ABSTRACT

Title of Document: PERCEPTUAL CONSEQUENCES OF
EARLY-ONSET HEREDITARY HEARING
LOSS IN THE BELGIAN WATERSLAGER
CANARY (*SERINUS CANARIUS*)

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Psychology

Belgian Waterslager canaries (BWS) are bred for a distinctive low-pitched

song that includes sounds that are thought to resemble water. This strain of canary

has been used in multiple neurobiological and behavioral studies of song learning.

These birds have a permanent hereditary hearing loss associated with missing and

abnormal hair cells. The hearing deficit develops after hatch, but is present when the

birds learn their song. The manner in which these birds process complex sounds

indisputably affects the content of their vocalizations; however, no studies have

looked at BWS canaries' ability to detect and discriminate sounds other than

detection of pure tones in quiet. Thus, the BWS canary provides a unique opportunity

to investigate the relationship between the form and function of an auditory system

involved in vocal learning. Here I describe a series of psychoacoustic experiments

that investigate differences in masking, discrimination, temporal processing, and

perception of song elements in BWS canaries and normal-hearing non-BWS canary strains. Spectral and temporal studies of masking showed that frequency resolution and the phase response of the basilar papilla are impaired in BWS canaries. Frequency discrimination was superb at low frequencies, but worse than normal at high frequencies in BWS canaries. Duration and intensity discrimination was not adversely affected by the hearing loss. Temporal resolution was normal or better than normal under some conditions in BWS canaries. Despite the hearing loss, BWS canaries are able to accurately discriminate among strain-specific song syllables as well as syllables of other canary strains. In fact, BWS canaries are actually better than non-BWS canaries at discriminating among BWS canary syllables. These perceptual predispositions in BWS canaries are presumably related to the structural abnormalities of the inner ear, and are likely to play a role in song learning and song maintanence by enhancing the birds' ability to attend to important acoustic features that are characteristic of BWS vocalizations.

PERCEPTUAL CONSEQUENCES OF EARLY-ONSET HEREDITARY
HEARING LOSS IN THE BELGIAN WATERSLAGER CANARY (*SERINUS
CANARIUS*)

By

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Dissertation submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
2006

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Acknowledgements

First, I thank my dissertation committee for insightful comments and discussions. I am lucky to have such an esteemed panel of experts involved in the dissertation process. I am especially grateful to my official advisor, Dr. Robert Dooling, and my unofficial advisor, Dr. Marjorie Leek, for six years of mentorship. I am also eternally indebted to Dr. Elizabeth Brittan-Powell for the guidance, wisdom, and sympathy she has provided to me. I also need to thank my husband, my family, the Integrative Neuroscience faculty and the Dooling lab members (past and present) for their support. Finally, the birds deserve the biggest thanks for being cooperative subjects.

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Chapter 1: Introduction

This dissertation examines several aspects of hearing in the Belgian Waterslager canary--the only bird known to suffer from a permanent hereditary hearing loss.

Canaries (*Serinus canarius*) are a species of Carduline finch that are bred for either particular song characteristics popular or body shape and plumage. Canaries have been popular songbirds for the study of vocal learning and vocal production. The strain of canary used in many of these studies, the Belgian Waterslager (BWS) canary, has since been found to have a permanent hereditary high-frequency hearing loss linked to hair cell abnormalities (Gleich et al., 1994a; Okanoya and Dooling, 1985, 1987a; Okanoya, et al., 1990). This strain has been bred for hundreds of years for a distinctive low-pitched song. Intriguingly, the hearing deficit develops after hatch, but before the bird reaches adulthood (Okanoya and Dooling, 1985, 1987a; Okanoya, et al., 1990; Brittan-Powell et al., 2002; Wright et al., 2004).

There is a substantial body of research describing the peripheral auditory system pathology in BWS canaries, as well as behavioral and neurobiological studies of song learning. However, there is little information about how the anatomical abnormalities of the peripheral auditory system affect the perception of sounds in these birds. The structural abnormalities of the BWS canary auditory system are likely to result in strain-specific perceptual capabilities that impose constraints on vocal learning and communication. Thus, a description of the limitations and capabilites of auditory perception in BWS canaries provides a unique opportunity (in

a non-human animal) to investigate the relationship between the form and function of an auditory system involved in vocal learning.

Hearing in Birds with a Focus on Belgian Waterslager Canaries

Birdsong is characterized by rapid frequency and amplitude changes over time (Greenwalt, 1968). The ability of a bird to resolve these acoustic cues must directly influence the content of vocalizations in species that learn their songs and rely on auditory feedback for song maintenance. The bird must use these cues to distinguish one call or note from another in order to develop and maintain a normal vocal repertoire, and it must be able to focus its attention on important acoustic information to effectively communicate with other members of its species. More specifically, the bird must be able to resolve differences in frequency, time, and intensity. It must also be able to combine complex interactions of these acoustic dimensions to perceive and respond to naturally occurring sounds.

The act of hearing involves the whole, awake animal. Behavioral estimates of hearing function in animals have traditionally been obtained using well-established psychoacoustic methods (Stebbins, 1970; Klump et al., 1995). Psychoacoustic measures may provide more sensitive estimates of hearing function than physiological methods because they involve the whole awake, responding organism rather than the response of one cell or a population of cells. These methods also provide a link between structure and function in the auditory system, and in some cases allow for direct comparison with data from humans. Many behavioral studies have investigated hearing abilities in birds (reviewed in Dooling et al., 2000).

Relevant studies are discussed in each section of this dissertation, rather than reviewing them here.

The relationship between the spectral characteristics of vocalizations and audible bandwidth in birds has been well established (e g., Dooling et al., 1971; Konishi, 1969, 1970; Saunders et al., 1974). Songbirds typically hear best between about 500 and 6000 Hz (reviewed in Dooling et al., 2000). The average power spectrum of most songbird vocalizations falls within this range, and the highest frequencies contained in a certain species' vocalizations correlates with hearing sensitivity at high frequencies (Dooling 1980, 1982).

The close link between audible bandwidth and vocalization spectra has been well established in canaries. Dooling et al. (1971) first showed that the acoustic power in the vocalizations of the "Common" canary falls primarily in the range of best auditory sensitivity (i. e., lowest absolute thresholds). Presumably, these birds were not bred for particular song characteristics. Spanish Timbrado canaries, which are bred to sing both high-pitched and low-pitched song elements, have slightly better absolute sensitivity at low and high frequencies compared to canaries not bred for song (Lohr et al., 2004). Thus, canaries of the Timbrado strain show a broader frequency range in their vocalizations as well as their audiograms.

Several studies have confirmed the unusual auditory sensitivity of the BWS canary compared to non-BWS strains. Behavioral audiograms, compound action potential recordings, and auditory brainstem response measurements reveal pure tone thresholds that are approximately 20-40 dB higher in BWS canaries than in other strains of canaries at frequencies above 1500 to 2000 Hz (Okanoya and Dooling

1985, 1987a; Okanoya et al., 1990; Gleich et al., 1995; Brittan-Powell et al., 2002; Wright et al., 2004). The hearing impairment is hereditary and sex-linked (Okanoya et al., 1990; Wright et al., 2004), but is not present at hatch (Brittan-Powell et al., 2002).

The loss of sensitivity at high frequencies results in a shifted region of best sensitivity in BWS canaries compared to non-BWS canaries. BWS canary song contains little energy above 4000 Hz (Güttinger, 1985), and the hearing loss is greatest above 4000 Hz (Okanoya and Dooling 1985, 1987a). The region of best sensitivity in BWS canaries (approximately 1000-2000 Hz) corresponds to the spectral peaks in their calls (Okanoya et al., 1990). Similarly, the amount of energy in BWS canary song and Border canary song at 4000 Hz relative to 1000 Hz correlates with their ABR thresholds (Wright et al., 2004). Hybrid BWS-Border canaries show intermediate auditory sensitivity and vocalization spectra (Okanoya et al., 1990; Wright et al., 2004).

Role of Hearing in Song Learning

The role of hearing in song learning has been extensively studied and will only be reviewed briefly here. Auditory information is crucial for the development and maintenance of species-specific vocalizations in songbirds. Although the song learning process differs somewhat across species, the basic premise is similar (reviewed in Brainard and Doupe, 2000; Konishi, 2004). The bird must first learn song elements from listening to and interacting with a tutor, usually its father. Certain perceptual predispositions and biological processes affect when, how, and

what the bird learns. Modifications to its song occur following auditory feedback and from further experience with tutors. Altering normal auditory experience by removing the tutor or disrupting auditory feedback can have significant consequences on the animal's vocalizations. Birds raised in isolation develop relatively simple songs compared to birds raised in the presence of tutors (e.g., Thorpe, 1958; Dittus and Lemon, 1969; Marler, 1970; Marler and Waser, 1977; Eales, 1985). Perturbed auditory feedback results in the slow deterioration of song in zebra finches (Leonardo and Konishi, 1999). Surgical deafening in juveniles results in the development of an abnormal song (Konishi, 1965; Nottebhom, 1968; Price, 1979). Surgical deafening during adulthood can result in the degradation of song in canaries (Nottebohm et al., 1976), and other songbird species (Nordeen and Nordeen, 1992; Okanoya and Yamaguchi, 1997; Woolley and Rubel, 1997; Lombardino and Nottebohm, 2000). Similarly, the warble song and contact calls of budgerigars degrade after surgical deafening (Heaton et al., 1999).

Vocalizations are also affected by hair cell damage due to noise and ototoxic drug exposure in birds. Loss of high frequency hearing after treatment with the ototoxic drug amikacin does not disrupt song in Bengalese finches (Woolley and Rubel, 1999). However, extensive damage to the basilar papilla resulting from combined noise and ototoxic drug exposure in Bengalese finches results in degradation of song features, including decreased syllable sequence stereotypy, decreased phonology of some syllables, reduction in the number of syllable types, and combined, new or unrecognizable syllables (Woolley and Rubel, 1999, 2002). Budgerigars (*Melopsittacus undulatus)* exhibit a decrease in the precision of matching

a template contact call with an elicited call following kanamycin-induced hair cell damage (Dooling et al. 1997). In both species, normal functioning occurs following regeneration of hair cells (Dooling et al. 1997; Woolley and Rubel, 2002).

Song Learning and Production in the Belgian Waterslager Canary

Canaries produce intricate songs during a yearly breeding season. Domesticated canary song is noted for its sequences, or tours, consisting of consecutive repetitions of notes or syllables (Güttinger, 1985). Several strains of canaries are bred for particular song characteristics, while other strains are bred for body shape or plumage. BWS canaries are one of the types bred for song. Their song contains distinct syllables referred to by breeders as "water notes." The frequency range of BWS canary song lies mainly between 1000 and 3000 to 4000 Hz (Nottebohm and Nottebohm, 1978; Güttinger, 1985; Wright et al., 2004). The song repertoire consists of approximately 20-35 different syllable types (Marler & Waser, 1977; Nottebohm and Nottebohm, 1978; Güttinger, 1985). In contrast, songs produced by non-BWS strains typically have broader frequency ranges (up to 6500 Hz) and more syllable types.

Male BWS canaries normally learn their songs from their fathers and male siblings, though they can learn from other sources of acoustic input (Waser and Marler, 1977). Initial song learning occurs after weaning, at about 30 days of age. Males begin to sing plastic song at about 60 days of age, and stable song begins to appear at about 4 months (Nottebohm and Nottebohm, 1978). The time-course of

song learning in BWS canaries is notable, since the birds have restricted auditory input by the time they must memorize their tutors' song (Brittan-Powell et al., 2002).

Several studies have shown that song learning by juveniles is largely influenced by tutor song, and that young birds are capable of imitating synthesized song with a structure that does not resemble normal adult song, emphasizing the importance of hearing during song learning (Gardner et al., 2005). Birds raised in the presence of constant masking noise prior to weaning develop normal song when tutored by an adult male from weening onward (Marler and Waser, 1977; Waser and Marler, 1977). However, birds raised normally until weaning do not develop normal song if they are isolated from tutors after weaning (Marler and Waser, 1977; Waser and Marler, 1977).

Adult canaries are seasonal learners and experience a period of plastic song following each breeding season during which syllables are added, deleted, and modified (Nottebohm and Nottebohm, 1978). Though the size and composition of the syllable repertoire changes over successive breeding seasons, certain features of the song, such as mean syllable duration and frequency range, remain constant across seasons. This seasonal song learning is associated with changes in gonadal hormone levels (Nottebohm et al., 1987) and increases in the volumes of song control nuclei (Nottebohm et al, 1986).

All of the studies of song learning in canaries have been conducted exclusively with male birds. However, female canaries sing following administration of testosterone (Nottebohm and Arnold, 1976; Nottebohm, 1980) and sometimes sing spontaneously (Pesch and Güttinger, 1985), although their songs are somewhat

simpler than male songs.   It is possible that females undergo song learning processes that are similar to those identified in males. Mates are selected by females partly by their songs (Payne, 1983), and these choices are likely guided by songs heard early in life (Nagle and Kreutzer 1997). Thus, female BWS canaries are also "learning" song by the time the hearing loss develops.

Canary song, like all birdsong, is produced via airflow through a bifurcated syrinx; however, there are strain differences in the way the song is produced. A French non-BWS canary strain, called Common Domestic canaries, produce many multi-note syllables using both sides of the syrinx (Suthers, et al., 2004). Low frequency notes, or elements, are produced through the left side of the syrinx and high frequency notes are produced through the right side of the syrinx in these birds (Suthers, et al., 2004). In contrast, BWS canaries produce notes almost exclusively through the left side of the syrinx (Nottebohm and Nottebohm, 1976; Hartley and Suthers, 1990). These notes are low pitched, and often consist of only one note (Nottebohm and Nottebohm, 1976; Hartley and Suthers, 1990). Interestingly, the proportion of one-note song syllables in the repertoire of BWS canaries increases over successive breeding seasons (Nottebohm and Nottebhom, 1978). One can speculate that BWS canary males increase the proportion of one-note syllables in part because female BWS canaries prefer those song syllables and/or because they themselves can hear the one-note syllables more easily.

Anatomical and Developmental Studies of Hearing Loss in the Belgian Waterslager Canary

The Normal Canary Basilar Papilla

Unlike mammals, hair cells in the avian basilar papilla are not divided into two distinct types according to their shape and innervation. In birds, there is a gradient of hair cell shape and innervation pattern across the basilar papilla (reviewed in Gleich and Manley, 2000). Though the precise distribution of hair cells types in the basilar papilla is species-specific, the general arrangement is consistent across bird species. Hair cells along the neural edge of the papilla receive contacts from mostly afferent auditory nerve fibers and have slightly elongated shapes. These cells have traditionally been termed "tall hair cells," and are similar to mammalian inner hair cells. Hair cells along the abneural edge of the papilla receive almost exclusively efferent contacts and have shorter, wider shapes. These cells have traditionally been termed "short hair cells," and are similar to mammalian outer hair cells. Cells with intermediate degrees of afferent and efferent contacts lie in the middle of the basilar papilla. For simplicity's sake, the term "tall hair cell" will refer to cells with primarily afferent contacts, and the term "short hair cells" will refer to cells with primarily efferent contacts in the following text.

Gleich et al. (1994b) described in detail the morphology of the non-BWS canary basilar papilla. The basilar papilla of non-BWS canaries is approximately 1.6 mm in length, with a total of about 3000 hair cells. As in other species, the canary basilar papilla is slightly bent. The basal end of the papilla is narrow, and the width of the papilla increases toward the apical end, reaching a maximum width (on average 140 μm) at about 60 to 80% distance from the base. Accordingly, the number of hair

cells across the width of the papilla increases with increasing width (approximately

10 cells across the base and 20 hair cells across the papilla at 60-80% from the base).

Hair cells surface area systematically varies across the papilla, such that cells near the

neural edge of the papilla have smaller surface areas than cells near the abneural

edge. The amount of the difference in surface area across the papilla increases with

greater distances from the base. Neural hair cells have more stereocilia than abneural

cells, and the number of stereocilia decreases from base to apex. Gradients in

stereociliary bundle (SB) surface area, length, shape, position, and orientation also

occurred along and across the papilla. The numerous morphological gradients along

and across the canary basilar papilla essentially make every hair cell unique.

The Belgian Waterslager Basilar Papilla

Several studies have described abnormalities of the basilar papillae of BWS

canaries. The papillae of BWS canaries are slightly longer and narrower than non-

BWS canary basilar papillae (Gleich et al., 1994a). There are fewer (approximately

30%) hair cells on the BWS canary basilar papilla compared to non-BWS canaries

(Gleich et al., 1994a; Weisleder and Park, 1994; Weisleder et al., 1996). Many of the

remaining hair cells are misshapen, have enlarged nuclei, and have fewer stereocilia

and disorganized, disoriented or missing SBs (Gleich et al., 1994; Weisleder and

Park, 1994; Weisleder et al., 1996). The short hair cells appear to be the most

severely affected (Gleich et al., 1994; Weisleder and Park, 1994; Weisleder et al.,

1996). The 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, 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., 1994a; Weisleder and Park, 1994; Weisleder et al., 1996).

These anatomical abnormalities are likely to be associated with irregularities in the mechanical and electrical properties of the BWS canary basilar papilla, although the physiological functioning of the BWS canary basilar papilla has not been tested directly. The hair cell abnormalities may result in a reduced stiffness gradient along the papilla in BWS canaries. A change in the stiffness of the basilar papilla could decrease the resonant frequency of the SBs, which would limit the highest frequency that is represented along the papilla (Gleich et al., 1994a; Weisleder et al., 1996). In aggregate, the change in stiffness could expand the representation of lower frequencies to larger than normal regions of the basilar papilla. Additionally, the decrease in stereocilia number could reduce the responsiveness of the basilar papilla to higher frequencies (Gleich et al., 1995).

Abnormal hair cells have also been found in the sacculus of BWS canaries, prompting the suggestion that they suffer from Sheibe's-like dysplasia (Weisleder and Park, 1994). However, both the endocochlear potential and the tegmentum vasculosum appear to be normal in BWS canaries, indicating that these birds are afflicted by a neuroepithelial rather than a cochleo-saccular defect (Gleich et al., 2000).

BWS canaries show a low rate of spontaneous hair cell proliferation and differentiation that is greater than in non-BWS canaries (Gleich et al., 1997). Despite

the continuous hair cell proliferation, the BWS canary papilla does not appear to repair itself. The appearance of new hair cells may be offset by continuous hair cell death that occurs at about the same rate as proliferation (Wilkins et al., 2001).

Noise overexposure and aminoglycoside-induced damage cause a further increase in supporting cell proliferation and differentiation in BWS canaries (Dooling et al., 1997; Gleich et al., 1997; Dooling and Dent, 2001). Despite the capability for hair cell regeneration in BWS canaries, the new hair cells do not seem to repair the basilar papilla and establish normal hearing. Hair cell damage induced by the ototoxic drug kanamycin results in a temporary elevation of absolute thresholds for high frequencies that return to near pre-treatment levels by 13 weeks after injections cease (Dooling et al., 1997; Dooling and Dent, 2001; Dooling et al, in prep). At some frequencies, recovered thresholds are slightly better (5 dB) than before kanamycin treatment; however, it is not clear if this improvement is permanent.

The effects of attenuated acoustic input on the rest of the auditory system in BWS canaries are largely unknown. Weisleder et al. (1996) reported that the perimeters of BWS canary hair cells were deformed in the area of the synapse with auditory nerve fibers, and that the number of myelinated fibers was reduced. Although about 30% of hair cells are missing in BWS canaries, there is only a 12% reduction in the number of auditory nerve fibers (Gleich et al., 2001). Intriguingly, there is a larger proportion of small and large diameter auditory nerve fibers in BWS canaries than in non-BWS canaries. The majority of small diameter fibers originate in the base of the papilla in other bird species (Köppl et al., 2000). It is, therefore, surprising that BWS canaries show a greater proportion of small diameter fibers given

their hearing loss at high frequencies. Relatedly, nucleus magnocellularis (NM) and nucleus laminaris (NL) show normal cell number and organization in adult birds. However, NM and NL have reduced volumes attributed to smaller cell size (Kubke et al., 2002). Presumably, the smaller cell size is associated with reduced afferent input from the auditory nerve that occurs early in post-hatch development.

The hair cell abnormalities and hearing loss in BWS canaries have been shown to develop after the birds hatch. Auditory brainstem response measurements indicate that hearing sensitivity develops normally until approximately 20 days post-hatch, at which point thresholds begin to deteriorate (Brittan-Powell et al., 2002; Ryals and Dooling, 2002). During this period, hair cell abnormalities begin to develop along the basilar papilla (Ryals & Dooling, 2002). Auditory brainstem response thresholds continue to increase as the birds develop, and show adult-like hearing loss by about 20-30 days post hatch (Brittan-Powell et al., 2002).

Behavioral Studies of Hearing Loss and Hair Cell Regeneration in Birds

Behavioral studies in several species of birds have shown that damage to hair cells by acoustic overstimulation or the administration of ototoxic drugs results in temporary changes in auditory sensitivity. Absolute (pure tone) thresholds temporarily increase following hair cell damage in budgerigars (Hashino et al., 1988; Hashino and Sokabe, 1989; Dooling et al., 1997; Dooling et al., 2006; Ryals et al., 1999), chickens (*Gallus domesticus:* Saunders et al., 1995), quail (*Coturnix japonica:* Niemiec et al., 1994), zebra finches (*Taeniopygia guttata:* Ryals et al., 1999), starlings (*Sturnis vulgaris:* Marean et al., 1993), and canaries (Ryals et al., 1999;

Dooling et al, in prep). In all of these studies, thresholds recover to normal or near-normal levels after hair cells regenerate.

