Recent Experience and Season Modulate Auditory Tuning in Canary Caudomedial Nidopallium

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And approved by

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ABSTRACT OF THE THESIS

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Nidopallium

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The caudomedial nidopallium (NCM) is an area of songbird telencephalon analogous to a portion of the mammalian auditory cortex and is important for conspecific song discrimination, memorization and individual recognition. The tuning properties of NCM neurons differ between canaries and zebra finches. To test whether these differences reflect recent experience, three groups canaries of each sex were housed for 9 days in different conditions: conspecific aviary (normal condition), zebra finch aviary (cross condition) or in isolation box (isolation condition). Extracellular multi-unit electrophysiological responses to simple pure tone stimuli (250-5000Hz) in NCM of awake birds were recorded. Both phasic and tonic responses were measured, and auditory tuning width was quantified as the number of contiguous frequencies at least 1 SD above baseline. Compared to normal birds, tuning was narrower in cross-housed birds and wider in isolated birds. This effect was more pronounced in female canaries than in males. Tuning width was narrower early in the year and broader later in the year and this effect was most pronounced in cross-housed males. These findings demonstrate that tuning properties of NCM neurons are not fixed, but change in response to recent experience. The acoustic and social environment and season-related hormonal change may have important effects on auditory tuning and thus affect the perception of complex songs.

Acknowledgement

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Introduction

Studies of vocal learning in songbirds

Understanding the neural substrate underlying language learning is one of the most challenging tasks in neuroscience. Most recent studies that have been done on human subjects are based on imaging methods and a top-down strategy. This research approach has yielded a good understanding of the large scale neural circuit involved in language learning and processing, although much less is known about how language signals are processed and stored in local networks and how language learning may depend on neurophysiological changes at the cellular level (Pulvermüller, 2003). Songbirds provide researchers a great opportunity to study components of language learning through bottom-up methods. Bird songs are long and complex vocalizations that consist of structures similar to human language (Fig. 1). Although bird songs differ from human language in that songs do not convey meaning as language does, song learning shares many similarities with human speech acquisition (Brainard and Doupe, 2002): Both infant birds and human babies have an innate bias for learning vocalizations from conspecific tutors. Both song learning in birds and speech acquisition in humans depend on exposure to conspecific vocalizations in an appropriate social context during a limited critical period. Songbirds are among the few non-human animals that learn their vocalizations through a process of vocal imitation. The others are parrots, hummingbirds, and cetaceans; of these, songbirds are the most accessible for laboratory study. Songbirds have been shown to have a specialized neural system for vocal learning and

production that may function analogously to that of humans. Moreover, to our knowledge, among all nonhuman animals only songbirds have the capacity to learn recursive syntax, inserting "phrases" within "sentences", which was considered to be the unique characteristic of human language (Gentner et al., 2006).

For these reasons, the study of songbirds has generated a great deal of interest in the past thirty years, during which period of time the motor pathway contributing to vocal learning and production has been under extensive study and has yielded many important findings (for recent reviews, see Zeigler and Marler, 2004). However, auditory processing and memory formation that are equally important in vocal learning remain mysteries. As for speech in humans, song learning in songbirds requires not only the ability to process basic acoustic features such as pitch and temporal duration, but also the capacity to process complex acoustic structures and sequences of acoustic elements, cf. syllables in human language, so a better understanding of neural substrates of auditory processing of songs may help us to understand basic principles of vocal learning. Moreover, memorization of tutor songs also plays an important role in song learning. There are two phases of song learning: a sensory period, in which tutors' songs are stored as templates for later motor learning, and a sensorimotor period, in which birds adjust their vocalizations by comparing their song with memorized information (Brainard and Doupe, 2002). Given that memory processes are also involved in human speech acquisition (Kuhl and Meltzoff, 1996), songbirds may provide a model for study of the sensory motor system of

speech acquisition and production. Furthermore, there is evidence that long-term memorization of conspecific songs and processing of auditory stimuli occur in the same area of songbird telencephalon, the caudomedial nidopallium (NCM, Chew et al., 1996). Moreover, a recent study even revealed that the tutor songs that the birds learned in their early life were stored in this area (Phan et al., 2006), suggesting that NCM plays an important role in sensory aspects of vocal learning.

<u>Physiology and gene expression of NCM and field L</u>

NCM receives projections from field L, an analogue of the primary auditory cortex of mammals, which in turn receives auditory input from the thalamus. Both field L and NCM show a dorso-ventral tonotopic gradient from low to high frequency stimuli (Fig. 2, Terleph et al., 2006). However, there is a large amount of evidence supporting the hypothesis that NCM plays an essential role in song processing and long-term memorization. Studies show the immediate early gene *ZENK* (also known as zif268), known to be expressed in mammalian hippocampus and clearly linked to synaptic plasticity (Cole et al., 1989), is also expressed in NCM in response to hearing songs. This *ZENK* induction declines after repeated exposure to an individual song and returns when birds are exposed to a novel song (Mello et at., 1995), suggesting that ZENK expression in NCM is involved in the formation of long-term memories of individual songs. Furthermore, the fact that ZENK induction in NCM is much higher in response to conspecific songs than heterospecific songs and that there is no ZENK expression in response to pure tones suggest that NCM is specialized for processing conspecific songs (Mello et at., 1992).

