_{20} values were slightly larger at 2000 Hz than at 1000 Hz in BWC canaries. No Q_{20} could be measured in BWC at 2860 Hz. Q_{10} and Q_{20} values were similar in BWC and non-BWC at 1000 Hz. Q_{10} values were smaller at 2000 Hz and 2860 Hz for BWC than non-BWC. The Q_{20} value at 2000 Hz was smaller in BWC compared to non-BWC.

Phase effects on masking

Average thresholds for detecting 2800 Hz tones in harmonic complex maskers for BWC are shown in Figure 4 along with mean thresholds replotted from Lauer et al. (2006) for three normal-hearing non-BWC tested using identical methods. Several of the non-BWC also participated in Experiments 1 and 2 of the present study. The scalar values used to create the maskers are plotted on the x-axis, and masked thresholds are plotted on the y-axis in units of dB signal level re masker component level. Thresholds closer to the top of the figure indicate more masking, while thresholds towards the bottom indicate less masking. A strain x scalar mixed factor ANOVA revealed significant effects of scalar value ($p < 0.001$), strain ($p = 0.004$), and a significant interaction of scalar value and strain ($p < 0.001$).

Discussion

Masking by broadband noise and critical ratios

The basilar membrane of mammals (as well as the basilar papilla of birds) is believed to function as a bank of overlapping bandpass filters, with each filter responding to a different range of frequencies estimated as the critical bandwidth or critical ratio (CR; Fletcher 1940).

The average CR at 2000 Hz for non-BWC in this study was consistent with the average CR reported in one study in German Roller canaries (no other frequencies were tested; Okanoya and Dooling 1985). CRs for higher frequencies were larger in BWC compared to non-BWC suggesting wider critical bandwidths and reduced frequency selectivity in the region of the birds' hearing loss. These results are consistent with those reported in humans with hearing loss (de Boer and Boumeester 1974; Margolis and Goldberg 1980; Hall and Fernandes 1983) and in birds with experimentally induced temporary hearing loss (Hashino and Sokabe 1989). These results indicate reduced frequency selectivity in BWC since a higher signal-to-noise ratio is required to detect sounds in broadband noise in the region of hearing loss.

Interestingly, CRs were smallest in the area corresponding to the spectral peaks of BWC vocalizations. Thus, BWC maintain excellent detection of frequencies that correspond to the range of vocalization frequencies (Güttinger 1985; Okanoya et al., 1990; Wright et al. 2004) in a difficult listening environment. In contrast, high frequencies are rendered nearly inaudible to BWC in a noisy background.

Masking patterns and derived psychophysical tuning curves procedures

CRs provide less than ideal estimate of auditory filter shape. These measures assume that the filters have a rectangular shape and that filter bandwidth is independent of level. Additionally, the critical band concept assumes that maskers with frequencies that are remote from the signal frequency cannot affect detection of the signal. Many studies since Fletcher's (1940) classic study have shown that these assumptions are inaccurate (reviewed in Moore 2003). Tone-on-tone masking patterns and PTCs provide a more accurate measure of spectral masking and auditory filter shape, and are akin to basilar membrane or neural tuning curves measured physiologically.

Tone-on-tone masking patterns were symmetrical and shaped like inverted Vs for non-BWC for all signal frequencies tested. This result is consistent with previous reports in other species of birds (Bock and Saunders 1975; Saunders and Else, 1976; Saunders et al., 1978; Kuhn and Saunders, 1980; Saunders and Pallone 1980; Dooling and Searcy 1980, 1985; Saunders and Salvi 1995; Brown et al. 2001). Maskers with frequencies that were close to the signal frequency produced the most masking for all signal frequencies tested in non-BWC. Masking patterns were nearly symmetrical at 2000 Hz, but were asymmetrical at 1000 Hz and 2860 Hz.

in BWC. Masking patterns also became flatter and broader at higher frequencies in BWC. Maskers with frequencies that were close to the signal frequency produced the maximum amount of masking for 1000 Hz and 2000 Hz in BWC; however, the maximum amount of masking was produced below the signal frequency for the 2860 Hz signal condition.