A handful of studies have looked at the effects of hair cell damage and regeneration on more complex auditory perceptual tasks. Frequency selectivity temporarily worsens in the area of damage in budgerigars and starlings, indicating reduced frequency selectivity (Hashino and Sokabe, 1989; Marean et al., 1998). Temporal processing may also be affected by hair cell damage in birds. Reduced temporal integration occurs in chickens immediately following experimentally induced hair cell damage (Saunders et al., 1995).   However, temporal modulation transfer functions are mostly unaffected by hair cell damage in starlings (Marean et al., 1998). Discrimination of contact calls also temporarily worsens in budgerigars after hair cell damage (Dooling et al., 1997). As with absolute thresholds, none of these effects are permanent.

Present Aims

The Belgian Waterslager canary presents an opportunity to study a unique combination of processes related to hearing and vocal learning in an animal model including auditory system abnormalities, song learning and vocal production, hearing loss and recovery, auditory perception, and potential genetic underpinnings of these processes in an animal system. There is no question that the inherited auditory pathology and hearing loss in these birds is affects the characteristics of their learned vocalizations. While BWS canaries demonstrate continuous hair cell regeneration, there is never complete repair of the basilar papilla, so the pathology persists in

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adulthood. The unusual hair cell pathology in BWS canaries leads to speculation about perceptual consequences. However, other than elevated high frequency thresholds, not much is known. Studies of song learning in BWS canaries clearly demonstrate that the birds rely on auditory input for normal song learning and maintenance. The hair cell abnormalities in BWS canaries are certain to shape the auditory input, and the unique auditory experience of BWS canaries must be linked to their distinctive vocalizations.

The pattern of damage along the BWS canary basilar papilla indicates that there might be problems with spectral coding, such as reduced frequency selectivity and frequency discrimination ability. Changes in the mechanical properties of the basilar papilla, reduced myelination of auditory nerve fibers, and changes in the filtering characteristics of the auditory system may affect temporal processing mechanisms such as phase effects on masking, temporal integration, and temporal resolution. The reductions in NM and NL volume also might be reflected in deficits in the accuracy of temporal coding of stimuli. Finally, the reduced number of hair cells and auditory nerve fibers may lead to abnormalities of intensity coding. All of these potential perceptual differences between BWS and non-BWS canaries may combine to affect perception of natural sounds such as vocalizations in a unique way.

The following experiments are designed to provide a detailed characterization of auditory perception in BWS canaries and normal-hearing non-BWS canaries. Perception of spectral, temporal, and intensity features of sounds, as well as susceptibility to masking and perception of natural vocal signals, were assessed in BWS and non-BWS canaries using operant conditioning and psychoacoustic

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procedures. These experiments will enhance our understanding of the links between the structure and function of the auditory system and vocalizations in BWS canaries.

Chapter 2: General Methods

Subjects

Three to 4 adult BWS canaries and 3 to 4 non-BWS canaries were used in each experiment. Some birds died before completing the experiments, so the same birds were not run in all experiments. A grand total of 12 BWS canaries and 7 non-BWS canaries were used. More BWS canaries were used because the strain is extremely inbred, and the birds easily succumb to diseases. Birds were housed in an avian vivarium at the University of Maryland and kept on a photoperiod correlated with the season. 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, MD approved the care and use of animals in this study (A3270).

Apparatus

All experiments were conducted in a wire test cage (23 x 25 x 16 cm^2) mounted in a sound-attenuated chamber (Industrial Acoustics Company, Bronx, NY, IAC-3) lined with acoustic foam. The test cage consisted of a perch, an automatic feeder (food hopper), and two response keys, as shown in Figure 1. The perch was mounted on the floor of the cage behind an opening in the floor through which seed was made accessible by activation of a solenoid that raised the food hopper. The two

response keys were mounted vertically on the front of the cage in front of the perch

and food opening, approximately 5 cm apart. Keys were made of red and green 8 mm

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 sound-attenuated chamber was illuminated with a 60-watt light

bulb mounted in a fixture at the top of the chamber. The animals were monitored at

all times by an overhead video camera system.

Figure 1. Photograph of a canary in the testing apparatus.

The experiments were controlled by an IBM Pentium III microcomputer

operating Tucker-Davis Technologies (TDT, Gainsville, FL) System 2 modules.

Stimuli were either stored digitally or generated online 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 a separate channel of the D/A converter to a

separate digital attenuator (TDT, Model PA4) and amplifier (TDT, Model HB6) to a

loudspeaker (KEF Model 80C, England) in the sound-attenuated chamber. 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. Stimulus calibration was performed periodically with 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 Visual Basic

computer program. Data was stored digitally and analyzed using commercially

available statistics software and a custom designed analysis program.

Training and Testing Procedures

Birds were trained to peck the keys for food reward using an operant auto-

shaping program. The phases of training are summarized in Table 1 below. Once the

bird completed all training stages of the auto-shaping program, a variable stimulus

presentation interval was gradually increased to 2 to 6 s, the maximum response

interval was decreased from 3 s to 2 s, and the percent food reinforcement was

gradually decreased to approximately 50-90% (depending on the task and the bird's

behavior). For all experiments, the bird was required to peck the observation key (left

key) for a random interval of 2 to 6 s during a repeating background sound or in quiet.

After this random interval, the background sound was alternated with a target sound.

The bird had to peck the report key (right key) within 2 s of this target/background alternation to receive a food reward. A report key peck during this time was recorded as a hit. If the bird failed to peck the report key within 2 s of the target/background alternation, it was recorded as a miss. Incorrect report key pecks were punished with a time-out period during which all of the room lights were extinguished. Thirty percent of all trials were sham trials, during which there were no target/background alternations. Pecks to the report key during these trials were recorded as false alarms and punished with variable time-out periods (approximately 1 to 10 s). Sessions with false alarm rates of 18% or higher were not used for analysis.

Table 1. Auto-shaping training phases.

Training Phase	Behavior Required to Move to Next Phase
Hopper Training	Food hopper is propped up, allowing free access to food. Bird must eat seeds out of the hopper.
Phase 1	At a predetermined interval, observation key LED blinks, tone plays, and hopper raises. Bird must peck the observation key 10 times.
Phase 2	Observation key LED is always on. Bird must peck the observation key 10 times.
Phase 3	Observation key LED is always on. A peck to the observation key results in the presentation of a tone and blinking of the report key LED. Bird must then peck the report key. The bird must perform sequence 10 times.
Phase 4	LEDs for both keys are always on; bird must peck the observation key, followed by the report key. A tone is presented after each observation key-peck. The bird must correctly perform sequence 10 times.
Phase 5	LEDs for both keys are always on. Random sham trials, in which no tone is presented following a peck to the observation key, are introduced. Bird runs until it refrains from pecking the report key during sham trials.

Sessions consisted of approximately 50 to 100 trials, and birds were tested twice a day, 5 days a week. Each bird ran a minimum of 300 trials on each

experimental condition, and the last 200 trails once behavior stabilized were used for analysis. For experiments measuring thresholds, target sounds were presented using the Method of Constant Stimuli at 7 different levels within a block of 10 trials. Behavior was considered stable if the threshold did not change by more than 1/3 of the increment step size within the last two 100-trial blocks. For experiments measuring discrimination abilities, behavior was considered stable if the percent correct for a given target did not change by more than 10% within the last two 100-trial blocks. To minimize response biases and practice effects, birds ran on different experimental conditions in a random order.

Chapter 3: Basic Sensitivity

It is important to define the basic sensitivity of an animal to determine the range of available spectral information. A songbird is unlikely to produce vocalizations that are not audible to itself and other members of its species. Behavioral audiograms for BWS and non-BWS canary strains have been reported in several studies.   These studies have shown that the region of best sensitivity falls between about 2000 to 4000 Hz for non-BWS canaries not bred for specific song characteristics (Dooling et al., 1971; Okanoya and Dooling, 1985, 1987a), 2000 to 5700 Hz for Spanish Timbrado canaries bred for a song with a wide frequency range (Lohr et al., 2004), and 1000 to 2500 Hz for BWS canaries bred for a low pitched song (Okanoya and Dooling, 1985, 1987a). Compared to non-BWS canary strains, BWS canaries always show elevated thresholds for frequencies above 1500 to 2000 Hz (Okanoya and Dooling, 1985; 1987a).

Experiment 1: Absolute Thresholds for Pure Tones

To ensure that the birds used in the following series of experiments showed absolute sensitivity comparable to that of previously tested BWS and non-BWS canaries, absolute thresholds for pure tones were measured for each subject.

Methods

Stimuli

Stimuli were 400 ms pure tones with frequencies of 500, 1000, 2000, 2860, 4000, 5700, and 8000 Hz. The tones had rise/fall times of 20 ms $\cos^2$, and were generated at a sampling rate of 40 kHz.

Procedures

Absolute thresholds for pure tones were measured at several frequencies ranging from 500 to 8000 Hz for all subjects prior to running in other experiments. In all, 12 BWS and 7 non-BWS canaries were tested. Not all birds were tested at all frequencies. Tones were presented at a range of levels bracketing the expected threshold according to the Method of Constant Stimuli, with an increment size of 3 or 5 dB. The order of frequencies tested was randomized for each individual bird. Thresholds were defined as the level of a tone detected 50% of the time, corrected for the false alarm rate [Pc*=(Pc-FA)/(1-FA)] (Gescheider, 1985; Dooling and Okanoya, 1995a).

Results and Discussion

Absolute thresholds for individual birds along with average thresholds reported for BWS and non-BWS canaries by Okanoya and Dooling (1985) are plotted in Figure 2. Thresholds for all BWS and non-BWS were consistent with previously published thresholds. Thresholds for non-BWS canaries did not differ across individuals by more than about 5-10 dB at any frequency. All BWS canaries showed elevated thresholds above 1000 or 2000 Hz, though the amount of hearing loss varied

considerably at some frequencies (by nearly 40 dB at 2000 and 4000 Hz and 25 dB at 2860 Hz). Variation in hearing threshold in BWS canaries is likely due to variation in the pattern of hair cell damage across individuals (Gleich et al., 1994).
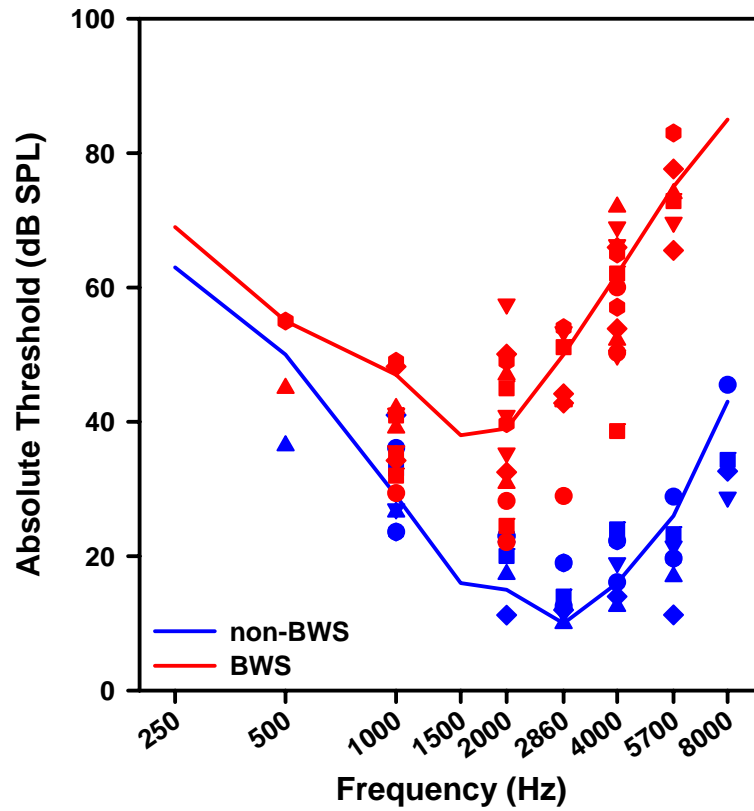
Figure 2. Absolute thresholds for BWS and non-BWS canaries. Individual thresholds from birds used in the present experiments are plotted as symbols. Average thresholds from Okanoya and Dooling (1985) are plotted as solid lines.

Chapter 4: Masking

In nature, sounds rarely occur against a quiet background, but rather as part of a complex acoustic milieu in which there are many background sounds present. While an animal may not attend to background sounds, the sounds are certain to affect the animal's ability to detect a signal. When the detection of one sound is made more difficult by the presence of another sound, it is referred to as masking. The spectral and temporal properties of the masking sound can both affect the amount of masking that occurs. Studies of masking can reveal how well an organism can "hear out" a signal in the presence of other sounds, as well as how the sensory epithelium processes complex sounds.

The majority of masking studies have focused on spectral masking phenomena to estimate place coding along the cochlea. Spectral masking depends on the ear's ability to separate components of a complex sound, or frequency selectivity. The general premise of these studies is that sounds with spectral components that are close to or the same as the signal frequencies will mask the signal more than sounds with frequencies farther away from the signal (e.g., Wegel and Lane, 1924; Fletcher, 1940). More recent studies have shown that when the spectra of the maskers are held constant, detection of a signal can also be affected by the temporal properties of the masker (e.g., Carlyon and Datta, 1997a, b; Smith et al., 1986; Kohlrausch and Sander, 1995; Summers and Leek, 1998; Lentz and Leek, 2001; Oxenham and Dau, 2001).

In humans, increased absolute thresholds are often accompanied by increased susceptibility to masking (reviewed in Moore, 1995). The changes in masking that accompany hearing loss of cochlear origin may be due to changes in the response

characteristics of the basilar membrane associated with hair cell damage, such as broadened auditory filters and reduced or absent nonlinear processing mechanisms. The experiments described in this chapter investigate the effects of spectral and temporal features of maskers on signal detectability in BWS and non-BWS canaries.

Experiment 1: Masking by Broadband Noise and Critical Ratio Estimates of Frequency Selectivity

The basilar membrane of mammals (as well as the basilar papilla of birds) is believed to function as a bank of overlapping bandpass filters that each respond to a different range of frequencies (Fletcher, 1940). A listener is assumed to use a filter with a center frequency close to the signal frequency when detecting a sound. Masker components that fall within this filter make the signal more difficult to detect. The threshold of a signal corresponds to a certain signal-to-noise ratio at the output of the filter. By measuring the threshold of a signal in the presence of maskers with a range of bandwidths, an estimate of the width of the filter, the critical bandwidth, can be obtained (Fletcher, 1940). Each critical band is assumed to represent an equal distance along the basilar membrane (Fletcher, 1940; Zwicker et al., 1957; Greenwood, 1961a, 1961b). Smaller critical bands indicate narrower filters. Narrower filters pass a smaller range of frequencies, and therefore exhibit better frequency selectivity than wider filters.

The ratio between the power of a tone at threshold and the power per Hz (spectrum level) of a flat-spectrum background noise corresponds to the critical ratio (CR), an indirect estimate of the critical band of the auditory filter (Fletcher, 1940). Frequency selectivity has most commonly been estimated by CR measures in animals

because an animal can learn to detect a tone in a constant noise background relatively easily and also because the data can be obtained in a fairly short amount of time. In starlings, critical bands calculated from CRs correspond closely to directly measured critical bands (Buus et al., 1995). One critical band corresponds to about 0.1 mm along the basilar papilla of the starling (Buus et al., 1995).

Cochlear damage in humans results in larger than normal critical bands and CRs in the area of hearing loss (de Boer and Bouwmeester, 1974; Margolis and Goldberg, 1980; Hall and Fernandes, 1983). Budgerigars with temporary hearing loss also show larger CRs (Hashino and Sokabe, 1989). Thus, a higher signal-to-noise ratio is required to detect sounds in broadband noise in the region of hearing loss in hearing-impaired humans and budgerigars with temporary hearing loss. In the present experiment, thresholds for tones in broadband noise were measured in BWS and non-BWS canaries to assess susceptibility of different regions along the basilar papilla to masking by noise with spectral energy spread over a wide range of frequencies.

Methods

Stimuli

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

Procedures

Masked thresholds for tones were measured in continuous broadband noise. Thresholds were measured for 3 non-BWS canaries and 3 BWS canaries in 65 dB SPL noise (spectrum level 25 dB). Thresholds for BWS canaries were also measured in 75 dB SPL noise (spectrum level 35 dB) because the 65 dB SPL noise did not produce sufficient masking at 4000 and 5700 Hz.

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, 1995a). The average false alarm rate was 2.79% for non-BWS canaries and 1.46% for BWS canaries. Data from sessions with false alarm rates larger than 18% are typically excluded from analysis. None of the data from non-BWS or BWS canaries were discarded.

Results and Discussion

Masked Thresholds

Masked thresholds determined using 65 dB SPL noise are shown in Figure 3 for non-BWS and BWS canaries. Thresholds for all frequencies were elevated by about 20-30 dB in non-BWS canaries. Thresholds in BWS canaries were elevated by about 5-20 dB for frequencies 4000 Hz and below for the 65 dB SPL noise level. There was little to no masking at 5700 Hz for BWS canaries by the 65 dB SPL noise. Even a noise level of 75 dB SPL did not produce sufficient masking at 5700 Hz in BWS canaries. This is likely due to the fact that the noise levels used approach threshold levels at these higher frequencies, and may even be below threshold for

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some individual birds. Higher noise levels were not tested due to the risk of incurring

more hair cell damage.
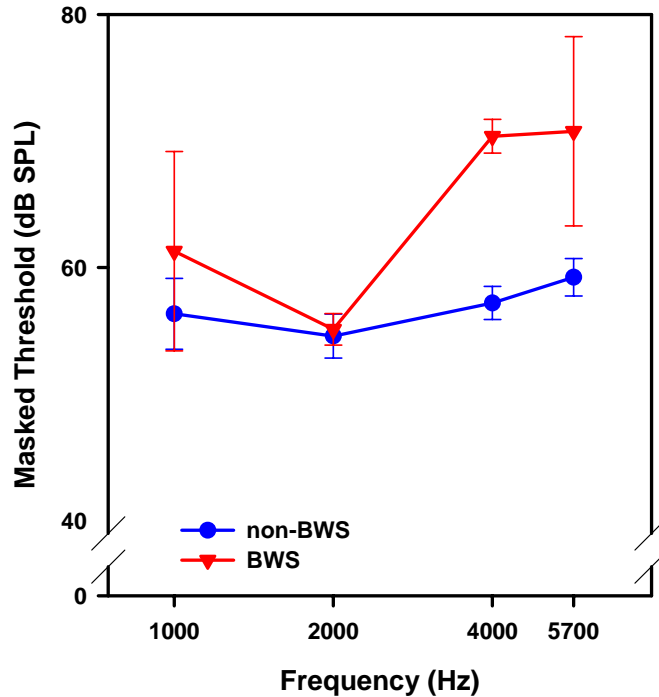
Figure 3. Masked thresholds for non-BWS and BWS canaries measured using 65 dB
SPL flat spectrum noise. Error bars indicate standard error.

Critical Ratios

CRs measured in 65 dB SPL noise for BWS and non-BWS canaries are shown

in Figure 4. Non-BWS canaries showed an average CR at 1000 Hz that was similar

to CRs at 1000 Hz reported in Spanish Timbrado canaries and German Roller

Canaries (Okanoya and Dooling, 1987b; Lohr et al, 2004). The CR at 2000 Hz for

non-BWS canaries was consistent with CRs reported in one study in German Roller

canaries (no other frequencies were tested; Okanoya and Dooling, 1985). However,

CRs for frequencies above 1000 Hz measured in non-BWS canaries in the present

study were smaller and changed less with frequency than in two other previous studies (Okanoya and Dooling, 1987b; Lohr et al., 2004). It is not clear if these differences are related to the strain of the canary, or just individual variation. One of the studies mentioned above (Okanoya and Dooling, 1987b) only measured CRs in one canary.
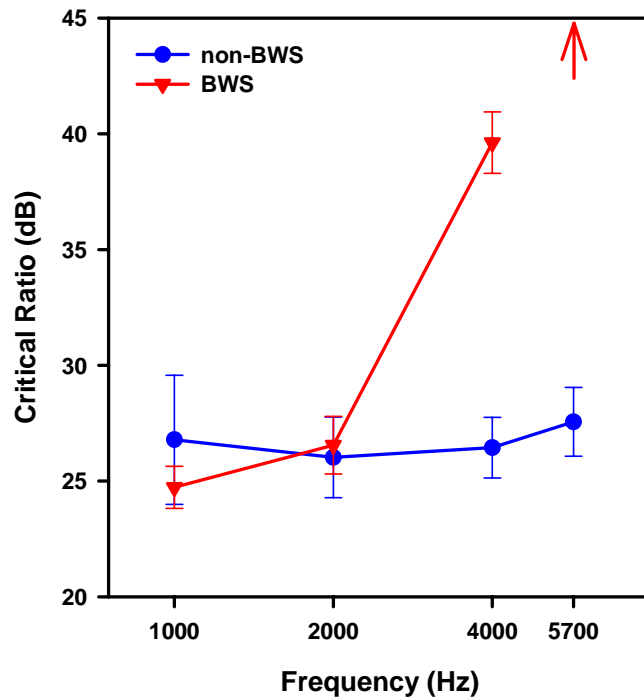
Figure 4. Critical ratios for non-BWS and BWS canaries. Error bars indicate standard error. The arrow indicates that a measurement could not be obtained at 5700 Hz for BWS canaries, and likely exceeded 40 dB.

No CR could be calculated for BWS canaries 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. The CR at 2000 Hz in BWS canaries was consistent with CRs previously reported at 2000 Hz

in this strain (Okanoya and Dooling, 1985). CRs increased by about 15 dB between 1000 and 4000 Hz in BWS canaries. Interestingly, CRs were smallest in the area corresponding to the spectral peaks of BWS canary vocalizations.