Electrophysiological studies on NCM in awake animals are consistent with ZENK expression studies. The electrophysiological response in NCM to songs decreases following repeated exposure and this adaptation effect is specific to individual songs (Chew et al., 1995). Moreover, the relative adaptation rates are good indicators of long term memory to songs (Chew et al., 1995). The electrophysiological response in NCM also appears to be selective to conspecific songs. The response to conspecific songs is more robust than heterospecific songs and other sounds. In addition, adaptation rates show a long-term change (24-48 hr) for conspecific songs but only a shorter period of adaptation (4-6 hr) for other acoustic stimuli. Therefore, both *ZENK* expression and electrophysiological studies suggest that NCM participates in conspecific song discrimination and memorization.

In contrast, the electrophysiological response in field L is robust to simple tone stimuli but is not selective for complex stimuli (Muller and Leppelsack, 1985) and responses do not adapt to repeated presentations of the same acoustic stimuli. Consistent with electrophysiological studies, *ZENK* is also not seen in field L after exposure to acoustic stimuli (Mello et al., 1992, Mello and Clayton, 1994). Therefore, auditory input does not induce a species-specific response and a memorization process in field L, but it is selectively processed and stored in NCM.

Responses to tone stimuli differ between NCM and field L

To understand what enables the complex processing capacity in NCM that differs from field L, a study using simpler stimuli has been done to compare the

electrophysiological tuning properties in NCM and field L (Terleph et al., 2006) in which muli-unit neuronal responses to pure tone stimuli with different frequencies were recorded from multiple sites along the dorsal-ventral axis of NCM and field L. Both the tuning width and response amplitude to the best frequency at each site were measured. This study reveals two important facts: First, NCM shows broader tuning functions than field L (Fig. 2). Second, field L shows strong responses to stimuli in a phasic time window (10ms -60ms from the onset of stimuli, Fig. 3) and the responses decrease rapidly during a tonic time window (70ms-140ms from the onset of stimuli, Fig. 3). In contrast, NCM shows both phasic and tonic responses and even shows a strong response to the offset of stimuli at some sites (Fig.3). The authors suggested that the broader tuning function in NCM neurons may enable the combination of different frequency components in complex sounds and the sustained response and offset response in NCM may contribute to combining multiple components in a sequence of sounds. These two unique properties in NCM may enable the spectro-temporal integration of complex acoustic stimuli that is necessary for selective responses and memorization of conspecific songs. In addition, these results also suggest that using simple pure tone stimuli to study the tuning properties of the auditory forebrain of songbirds may shed light on the mechanism of auditory processing of complex acoustic stimuli like songs.

Responses to tone stimuli in NCM and field L differ between songbird species

A further experiment compared the tuning properties in NCM and field L of canaries and zebra finches (Terleph et al., 2007). The reasoning was that, if

tuning properties of NCM enable it to be selective to conspecific vocalizations, then this selectivity in a species may depend on the unique tuning properties in NCM that are specialized to process the acoustic features of vocal repertoires within a species. The results show two facts. First, much greater species differences were seen in NCM than field L, which is consistent with the fact that field L does not participate in selectivity for conspecific songs. The more important fact is that canary NCM is more narrowly tuned (Fig. 4) and has a lower tonic response compared to that of zebra finches (Fig. 5). These tuning properties reflect the species-specific acoustic features of vocal repertoires: canaries' vocal repertoire consists of many narrow-band whistles and trills, while most vocalizations of zebra finches are broad-band, harmonically-structured notes (Fig. 6). Furthermore, the authors suggested that the narrowly-tuned NCM of canaries is suitable to process narrow-band whistles and trills and responses and the weaker tonic component could enhance the processing of sequences of rapid trills. In contrast, the more widely tuned NCM of zebra finches may allow it to integrate information at different frequencies in a broad-band harmonic note. In addition, the authors suggested three possibilities that may contribute to the species-specific tuning differences in NCM: genetic factors, developmental factors related to early rearing environment, and recent auditory experience.