Recall that masking patterns had to be measured at higher signal levels for the BWC than for the non-BWC to overcome the sensitivity loss, and so PTCs were necessarily elevated at all frequencies for BWC compared to non-BWC. The shapes of the PTCs for non-BWC were V-shaped and relatively symmetrical. PTCs for BWC were generally symmetrical for 1000 Hz and 2000 Hz probe signals. The PTC with a center frequency of 2860 Hz was asymmetrical and very flat in BWC. This suggests that channels with characteristic frequencies below 2860 Hz were responding more to the signal tone than channels with characteristic frequencies near 2860 Hz in BWC. This effect may arise because the hair cells in the region of the basilar papilla that normally would respond to frequencies around 2860 Hz are essentially non-functional. The high sound levels that are necessary for BWC to detect a 2860 Hz tone may instead have been exciting channels that are distant in frequency. This finding suggests that regions of the basilar papilla that are normally responsive to higher frequencies may be functionally dead in BWC.

Broadening of PTCs indicated by reduced Q values in BWC compared to non-BWC is consistent with reports in hearing-impaired humans (Leshowitz and Lindstrom 1977; Hoekstra and Ritsma 1977; Zwicker and Schorn 1978; Bonding 1979; Florentine et al. 1980; Carney and Nelson 1983; Festen and Plomp 1983; Stelmachowicz et al. 1985; Nelson 1991). Chinchillas and patas monkeys with experimentally induced hearing loss also show broadened PTCs (Ryan et al. 1979; Salvi et al. 1982; Smith et al. 1987). Broadening of the PTCs in mammals is typically associated with loss of active processing due to outer hair cell damage, similar to the extensive damage to abneural hair cells and stereocilia in BWC.

The reduced frequency selectivity at higher frequencies in BWC is likely to have a profound impact on other aspects of perception that are related to or limited by the bandwidth of the auditory filters. Changes in the shape of the auditory filters may result in reduced ability to “hear out” high frequency sounds that occur in complex acoustic environments. The reduced frequency selectivity shown here may be related to the reduced frequency discrimination ability and changes in temporal resolution previously shown in BWC (Lauer et al. 2007). On the other hand, good frequency resolution at low frequencies coupled with poor frequency resolution at high frequencies may support learning of the low-pitched vocalizations characteristic of the BWC strain. One could envision a scenario where BWC are more likely to learn strain-specific low frequency notes in part because high-frequency notes are less detectable and poorly resolved.

Phase effects on masking

Recent studies in birds and humans have examined the effects of temporal waveform shape on masking when long-term frequency cues are held constant but component starting phases are selected according to the Schroeder algorithm (Schroeder 1970; Leek et al. 2000; Dooling et al. 2001; Lauer et al. 2006). The complex that produces the least amount of masking within a set of Schroeder-phase maskers is thought to mirror the phase response of the cochlea, at least in mammals. Humans with sensorineural hearing loss show much less change in the amount of masking by complexes with different component starting phase selections compared to normal-hearing listeners (Lentz and Leek 1999; Oxenham and Dau 2004).

There were three primary differences in performance on the phase effects on masking task by BWC and non-BWC. First, BWC showed higher thresholds overall compared to non-BWC,

except at $C=-1.0$. This is only partially because of the higher absolute thresholds of the hearing-impaired birds. The dashed line shown on the figure reflects the mean absolute threshold at 2860 Hz for these three birds. This would be the lowest possible level of the signal that would be audible if the masker were not present, and might be thought of as a “hard limit” for the possible release from masking available to the BWC subjects. Clearly, they did not achieve that degree of release from masking.