A mixed factor (frequency x strain) ANOVA showed a significant effect of frequency [$F(2, 8)=21.73$, $p=0.001$] and strain [$F(1, 4)=4.272$, $p<0.001$] and a significant interaction of frequency and strain [$F(2, 8)=21.957$, $p=0.001$]. CRs were not significantly different between BWS and non-BWS canaries at 1000 Hz and 2000 Hz. At 4000 Hz, CRs for BWS canaries were much larger than those of non-BWS canaries [$q(3,23)=-10.0$, $p<0.05$], 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 (Margolis and Goldberg, 1980; Hall and Fernandes, 1983) and in birds with experimentally-induced temporary hearing loss (Hashino and Sokabe, 1989).

Experiment 2: Frequency Selectivity Estimated with Psychophysical Tuning Curves

Though noticeably simple to measure, critical bands and CRs do not provide a good 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) momentous study have shown that these assumptions are inaccurate (reviewed in Moore, 2003). Since then, several other psychoacoustic measures of frequency selectivity have been developed.

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Psychophysical tuning curves (PTCs) provide a more accurate measure of spectral masking and auditory filter shape. Instead of varying the signal level in a constant background noise as in CR measurements, signal level is held constant and the background level is varied when measuring PTCs (reviewed in Patterson and Moore, 1986). The masker level needed to just mask the signal is determined for maskers with a range of frequencies. Lower masker levels are required to just mask the signal tone if the maskers are close in frequency to the signal. As the masker frequency is moved farther away from the signal frequency, higher masker levels are required to just mask the signal. A low signal level is normally used (approximately 10 to 15 dB sensation level) when measuring PTCs to produce excitation primarily at one filter. The masker level at threshold is assumed to produce a constant output from that filter. The resulting PTCs are akin to basilar membrane or neural tuning curves measured physiologically.

In mammals, PTCs are typically asymmetrical, with a pronounced low frequency tail (e. g. Moore, 1978; Ryan et al., 1979). That is, the masker function is steeper on the high frequency side (maskers above the signal frequency) than on the low frequency side (maskers below the signal frequency). The absolute bandwidth of the tuning curves increases with increasing frequency when PTCs are plotted on a log frequency scale; however, the relative bandwidth decreases with increasing frequency (linear scale). Thus, frequency selectivity is better for higher frequencies than for low frequencies. Additionally, tuning curves become less sharp with increasing level, resulting in reduced frequency selectivity at higher sound levels. This is thought to occur because the outer hair cells produce active movements in response to low sound

levels that amplify components of a sound that are close to the characteristic frequency for a given auditory channel. This active gain mechanism ceases to operate at higher levels.

Cochlear damage in humans typically causes PTCs to broaden (Leshowitz et al., 1975; 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 if hair cell damage is severe enough (Ryan et al., 1979; Salvi et al., 1982; Smith et al., 1987).

Psychophysical tuning curves have only been measured in one bird species, the budgerigar. Budgerigar PTCs are more symmetric, more narrowly tuned in the area of best sensitivity, and vary less as a function of level than those of mammals (Bock and Saunders, 1975; Saunders et al., 1979; Saunders and Pallone, 1980). However, budgerigars have unusually good frequency resolution in the area of 2860 Hz compared to other birds (Okanoya and Dooling, 1987b). To the author's knowledge, there are no other reported PTCs in birds. Tuning curves measured from auditory nerve fibers in the starling are generally V-shaped and symmetrical (Manley et al., 1985). There is a lot of variability in frequency selectivity at a given characteristic frequency, but on average frequency selectivity increases with increasing characteristic frequency (Manley et al., 1985). In fact, these tuning curves are narrower in starlings than in mammals, even when comparing frequency selectivity at 40 dB above threshold (Manley et al., 1985). Thus, we might expect a similar pattern of results for behaviorally measured PTCs in songbirds.

In this experiment, PTCs with center frequencies of 1000, 2000 and 2860 Hz were estimated from tone-on-tone masking patterns measured in BWS and non-BWS canaries. These measures provide a more precise estimate of frequency selectivity and auditory filter shape in the two canary strains. The results have implications for the effects of abnormal hair cell pathology on the functioning of the basilar papilla in BWS canaries, as well as for the function of the active mechanism associated with efferently innervated hair cells in non-BWS canaries. If the short hair cells increase frequency selectivity in birds as do the outer hair cells of mammals, then frequency selectivity should be reduced in BWS canaries as indicated by their larger than normal relative bandwidths.

Methods

Stimuli

The signals were 20 ms 1000, 2000, and 2860 Hz pure tones with $\cos^2$ rise/fall times of 5 ms. Maskers were 300 ms tones with rise/fall times of 20 ms. Maskers were 700, 800, 900, 950, 1050, 1100, 1200, 1400, 1600, and 1800 Hz for the 1000 Hz signal; 1400, 1600, 1800, 1900, 1950, 2050, 2100, 2200, 2400, 2600, and 2700 Hz for the 2000 Hz signal; and 2060, 2260, 2460, 2660, 2760, 2810, 2910, 2960, 3060, 3260, 3460, and 3660 Hz for the 2860 Hz signal.

Procedures

Three BWS canaries and 3 non-BWS canaries were used as subjects in the experiment. Prior to measuring masked thresholds, absolute thresholds for the pure

tone signals of 1000, 2000, and 2860 Hz were measured to establish a 10 dB SL for each signal tone. Psychophysical tuning curves 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, a somewhat unorthodox method of measuring PTCs was used to make the task easier for the birds and to ensure that they were responding only when they detected the signal tone. A similar procedure was used to measure tone-on-tone masking patterns in chickens (Saunders and Salvi, 1995).

Instead of keeping the signal at a constant level and varying the masker level on each trial, 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 levels to generate masking functions. The level of the signal tones were presented in 5 dB steps according to the Method of Constant Stimuli and varied randomly from trial-to trial. Maskers for the 1000 Hz signal were presented at 40, 50, and 60 dB SPL for non-BWS canaries and at 60, 70, and 80 dB SPL for BWS canaries. Maskers for the 2000 Hz signal were presented at 30, 40, and 50 dB SPL for non-BWS canaries and 60, 70, and 80 dB SPL for BWS. Maskers for the 2860 Hz signal were presented at 30, 40, and 50 dB SPL for non-BWS canaries and 70, 80, and 85 dB SPL for BWS canaries. The stimulus presentation system was

unable to accommodate masker levels greater than 85 dB SPL. These masker levels were chosen to ensure that at least 10 dB of masking was produced by 6 masker frequencies.

A nonsimultaneous masking paradigm was used, in which the signal tone immediately followed the masker tone. Nonsimultaneous masking has the benefit of avoiding two-tone suppression effects that may occur, in which the basilar membrane or basilar papilla response to one tone may be suppressed by the presence of another tone (Sachs and Kiang, 1968; Sachs et al., 1974).

Thresholds were defined as the level of the tone detected 50% of the time, adjusted by the false alarm rate [Pc*=(Pc-FA)/(1-FA)] (Gescheider, 1985; Dooling and Okanoya, 1995a). The average false alarm rate was 2.33% for non-BWS canaries and 3.09% for BWS canaries. Data from sessions with false alarms larger than 18% were excluded from analysis. In all, 10% of all non-BWS canary sessions and 4% of all BWS canary sessions were discarded.

The amount of masking of each signal frequency was plotted as a function of masker frequency for each level tested. A separate masking function was generated for each signal frequency. Psychophysical tuning curves were derived from the masking functions by plotting the masker level and interpolated masker frequency required to produce a constant amount of masking (10 dB).

Results and Discussion

The amount of masking was calculated as the difference in threshold of a signal tone in the presence of a masker and in quiet for each masker-signal

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combination at each level tested. The average amount of masking as a function of masker frequency is shown for signal frequencies of 1000Hz (A), 2000 Hz (B), and 2860 Hz (C) for non-BWS and BWS in Figure 5. Higher masker levels were required in BWS canaries to mask the signal than in non-BWS canaries for all signal frequencies.

The general shape and symmetry of the tone-on-tone masking functions for non-BWS were consistent with tone-on-tone masking functions reported in chickens (Saunders and Salvi, 1995) and budgerigars (Dooling and Searcy, 1985a), and with narrow-band masking patterns measured in non-BWS canaries and other species of birds (Saunders et al., 1978; Saunders and Pallone, 1980; Brown et al., 2001). There was a greater change in the overall amount of masking by maskers with different frequencies in non-BWS canaries compared to BWS canaries. Maskers with frequencies that were close to the signal frequency produced the maximum amount of masking for all signal frequencies tested in non-BWS canaries. Maskers with frequencies that were close to the signal frequency produced the maximum amount of masking for 1000 Hz and 2000 Hz in BWS canaries; however, the maximum amount of masking was produced below the signal frequency for the 2860 Hz signal condition. This finding indicates that channels with characteristic frequencies below 2860 Hz may have been responding more to the signal tone than channels with characteristic frequencies near 2860 Hz in BWS canaries. This may be because the
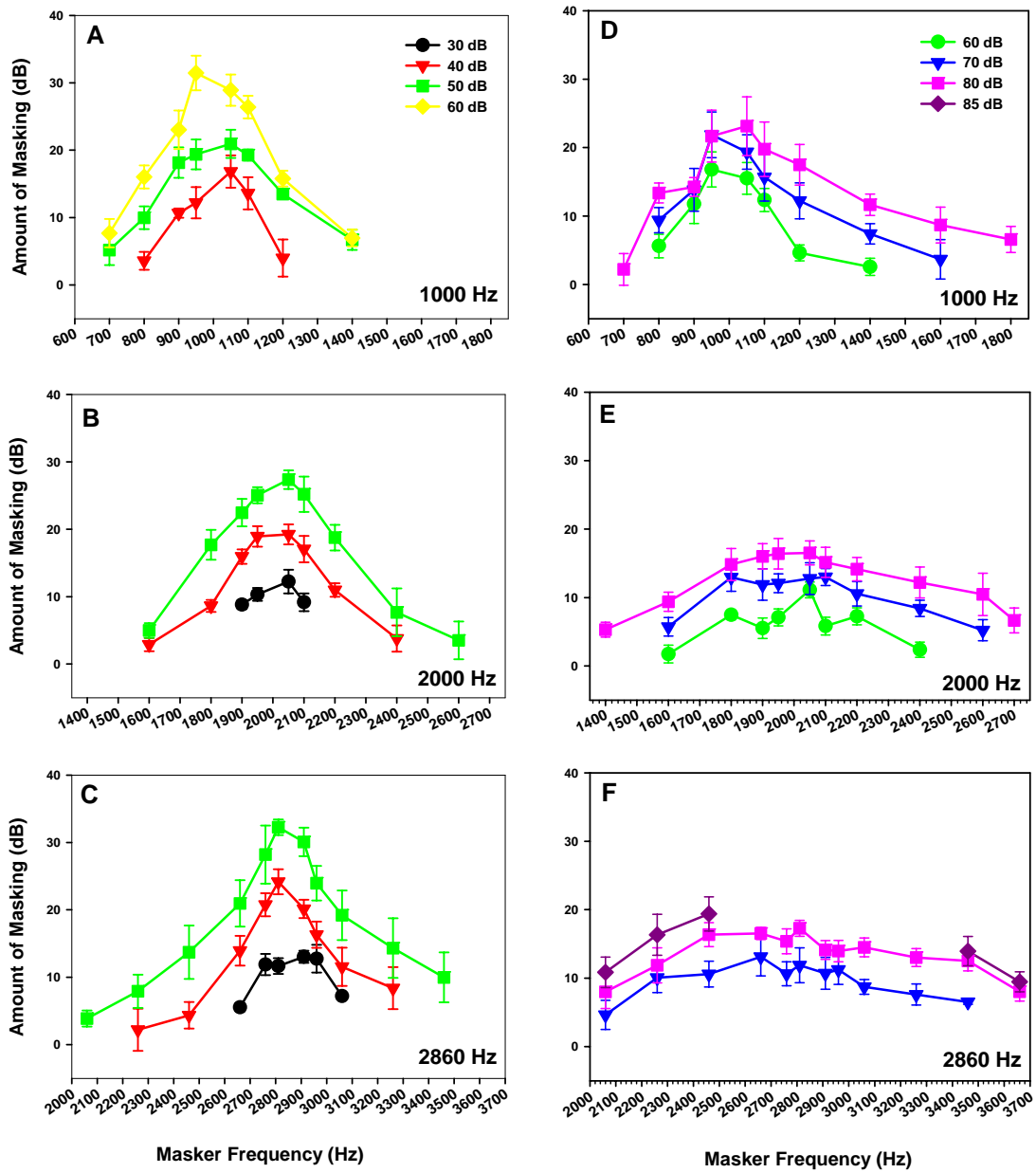
Figure 5. Tone-on-tone masking patterns for non-BWS canaries: A) 1000 Hz, B) 2000 Hz, C) 2860 Hz, and BWS canaries: D) 1000 Hz, E) 2000 Hz, F) 2860 Hz. Error bars indicate standard error.

tall 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 BWS canaries 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 BWS canaries.
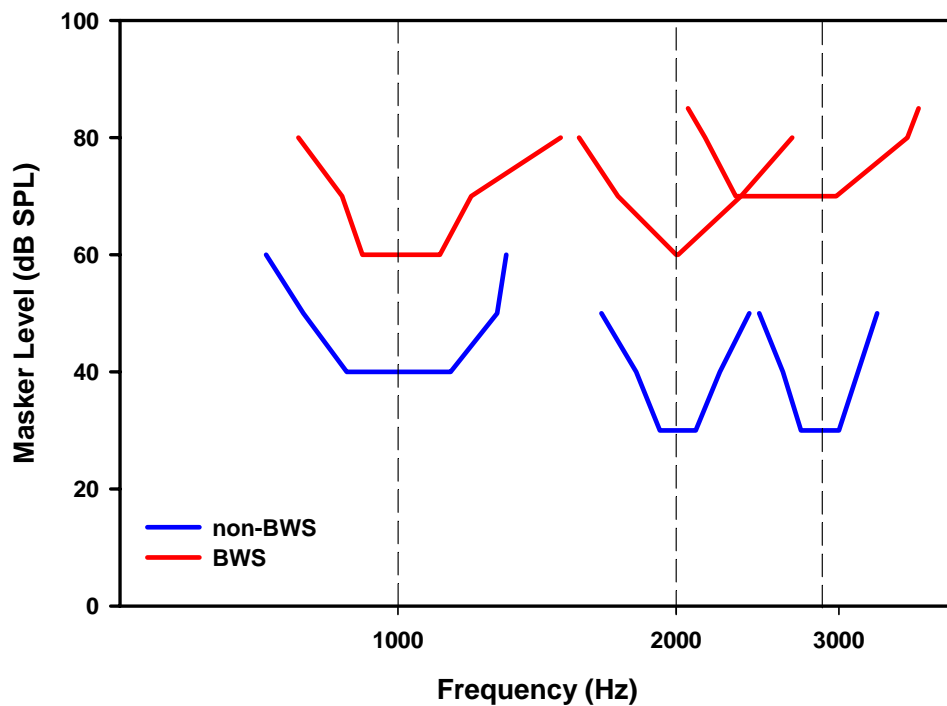
Figure 6. Psychophysical tuning curves for non-BWS and BWS canaries. Dotted lines indicate signal frequencies.

Psychophysical tuning curves were derived from the masking functions by plotting the masker level and frequency required to produce a constant amount of masking (10 dB; shown in Figure 6). PTCs were elevated at all frequencies for BWS canaries compared to non-BWS canaries. The shapes of the PTCs for non-BWS

canaries were V-shaped and relatively symmetrical. This result is consistent with

previous reports in budgerigars (Saunders and Else, 1976; Saunders et al., 1978;

Saunders et al., 1979; Kuhn and Saunders, 1980) in the range of frequencies tested

here. PTCs for BWS canaries were more asymmetrical than non-BWS canary PTCs.

The PTC with a center frequency of 2860 Hz was the most asymmetrical, once again

indicating that channels tuned to lower frequencies were responding to the 2860 Hz

signal tone.

One further benefit to measuring PTCs is that the results can be used to

identify dead regions (regions with no functioning hair cells) in the inner ear (Moore

et al., 2000; Moore and Alcantara, 2001). The tip of a PTC may be shifted away from

the signal frequency when there are no functioning hair cells in the region of the

basilar membrane corresponding to the signal frequency. Detection of the tone will

rely on excitation of healthy hair cells above or below the dead region. A low

frequency dead region is indicated by a PTC tip that is above the signal frequency. A

high frequency dead region results in PTC tips that are below the signal frequency.

Finally, a mid-frequency dead region may result in the PTC tip being shifted above or

below the signal frequency. Though there is no distinct tip for the PTC for a 2860 Hz

signal in BWS canaries, the region near the signal frequency is clearly skewed toward

lower frequencies. This might indicate that BWS have a high frequency dead region.

A measure of the sharpness of tuning, the quality factor (Q), was calculated

for each PTC.   Q is the reciprocal of relative (to the center frequency) bandwidth at a

given amount of masking. Larger Q values indicate sharper tuning, or increased

frequency selectivity. Q values were calculated at 10 dB of masking (Q_{10}) and at 20

dB of masking (Q_{20}). Average Q values for non-BWS and BWS canaries are shown in Figure 7 for 10 dB (A) and 20 dB (B) bandwidths.
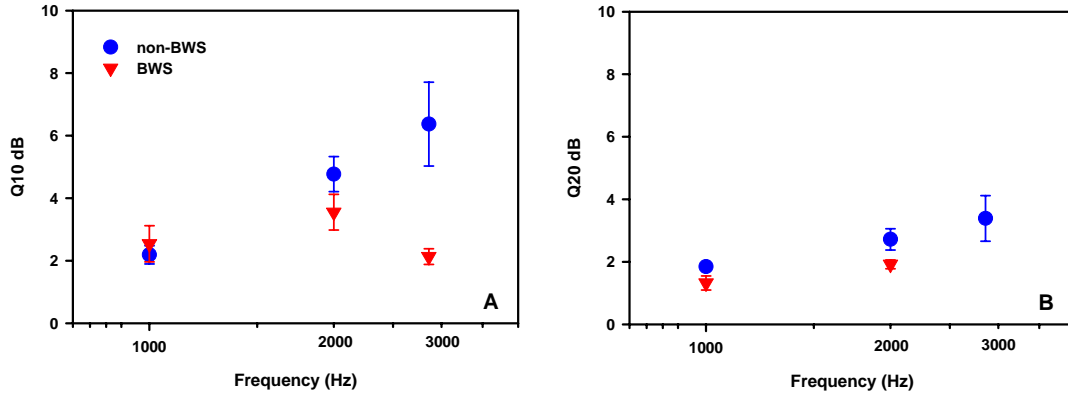
Figure 7. Average quality factor (Q) values for BWS and non-BWS canaries for 10 dB (A) and 20 dB (B) bandwidths. Error bars indicate standard error.

Both Q_{10} and Q_{20} values increased with increasing frequency in non-BWS canaries, indicating increased frequency selectivity at higher frequencies compared to lower frequencies. The non-BWS canary Q_{10} values are smaller than those reported in budgerigars (Kuhn and Saunders, 1980) and humans (Moore, 1978) using forward masking procedures. This result indicates poorer frequency selectivity in non-BWS canaries. Q_{10} values were larger than Q_{20} values in non-BWS, reflecting the widening of the skirts of the PTC as the masker frequency moves away from the center frequency. CR estimates of frequency selectivity also indicate that frequency selectivity is worse in canaries compared to budgerigars and other bird species (Okanoya and Dooling, 1987b; Lohr et al., 2004).

In BWS canaries, Q_{10} values were larger for PTCs with center frequencies of 2000 Hz compared to 1000 and 2860 Hz. This result indicates that frequency

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selectivity increases between 1000 and 2000 Hz, but then decreases for higher frequencies. Q_{20} values were slightly larger at 2000 Hz than at 1000 Hz in BWS canaries. No Q_{20} could be measured in BWS at 2860 Hz.

A strain x frequency mixed factor ANOVA performed on the Q_{10} data revealed a significant effect of frequency [$F_{(2, 8)}$=6.073, p=0.025] and a significant interaction between strain and frequency [$F_{(2, 8)}$=7.313, p=0.016], but no significant main effect of strain. Post hoc analyses were performed using Tukey's HSD test. Q_{10} values for BWS canaries were not significantly different from Q_{10} values for non-BWS canaries at 1000 and 2000 Hz. Q_{10} values were larger at 2860 Hz for BWS canaries than non-BWS canaries [$q_{(3, 17)}$= 4.392, p<0.05]. This broadening of the PTC is consistent with reports in hearing-impaired humans (Leshowitz et al., 1975; 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).

Neural tuning curves measured for auditory nerve fibers in cats with experimentally-induced hair cell damage show that the exact shape of the neural tuning curve depends on the pattern of hair cell damage (reviewed in Liberman et al., 1986). A pattern of damage in which there is damage to some outer hair cells but no inner hair cell damage results in a raised tip, presumably due to damage to the active mechanism. A pattern of damage with a total loss of outer hair cells and intact inner hair cells results in tuning curves with no distinct tip and a very broad tuning, probably due to the complete loss of the active mechanism. Moderate damage to inner hair cell stereocilia combined with minimal damage to outer hair cells results in

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a tuning curve that is elevated, but has an almost normal shape. Severe damage to both inner and outer hair cell stereocilia results in an elevated and broadened tuning curve with no distinct tip.

Based on the findings of Liberman et al. (1996), the PTC characteristics found in BWS canaries suggest several things about the pattern of hair cell damage along the basilar papilla. The elevated PTCs at all center frequencies tested may be due to THC damage along the basilar papilla of BWS canaries. The extremely broad PTC with a 2860 Hz center frequency may result from loss of active processing mechanisms supported by SHCs. Alternatively (additionally), there may not actually be any channels tuned to 2860 Hz. Instead, channels with lower center frequencies may be excited by higher frequencies presented at higher levels. The sound levels used to test BWS canaries at high frequencies may be recruiting these lower-frequency channels.