Effects of recent auditory experience on tuning properties

To test whether recent auditory experience contributes to the species-specific tuning differences, a preliminary experiment was performed. In this experiment, male and female zebra finches and female canaries were housed in

one of three conditions for 9 days: 1) normal conspecific aviary, 2) cross-specific aviary (zebra finch aviary for canaries and canary aviary for zebra finches), 3) in individual isolation. The original expectation was that, if housing condition had any effect on tuning width, then the exposure to wide-band zebra finch songs would broaden canary NCM tuning width. The opposite effect was predicted for zebra finches exposed to the narrow bandwidth canary songs. However, the results show that, in female canaries, exposure to zebra finch songs narrowed NCM tuning width and isolation widened NCM tuning (Fig. 7). The same trend of changes was also seen in both male and female zebra finches. The current study used the same housing paradigm to test the effects of recent experience on NCM tuning width in male canaries in different seasons. We predicted that male canaries would show narrowed tuning in the cross condition and broadened tuning in the isolation condition, as was seen in female canaries. Furthermore, since several studies have shown that sex differences exist in NCM cell composition (Pinaud et al., 2006) and ZENK response to songs (Maney et al, 2006), we also compared the recent experience effect on male and female canaries. Lastly, adult male canaries change their song every year by adding new syllable types and discarding others. Moreover, the testosterone level in male canaries changes across the year and appears to have an important effect on neural plasticity in the song control system. Therefore, we expected that season would have an effect on auditory plasticity in NCM.

Materials and Methods

<u>Subjects</u>

For the current study of NCM tuning, 26 male canaries were obtained from the Rockefeller University Field Research Station. These canaries were assigned to three groups (10 in the cross group, 2 in the normal group and 14 in the isolation group). In the analysis, data collected from the 26 male canaries were combined with data obtained in the preliminary experiment, including 5 males in the normal group and 15 females in three groups (3 in the cross group, 7 in the normal group and 5 in the isolation group). All canaries were housed with conspecifics in our aviary under the natural light-dark cycle. Food and water were provided *ad libitum*, and all procedures conformed to a protocol approved by the Animal Care and Use Committee of Rutgers University. All animals were tested from February to December.

<u>Isolation and Cross housing condition</u>

Nine days before recording, canaries in the isolation group were individually isolated in sound-proof boxes and canaries in the cross group were moved to the zebra finch aviary that houses about 200 adult zebra finches. Both the zebra finch aviary and isolation boxes were kept under a 12-12 light cycle.

<u>Apparatus and Procedure</u>

Two days before the electrophysiological recording, the animal was anesthetized with Nembutal (50-55mg/kg IM). Then a round window was made on the skull in the area of the bifurcation of the midsagittal sinus for later

recording, a chamber was formed and a head post was attached to the skull with dental cement.

To allow for full recovery from anesthesia, the electrophysiological recording was made 48 hr after the surgery. Recordings were made in an acoustically isolated sound booth. The awake animal was immobilized in a comfortable tube and the head of the animal was fixed to the stereotaxic device using the head post. A 150 square grid (Electron Microscopy Sciences, Fort Washington, PA) was attached over the surface of the brain to define the position of electrode penetrations and drug injection along the caudo-rostral and medio-lateral axes. Recordings were made at 7 sites (3 in the left hemisphere, 4 in the right) using seven quartz-platinum/tungsten microelectrodes (1-4 MOhms impedance, Thomas Electronics, Ekhorn design, Germany) controlled by a multielectrode microdrive. White noise stimuli with the amplitude envelope of canary song were presented to search for responsive sites that indicated the dorsal border of NCM. Recordings were made in the first set of these responsive sites by presentation of 20 tone stimuli and then recordings with the same stimuli were repeated at two other depths (in 300um increments) along the dorso-ventral axis. After recording from these sites, all electrodes were moved more rostrally and the recording procedure was repeated two times to record at two other locations. After all recording sessions were completed, three small electrolytic lesions were made in each hemisphere to enable histological reconstruction of recording sites.

Animals were then sacrificed and perfused with saline, followed by paraformaldehyde. Their brains were then cut into 50um sections that were

stained with cresyl violet. NCM is located within the caudo-medial area of the forebrain and has larger, more loosely packed cells compared to field L, located rostral to NCM (Fortune and Margoliash, 1992). The lesion sites were confirmed histologically and then the other recording sites were located through their relative distance to the confirmed lesion sites, using grid position and depth coordinates. Recording sites found to be in field L and the border between field L and NCM were excluded from analyses.

Stimuli

Tone stimuli were sine wave bursts with tapered onsets and offsets (duration: 260ms; frequency range: 250-5000Hz in 250 Hz increments; sample rate: 40 KHz). All stimuli were modified to have equal root-mean-square (RMS) amplitude. Stimuli were presented in pseudo-random order with a 6s inter stimulus interval. Each frequency was repeated 3 times at each recording depth.

Data analysis

To quantify the multi-unit firing value, the firing values during a given interval were squared and averaged and then the root of this mean value was taken. This quantity, named the root-mean-square (RMS), reveals the average amplitude of neuronal firing during a given interval. To quantify the response at a given site to a single trial of a tone stimulus, the baseline firing RMS was measured in a 500 ms window occurring prior to stimulus onset. Then the response amplitude was measured in two time windows: 1) phasic window: 10 ms-60 ms from the onset of the stimulus; 2) tonic window: 60 ms – 130 ms from the onset of the stimulus.