A second distinction shown is the overall shape of the masking curve due to phase selection. BWC showed less change in the amount of masking by stimuli with different phase spectra than non-BWC. Non-BWC showed a large release from masking for maskers with peakier envelopes (C values near 0) that did not occur for BWC. There was a slight decrease in the amount of masking produced by maskers with peakier envelopes in BWC; however, the release from masking was not nearly as prominent as it was in non-BWC. The difference in the maximum and minimum amounts of masking was only 8.38 dB in BWC, while the difference was 18.57 dB in non-BWC. In non-BWC, the least effective masker corresponded to a C value of -0.2 . The least effective masker is less obvious in BWC, but appears to fall nearby, at $C=-1$.

Thirdly, previous studies in normal-hearing birds have shown that maskers with C values of $+1.0$ and -1.0 produce similar amounts of masking (Leek et al. 2000; Dooling et al. 2001; Lauer et al. 2006). Interestingly, BWC show a larger difference in the amount of masking by these maskers (4.94 dB) than non-BWC (1.07 dB) (Dooling et al. 2001; Lauer et al. 2006). Based on reports in hearing-impaired human listeners, this finding was unexpected. The reason for this result in birds is unclear. It indicates that maskers with downward frequency sweeps within periods are actually more effective than upward-going sweeps, in stark contrast to Schroeder harmonic complex masking asymmetries reported in humans.

The smaller release from masking due to component phase selection seen in BWC is consistent with reports in hearing-impaired human listeners tested using similar stimuli (Lentz and Leek 1999; Oxenham and Dau 2004). When a change in the amount of masking does occur in hearing-impaired listeners, the minimum amount of masking is produced by the masker with equal component starting phases ($C=0.0$; Lentz and Leek 1999; Oxenham and Dau 2004). The differences in masking effectiveness between normal-hearing and hearing-impaired humans have been attributed in part to the loss of active processing mechanisms that result from damage to the cochlea. It is possible that similar changes in the phase response of the BWC papilla that could produce these masking results may be tied to loss of active processing mechanisms as well as changes in the movement of the basilar papilla that result from structural abnormalities.

Loss of active processing in BWC

There is little known about the functional significance of active processing in the avian auditory system. BWC show stereociliar bundle abnormalities, and the most severe damage to the basilar papillae of BWC occurs in the abneural side that contains hair cells with primarily efferent innervation. Thus the three auditory deficits experienced by BWC, loss of sensitivity to low-level sounds, loss of frequency selectivity, and possible alterations in the representation of phase information along the basilar papilla, most likely are related to deficits associated with abnormal stereociliar bundle function and/or abnormal efferent feedback mechanisms. Direct investigations of efferent innervation patterns or functionality have not been reported in BWC, though the active modulation of cochlear activity has been posited as a primary function of the efferent system in mammals (see Guinan 1996, for a review of the efferent system).

Active cochlear mechanisms enhance responses to low-level sounds and frequency selectivity in both humans (e.g., Moore and Oxenham 1998) and in birds (Köppl 1995; Manley 2001).

We have now shown that BWC are deficient in both of these areas, at least for high frequency stimuli. BWC also show reduced phase effects in masking by Schroeder harmonic complexes, which has been associated with nonlinear compression in humans (Carlyon and Datta 1997; Oxenham and Dau 2004). It is striking that despite decidedly different mechanics of cochlear active processing in birds and mammals (Manley 2001; Köppl et al. 2004), the behavioral manifestations of damage to these active mechanisms are similar. These across-species similarities further support the existence of a ubiquitous presence of active nonlinear cochlear processing across multiple vertebrate taxa.

Acknowledgments

This work was supported by NIH Grants DC-01372 to RJD, DC-005450 to AML, DC-00626 to MRL, and DC-04664 (Core Center grant) to the University of Maryland. These experiments comply with the “Principles of care,” publication No. 86-23, revised 1985 of the National Institutes of Health and with the Animal Welfare Act of the United States.

Abbreviations

BWC	Belgian Waterslager Canary
CR	critical ratio
PTC	psychophysical tuning curve
dB	decibel
SPL	sound pressure level

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Figure 1. Critical ratios for non-BWC (closed circles) and BWC (open circles). Error bars indicate standard errors.