Weisleder et al. (1996) suggested that changes in the stiffness gradient of the BWS basilar papilla due to structural abnormalities results in a shift in the resonance of the basilar papilla to lower frequencies. In pigeons (*Columbia livia*), sensitivity, frequency selectivity, and tonotopic mapping of the basilar papilla is reduced in preparations in poor physiological condition (Gummer et al., 1987; Smolders et al., 1986).

The reduced frequency selectivity at higher frequencies in BWS canaries 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, reduced frequency discrimination ability, changes in temporal resolution, and difficulty with the perception of natural sounds that fall within the range of hearing loss in BWS canaries.

Experiment 3: Phase Effects on Masking

Most masking studies in birds have focused on the effects the spectral content of the masker has on signal thresholds, but some more recent studies have examined the effects of temporal waveform shape on masking when long-term frequency cues are held constant. Harmonic complexes with identical long-term frequency spectra, but varied phase spectra, have been used to elucidate differences in temporal masking effects between birds and humans (Leek et al., 2000; Dooling et al., 2001; Lauer et al., 2006). These complexes were constructed according to an algorithm developed by Schroeder (1970) to produce harmonic structures with component starting phases selected to form a waveform with a flat temporal envelope. Negative and positive versions of the algorithm produce complexes with identical long-term frequency spectra and envelope shapes but opposite temporal fine structure. Negative-phase and positive-phase Schroeder harmonic complexes are differentially effective as maskers in birds and humans. Positive-phase Schroeder waveforms are less effective maskers than negative-phase Schroeder waveforms in humans; whereas, the two complexes produce similar amounts of masking in birds (Leek et al., 2000; Dooling et al., 2001; Lauer et al., 2006).

Modified versions of Schroeder complexes with systematic variations in waveform shape produced by different selections of component starting phases have

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been used to estimate the phase response of the basilar membrane in humans and the

basilar papilla in birds (Lentz and Leek, 1999, 2001; Oxenham and Dau, 2001, 2004;

Lauer et al., 2006). The temporal waveform shape of the complex that produces the

least amount of masking is thought to mirror the phase response of the cochlea.

Complexes which have more modulated envelopes are generally less effective

maskers than complexes with relatively flat envelopes in normal-hearing species of

birds, and positive-phase and negative-phase maskers are similarly effective as

maskers (Lauer et al., 2006). However, the most modulated waveform does not

produce the least amount of masking. Rather, a negative-phase waveform that is

slightly less modulated results in the least amount of masking, and this remains

unchanged across the range of signal frequencies tested. In normal-hearing humans,

more modulated maskers are not always less effective maskers than flatter

waveforms, and positive-phase maskers generally produce less masking than

negative-phase maskers for signal frequencies of 1000 Hz and above (Lentz and

Leek, 2001; Oxenham and Dau, 2001; Lauer et al., 2006). The least effective

maskers in normal-hearing humans are typically positive-phase maskers that vary

with signal frequency. The masking differences and associated differences in the

cochlear phase response between birds and humans have been attributed to

differences in cochlear structure and function, such as the size (length and width), the

spatial arrangement of hair cells, the coupling of hair cells with the tectorial

membrane, and active processing mechanisms.

   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). Often, there is no discernable least effective masker. When there is a prominent least effective masker, the cosine-phase waveform produces the minimum amount of masking.

Differences in masking between normal-hearing and hearing-impaired human listeners have been attributed in part to the loss of active processing mechanisms associated with cochlear damage. Nonlinear processing mechanisms are thought to alter the internal representation of the positive-phase waveforms along the basilar membrane in normal-hearing humans, producing waveforms that are more modulated internally than the input waveforms. More modulated waveforms have longer low-energy portions in which the signal may be more easily detected. These nonlinear processing mechanisms are usually impaired or lost with outer hair cell damage, so the internal alteration of positive-phase waveforms does not occur in listeners with sensorineural hearing loss. Without alterations due to nonlinear processing, the cosine-phase masker has the longest low-energy portions within each period. Presumably, hearing-impaired listeners detect the signals within these long low-energy portions.

Efferently innervated cells that presumably underlie active processing mechanisms in birds are the most severely affected along the BWS canary basilar papilla (Gleich et al., 1994a; Weisleder et al., 1996). Therefore, a decrease or loss of active processing mechanisms would be expected in these birds. Based on reports in human listeners with cochlear damage, BWS canaries were expected to show less effect of temporal waveform shape on masking due to loss of active processing and

other changes in the mechanical response properties of the ear that accompany hearing loss.

Methods

Stimuli

Stimuli were harmonic complex maskers and maskers with an embedded signal tone. The masking stimuli were constructed by summing equal-amplitude cosine tones from 200 to 5000 Hz, with a fundamental frequency of 100 Hz. The phases of the tones were selected according to a modification of the Schroeder (1970) algorithm:

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

where θ_n represents the nth harmonic, N is the total number of harmonics, and C is a scalar (Lentz and Leek, 2001).

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. 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, so that scalars closer to zero produce more rapid frequency changes than those close to ±1.0. Phase

spectra and time waveforms for several of the harmonic complexes are shown in Figure 8.

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.
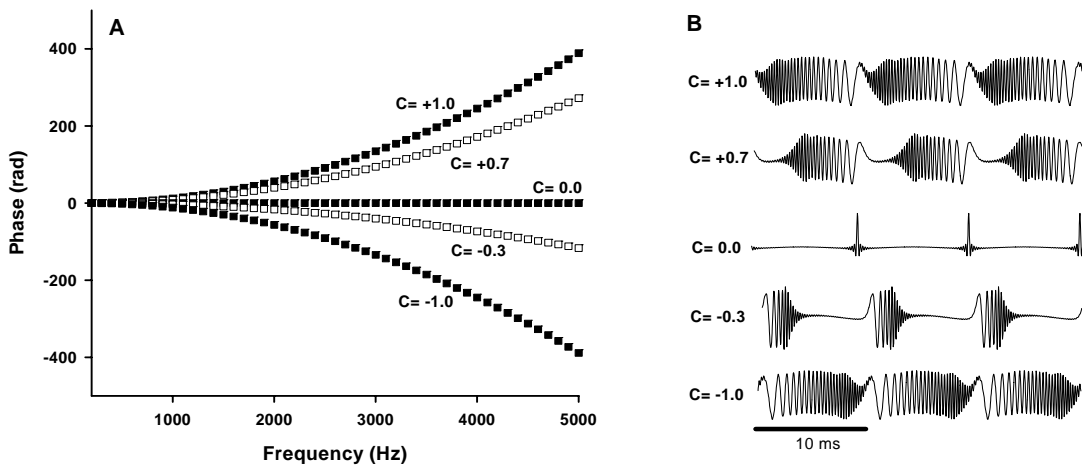
Figure 8. Phase spectra (A) and temporal waveforms (B) for selected harmonic complex maskers.

Procedures

The procedures were identical to those described by Lauer et al. (2006) and are only briefly discussed here. Three BWS canaries were used as subjects. The data from BWS canaries were compared to data from Lauer et al. (2006) for non-BWS canaries. 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

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were the masker plus the tone. Tones of different levels relative to the masker

component were presented using the Method of Constant Stimuli at 7 different levels

within a block of 10 trials. Step sizes were either 1 or 2 dB, depending on the bird's

behavior. Behavior was considered stable if the threshold did not change by more

than 1/3 of the increment step size within the last two 100 trial sessions. Birds were

tested with different maskers in a random order. Threshold was defined as the level

of the 2800 Hz tone relative to the 2800 Hz masker component that was detected 50%

of the time corrected for the false alarm rate [$Pc*=(Pc-FA)/(1-FA)$] (Gescheider,

1985; Dooling and Okanoya, 1995a). The average false alarm rate was 2.56% for

BWS canaries. Data from sessions with false alarm rates larger than 18% were

excluded from analysis. Two percent of the data from BWS canaries was discarded.

Results and Discussion

   Average thresholds (expressed in dB re. masker component) for detecting

2800 Hz tones in harmonic complex maskers for BWS canaries are shown in Figure

9, along with thresholds replotted from Lauer et al. (2006) for normal-hearing non-

BWS canaries tested using identical methods. The scalar values used to create the

maskers are plotted on the x-axis, and masked thresholds are plotted on the y-axis.

Thresholds closer to the top of the figure indicate that more masking occurred, while

thresholds towards the bottom indicate that less masking occurred. A strain x scalar

mixed factor ANOVA revealed significant effects of scalar value (C)

[$F_{(12,48)}=13.248$, $p<0.000$], strain [$F_{(1,4)}=37.062$, $p=0.004$], and a significant

interaction of scalar value and strain [$F_{(12,48)}=4.465$, $p<0.000$].
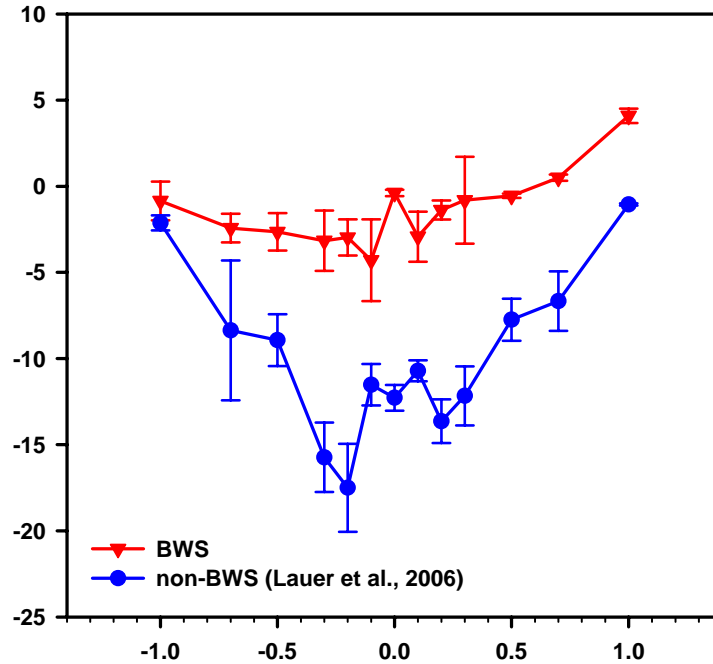
Figure 9. Masked thresholds for a 2800 Hz tone embedded in harmonic complex maskers. Error bars indicate standard deviation. Thresholds for non-BWS canaries are replotted from Lauer et al. (2006).

BWS canaries showed higher thresholds overall compared to non-BWS canaries, except at C=-1.0. BWS canaries showed less change in the amount of masking by stimuli with different phase spectra than non-BWS canaries. Non-BWS canaries showed a large release from masking for maskers with peakier envelopes (C values near 0) that did not occur for BWS canaries. There was a slight decrease in the amount of masking produced by maskers with peakier envelopes in BWS canaries; however, the release from masking was not nearly as prominent as it was in non-BWS canaries. The difference in the maximum and minimum amounts of masking was only 8.38 dB in BWS canaries while the difference was 18.57 dB in non-BWS

canaries. In non-BWS canaries, the least effective masker corresponded to a C value of -0.2. The least effective masker is less prominent in BWS canaries, but appears to fall just to the left of 0 (C=-0.1).

The smaller release from masking seen in BWS canaries is consistent with reports in human listeners with sensorineural hearing loss 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 results from damage to the cochlea (Lentz and Leek, 1999; Oxenham and Dau, 2004).

Measurments of BM movements in several mammalian species indicate that the normal phase response depends on the integrity of active processing mechanisms that enhance sensitivity to low and moderate sound levels (e g., de Boer and Nuttall, 2000; Cooper and Rhode, 1992; Ruggero et al., 1996; Russell and Nilsen, 1997; Fridberger et al., 2002). Experimentally induced damage to the BM that results in the loss of active processing mechanisms can result in an increased phase lag (Ruggero et al., 1996; Cooper and Rhode, 1992; Fridberger et al., 2002). Damage to the pigeon basilar papilla has been shown to disrupt traveling wave motion (Gummer et al., 1987; Smolders et al., 1987). Thus, the changes in the phase response of the BWS canary papilla 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.

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). When differences in the amount of masking do occur, they are very small. Interestingly, BWS canaries show a larger difference in the amount of masking by these maskers (4.94 dB) than non-BWS canaries (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 is unclear, but it may be that the waveform of the -1.0 masker is more normally represented along the BWS ear than the +1.0 masker.

Chapter 5: Frequency, Duration, and Intensity Discrimination

Birdsong is characterized by rapid frequency and amplitude changes over time (Greenwalt, 1968). The ability of a bird to resolve these changes must directly influence the content of vocalizations. The animal must distinguish one call or note from another in order to develop and maintain a normal vocal repertoire, and it must be able to focus its attention on important acoustic information to effectively communicate with other members of its species. To accomplish this, the bird must be able to discriminate changes in the acoustic dimensions of a song in order to produce a similar song (Greenwalt, 1968). More specifically, the bird must be able to resolve differences in frequency, time, and intensity. The experiments described in this chapter investigate the ability of BWS and non-BWS canaries to discriminate changes in the frequency, duration, and intensity of tones over time.

Experiment 1: Frequency Discrimination

Frequency is an essential cue for identifying a sound source. In songbirds, the spectral content of vocalizations conveys information about individual and species recognition, territoriality, male fitness, and functional referentiality (e.g., alarm vs. contact calls). Frequency may also provide information regarding the position of one bird relative to another, since the spectral content of a vocalization may become distorted or degraded by environmental factors when being transmitted over long distances.

While measures of frequency selectivity estimate how efficiently an organism can resolve the components of simultaneously occurring sounds, frequency discrimination measures the ability to distinguish changes in frequency over time. Despite the importance of frequency as a cue for recognition of vocalizations, most tests reveal that birds are not more sensitive to frequency changes than humans. Some birds are able to detect less than a 1% change in frequency between 1000 and 4000 Hz, while humans can detect less than a 0.5% change (reviewed in Dooling et al., 2000).

Spectral coding theories of frequency discrimination predict that frequency discrimination is directly related to frequency selectivity (Henning, 1967; Siebert, 1970; Zwicker, 1970). These theories assume that frequencies falling within the same auditory filter cannot be discriminated. Thus, more sharply tuned filters (narrower bandwidth) will result in smaller frequency difference limens (FDLs) because the range of frequencies falling within a single channel is reduced. In contrast, temporal coding theories assume no relationship between frequency selectivity and frequency discrimination (Goldstein and Srulovicz, 1977; Wakefield and Neslon, 1985; Loeb et al., 1983; Shamma, 1985). Instead, these models predict that phase locking in the auditory nerve determines FDLs. However, phase locking in mammals and songbirds deteriorates rapidly for frequencies above 1000 Hz (Rose et al., 1968; Gleich and Narins, 1988). Therefore, temporal coding theories cannot account for FDLs at higher frequencies. It is most likely that the two mechanisms operate at different frequency ranges—temporal mechanisms for low frequencies and spectral mechanisms for high frequencies.

FDLs are generally larger in hearing-impaired humans for frequencies in the area of hearing loss, though there is quite a bit of variability among listeners (Gengel, 1973; Tyler et al., 1983; Hall and Wood, 1984; Freyman and Nelson, 1986; Moore and Glasberg, 1986; Freyman and Nelson, 1987; Freyman and Nelson, 1991; Moore and Peters, 1992). However, there is no clear relationship between the size of FDLs and absolute thresholds (Simon and Yund, 1993) or frequency selectivity (Tyler et al., 1983).

In this experiment, FDLs at 1000, 2000, and 4000 Hz were measured in BWS and non-BWS canaries at a range of sound levels. If spectral coding mechanisms are responsible for frequency discrimination ability in canaries, then the broadened auditory filters in BWS canaries should result in larger FDLs at higher frequencies. Alternatively, if FDLs are better explained by temporal coding mechanisms in canaries, there may not be a clear relationship between reduced frequency selectivity and FDLs. Furthermore, if there is a decrease in the fidelity of temporal coding in BWS canaries, such as decreased precision of phase locking due to auditory nerve fiber degeneration, FDLs may be larger than normal in BWS canaries. Budgerigars with mild residual hearing loss 4 to 6 weeks following kanamycin exposure do not show significant increases in FDLs for 1000 Hz and 2860 Hz tones presented at 65 dB SPL (Dooling et al., 2006). Accordingly, BWS canaries may only show increased FDLs at frequencies where the hearing loss is more severe (4000 Hz).

Methods

Stimuli

Background stimuli were 1000, 2000, and 4000 Hz pure tones. Target stimuli were pure tones with frequencies ranging from 10 to 700 Hz above the background frequency, with a step size of 10, 20, 50, or 100 Hz depending on the frequency and the bird's estimated threshold. All stimuli were 400 ms in duration with rise/fall times of 20 ms, sampled at 40 kHz. Birds were tested at a range of sound levels at each frequency. Stimuli were presented at 50, 60, 70, and 80 dB SPL for the 1000 Hz background condition; 40, 50, 60, 70, and 80 dB SPL for the 2000 Hz background condition; and 30, 40, 50, 60, 70, and 80 dB SPL for the 4000 Hz background condition. It was not possible to test BWS canaries at all of the levels that non-BWS were tested on due to their high absolute thresholds. To control for potential loudness cues, the sounds were randomly roved by +/-6 dB on each presentation.

Procedures

Thresholds for detecting increments in frequency were measured in 4 non-BWS and 4 BWS canaries. The increment size was 10 or 20 Hz for non-BWS canaries for all frequencies. The step size was 10 or 20 Hz for BWS at 1000 Hz and 2000 Hz and was 50 or 100 Hz at 4000 Hz depending on the individual bird's performance. Stable thresholds could not be obtained from BWS using smaller step sizes at 4000 Hz. Thresholds for detecting increments in frequency were measured at all levels (randomly) at a given frequency before a bird was tested on another frequency. The order of frequencies and levels tested was randomly chosen for each

individual bird. Frequency difference limens were defined as the minimum change in

frequency that was detected 50% of the time corrected for the false alarm rate

[Pc*=(Pc-FA)/(1-FA)] (Gescheider, 1985; Dooling and Okanoya, 1995a). The

average false alarm rate was 2.82% for non-BWS canaries and 3.64% for BWS

canaries. Data from sessions with false alarm rates larger than 18% were excluded

from analysis. Four percent of the data from non-BWS canaries were discarded, and

5% of the data from BWS canaries was discarded.

Results and Discussion

　　　　FDLs expressed as percent of the reference frequency, are shown in Figure 10

for BWS and non-BWS canaries for equal SPLs (A-C) and equal SLs (D-F). Data

points for individual BWS canaries are shown where no average data were available.

As reported in other songbird species, non-BWS canaries were able to detect a change

in frequency as small as 1-2 % at high sound levels (Dooling and Saunders, 1975;

Kuhn et al., 1980; Sinnott et al., 1980). Non-BWS canaries showed an increase of

about 3% in FDLs with decreasing level at 1000 Hz and less than 1% at 2000 Hz and

4000 Hz. The largest FDLs were at 1000 Hz for non-BWS canaries. Of the three

frequencies tested, non-BWS show the highest absolute thresholds at 1000 Hz.

　　　　BWS canaries showed larger FDLs than non-BWS canaries at 2000 Hz and

4000 Hz for both equal SPLs and equal SLs. The largest FDLs occurred at 4000 Hz,

where absolute thresholds are the most elevated. At 1000 Hz, BWS canaries showed

a trend toward slightly better FDLs at the two lowest levels tested. A one-tailed t-

test revealed that this difference was significant at 60 dB SPL [t(6)=-2.687, p<0.05].
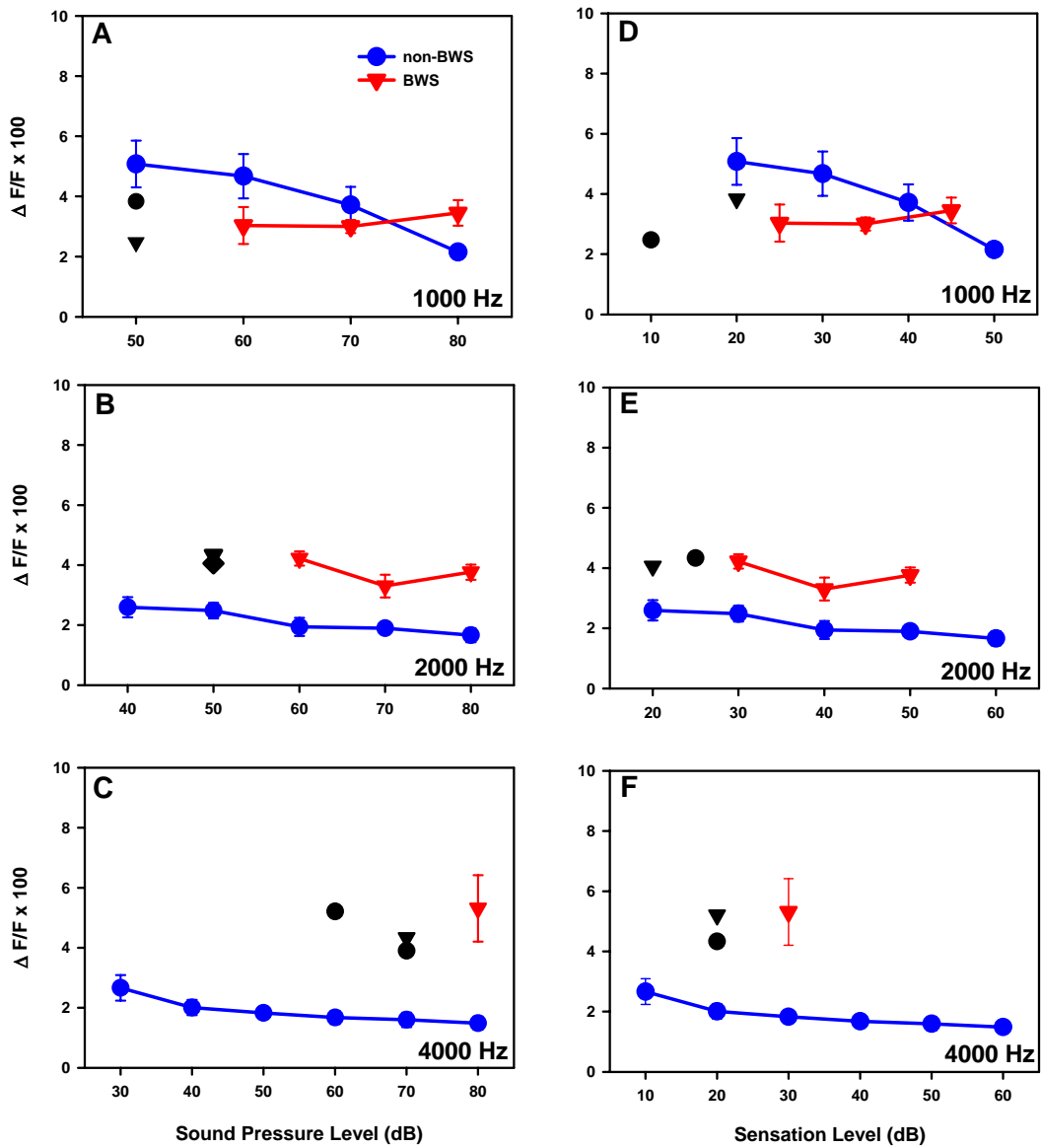
Figure 10. Frequency difference limens for BWS and non-BWS canaries at equal sound pressure levels: A) 1000 Hz, B) 2000 Hz, C) 4000 Hz, and equal sensation levels: D) 1000 Hz, E) 2000 Hz, F) 4000 Hz. Error bars indicate standard error.