These time windows were defined in the same way as those used in earlier studies (Terleph et al., 2006).

Tuning curves for phasic responses and tonic responses were computed separately. The responses to the 3 repeats of each stimulus frequency at a recording site were averaged. Tuning width was defined in the same manner as in previous work (Terleph et at., 2006): the frequency range of a contiguous set in which each stimulus frequency elicits a response at least 1 SD above baseline. This measure was adopted because many NCM sites do not have a simple single-peak tuning function.

Tuning widths for phasic and tonic response were analyzed separately by ANCOVA. Since the data on tuning width were collected from February to December, the date of recording was included as a covariate in an ANCOVA to analyze the seasonal effect on tuning width. All ANCOVAs treated different housing conditions and sex as independent variables.

If the tuning widths recorded from sites within a bird are related to each other, using recording sites as independent samples can overestimate the degrees of freedom. To test this possibility, sites within each bird in the same group were randomly paired and the tuning widths recorded from each pair of the sites were randomly assigned to the independent variable and the dependent variable in a linear regression that included sites from all birds in the group. The test showed no significant relationship between the pairs of sites. For comparison, all sites from these same birds were combined into a pool of sites. Sites were randomly paired from the entire pool and the tuning widths of the pairs were

randomly assigned to the independent variable and the dependent variable in another linear regression. This test showed no significant relationship and a comparison of correlation coefficients from the two tests showed no difference between sites paired within each bird and sites paired across birds. Therefore sites within each bird appear to be independent samples.

HSD post hoc tests were performed after ANCOVA to analyze the difference between each group. Linear regressions that treat date as the independent variable and tuning width as the dependent variable were performed for all canaries and for each condition and each sex to analyze the interactions involved in the seasonal effect.

Results

Muli-unit activity was analyzed only for sites that were located in NCM, based on the histological reconstruction. A total of 893 sites were recorded in NCM in the current study and the preliminary experiment as follows: male canaries: 165 sites from 10 birds in cross group, 206 sites from 7 birds in normal groups, 284 sites from 14 birds in isolation group, female canaries: 41 sites from 3 birds in cross group, 85 sites from 7 birds in normal group and 112 sites from 5 birds in isolation group.

We found that the condition, date and the interaction of these two factors have significant effects on phasic and tonic tuning widths of canary NCM. In both sexes, birds in the cross condition have narrower tuning width than birds in the isolation condition. The tuning width increased from the spring to later in the year

and this overall seasonal effect is significant only in the cross condition, but not in the normal condition or isolation condition.

Recent Experience Effect

The ANCOVA that analyzed phasic response data and the ANCOVA that analyzed tonic response data showed significant main effects for condition (Phasic: $F=20.0$, $p<0.001$, Tonic: $F=9.1$, $p<0.001$). The general trend is narrower tuning in cross groups and broader tuning in isolation groups, compared to normal groups (Fig. 8). Post hoc tests for phasic responses reveal that tuning is wider for the isolation group than the cross group for males ($p<0.01$), but the normal group is not significantly different from each of the other two groups. In contrast, all three groups of females are significantly different from each other ($p<0.0001$), although there is no significant main effect for sex or sex by condition interaction. Post hoc tests for the tonic responses showed no difference across groups in males but showed significant differences between each group of females ($p<0.0001$). Therefore, the housing condition appears to have a greater effect on females than on males and have a greater effect on phasic tuning width than on tonic tuning width.

Seasonal Effect

Both of the ANCOVA analyses of phasic and tonic responses showed a significant main effect for date (Phasic: $F=40.6$, $p<0.001$, Tonic: $F=39.9$, $p<0.001$). Both the linear regression that treats phasic response as the dependent variable and the linear regression that treats tonic response as the dependent variable showed tuning width increased from the spring to later in the year (Phasic:

$R^2=0.005$, $p<0.05$, Tonic: $R^2=0.016$, $p<0.001$, Fig 9). Both the ANCOVAs also showed an interaction between date and condition (Phasic: F=23.6, $p<0.001$, Tonic: F=8.07, $p<0.001$). The linear regressions for each condition showed that the seasonal effect is only significant in the cross group (Phasic: $R^2=0.275$, $p<0.0001$, Tonic: $R^2=0.302$, $p<0.0001$, Fig. 10). Therefore the overall seasonal effect can mainly be explained by the seasonal effect in the cross group.

Sex Effect

An ANCOVA that analyzed phasic responses showed a significant effect of the sex by date interaction (F=18.42, $p<0.001$). This is consistent with the result of linear regressions that analyzed the phasic response for each sex, which showed that the date effect was significant in males ($R^2=0.037$, $p<0.0001$) but not in females. In contrast, the ANCOVA that analyzed the tonic response showed no significant effect of the sex by date interaction, although the linear regression for the tonic response showed the date effect exists only in males ($R^2=0.036$, $p<0.0001$). Apparently, the variance due to the interaction does not reach significance in the ANCOVA model, perhaps due to association with other factors in the model.