Figure 2.

Tone-on-tone nonsimultaneous masking patterns for non-BWC (a-c) and BWC (d-f) measured for center frequencies of 1000, 2000, and 2860 Hz. Masking patterns were measured for several different masker levels. BWC could not be tested at all masker frequencies at all levels due to high thresholds at the limits of the experimental system's output. Psychophysical tuning curves were derived from these masking patterns by plotting the interpolated values that fall on a line drawn at 10 dB of masking (dashed line and inset in panel a). If no derived PTC point fell at or near the signal frequency, a tip was derived by calculating the difference in amount of masking between the data point just below or above the signal frequency and the masking at the signal frequency, and placing the tip of the derived tuning curve that amount of dB less

than the masker level for the point just below or above the signal frequency on the PTC, demonstrated in the inset.

Figure 3.

Derived PTCs with center frequencies of 1000, 2000, and 2860 Hz for non-BWS (closed circles) and BWS (open circles). Bold dashed lines show estimated values at the tips of some of the PTCs. Dashed vertical lines indicate signal frequencies. Corresponding Q10 and Q20 values are shown.

Figure 4.

Masked thresholds for a 2800 Hz signal embedded in harmonic complex maskers with different phase spectra for non-BWC (closed circles) and BWC (open circles). Examples of the harmonic complex masker waveforms (two periods of each) are displayed below the abscissa, corresponding to $C=-1.0$, 0.0 , $+0.7$, and $+1.0$, respectively. The horizontal dashed line indicates the mean absolute threshold of a 2860-Hz tone for the BWC, relative to the level per component of the maskers.

Thresholds (in dB SPL) for 400 ms pure tones in quiet for individual canaries used in the experiments. Pure tone thresholds are measured for each subject as a routine lab procedure to determine the presence of normal hearing or hearing loss. The experiments that each bird participated in are indicated at the bottom of each section of the table. The non-BWC thresholds for birds in Experiment 3 are taken from Lauer et al. (2006)

Table 1

Non-BWC						
Subject Name:	Carrie	Foghorn	Marsala	Rufus	Jlo	
Frequency (Hz)						
1000	36	27	33	32	24	
2000	23	21	20	25	23	
2860	13	12	14	20	19	
4000	16	19	24	17	22	
5700	29	21	23	12	20	
8000	46	29	34	36	--	
Experiment	1,2,3	1,2,3	1,3	2	2	
BWC						
Subject Name:	Schokkel	Indruk	Woeten	Knorr	Tjok	
Frequency (Hz)						
1000	41	41	48	34	39	
2000	41	45	50	33	31	
2860	53	51	43	44	43	
4000	66	62	66	54	52	
5700	70	73	78	65	--	
Experiment	1,2	1	1	2	2	
BWC (continued)						
Subject Name:	Bell	Popcorn	Perdue	Prince		
Frequency (Hz)						
1000	36	42	49	--		
2000	35	47	49	28		
2860	46	43	54	29		
4000	50	72	65	60		
5700	--	74	83	--		
Experiment	2	3	3	3		

Table 2Average ($\pm$ 1 SD) absolute and masked thresholds for non-BWC and BWC for Experiment 1

Frequency		Absolute threshold	Masked threshold
1000 Hz	non-BWC	32.04 (4.60)	55.98 (4.49)
	BWC	43.47 (4.11)	54.35 (2.32)
2000 Hz	non-BWC	21.45 (1.45)	53.13 (3.94)
	BWC	45.34 (4.57)	51.52 (0.44)
4000 Hz	non-BWC	19.70 (3.99)	53.28 (5.31)
	BWC	64.79 (2.37)	68.71 (3.32)
5700 Hz	non-BWC	24.53 (3.85)	55.46 (5.83)
	BWC	73.37 (3.99)	71.46 (1.87)