BWS canaries showed very little change in FDLs with increasing level at 1000 Hz and 2000 Hz. The two BWS canaries that were tested at multiple levels at

4000 Hz showed a decrease in FDL with increasing sound level. Because BWS canaries were not tested at all of the levels non-BWS were tested on, it was not possible to run an ANOVA on the data. Thus, a mixed factor ANOVA (strain x frequency) was conducted on the FDLs for the 80 dB SPL condition only. There was no significant main effect of frequency; however, there was a significant effect of strain [$F(1, 6)=30.484$, $p=0.001$]. The interaction between factors was not significant.

The increased FDLs in the region of hearing loss in BWS canaries are consistent with reports of reduced frequency discrimination abilities in humans with sensorineural hearing loss (Gengel, 1973; Tyler et al., 1983; Hall and Wood, 1984; Freyman and Nelson, 1986; Moore and Glasberg, 1986; Freyman and Nelson, 1987; Freyman and Nelson, 1991; Moore and Peters, 1992). FDLs are usually larger than normal in human listeners with sensorineural hearing loss, but the results vary widely across studies. Interpretation of these results is difficult because the authors' use different testing procedures and presentation levels, and include listeners with different configurations of hearing loss. The amount of increase in FDLs often differs between low and high frequencies, suggesting different encoding mechanisms. There is not a strong correlation between the amount of hearing loss and the FDL across both low and high frequencies; however, FDLs are more closely linked to hearing thresholds at 1000 Hz and above (Freyman and Nelson, 1986; Freyman and Nelson, 1991; Simon and Yund, 1993). Further, some listeners show very large differences in FDLs between the left and right ears, despite similar audiograms in both ears (Simon and Yund, 1993). Conversely, listeners with asymmetrical losses sometimes show no difference in FDLs between the ears (Simon and Yund, 1993). In aggregate, these

results suggest that FDLs are determined by different mechanisms at low and high frequencies. The pattern of hair cell damage unique to each individual may also explain some of the variability seen across listeners and across studies.

Complete destruction of OHCs in the region of the basilar membrane corresponding to the test frequency in cats and chinchillas does not result in increased FDLs; however, damage that results in the destruction of over 50% of IHCs *and* complete destruction of OHCs does result in increased FDLs (Nienhuys and Clark, 1978; Prosen et al., 1989). BWS canaries show damage primarily to efferently innervated SHCs, but also show abnormal THCs. Thus, the decreased sensitivity to changes in frequency over time at 2000 Hz and 4000 Hz is not surprising. However, the fact that frequency discrimination is better than normal in BWS canaries at 1000 Hz at some levels despite the presence of significant hair cell abnormalities across the entire basilar papilla suggests that 1) there is not enough damage to hair cells with characteristic frequencies near 1000 Hz to impair frequency discrimination, or 2) frequency discrimination is accomplished through different mechanisms at low and high frequencies. These two possibilities are not necessarily mutually exclusive. It may be the case that spectral coding mechanisms aid temporal coding mechanisms in the frequency region where phase locking declines in canaries.

Increased FDLs at 2000 and 4000 Hz relative to 1000 Hz in BWS canaries may be related to differences in frequency selectivity that accompany the hair cell damage in BWS canaries. If FDLs are determined by spectral coding mechanisms, then FDLs should be larger in the region where auditory filters are broader in BWS canaries. This is indeed the case. CRs and PTCs measured in earlier experiments

indicate that frequency selectivity is reduced at higher frequencies in BWS canaries. However, PTC tuning does not explain why BWS canaries are better at lower levels at 1000 Hz. It is possible that there is some compensatory mechanism in the auditory system, such as increased hair cell innervation density, increased size or number of synaptic vesicles, or increased size or number of neurotransmitter binding sites.

The relationship between absolute threshold and frequency discrimination ability in BWS and non-BWS canaries also indicates that spectral mechanisms are in play for higher frequencies and temporal mechanisms support frequency discrimination at lower frequencies. FDLs at 80 dB SPL are significantly correlated with absolute threshold in BWS and non-BWS canaries when all reference frequencies are taken into account (r^2=0.627, p<0.0001). However, a stronger correlation occurs between FDLs at 80 dB SPL and absolute threshold if the 1000 Hz data are excluded (r^2=0.814, p<0.0001). Furthermore, an analysis of the 1000 Hz data alone reveals no significant correlation between FDLs and absolute thresholds (r^2=-0.021, p=0.732). These correlations, coupled with the fact that damage along the BWS canary basilar papilla is worse in the apex and midsection than in the base, strongly suggests that temporal coding mechanisms are responsible for frequency discrimination at low frequencies in canaries.

Differences in frequency discrimination ability between BWS and non-BWS canaries may be related to differences in vocalizations. The vocalizations of BWS canaries contain most of their energy below 4000 Hz (Nottebohm and Nottebohm, 1978; Güttinger, 1985; Okanoya and Dooling, 1990; Wright et al., 2004). In contrast, the vocalizations of non-BWS canaries often have a significant amount of energy

present at frequencies up to 6000 or 7000 Hz (Güttinger, 1985; Lohr et al., 2004).

Reduced frequency discrimination ability at higher frequencies and enhanced

discrimination ability at lower frequencies might actually help BWS canaries attend

to strain-specific vocalizations and "filter out" high-pitched sounds that are not BWS

vocalizations.

Experiment 2: Duration Discrimination

Analysis of canary song structure reveals components that occur on several

time scales (Güttinger, 1979, 1981, 1985). Singing bouts can last for many minutes,

tours (repetitions of a single syllable) typically last several seconds, syllables range

from approximately 50 to 300 ms, and individual notes range from about 10 to 300

ms. Despite the wide range of durations of song elements in birds, few studies have

investigated the ability to detect changes in sound duration in birds. Only two species

have been tested. Budgerigars and starlings can detect about a 10-20% increase in the

duration of tones (Dooling and Haskell, 1978; Maier and Klump, 1990). The duration

difference limens reported in birds are about as good as those in humans (Creelman,

1962; Abel, 1971; Divenyi and Danner, 1977).

Hearing loss does not appear to affect duration discrimination in humans;

however, duration difference limens (DDLs) are sometimes increased in older

listeners (Abel et al., 1990; Gordon-Salant and Fitzgibbons, 1993; Fitzgibbons and

Gordon-Salant, 1994; Fitzgibbons and Gordon-Salant, 1995). Because reduced

duration discrimination ability occurs in older listeners with and without hearing loss,

the effect is likely due to changes in the central auditory system that accompany age

(Gordon-Salant and Fitzgibbons, 1993; Fitzgibbons and Gordon-Salant, 1994; Fitzgibbons and Gordon-Salant, 1995).

Duration discrimination is thought to be mediated by a neural counting mechanism that functions by counting random input pulses during the duration of a stimulus (Creelman, 1962). The probability of a pulse occurring increases with increasing duration; therefore, longer stimuli are associated with more pulses. This model predicts that the neural counter is independent of changes in stimulus parameters, such as amplitude and energy spectrum, for clearly detectible signals (i.e., above threshold). Discrimination of tone pulses and noise bursts in humans is in accordance with this model for durations above 5 ms (Creelman 1962; Abel 1971; Divenyi and Danner 1977).

In this experiment, duration difference limens were measured for 1000, 2000, and 4000 Hz tones in BWS and non-BWS canaries for a range of reference durations similar to the range of durations that are characteristic of canary song syllables. Provided the stimuli in the present experiment were clearly audible, BWS canaries were expected to detect increases in duration as well as non-BWS canaries at all frequencies tested.

Methods

Stimuli

Stimuli were 1000, 2000, and 4000 Hz pure tones created with a 40 Hz sampling rate and 5 ms $\cos^2$ rise/fall times. Reference durations of 10, 20, 40, 80, and

160 ms were used. Stimuli were presented at 80 dB SPL to ensure that the tones were at least 10 dB above absolute thresholds for BWS canaries at all frequencies.

Procedures

Four BWS canaries and 4 non-BWS canaries were used in this study. Birds were trained to detect increments in the duration of a repeating tone. Increments of 10 or 20% of the reference durations were used, depending on the bird's performance. Birds were tested on one frequency at all stimulus durations before being tested on another frequency. The durations were tested in a random order, and the order of frequencies was randomized for each bird. DDLs were defined as the smallest detectible increase in duration that was detected 50% of the time corrected for the false alarm rate [$Pc^*=(Pc-FA)/(1-FA)$] (Gescheider, 1985; Dooling and Okanoya, 1995a). The average false alarm rate was 2.69% for non-BWS canaries and 3.08% for BWS canaries. Data from sessions with false alarm rates larger than 18% were excluded from analysis. Two percent of the data from non-BWS canaries were discarded, and 4% of the data from BWS canaries were discarded.

Results and Discussion

DDLs for 1000, 2000, and 4000 Hz tones for BWS and non-BWS canaries are shown in Figure 11. Non-BWS canaries were able to detect an increase of approximately 25-30% in duration for stimuli that were longer than 10 ms at 2000 and 4000 Hz, and 50-60% at 1000 Hz. These thresholds are somewhat larger than DDLs reported in other bird species (Dooling and Haskell, 1978; Maier and Klump,

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1990). BWS canaries were able to detect increases of 20-30% in duration above 10 ms for all frequencies. DDLs increased at the shortest duration tested (10 ms) for both non-BWS canaries (50-140%) and BWS canaries (40-70%) and varied more among individual birds than at other durations. Humans and birds also show increased DDLs for very short durations (Creelman, 1962; Abel, 1971; Divenyi and Danner, 1977; Dooling and Haskell, 1978; Maier and Klump, 1990).
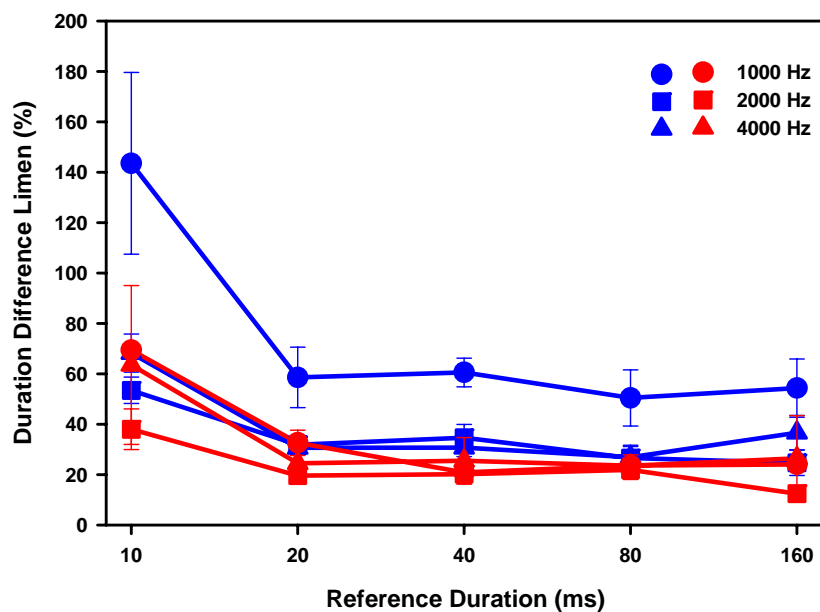
Figure 11. Duration difference limens for BWS (red) and non-BWS canaries (blue). Error bars indicate standard error.

A strain x frequency x duration mixed factor ANOVA revealed significant effects of strain [$F_{(1, 6)}=26.897$, $p=0.002$] and duration [$F_{(4, 24)}=28.128$, $p<0.0001$] and significant interactions between duration and strain [$F_{(4, 24)}=5.903$, $p=0.002$] and duration and frequency [$F_{(8, 48)}=24.752$, $p<0.0001$]. There was not a significant main effect of frequency, and no other interactions were significant.

These results show that the inner ear abnormalities have no detrimental effect on duration discrimination in BWS canaries. Given the stimulus presentation level of 80 dB SPL, BWS canaries were listening at a reduced SL compared to non-BWS canaries. However, this reduced audibility of the stimuli did not have a negative effect on performance. Surprisingly, BWS canaries were actually better than non-BWS canaries at discriminating changes in the duration of a 1000 Hz tone. Differences in DDLs as a function of frequency have not been reported in humans or other nonhuman animals. The reason for this effect is unknown. One possibility is that BWS canaries have more neurons responding to 1000 Hz than normal. There may be some abnormal tonotopic mapping that occurs as a result of reduced afferent input at higher frequencies. If the counter mechanism is correct, then more neurons responding to 1000 Hz would result in more pulses being generated during the stimulus presentation. More pulses would increase the likelihood of detecting duration differences. Alternatively, BWS canaries might be better at discriminating changes in the duration of a 1000 Hz tone because they have more experience listening to vocalizations with a significant amount of energy near 1000 Hz. BWS canary song often includes syllables that have similar frequency structure but are of different durations. Enhanced duration discrimination ability for sounds with energy around 1000 Hz might help BWS canaries to learn such syllables.

Experiment 3: Intensity Discrimination

The healthy avian auditory system responds to a wide range of sound intensities without incurring damage, with a dynamic range of at least 100 dB

(Gleich, 1989; Manley et al., 1985). Intensity resolution, the ability to detect changes in the intensity of sounds, is quite remarkable in its sensitivity relative to this range. Most avian species can detect intensity changes as small as 1 to 4 dB (reviewed in Dooling et al., 2000). This ability is assumed to be based on the perceived loudness of sounds. Clearly, the dynamic range of the BWS canary auditory system is restricted at higher frequencies as a consequence of the hearing loss. However, it is unknown how the auditory system abnormalities affect other aspects of the perception of sound intensity.

Intensity discrimination of gated or pulsed stimuli is commonly evaluated by measuring intensity difference limens (IDLs) in animals, defined as the smallest detectible change in intensity expressed in dB. Intensity difference limens decrease with increasing sound level in all songbird species tested to date. That is, IDLs become smaller when measured at higher sound levels, with no consistent effect of frequency on IDLs across species (Hienz et al., 1980; Klump and Baur, 1990).

Weber's law states that the smallest detectible change in stimulus intensity is approximately a constant proportion of the intensity of the stimulus ($\Delta I/I$ is constant). In humans, Weber's law holds true for sound levels of about 20 to 100 dB above threshold for wideband stimuli (Miller, 1947). However, for pure tones and narrowband stimuli, the ability to detect changes in intensity actually improves with increasing sound level (Riesz, 1928; Harris, 1963; Viemeister, 1972; Jesteadt et al., 1977; Florentine, 1983; Florentine et al., 1987; Viemeister and Bacon, 1988; Wojtczak and Viemeister, 1999). As the level of a tone increases, the smallest detectible change in intensity decreases slightly. This effect has been termed the

'near miss' to Weber's law. It is thought to occur because of the nonlinear growth of the excitation pattern of the cochlea and by combining information from multiple auditory channels.

The excitation pattern produced in response to a tone grows in a nonlinear, compressive way near its center, but grows in a more linear fashion on the high-frequency side in humans (Nelson and Shroder, 1997). Thus, the rate of growth of the response with increasing stimulus level is larger on the high-frequency side of the excitation pattern than near its center. This means that smaller stimulus increments are needed to produce the same amount of change in excitation for higher sound levels. Furthermore, listeners may combine information from more than one channel to detect a change in stimulus level (Florentine and Buus, 1981). As the level of a tone increases, more channels with center frequencies that are distant from the stimulus frequency are activated. Thus, information from more neurons may be available to detect intensity changes.

The nonlinear growth of excitation that is seen in mammals does not occur in birds (Gleich, 1994; Brown et al., 2001). Buus et al. (1995) suggested that the small IDLs that occur over a large range of frequencies in the starling are possible because there is a large range of auditory nerve fiber thresholds for units tuned to a given characteristic frequency (Gleich, 1989). Even though single unit auditory nerve fiber responses have not been measured in the canary, we assume that the responses would be similar to other songbird species.

In hearing-impaired human listeners, sensitivity to low level sounds is reduced, but the levels at which sounds become uncomfortable remain normal. Thus,

a change in intensity is thought to correspond to a larger than normal increase in loudness in hearing-impaired human listeners, a phenomenon known as loudness recruitment. Given this abnormal growth of loudness, it might be expected that hearing-impaired human listeners would have better than normal intensity resolution. In general, this is true when intensity resolution is compared at equal SLs, but intensity resolution is not better when compared at equal SPLs. In humans with cochlear damage, IDLs for tone pulses are often smaller than in normal-hearing humans tested at similar SLs (Glasberg and Moore, 1989; Turner et al., 1989; Shroder et al., 1994). However, when compared at equal SPLs, IDLs for hearing-impaired subjects are often closer to those of normal-hearing listeners at moderate to high levels (Glasberg and Moore, 1989; Turner et al., 1989; Florentine et al., 1993: Shroder et al., 1994). At low SPLs, IDLs are usually higher for hearing-impaired listeners (Florentine et al., 1993; Glasberg and Moore, 1989; Turner et al., 1989; Shroder et al., 1994).

In an earlier experiment, IDLs for continuous broadband noise were measured as a function of SPL level in BWS canaries (Okanoya and Dooling, 1985). Intensity difference limens decreased from approximately 2.9 dB for 60 dB SPL noise to about 1 dB for 80-90 dB SPL noise. Between 70 and 90 dB SPL, IDLs changed very little with increasing level. This result is consistent with predictions from Weber's law and with reported IDLs for noise in budgerigars (Dooling and Searcy, 1981). Performance in BWS canaries was worse at the lower sound levels presumably because of the inaudibility of high frequency noise components. Fewer auditory channels were excited; therefore, intensity discrimination was worse. To test whether

discrimination of intensity changes in pure tones were affected by the inner ear pathology in BWS canaries, IDLs were measured as a function of frequency and level in BWS and non-BWS canaries. Intensity discrimination of tones in BWS canaries was expected to be slightly better than normal at equal SLs, but not at equal SPLs, as in human listeners with sensorineural hearing loss.

Methods

Stimuli

Stimuli were 1000, 2000, and 4000 Hz pure tones with durations of 400 ms. Tones had 20 ms $\cos^2$ rise/fall times generated at a sampling rate of 40 kHz.

Procedures

Four non-BWS canaries and 4 BWS canaries were used as subjects. Birds were trained to detect increments in the intensity of a repeating tone. The increment size was 1 or 2 dB, depending on the bird's performance. Thresholds for the smallest detectable increase in intensity in the pure tones were measured at levels of 10, 20, 30, and 40 dB SL. It was not possible to test BWS at all SLs for all frequencies due to the high levels of sound necessary to determine thresholds. The order of frequencies and sound levels tested was randomized for each individual bird.

Intensity difference limens were defined as the smallest increase in intensity that was detected 50% of the time corrected for the false alarm rate [Pc*=(Pc-FA)/(1-FA)] (Gescheider, 1985; Dooling and Okanoya, 1995a). The average false alarm rate was 2.51% for non-BWS canaries and 3.31% for BWS canaries. Data from sessions

with false alarm rates larger than 18% were excluded from analysis. Two percent of

the data from non-BWS canaries were discarded, and 4% of the data from BWS

canaries were discarded.

Results and Discussion

IDLs for BWS and non-BWS canaries at equal SLs are plotted in the left side

of Figure 12 for 1000 Hz (A), 2000 Hz (B) and 4000 Hz (C). Data points for

individual BWS canaries are shown where no average data were available. IDLs for

non-BWS ranged from approximately 3 to 6 dB. Overall, BWS canaries had

somewhat lower IDLs than non-BWS canaries, but IDLs decreased with increasing

SL for both strains. Non-BWS canaries showed more of a decrease (up to about 3

dB) than BWS canaries (up to about 1.5 dB).