Both of the ANCOVAs also showed significant interaction of sex by date by condition (Phasic: F=36.6, $p<0.001$, Tonic: F=22.6, $p<0.001$). The linear regressions for analysis of the phasic response showed a significant date effect in the male cross group and the male isolation group (Male Cross: $R^2=0.616$, $p<0.0001$; Male Isolation: $R^2=0.074$, $p<0.0001$). In contrast, in female canaries, the date effect is only significant in the isolation group ($R^2=0.139$, $p<0.001$). The

linear regressions for analysis of the tonic response showed a significant date effect on the male cross group and the female isolation group (Male Cross: R^2=0.163, p<0.0001; Female Isolation: R^2=0.045, p<0.05).

Since the seasonal effect only exists in the cross group, but the female cross group does not show the seasonal effect, the male cross group may contribute most of the overall seasonal effect. However, the lack of a seasonal effect in the female cross group may be due to its small sample size and very short sampling interval during the year. Only three female canaries were recorded from April 21-April 28 in the cross group. Furthermore, in the comparison of sex differences in the same season and same condition, the strongest contrast in tuning width exists between isolated males and isolated females recorded during the spring (F=146.7, p<0.0001, Fig. 11 C). In contrast, no sex difference exists in the normal condition (Fig. 11B) and only a weak sex difference exists between cross males and cross females in the spring (F=5.6, p<0.05, Fig11A). Therefore, the sex difference in the spring isolation group contributes most to the effect of sex by date by condition interaction. Since we only had recorded isolated females in the spring, this phenomenon may not be a real sex by date by condition interaction, but a sex by condition interaction.

Discussion

The present study investigated the effect of recent experience, sex and season on tuning width of neurons in the caudomedial nidopallium of canaries. We found that the housing condition can significantly change the tuning width of NCM during a very short period of time and this effect is stronger in females than

in males and more significant in the phasic response than in the tonic response. Moreover, our results suggest that season also affects tuning width, increasing the tuning width later in the year, and this effect is strongest in the cross male group.

The condition effect observed in our study is similar to what was seen in preliminary observations in zebra finches (Fig. 12). In zebra finches, ANOVAs show the significant condition effect (Phasic: $F=14.6$, $p<0.0001$; Tonic: $F=7.0$, $p<0.001$) and the same trend of change under manipulation of conditions was observed in both the phasic and the tonic windows, even if some differences between groups were not significant. During the phasic window, isolated male zebra finches had wider tuning than males in either normal or cross conditions, but females only showed a difference between cross and isolation groups. During the tonic window, only the cross females showed narrower tuning compared to those in the normal group. The most important observation in the present work is that the trend of tuning changes depends on the novelty and enrichment of the acoustic environment rather than the specific acoustic features of the species' vocal repertoire, as we expected initially. This result suggests that songbirds may have the capacity to adjust their tuning width rapidly in order to adapt to a novel acoustic environment or abnormal silent environment. It is possible that the effect of tuning width on quality of auditory coding depends on the noise level in the acoustic environment (Pauget et al., 1999). If the noise level is reduced in an environment, as occurs in the isolation condition, increasing tuning width may enhance the coding quality. On the other hand, in a heterospecific aviary in the

cross condition, discrimination of unfamiliar heterospecific vocalizations would be a challenge for a bird, so more vocalizations in the aviary may be interpreted as background noise. If this is true, the increased noise level experienced by a cross bird may sharpen its tuning width so that the capacity to discriminate attended vocalizations can be improved.

However, an alternative explanation is that the changes of social environment affect auditory tuning of songbirds. Moving a bird to an isolation box or an unfamiliar room of heterospecific animals can have complex effects on the internal state of the bird and its brain. For example, this manipulation may be stressful and induce changes in hormone or norepinephrine levels, changes that may affect the tuning width observed in cross and isolation birds. Further studies that selectively manipulate acoustic stimuli in a familiar or constant environment may clarify which possibility is true.

Compared to zebra finches and male canaries, female canaries show a strong condition effect because there are clear differences between each of the three groups that occurred in both the phasic and tonic windows. A possible explanation for this strong effect is that females need to select amongst the songs of male suitors, thus attending to different individuals' vocalizations, while males may attend more closely only to the sounds of their own song. However, this may not be the case due to the fact that no significant sex difference is seen in the normal condition. Given that there is a significant difference between isolation males and isolation females recorded during the same period of the year,

an alternative explanation is that female canaries are more sensitive to the isolation condition than male canaries and zebra finches.

The present results also showed more plasticity in the phasic response than in the tonic response. This sensitivity to the acoustic environment in the phasic response may suggest that the changes in tuning width may function to improve the temporal precision of auditory processing.