A mixed factor ANOVA (SL x frequency) showed that there was no

significant effect of frequency or significant interaction of SL and frequency;

therefore, data were collapsed across frequency for further analysis. There was a

significant effect of SL [$F_{(4, 52)}=9.572$, $p<0.0001$] and strain [$F_{(1, 13)}=719.056$,

$p<0.0001$], but the interaction between factors was not significant. The finding that

IDLs do not vary systematically with tone frequency in both canary strains is

consistent with previous reports in other songbird species (Hienz et al., 1980; Klump

and Baur, 1990). The similar IDLs across frequency indicate that the same intensity

coding mechanism is used throughout the entire basilar papilla.

For comparison at equal sound levels, the IDLs for BWS and non-BWS

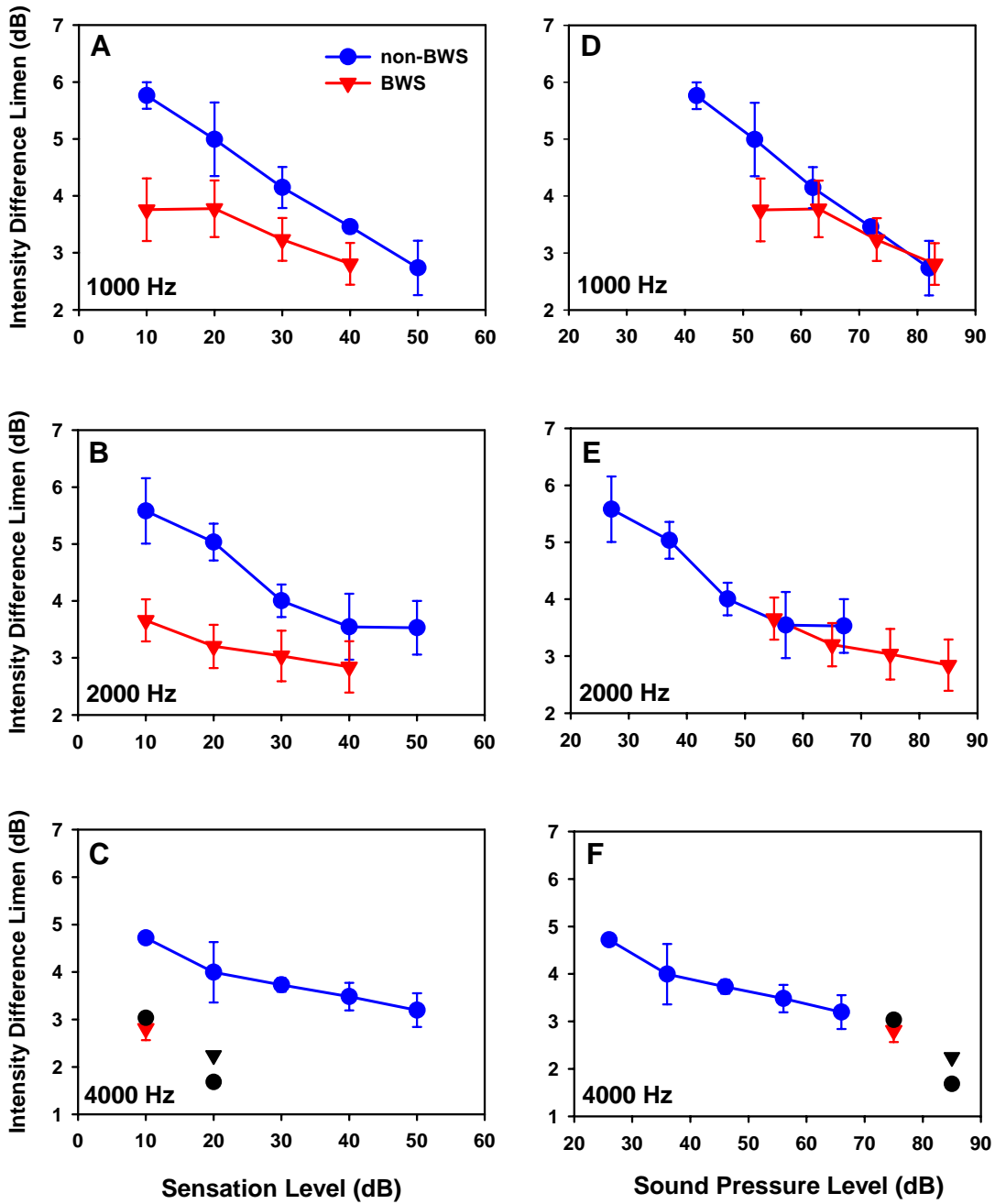canaries are shown at equal SPLs in the right side of Figure 12 for 1000 Hz (D), 2000

Figure 12. Intensity difference limens in non-BWS and BWS canaries for sensation level: A) 1000 Hz, B) 2000 Hz, C) 4000 Hz, and sound pressure level: D) 1000 Hz, E) 2000 Hz, F) 4000 Hz. Error bars indicate standard deviation.

Hz (E), and 4000 Hz (F). IDLs were not notably different between BWS and non-BWS canaries when compared at equal SPLs. Thus, intensity discrimination is not necessarily enhanced in BWS canaries as the SL data suggest. However, it is important to note again that BWS canaries typically listen at lower SLs than non-BWS canaries in everyday situations.

The range of IDLs reported here for non-BWS canaries is consistent with those reported for pure tones in other bird species [budgerigar-Dooling and Saunders, 1975, Dooling and Searcy, 1979; redwing blackbird (*Agelaius phoeniceus*)-Hienz et al., 1980; pigeon- Hienz et al., 1980; starling-Klump and Baur, 1990; orange-fronted conure (*Aratinga canicularis*)-Wright et al., 2003]. Despite having more hair cells across the width of the basilar papilla (reviewed in Gleich et al., 2000), birds are not typically better at intensity discrimination than mammals (Dooling et al., 2000).

The smaller IDLs at equal SLs in the hearing-impaired BWS canaries are consistent with findings in humans with sensorineural hearing loss (Riesz, 1928; Harris, 1963; Jesteadt et al., 1977; Viemeister, 1972; Viemeister and Bacon, 1988; Florentine, 1983; Florentine et al., 1987; Wojtczak and Viemeister, 1999). Despite the significant amount of missing and damaged hair cells along and across the BWS basilar papilla and the reduced number of auditory nerve fibers, BWS canaries must have enough afferent input to properly encode sound intensity and to detect intensity changes. Viemeister (1988) suggested that changes in firing rate in a small number of neurons can account for intensity discrimination abilities in mammals. Thus, the reduction in the number of auditory nerve fibers in BWS canaries may not be severe enough to affect intensity resolution.

Chapter 6: Temporal Processing

The auditory system is particularly well-suited for processing stimuli that change rapidly over time (Viemeister and Plack, 1993). In some birds, species recognition depends on the temporal structure of song (Emlen, 1972; Brenowitz, 1983). Disruption of the temporal sequence of song syllables occurs prior to degradation of spectral content in deafened Bengalese finches (Okanoya and Yamaguchi 1997; Woolley and Rubel 1997). Thus, temporal processing in the avian auditory system likely plays an important role in song maintenance.

In humans, cochlear damage can adversely affect temporal processing. This seems contrary to what one might expect based on theories of the spectral filtering properties of the ear that predict an inverse relationship between temporal and spectral resolution (Duifhuis, 1973; de Boer, 1985). In listeners with sensorineural hearing loss, the broadened auditory filters should lead to enhanced temporal resolution. However, temporal resolution is rarely better than normal in hearing-impaired humans and can severely deteriorate under certain conditions.

It is unclear how damage to the ear affects temporal processing in birds, as only two studies have directly addressed this issue. Marean et al. (1998) measured temporal modulation transfer functions (TMTFs) following hair cell regeneration in starlings. Immediately following treatment with the ototoxic drug kanamycin, two of four birds showed a loss of sensitivity in the TMTF at 5000 Hz soon after the dosing period. The remaining two birds showed no significant change in TMTF bandwidth. Presumably, the change in sensitivity in two of the four birds occurred because frequencies above 4000 Hz became inaudible following kanamycin treatment. TMTF

bandwidths for the two impaired birds quickly returned to normal as thresholds

recovered for frequencies above 4000 Hz. These results indicate that hair cell

damage has little to no effect on temporal resolution in birds. However, temporal

integration has been shown to be reduced in chickens with temporary hearing loss

(Saunders et al., 1995). The experiments described in this chapter investigate

temporal processing in BWS and non-BWS canaries.

Experiment 1: Temporal Integration

Temporal integration refers to the auditory system's ability to integrate

acoustical energy over time and is reflected in the relationship between the duration

of a sound and the threshold for detection of that sound (Hughes, 1946). Detection

thresholds decrease as the duration of a sound increases from a few milliseconds up to

a certain point referred to as the time constant τ (Plomp and Boughman, 1959). This

point is around 200 ms in most species tested (Fay, 1988). For durations longer than

this time constant, there is no further improvement in threshold. Birds typically show

about a 10 to 20 dB threshold improvement with increasing stimulus duration

(Dooling, 1979, Dooling and Searcy, 1985b; Klump and Maier, 1990; Saunders and

Salvi, 1993).

The change in threshold with increasing duration is often smaller than normal

in hearing-impaired humans (Gengel and Watson, 1971; Pedersen and Eberling,

1973; Elliott, 1975; Chung, 1981; Hall and Fernandes, 1983; Carlyon et al., 1990).

The cause of reduced temporal integration is unclear, but authors have suggested that

it may be due to increased sound level at threshold, detection of spectral changes

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associated with changes in duration, or the reduction in the compressive nonlinearity in the basilar membrane. A recent re-analysis of temporal integration data from cats with sensorineural hearing loss suggests that the mechanism underlying maximum temporal integration remains unchanged by cochlear damage, but the effective portion of a stimulus is reduced as a result of hair cell damage (Neubauer and Heil, 2004).

Temporal integration in BWS and non-BWS canaries was measured for several tone frequencies. Saunders et al. (1995) demonstrated that temporal integration is reduced in chickens with temporary hearing loss resulting from hair cell damage. Similarly, BWS canaries were expected to show smaller changes in threshold with increasing duration compared to non-BWS canaries.

Methods

Stimuli

Pure tones of 1000, 2000, and 4000 Hz with durations of 5, 10, 20, 40, 80, 160, and 240 ms were used as target stimuli presented in a quiet background. Tones had a sampling rate of 40 kHz, with $\cos^2$ rise/fall times of 2 ms.

Procedures

Thresholds for detecting tones of different durations were measured in 4 non-BWS canaries and 4 BWS canaries. The tones were presented using the Method of Constant Stimuli at 7 different levels in increments of 5 dB within a block of 10 trials. Birds were tested on one frequency at all stimulus durations before moving on to

another frequency, but the durations were tested in a random order. The order of the frequencies tested was randomized for each bird. Threshold was defined as the level of tone that was detected 50% of the time corrected for the false alarm rate [$Pc^*=(Pc-FA)/(1-FA)$] (Gescheider, 1985; Dooling and Okanoya, 1995a). The average false alarm rate was 3.0% for non-BWS canaries and 2.92% for BWS canaries. Data from sessions with false alarm rates larger than 18% were excluded from analysis. Thirteen percent of the data from non-BWS canaries were discarded, and 5% of the data from BWS canaries were discarded. The amount of discarded data is somewhat high for the non-BWS canaries because the birds initially had very unstable behavior when detecting the tones with the smallest durations.

Results and Discussion

To facilitate comparisons of the amount of threshold change in non-BWS and BWS canaries, relative thresholds (threshold at duration x – threshold at longest duration) were calculated for individual birds at each frequency. Average relative thresholds as a function of tone duration for 1000 (A), 2000 (B), and 4000 Hz (C) are shown in Figure 13 for non-BWS and BWS canaries. Non-BWS canaries showed a decrease in threshold of about 10 to 15 dB with increasing stimulus duration for all frequencies. This amount of threshold change is consistent with reports in other species of birds (Dooling, 1979; Dooling and Searcy, 1985b; Klump and Maier, 1990; Saunders and Salvi, 1993). There was no evidence of a critical duration beyond which temporal integration ceased in non-BWS canaries. Because data for longer test durations are required to estimate the integration time constant properly, these data do

not lend themselves well to analysis using traditional models of temporal integration (Hughes, 1946; Plomp and Boughman, 1959; Zwislocki, 1960).

Thresholds improved about 7 dB with increasing duration at 2000 Hz in BWS canaries, but showed less than 5 dB of improvement at 1000 and 4000 Hz. The amount of temporal integration in BWS canaries is consistent with the amount of temporal integration reported in chickens with temporary hearing loss (Saunders et al., 1995). A strain x frequency x duration mixed factor ANOVA revealed significant effects of duration [$F(8, 48)=28.409$, $p<0.0001$] and strain [$F(1,6)=14.099$, $p=0.009$], and significant interactions of duration and strain [$F(8,48)=7.264$, $p<0.0001$] and frequency, duration, and strain [$F(16, 96)=2.017$, $p=0.019$]. No other interactions were significant. There was no significant effect of frequency, indicating that temporal integration does not change systematically with frequency. Two stimulus durations, 5 ms and 240 ms, were chosen for post hoc comparisons using the Bonferroni method. Relative thresholds at 5 ms were higher in non-BWS at 1000 Hz [$t(6)=5.166$, $p=.002$] and 4000 Hz [$t(6)=3.905$, $p=.008$], but not at 2000 Hz. Relative thresholds at 240 ms were not significantly different between non-BWS and BWS at any frequency.

In general, non-BWS canaries showed temporal integration functions with steeper slopes than BWS canaries at 1000 and 4000 Hz, but not at 2000 Hz. Thus, thresholds improved more with increasing duration (indicating increased temporal integration) for non-BWS than BWS canaries at 1000 and 4000 Hz, but not at 2000 Hz.

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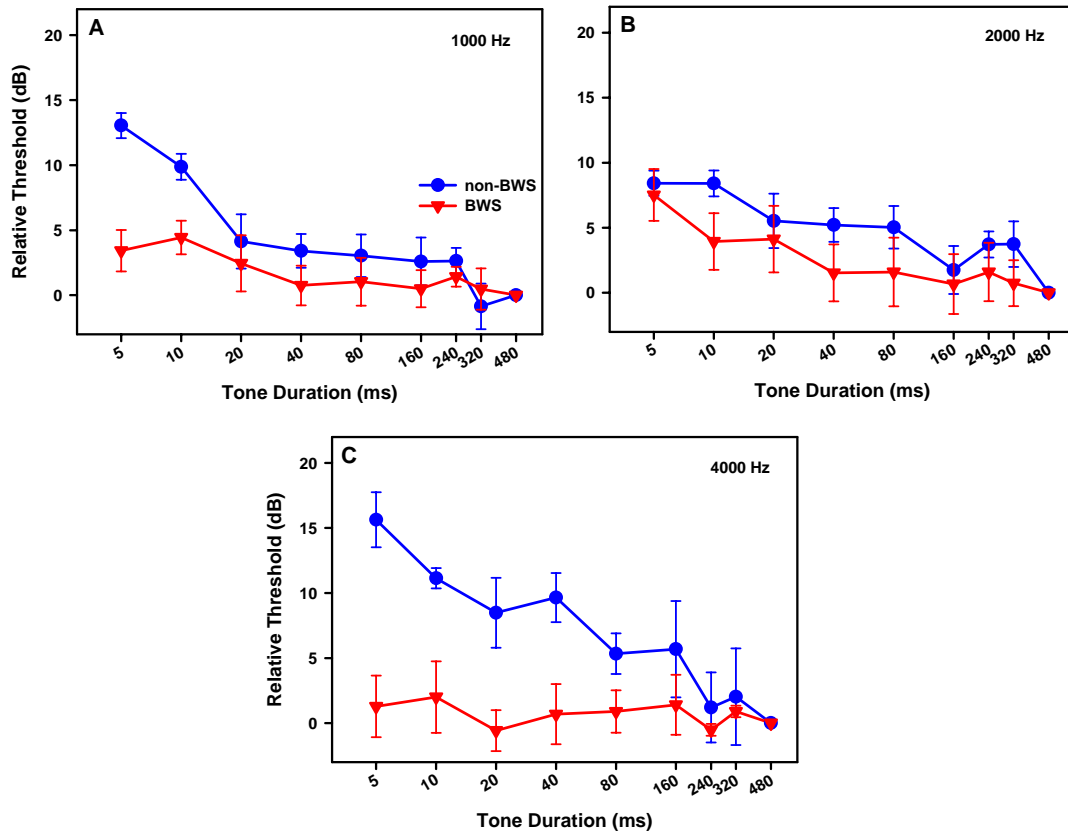
Figure 13. Absolute thresholds for pure tones as a function of duration for non-BWS and BWS canaries for 1000 Hz (A), 2000 Hz (B), and 4000 Hz (C). Error bars indicate standard deviations.

Smaller than normal changes in threshold with increasing stimulus duration have typically been attributed to reduced temporal integration resulting from reduced peripheral compression in hearing-impaired human listeners (Gengel and Watson, 1971; Pedersen and Eberling, 1973; Elliott, 1975; Chung, 1981; Hall and Fernandes, 1983; Carlyon et al., 1990) and cats with experimentally induced hearing loss (Solecki and Green, 1990). However, a new analysis of the data from cats indicates that the reduction in threshold change associated with hearing loss is actually due to

changes in the effective portion of the stimulus rather than changes in the temporal

integration mechanism (Neubauer and Heil, 2004). That is, not only is there a

reduction in sensitivity with hearing loss, but also an elevation in the baseline above

which sound pressure is effective in exciting the auditory system. A similar cause of

reduced threshold change with increasing stimulus duration may be at play in BWS

given that there is little evidence for peripheral compression in birds. The amount of

threshold improvement at 2000 Hz was similar in BWS and non-BWS canaries. The

audiogram shows the best sensitivity at about 2000 Hz in BWS canaries. It is

possible that the effective bandwidth of the stimulus is most normal in this region.

Experiment 2: Gap Detection Thresholds

While measures of temporal integration describe how an organism combines

auditory information over a period of time, these measures do not describe the ability

to resolve temporal fluctuations in sounds. A simple and convenient measure of

temporal resolution of the auditory system can be obtained by measuring thresholds

for detecting temporal gaps, or brief silent periods, in noise.

Starlings, zebra finches, budgerigars, and barn owls can detect about a 2 to 3

ms gap in noise with levels exceeding 20 dB SL (Okanoya and Dooling, 1990; Klump

and Maier, 1989; Klump et al., 1998). Gap detection thresholds (GDTs) for birds

increase at lower noise levels (Okanoya and Dooling, 1990; Klump and Maier, 1989;

Klump et al., 1998). Okanoya and Dooling (1990a) found that a reciprocal

relationship between resolution of gaps in noise and spectral resolution exists in the

zebra finch. Thresholds for gaps in octave-band noise were easily predicted from

CRs in zebra finches, where larger GDTs corresponded to smaller CR values. These results are consistent with the time/frequency resolution trade-off described in theories of the filtering properties of the mammalian ear (Duifhuis, 1973; de Boer, 1985). A similar relationship was not found in budgerigars, probably due to their unusual CR function (Okanoya and Dooling, 1990). These species differences in the relationship between frequency selectivity and temporal resolution may reflect more general differences between songbirds and parrots.

In hearing-impaired human listeners, GDTs for broadband signals are larger than normal when compared at equal SPLs, but are only slightly worse at equal SLs (Irwin et al., 1981; Fitzgibbons and Wightman, 1982; Irwin and Purdy, 1982; Tyler et al., 1982; Florentine and Buus, 1984; Buus and Florentine, 1985; Fitzgibbons and Gordon-Salant, 1987; Glasberg et al., 1987). These findings are inconsistent with a simple time-frequency tradeoff hypothesis.

Thresholds for detecting gaps in broadband noise-bursts were measured in BWS and non-BWS canaries to determine if the damaged basilar papilla and broadened auditory filters adversely affects temporal resolution or whether the broadened auditory filters result in enhanced gap detection. If an inverse relationship between frequency selectivity and temporal resolution exists in canaries as it does in zebra finches, then BWS canaries should have smaller GDTs than non-BWS canaries provided the stimuli are clearly audible. However, if the inner ear abnormalities affect temporal coding of stimuli, then BWS canaries should show larger GDTs than non-BWS canaries. GDTs were expected to decrease with increasing sound level in both strains.

Methods

Stimuli

Background stimuli were 300 ms bursts of Gaussian noise with 5 ms $\cos^2$ rise/fall times, sampled at 40 kHz and lowpass filtered at 15 kHz. The target sounds were noise-bursts with gaps of different durations centered within the noise-burst, with rise/fall times of .01 ms. The total duration of the target sounds, including gaps and noise-bursts, was 300 ms. GDTs were measured for noise-bursts with levels of 60, 65, 70, and 75 dB SPL in BWS canaries and 40, 50, 60, 65, 70, and 75 dB SPL in non-BWS canaries.

Procedures

Four BWS canaries and 4 non-BWS canaries were used as subjects. Absolute thresholds for noise-bursts were measured using the procedures described in Experiment 1 for pure tone thresholds. GDTs were measured by randomly alternating target sounds (noise-bursts containing gaps) with repeating background noise-bursts. Noise-bursts with gaps of different durations were presented according to the Method of Constant Stimuli with 7 different gap durations within a block of 10 trials. The step size was 1 or 2 ms, depending on the birds' behavior. Performance was considered stable if the duration of the minimum detectible gap did not change by more than 1/3 of the increment step size within the last two 100 trial sessions. Gap detection thresholds were measured for noise-bursts of different SPLs in a random order.

Threshold was defined as the level of the 2800 Hz tone relative to the 2800 Hz masker component that was detected 50% of the time corrected for the false alarm rate [Pc*=(Pc-FA)/(1-FA)] (Gescheider, 1985; Dooling and Okanoya, 1995a). The average false alarm rate was 2.58% for non-BWS canaries and 4.04% for BWS canaries. Data from sessions with false alarm rates larger than 18% were excluded from analysis. Non-BWS canaries initially showed unstable behavior at the lower sound levels tested. Seventeen percent of the data from non-BWS canaries were discarded, and 7% of the data from BWS canaries were discarded.