Another interesting result in our study is that there is a strong effect of season on the auditory tuning of canaries and this effect is strongest in cross males. This seasonal effect may mainly be due to the contrast between the lower tuning width recorded from February to May and the higher tuning width recorded later in the year. This lower tuning period overlaps with the early breeding season of canaries, during which new song syllables are added to individual song repertoires and testosterone level changes (Nottebohm, 2004). It is therefore possible that the auditory plasticity is modulated both by sex hormones and novel stimuli. However, considering that this seasonal effect is not seen in normal canaries, testosterone may only optimize the potential for plasticity, without affecting the tuning width directly. Although the seasonal effect is not seen in the female cross group or isolation group, we cannot rule out the possibility that this effect also exists in females, but was undetected, because of the small sample and narrow seasonal range sampled in cross females. Further studies that record females in other seasons may clarify this possibility.

The current study on tuning plasticity of canary NCM may contribute to the understanding of auditory processing. The results show that the tuning properties

of NCM should not be treated as stable constants. Instead, they may undergo rapid modulation by various possible factors, including signal-to-noise level in the environment, stress and hormones. This rapid neural plasticity in tuning properties may have an important effect on discrimination and memorization of complex song stimuli, especially in migrating birds that move to a new environment at the start of the breeding season. The current results also suggest that the NCM neurons of canaries and zebra finches are not tuned to simply match acoustic features of their vocal repertoires. A combination of effects of acoustic background, hormones and other factors may determine the NCM tuning differences between the two species.

Appendix
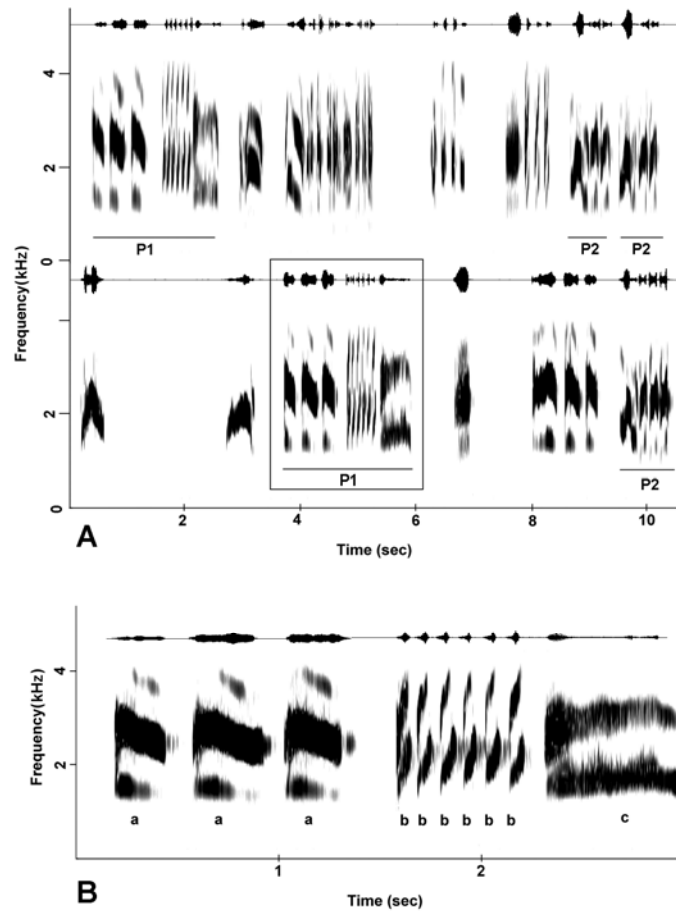
Figure 1. Descriptions of song structures. (A) The sonogram indicates a portion of two individual songs of a Plain Laughingthrush (*Garrulax davidi*) that are shown in the above and the below row, respectively. Sound amplitude in the form of an oscillogram is shown in the top of each row. Phrase P1 is repeated in the second sentence, while phrase P2 is repeated once in the same sentence and the second sentence. Phrases that do not repeat in the shown segments are not labeled in A. (B) Expanded phrase P1. Phrase P1 is made up of three 'a ' syllables, six 'b' syllables and one 'c' syllable or note. Figure courtesy of Zeng et al. (2007)
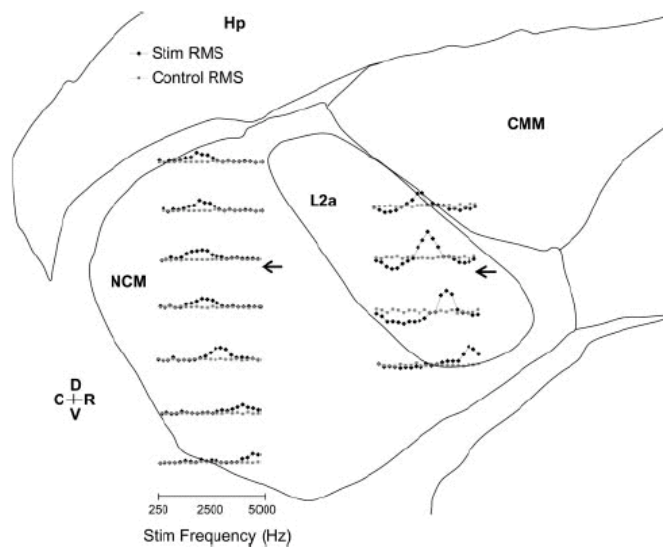
Figure 2. Tuning functions recorded at different sites along two representative