Results and Discussion

Average absolute thresholds for noise-bursts for BWS and non-BWS canaries are listed in Table 2. Thresholds were significantly lower for non-BWS canaries (mean=22.08 dB SPL, SD=3.91) than for BWS canaries (47.23 dB SPL, SD=5.89) [$t(6)$=-7.089, $p<0.0001$]. This difference in thresholds for noise-bursts between non-BWS and BWS canaries is comparable to differences in pure tone thresholds between the two strains for frequencies above 2000 Hz. Presumably, thresholds for noise-bursts are higher in BWS canaries than in non-BWS canaries due to the inaudibility of the high frequency components of the noise. Noise-burst thresholds were used to determine SLs so that gap detection ability could be compared at equal SPLs and equal SLs.

Table 2. Absolute thresholds for noise-bursts for non-BWS and BWS canaries.

Strain	Threshold	SD	SE
Non-BWS	22.08	3.91	1.95
BWS	47.23	5.89	3.40

Average gap detection thresholds for BWS and non-BWS canaries at equal SPLs are shown in Figure 14A. Thresholds ranged from 3.62 ms at 75 dB SPL to 6.49 ms at 40 dB SPL for non-BWS canaries, increasing somewhat with decreasing level. These GDTs are within the range of those reported in other species of birds (Okanoya and Dooling, 1990; Klump and Maier, 1989; Klump et al., 1998). Thresholds for BWS canaries ranged from 1.78 ms at 75 dB SPL to 11.21 ms at 60 dB SPL. BWS canaries were not tested at lower SPLs due to audibility constraints. In the range of 60 to 75 dB SPL, BWS canaries showed a much larger change as a function of level than non-BWS canaries.

A strain x SPL mixed factor ANOVA revealed significant effects of strain [$F(1,16)=224.464$, $p<0.0001$] and SPL [$F(3,18)=62.909$, $p<0.0001$], and a significant interaction between strain and SPL [$F(3,18)=48.023$, $p<0.0001$]. Post hoc analyses using the Bonferroni method showed that GDTs for BWS canaries were not significantly different from GDTs for non-BWS canaries at 65 and 70 dB SPL. GDTs for BWS canaries were larger than GDTs for BWS canaries at 60 dB SPL [$t(4)=8.875$, $p<0.05$] , and were lower than in non-BWS canaries at 75 dB SPL [$t(4)=-3.851$, $p<0.05$].
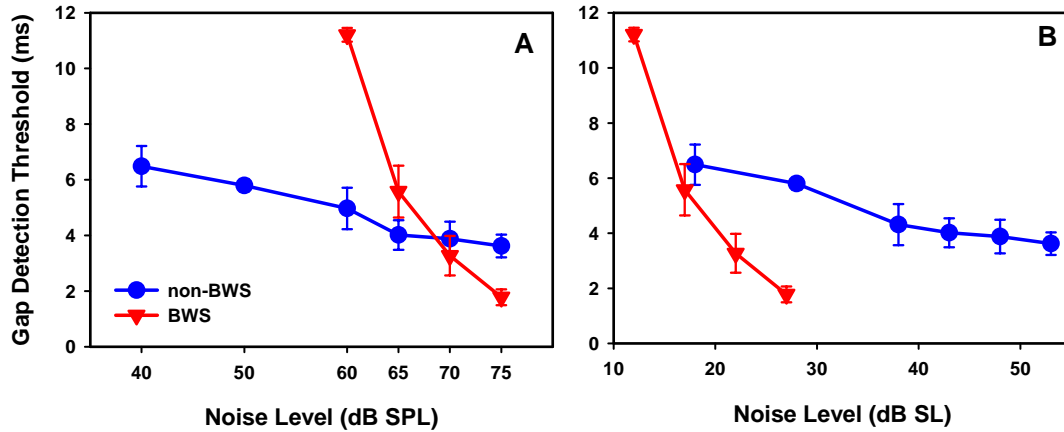
Figure 14. Average gap detection thresholds as a function of sound pressure level (A) and sensation level (B) for BWS and non-BWS canaries. Error bars indicate standard error.

The sharp decrease in resolution of gaps between 75 and 60 dB SPL in BWS canaries may be related to the low SLs of the noise-bursts at 60 and 65 dB. Average GDTs at equal SLs are shown in Figure 14B. GDTs were much lower than normal in BWS canaries for SLs above 20 dB. Non-BWS canaries had thresholds for noise-bursts that were approximately 25 dB lower than in BWS canaries. Thus, 60 dB SPL is approximately 35 dB SL in non-BWS canaries but only 10 dB SL in BWS canaries. As the higher frequency components become less audible, BWS canary performance decreases.

The enhanced sensitivity to gaps shown in BWS canaries at high sound levels is not consistent with thresholds for gaps in noise reported in human listeners with sensorineural hearing loss (Irwin et al., 1981; Fitzgibbons and Wightman, 1982; Irwin and Purdy, 1982; Tyler et al., 1982; Florentine and Buus, 1984; Buus and Florentine, 1985; Fitzgibbons and Gordon-Salant, 1987; Glasberg et al., 1987). GDTs in

hearing-impaired humans approach normal levels at higher SPLs but are not better than those of normal-hearing listeners.

The superior temporal resolution at 75 dB SPL in BWS canaries may be related to the wider bandwidth of auditory filters in the area of hearing loss. An earlier experiment (Chapter 2) showed that PTCs are larger in BWS canaries than in non-BWS canaries at higher frequencies. The wider filters may result in less smoothing of the input waveform over time, thus preserving more of the fine details of the signal that aid gap detection. Additionally, wider filters have less ringing than narrow filters. Ringing may obscure the abrupt offset of the noise when a gap occurs, thereby increasing GDTs for non-BWS canaries.

Experiment 3: Discrimination of Temporal Fine Structure

The vocalizations of birds are often temporally complex, with both slow envelope fluctuations and fast within-period fluctuations. The majority of hearing studies of temporal resolution in birds have focused on the detection or discrimination of slow overall changes in the envelope of sounds, such as the detection of brief silent periods (gaps). Recently, Dooling et al. (2002) presented evidence that birds are superior to humans when envelope and frequency cues are removed and discrimination of sounds must rely on cues in the temporal fine structure (within period temporal fluctuations). Budgerigars, zebra finches, non-BWS canaries, and humans discriminated between harmonic complexes constructed according to the Schroeder (1970) algorithm. Negative-phase and positive-phase versions of these complexes have similar envelopes and long-term frequency spectra, but different

temporal fine structure. Harmonic complexes with higher fundamental frequencies had shorter periods. While humans were unable to discriminate well between complexes with fundamental frequencies of approximately 400 Hz and above, birds were able to make the discrimination between complexes with fundamental frequencies of up to 800 Hz and sometimes 1000 Hz. The birds were able to resolve the temporal fine structure of stimuli with periods as short as 1 ms, while humans were limited at approximately 2.5 ms.

This difference in resolution of within-period temporal changes between birds and humans is thought to be related in part to the width of the auditory filters. Broader auditory filters should lead to better within-channel temporal resolution in a linear system because there is better preservation of phase relationships as more components fall within one channel (Duifhuis, 1973; de Boer, 1985). Behavioral estimates of auditory filter bandwidth suggest that birds generally have broader filters than humans (reviewed in Dooling et al., 2000). However, tuning curves of some auditory nerve fibers in birds are more narrowly tuned than in mammals (reviewed in Gleich and Manley, 2000). Thus, the relationship between temporal acuity and peripheral auditory filtering mechanisms remains unclear.

The previous experiment measuring resolution of gaps in noise in non-BWS and BWS canaries demonstrated that under some conditions, BWS canaries are actually better than non-BWS canaries at detecting changes in the envelope of sounds. This experiment addresses the question of whether or not resolution of fast within-period temporal changes (temporal fine structure) is affected by the inner ear abnormalities in BWS canaries. The ability to discriminate between stimuli identical

to those used by Dooling et al. (2002) was measured in BWS and non-BWS canaries. If the efficiency of encoding fast temporal changes is disrupted by the pathology in BWS canaries, then their discrimination ability should be worse than non-BWS canaries. However, it is also possible that the broadened auditory filters in BWS canaries could lead to enhanced within-channel preservation of the temporal waveforms of the stimuli, thereby leading to better discrimination ability compared to non-BWS canaries.

Methods

Stimuli

Harmonic complexes were constructed with component starting phases selected according to the Schroeder (1970) algorithm [$\theta_n = C\pi n (n + 1)/N$], with C= -1.0 and +1.0. These scalar values produce complexes with very flat temporal envelopes (see Figure 8, p. 47). Complexes with fundamental frequencies of 150, 200, 300, 400, 600, 800, and 1000 Hz were created. Complexes with larger fundamental frequencies have shorter period durations. The periods of these stimuli ranged from 1 ms to 6.67 ms. The waveforms were 260 ms in duration, with 20 ms $\cos^2$ onset/offset ramps. Stimuli were presented at 80 dB SPL.

Procedures

Three BWS canaries were tested. The data from BWS canaries were compared to data from 3 non-BWS canaries reported by Dooling et al. (2002). Birds were trained to discriminate between negative-phase (C=-1.0) and positive-phase

(C=+1.0) waveforms. Percent correct discrimination values were taken as the mean percent correct over the last 200 trials once the bird's behavior stabilized for each experimental condition. Percent correct values were averaged over these two measures for each fundamental frequency. Both negative-phase and positive-phase variations of the waveforms were tested as both target and background sounds, and so birds were tested twice at each fundamental frequency. Thus, the reported percent correct value reflects a total of 400 trials for each fundamental frequency.

Birds ran a minimum of 300 trials on each condition, or until behavior stabilized. Behavior was considered stable if the percent correct for a given target did not change by more than 10% within the last two 100 trial blocks. If the bird's percent correct remained at 100% for the first two blocks of 100 trials, the bird was not run on more trials for that condition. The order of fundamental frequencies was randomized for each individual bird. Since birds required extra training when switching between negative-phase and positive-phase background sounds, all fundamental frequencies were tested for a given phase selection (negative or positive) before switching to the opposite phase selection. Whether a bird began with the negative-phase or the positive-phase background sounds was chosen randomly. The average false alarm rate was 4.49% for BWS canaries. Data from sessions with false alarm rates larger than 18% were excluded from analysis. Seven percent of the data from BWS canaries were discarded.

Results and Discussion

Figure 15 shows percent correct discriminations for harmonic complexes with different fundamental frequencies for BWS canaries along with data from non-BWS canaries tested using identical methods replotted from Dooling et al. (2002). Average BWS canary discrimination ability was high (80% correct or better) for complexes with fundamental frequencies up to 1000 Hz, whereas non-BWS discrimination performance fell to chance for complexes with fundamental frequencies of 1000 Hz. Thus, BWS canaries were able to make the discrimination even for stimuli with periods as short as 1.0 ms. This is well beyond the human limit, which falls at about 300 to 400 Hz (Dooling et al., 2002).
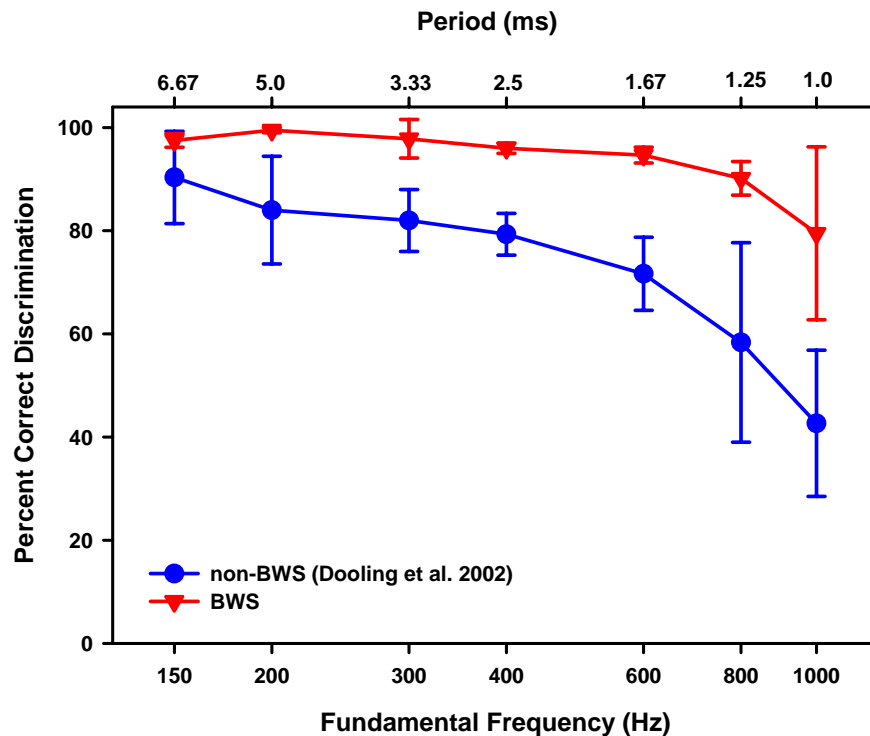

Figure 15. Percent correct discriminations of harmonic complexes for non-BWS (Dooling et al., 2002) and BWS canaries. Error bars indicate standard error.

BWS canaries showed slightly higher percent corrects than non-BWS canaries at most fundamental frequencies; however, a mixed factor (strain x fundamental frequency) ANOVA showed that the main effect of strain was not significant. There was a significant effect of fundamental frequency [$F_{(6,24)}=16.056$, $p<0.000$] and a significant interaction between fundamental frequency and strain [$F_{(6,24)}=2.667$, $p=0.040$]. Post hoc comparisons using the Bonferroni method showed that BWS canaries had higher percent correct discriminations only for complexes with fundamental frequencies of 300 Hz [$t_{(4)}=3.354$, $p<0.05$] and 600 Hz [$t_{(4)}=3.137$, $p<0.05$].

These data show that the ability to discriminate fast temporal changes in sounds is not impaired in BWS canaries, despite the disruption of the phase response of the basilar papilla. The excellent resolution of fast temporal changes seen in BWS canaries may be attributed in part to wider filter bandwidths at higher frequencies. Wider filters allow more components of the harmonic complexes to fall within one channel, thereby preserving more of the phase relationships between components than in narrower filters. Alternatively, the internal waveform shapes of the negative-phase complexes and the positive-phase complexes in BWS canaries might be more different than in non-BWS canaries, owing to changes in basilar papilla motion.

Chapter 7: Perception of Vocalizations

Experiment 1: Song Syllable Discrimination

Investigating the perception of various acoustic parameters in BWS canaries is necessary to understand the functional consequences of the abnormal inner ear pathology in this strain. Still, we know in humans that audiometric thresholds (or discrimination abilities) are not always well correlated with the ability to perceive and understand sounds containing complex interactions of spectral, temporal, and intensity cues, such as speech. Thus, the basic auditory capability in canaries is undoubtedly different than the ability to perceive complex sounds, such as bird vocalizations. Hearing impairment may alter higher level pattern encoding in ways that do not correlate directly with detection or discrimination thresholds in BWS canaries.

Songbirds like canaries learn, produce, and use complex vocalizations ranging from short notes and calls to longer, elaborate songs. Such sounds convey relevant information for individual, kin, and species recognition, mate selection, and territoriality. Males must be able to hear their own complex communication signals for learning and maintenance of song to occur (reviewed in Brainard and Doupe, 2002; Konishi, 2004). Females also memorize songs heard early in life and may use these templates to select mates (Nagle and Kreutzer, 1997). Thus, there is every reason to think there is a close relationship between hearing sensitivity and vocalization learning in the BWS canary.

Canaries bred for plumage or body shape show song characteristics that are more similar to wild canaries than songs of strains long bred for specific song

characteristics (e.g., BWS canaries, German Harz Roller canaries; Güttinger, 1985).

Song canaries typically vocalize at a lower range of frequencies, sing more frequent

and longer tours, prefer shorter syllables, and have fewer syllable types in their

repertoires (Güttinger, 1985). Thus, selective breeding for particular song qualities

may also have affected the mechanisms underlying memorization and production of

acoustic information in these canaries. In doing so, perceptual predispositions that

allow the birds to effectively attend to strain-specific vocalizations may have also

been selected for.

The calls and songs of BWS canaries contain energy primarily at lower

frequencies (Güttinger, 1985; Nottebohm and Nottebohm, 1978; Okanoya et al.,

1990; Wright et al., 2004). In contrast, non-BWS canary calls and songs contain

more high frequency energy than those of BWS canaries (Güttinger, 1985; Okanoya

et al., 1990; Wright et al., 2004). Absolute thresholds at 4000 Hz are correlated with

the relative amount of song energy at 4000 Hz in BWS, non-BWS, and hybrid

canaries (Wright et al., 2004). One reason that BWS canaries do not incorporate high

frequency information into their vocal repertoires is most likely because they cannot

detect or discriminate high frequency sounds well.

An early operant conditioning study found that BWS canaries are more

proficient at learning to classify BWS canary calls than are non-BWS canaries (Park

et al., 1989). However, this study did not test how non-BWS canaries perceive non-

BWS canary vocalizations. Past studies have used psychophysical procedures to

measure response latencies followed by multidimensional scaling (MDS) techniques

to show that birds have perceptual categories for vocalizations (e.g., Brown et al.,

1988; Dooling et al., 1987) and that birds are generally more adept at discriminating between vocalizations of their own species than between the vocalizations of other species (Dooling et al., 1992). Budgerigars, zebra finches, and non-BWS canaries all show shorter response latencies, indicating an easier discrimination, when discriminating among species-specific calls than among calls of other species (Dooling et al., 1992).

In this experiment, discrimination of BWS canary and non-BWS canary song syllables was measured in both strains to determine whether there are strain-specific perceptual predispositions for song elements. If BWS canaries have a perceptual advantage for discriminating between BWS canary syllables, then their response latencies should be shorter compared to non-BWS canaries when discriminating between pairs of BWS canary syllables. Conversely, if non-BWS canaries have a perceptual advantage for discriminating between non-BWS canary syllables, then their response latencies should be shorter compared to BWS canaries when discriminating between pairs of non-BWS canary syllables. The two strains were expected to perform equally well when discriminating BWS canary syllables from non-BWS canary syllables.

Method

Stimuli

Four BWS and 4 non-BWS canaries from our flock were recorded in a sound-attenuated chamber using a Marantz digital recorder (PMD670) or a Marantz analog recorder (PMD740). None of the birds used as subjects in this study were recorded.

al recorder.

Audition

0 Hz using

ape

syllables

and 4 non-BWS canary syllables were used as stimuli. Spectrograms of the stimuli
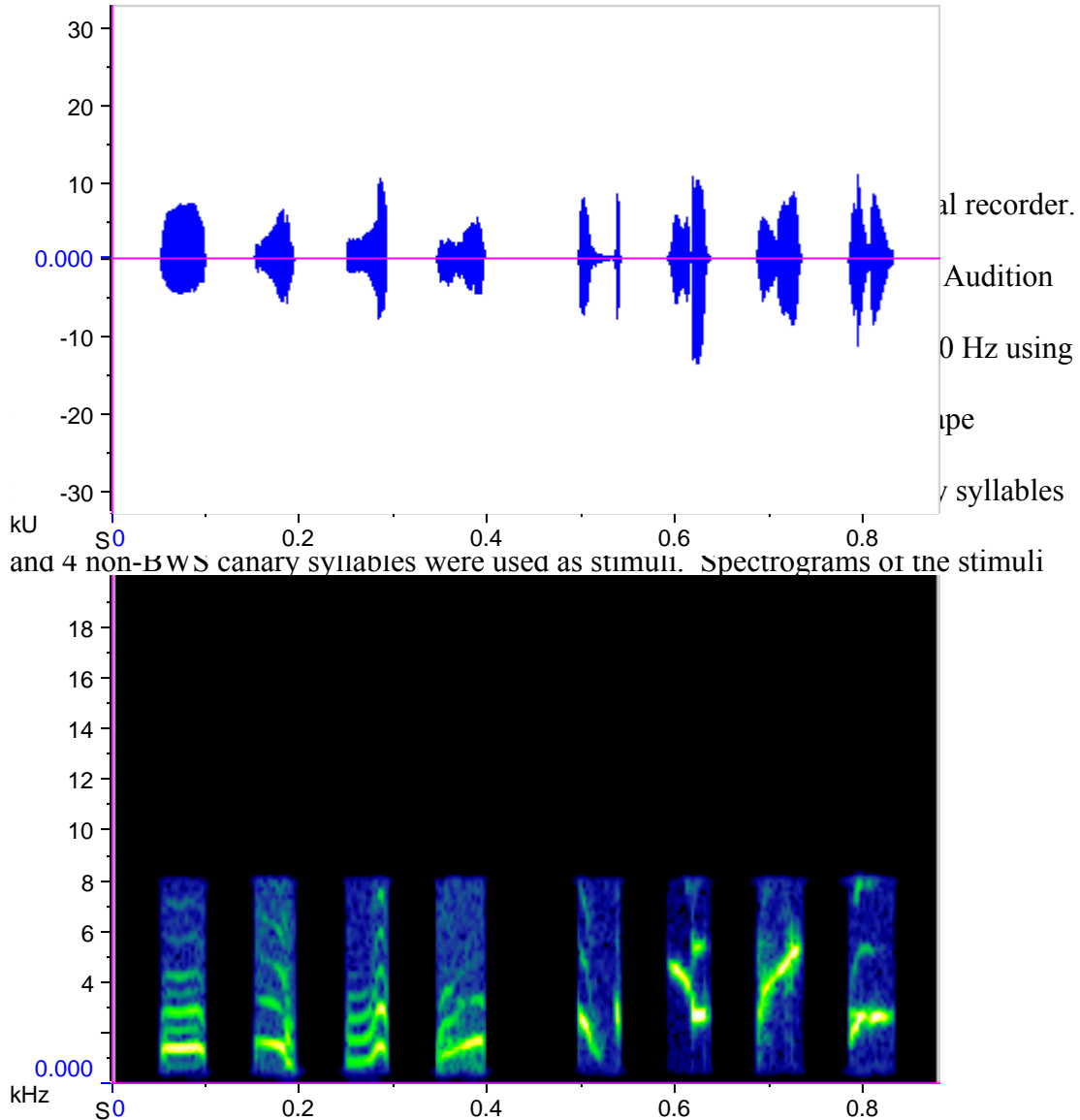
Figure 16. Spectrograms of the BWS and non-BWS canary song syllables used as stimuli.