dorso-ventral penetrations in NCM and field L. The dark symbols show response

amplitude to different stimulus frequencies (tuning curves) and the grey line

shows baseline activity. Figure courtesy of Terleph et al. (2006)
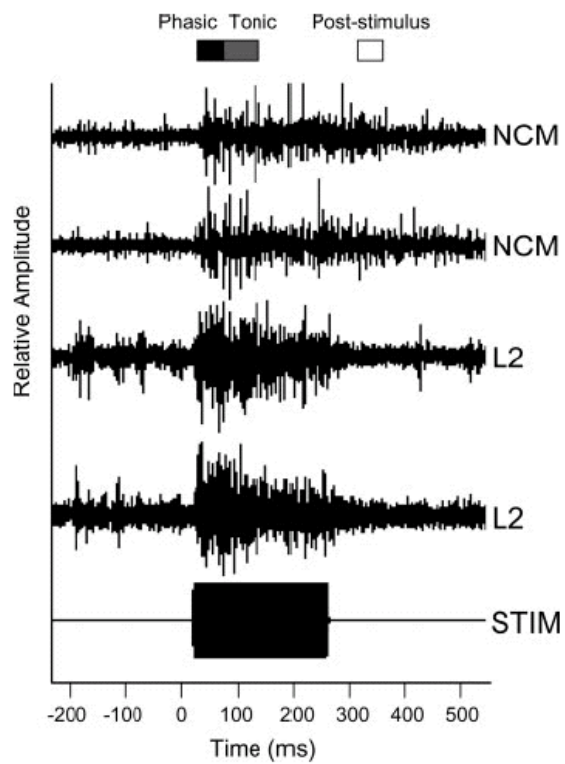
Figure 3. Raw multi-unit responses to a tone stimulus recorded in at two sites in

NCM and two sites in field L. Phasic and tonic windows are show at the top.