Individual syllable characteristics are summarized in Table 3. The peak

frequencies of the BWS canary syllables were lower than those of non-BWS canary

syllables. The bandwidth and the within-syllable amplitude difference (maximum

amplitude – minimum amplitude) were smaller in BWS canary syllables compared to

non-BWS canary syllables. All of the BWS canary syllables consisted of only one

note; whereas, the non-BWS canary syllables consisted of two notes. In some cases,

the two notes overlapped in time. All syllables were all approximately 45 ms in

duration (+/- 5 ms) and were presented at a sampling rate of 40 kHz. The stimuli

were presented at a level of approximately 70 dB SPL, and the level was randomly

roved up to +/ 5 dB on each trial to control for loudness cues.

Table 3. Summary of canary song syllable characteristics.

Syllable	Strain	# Notes	Peak Frequency (Hz)	Bandwidth (Hz)	Max-Min Amplitude (dB)
B1	BWS	1	1406	3867	7
B2	BWS	1	1562	1719	20
B3	BWS	1	937.5	2890	13
B4	BWS	1	1445	2695	13
AVG	**BWS**	**1**	**1337.6**	**2792.8**	**13.3**
N1	non-BWS	2	1250	2070	18
N2	non-BWS	2	2773	3945	19
N3	non-BWS	2	4531	3125	17.5
N4	non-BWS	2	2617	3907	19.5
AVG	**non-BWS**	**2**	**2792.8**	**3261.8**	**18.5**

Procedures

Four BWS canaries and 4 non-BWS canaries were used in this experiment.

The procedures were similar to those described by Dooling and Okanoya (1995b).

Birds were trained to discriminate among BWS and non-BWS canary song syllables.

Within a session, one syllable served as the repeating background. The background

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was periodically alternated with one of the other 7 syllables. All 7 target syllables were presented in a random order within a block of 10 trials. The birds were run for 200 trials on each background, with each syllable serving as a background one time. Thus, each syllable was paired with each other syllable twice: once as a target and once as a background. Responses from these two target-background pairings for each syllable combination were pooled for analysis, and a half-matrix of response latencies was created. Percent correct discriminations and mean response latencies were calculated for analysis. The average false alarm rate was 4.43% for non-BWS canaries and 1.46% for BWS canaries. No data were excluded from analysis.

Results and Discussion

Percent correct discriminations are shown in Figure 17A for BWS and non-BWS canaries. A mixed factor (strain x syllable comparison type) ANOVA revealed a significant effect of syllable comparison type [$F_{(2,12)}=4.314$, $p=0.039$] and a significant interaction of strain and syllable comparison type [$F_{(2, 12)}=4.104$, $p=0.044$] but no significant effect of strain. Post hoc comparisons using Tukey's HSD showed that BWS canaries had higher percent correct discriminations than non-BWS canaries for BWS/non-BWS syllable comparisons [$q_{(3, 12)}= -7.917$, $p<0.05$]. There was no significant difference between strains for BWS/BWS comparisons or non-BWS/non-BWS comparisons.
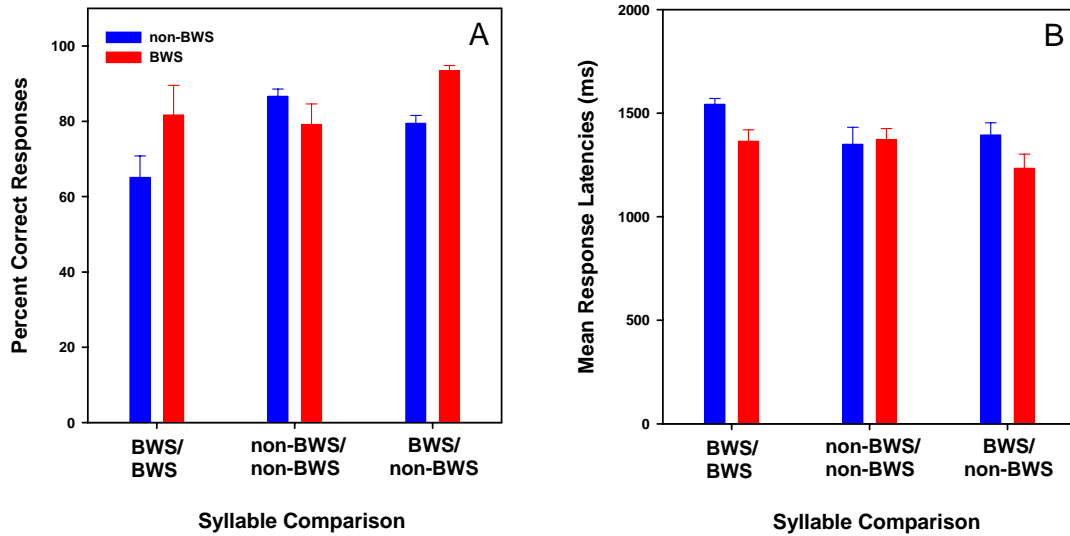
Figure 17. Percent correct responses (A) and mean response latencies (B) for BWS and non-BWS canaries. Error bars indicate standard error.

Mean response latencies are shown in Figure 17B. BWS canaries showed consistent mean response latencies across all conditions, while non-BWS canaries showed slightly longer response latencies for BWS canary syllable pairs. A mixed factor (strain x syllable comparison) ANOVA revealed a significant effect of syllable comparison [$F_{(2, 12)}=5.336$, $p=0.022$] but no significant overall effect of strain or significant interaction. Post hoc comparisons using Tukey's HSD showed that response latencies were shorter for BWS canaries compared to non-BWS canaries for BWS/BWS syllable comparisons [$q_{(3, 12)}=4.10$, $p<0.05$], but there were no significant differences between strains for non-BWS/non-BWS or BWS/non-BWS syllable comparisons. These results indicate that BWS could more easily discriminate between BWS song syllables than non-BWS canaries.

As expected, BWS canaries were faster than non-BWS canaries when discriminating among pairs of BWS syllables. This result is consistent with an earlier experiment that showed that BWS canaries learn to classify BWS canary calls faster and more accurately than non-BWS canaries (Park et al., 1989). Most of the spectral information contained in these syllables falls within the area of best sensitivity and best frequency selectivity in BWS canaries (1000 to 2000 Hz) and below the region of best sensitivity for non-BWS canaries (2000 to 4000 Hz). The superior temporal resolution and frequency discrimination at 1000 Hz demonstrated in earlier experiments in BWS canaries may also provide a perceptual advantage for discriminating BWS canary song syllables.

Presumably, the strain differences in perception of BWS canary vocalizations is not reflective of some general difference in learning abilities, since BWS canaries did not show shorter response latencies than non-BWS canaries for non-BWS/non-BWS syllable comparisons or BWS/non-BWS comparisons. It is not surprising that BWS and non-BWS canaries are equally good at discriminating BWS canary syllables from non-BWS canary syllables, given the different frequency ranges and number of elements characteristic of the two syllable types.

The fact that BWS canaries could discriminate the higher-pitched non-BWS canary syllables as well as non-BWS canaries was intriguing because of the reduced sensitivity and reduced frequency selectivity at higher frequencies in BWS canaries. A large amount of the spectral content of non-BWS canary syllables falls in the range of reduced sensitivity and reduced frequency selectivity in BWS canaries (above 2000 Hz). Perhaps the only reason that BWS canaries are not worse than non-BWS

canaries when discriminating between non-BWS syllables is that non-BWS canary syllables are somewhat more complex (broader frequency range, more elements, more change in amplitude). In other words, non-BWS canary syllables might simply be easier to discriminate in general because they contain more cues to utilize.

There is one obvious cue that is typically present in non-BWS canary song syllables that is absent from BWS canary song syllables. For example, BWS canary song is primarily produced via the left side of the syrinx, and, consequently, contains a large proportion of one-note syllables (Hartley and Suthers, 1990). The song of a non-BWS canary strain, called the "Common Canary" in France, is produced using both sides of the syrinx and contains many two-note syllables (Suthers et al., 2004). Females of this type of non-BWS canary have been shown to prefer a certain two-note syllable type (Vallet et al., 1998; Pasteau et al., 2004), and they may learn this preference early in life (Nagle and Kreutzer, 1997). Similarly, BWS canaries may learn a preference for one-note (low frequency) syllables early in life. No study has directly addressed this question; however, indirect evidence for a preference for one-note syllables comes from the observation that BWS increase the proportion of one-note syllables in their repertoires over successive breeding years (Nottebohm and Nottebohm, 1978).

Individual differences multidimensional scaling (MDS) procedures similar to those used in previous studies (Dooling et al, 1990; Okanoya and Dooling, 1990; Kreutzer et al., 1991) were conducted on the matrix of mean response latencies for BWS and non-BWS canaries to determine how the two strains perceptually grouped the song syllables. MDS places the stimuli in multidimensional space (perceptual

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space) so that distances between stimuli correspond to perceived stimulus similarity.

A MDS analysis using 5 dimensions accounted for 0.59% of the variance of the BWS

data. Adding more dimensions did not significantly improve the variance accounted

for. For visual simplicity, 3-dimensional MDS plots are shown in Figure 18 for the

derived stimulus configuration (A) and the derived subject weights (B). The stimulus

configuration plot BWS and non-BWS stimuli were not grouped as two distinct

perceptual groups. The individual subject weights plot indicates that each individual

subject may weighed each dimension differently. Of all the acoustic characteristics

of the stimuli described in Table 3, only one parameter, Maximum–Minimum

Amplitude, was significantly correlated with a dimension ($r=0.747$, $p=0.033$). Thus,

it may not be particular acoustic dimensions of the song syllables that are important

for discrimination, but rather the combinations of spectral, temporal, and intensity
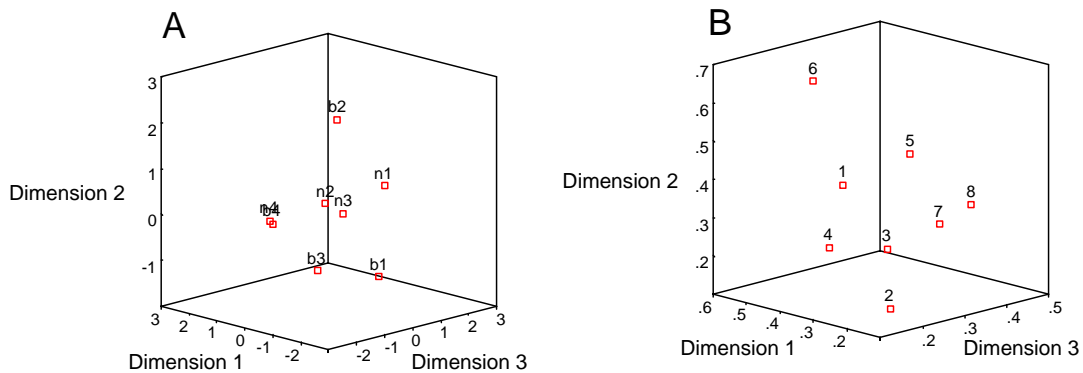
cues that are unique to each syllable.

Figure 18. A three-dimensional spatial representation of the derived stimulus configuration (A) and individual subject weights (B).

Chapter 8: Summary and General Discussion

Summary

Here, I have shown that a number of aspects of hearing and auditory perception are different in BWS canaries than in non-BWS canaries. These experiments have provided a multifaceted behavioral assessment of hearing abilities and auditory perception in BWS and non-BWS canaries. The differences in perceptual abilities demonstrated in BWS canaries highlight the uniqueness of this animal system.

The first set of experiments investigated spectral and temporal masking effects. These experiments showed that frequency selectivity is reduced at high frequencies in BWS canaries. Phase effects on masking are also reduced in BWS canaries. The second set of experiments investigated discrimination of changes in frequency, duration, and intensity over time. Frequency discrimination was better than normal at some sound levels for a 1000 Hz reference frequency in BWS canaries; however, discrimination of changes in higher frequencies was worse in BWS canaries than in non-BWS canaries. BWS canaries were also better than non-BWS canaries at discriminating changes in stimulus duration at 1000 Hz; however, duration discrimination was similar in BWS and non-BWS canaries at higher frequencies. Intensity discrimination was not adversely affected in BWS canaries. The third set of experiments investigated temporal processing. Temporal integration was reduced in BWS canaries at 1000 and 4000 Hz, but not at 2000 Hz. Temporal resolution of envelope changes was especially good in BWS canaries, provided the

high frequency components of the stimuli were clearly audible. Similarly, discrimination of fast within-period temporal changes was excellent in BWS canaries.

Studies of the discrimination of changes in single dimensions of a simple acoustic stimulus do not illustrate how a bird perceives naturally occurring sounds such as vocalizations which have complex and unique arrangements of spectral, temporal, and intensity cues. Thus, the final experiment investigated perception of natural vocal signals in BWS and non-BWS canaries. BWS canaries were faster than non-BWS canaries at discriminating among pairs of BWS canary song syllables. Interestingly, BWS canaries were as good as non-BWS canaries when discriminating among pairs of non-BWS canary song syllables. Both strains easily discriminated between BWS/non-BWS song syllable pairs.

It is likely that where BWS canaries are worse than non-BWS canaries are due to changes in the functioning of the basilar papilla as a consequence of hair cell damage. The converse may also be true. Some of the perceptual enhancements in BWS canaries may be due to the same inner ear damage. In addition, there may also be compensatory mechanisms in the BWS auditory system which have not yet been identified.   For instance, there may be differences in the morphology of the synapses between hair cells and auditory nerve fibers that support very precise temporal encoding. There also may be differences in the microstructure of hair cells that may enhance the processing of low frequency stimuli. Clearly, the behavioral differences between BWS and non-BWS canaries point to the need for further investigation into the mechanisms that underlie normal functioning of the avian peripheral and central auditory system.

In aggregate, these results demonstrate that there are significant perceptual consequences of the early-onset hearing loss in BWS canaries other than elevated pure tone thresholds. These perceptual differences between BWS and non-BWS canaries need not always be viewed as impairments. Instead, the unique perceptual profile in BWS canaries can, in some ways, be viewed as a specialization for attending to their unique low-pitched vocalizations. The perceptual differences between BWS and non-BWS canaries probably reflect the existence of inborn song learning preferences related to hair cell abnormalities.

The present study of hearing in BWS canaries now identifies even more clearly a number of elements in this animal model of hearing that are truly unique. These include: 1) the relation between papillar damage in a bird ear and perceptual consequences in a bird ear; 2) the effects of early-onset developmental hearing loss on higher auditory structures; 3) the effect of early-onset hearing loss on vocal development and song learning preferences; 4) artificial selection for hearing loss and specific vocalizations; 5) implications for the functioning of the normal bird ear; and 6) identification of genes involved in hair cell damage and loss and hair cell regeneration following experimentally induced damage.

Effects of Early-Onset Developmental Hearing Loss on Higher Auditory Structures

BWS canaries show a reduced number of auditory nerve fibers and reduced volume of auditory brainstem nuclei involved in temporal coding. From hatch until

approximately 2 weeks of age, BWS hearing develops normally (Brittan-Powell et al., 2002). This suggests that the structural deficits in the auditory nerve and brainstem nuclei are thought to be related to the post-hatch development of the hearing loss and hair cell pathology (Brittan-Powell et al., 2002; Ryals and Dooling, 2002). More in depth studies of the anatomy and physiology of higher auditory structures in BWS canaries are required to understand the effects of the developmental hearing loss on the auditory system.

Role of Early-Onset Developmental Hearing Loss in Song Learning and Song Preferences

Since elevated ABR thresholds and the abnormal hair cell pathology develop before song learning occurs in BWS canaries (Brittan-Powell et al., 2002; Ryals and Dooling, 2002), it is probably the case that the perceptual predispositions influence the content of what is learned. One way to test for the existence of genetic perceptual biases related to the hair cell pathology and hearing abilities is to raise juvenile BWS and non-BWS canaries with a choice of tutors: one a BWS canary and one a non-BWS canary. In all probability, BWS canaries tutored under these conditions would tend to sing mostly BWS canary song elements and non-BWS canaries would sing non-BWS canary elements.

The hearing loss also must affect the development of preferences for certain song characteristics in females. During the period when males are learning there songs, female BWS canaries are also memorizing characteristics of tutor song. The preferences learned early in life may be used by adult females to evaluate potential

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mates and possibly in production their own song. BWS canary males modify their song seasonally. Presumably, the addition, deletion, and modification of song syllables are affected by female song preferences. Females cannot hear high frequency song elements well; therefore, they are more likely to choose mates who sing lower frequency notes.

Artificial Selection: The Sensory Linkage Hypothesis

There is a question of whether the hair cell pathology and associated hearing abilities preceded the development of low-pitched vocalizations in the BWS canary strain over the course of time, or whether the hearing loss and hair cell pathology happened as a result of disuse (i.e, not singing and listening to high-pitched vocalizations). That is, did inbreeding within the strain result in a genetic mutation that caused a hearing loss, thereby resulting in the production of low-pitched vocalizations (bird can't learn to sing what it can't hear? Did the fact that BWS canaries produce primarily low-pitched vocalizations result in a loss of high-frequency hearing over time because of the lack of high frequency input to the auditory system? Or, did breeders inadvertently select for birds with a hearing loss because they produced more desirable, higher quality BWS songs?

Wright et al. (2004) proposed the *sensory linkage* hypothesis as a possible mechanism for the evolution of sexually selected traits (i.e., song) in BWS canaries. Changes in auditory perception due to inbreeding and artificial selection by breeders are likely to have affected song learning. This in turn could have simultaneously altered the expression of song in males and preference in females.

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Though it is impossible to know which came first, the low-pitched vocalizations or the hearing loss and hair cell pathology, the close link between hearing abilities and vocal characteristics suggests some interesting areas for further study. One approach is to test whether BWS canaries are capable of producing high frequency syllables. As discussed in a previous section, adult BWS canaries produce the majority of song elements with the left side of the syrinx, while non-BWS canaries produce song elements using both sides of the syrinx. If juvenile BWS canaries were tutored using high-pitched non-BWS songs altered to compensate for the hearing loss (i.e., amplify high frequency components), would they produce high-frequency song elements, or is the syrinx hard-wired to produce only low frequency song?

Structure and Function of the Avian Auditory System

Beyond the questions regarding the role of hearing abilities and genetics in song learning in BWS canaries, these birds provide a unique model in which to study the relationship between the structure of the auditory system, the physiological response of the auditory system, and behavior. In reality, we know very little about how the avian ear works compared to the wealth of research conducted on the functioning of the mammalian ear. The poor resolution and discrimination of high frequencies shown here in BWS canaries indicate that the basilar papilla may be abnormally tuned. This may be reflected in measurements of basilar membrane movement in response to sounds, single unit neural tuning curves, and place-frequency maps in higher auditory centers. The mechanisms underlying the

exceptional temporal resolution shown in BWS canaries could be explored via measures of phase locking in the auditory nerve and encoding of temporal information in higher auditory structures including the nucleus magnocellularis and nuclear laminaris.

Hair Cell Regeneration and the Genetics of Deafness

The BWS canary provides a potential model for the study of hair cell regeneration and the genetics of deafness. Despite continuous spontaneous hair cell death and regeneration and the ability to regenerate hair cells following experimentally induced damage, BWS basilar papillae are not repaired and "normal" hearing is not achieved. It is possible the hair cell abnormalities persist because the newly regenerated cells do not work. This possibility is highly intriguing, since regenerated hair cells other bird species are functional. It is likely the case that whatever genetic mutation results in the hair cell abnormalities continues to operate in new hair cells.

The mechanisms underlying cell death and the higher-than-normal rate of continuous hair cells proliferation and differentiation in BWS canaries are unknown. Investigations into these mechanisms could lead to possible therapeutic interventions, either in hatchling or adult BWS canaries, that could repair the existing hair cells or enable regenerated hair cells to repair the BWS canary basilar papilla. Related behavioral studies could identify how the "new" basilar papilla affects sound perception. If successful, similar treatments may one day be developed for humans with hearing loss.

Conclusions

In conclusion, the work completed in this dissertation has further developed the BWS canary model. These birds show a unique milieu of perceptual abilities that may enhance their ability to learn and produce strain-specific vocalizations. This model is exceptional in that it is the only animal in which we can investigate the link between genetics, auditory system structural abnormalities, vocal learning, vocal production, hair cell regeneration, and hearing abilities. The close correspondence between auditory perception and vocal characteristics in BWS canaries begs the question of whether or not the auditory pathology is a product of artificial selection. As breeders mated birds with desirable song characteristics, they may have inadvertently selected for auditory system abnormalities. In essence, the breeders may have artificially produced a specialization in BWS canaries over several hundred years. The BWS canary model has the potential to further our understanding of the evolution of vocal learning and production and the role of genetics in hearing and auditory pathology. Future studies should investigate the specific genes responsible for the hair cell abnormalities, the relationship between basilar papilla microstructure and hearing abilities and the physiological response of the auditory system, and the role of genetic predispositions in song learning in BWS canaries.

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