Figure courtesy of Terleph et al. (2006)
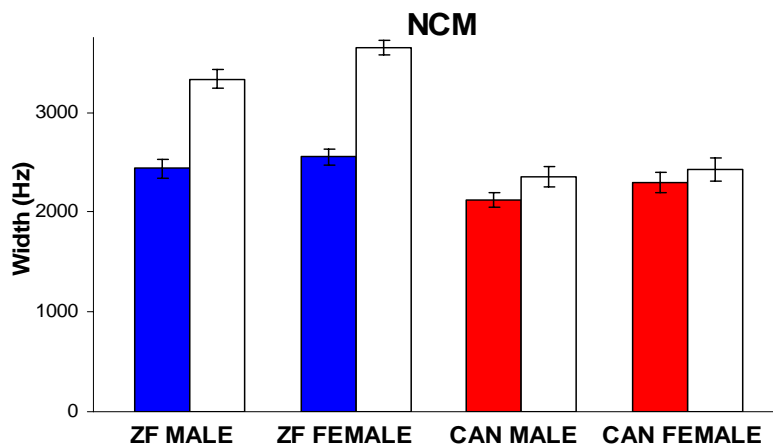
Figure 4. NCM tuning width (mean +-SE) in zebra finches (ZF) and canaries (CAN). The solid bars represent tuning width in the phasic window. The open bars represent tuning width in the tonic window. Figure courtesy of Terleph et al. (2007)
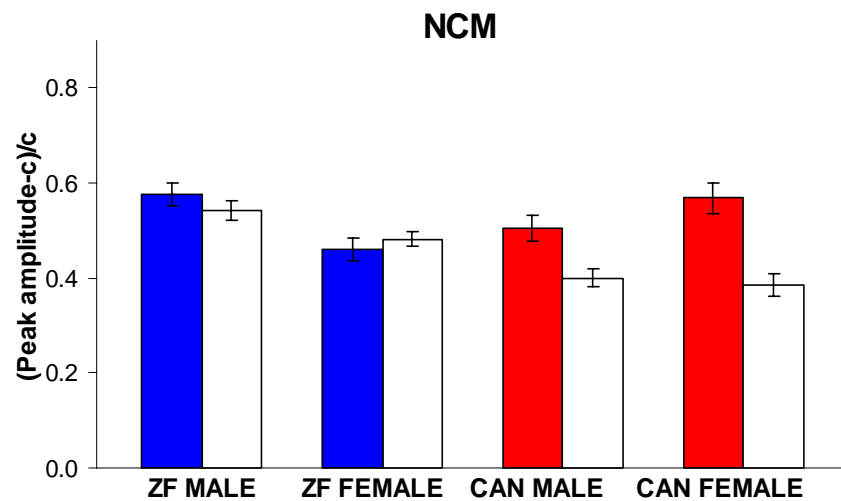
Figure 5. Peak amplitude in NCM of zebra finches (ZF) and canaries (CAN). The solid bars represent peak amplitude in the phasic window. The open bars represent peak amplitude in the tonic window. Figure courtesy of Terleph et al. (2007)
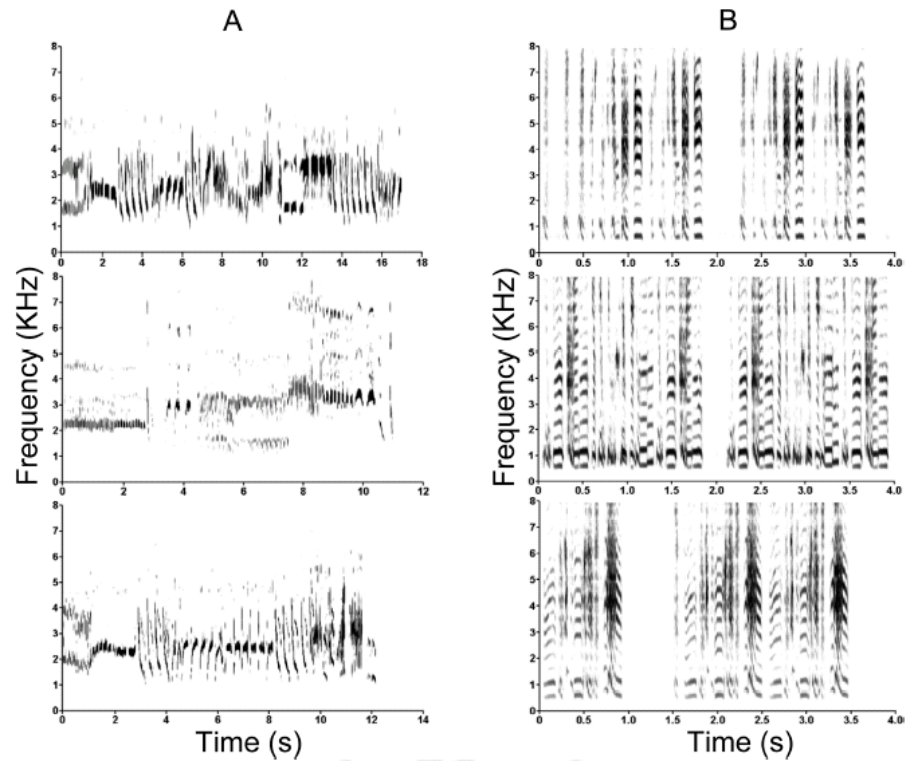
Figure 6. Sonograms show frequency as a function of time for 3 canary songs (A) and 3 zebra finch songs (B). The darkness represents the energy in each frequency component. Figure courtesy of Terleph et al. (2006)
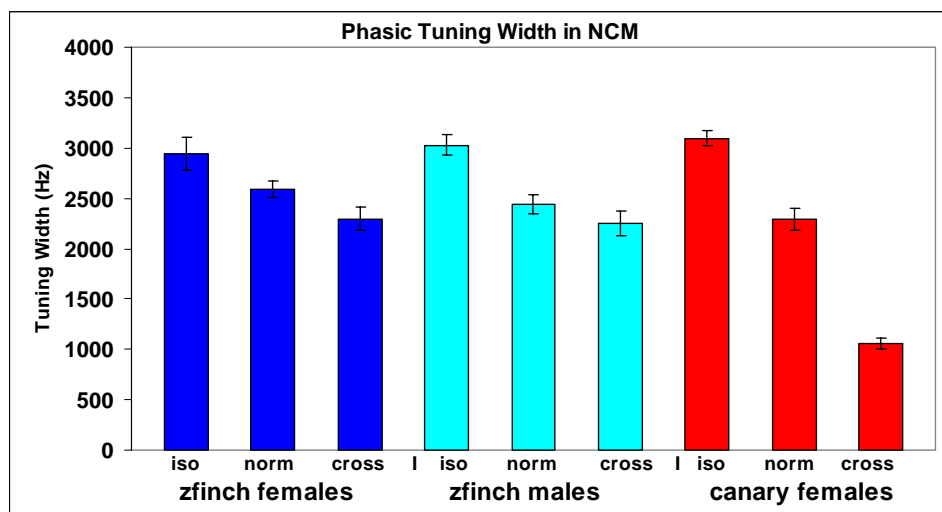
Figure 7. Effects of auditory environment (isolation vs. normal vs. cross) on NCM tuning width in zebra finches and female canaries. Figure courtesy of Terleph et al. (2007)
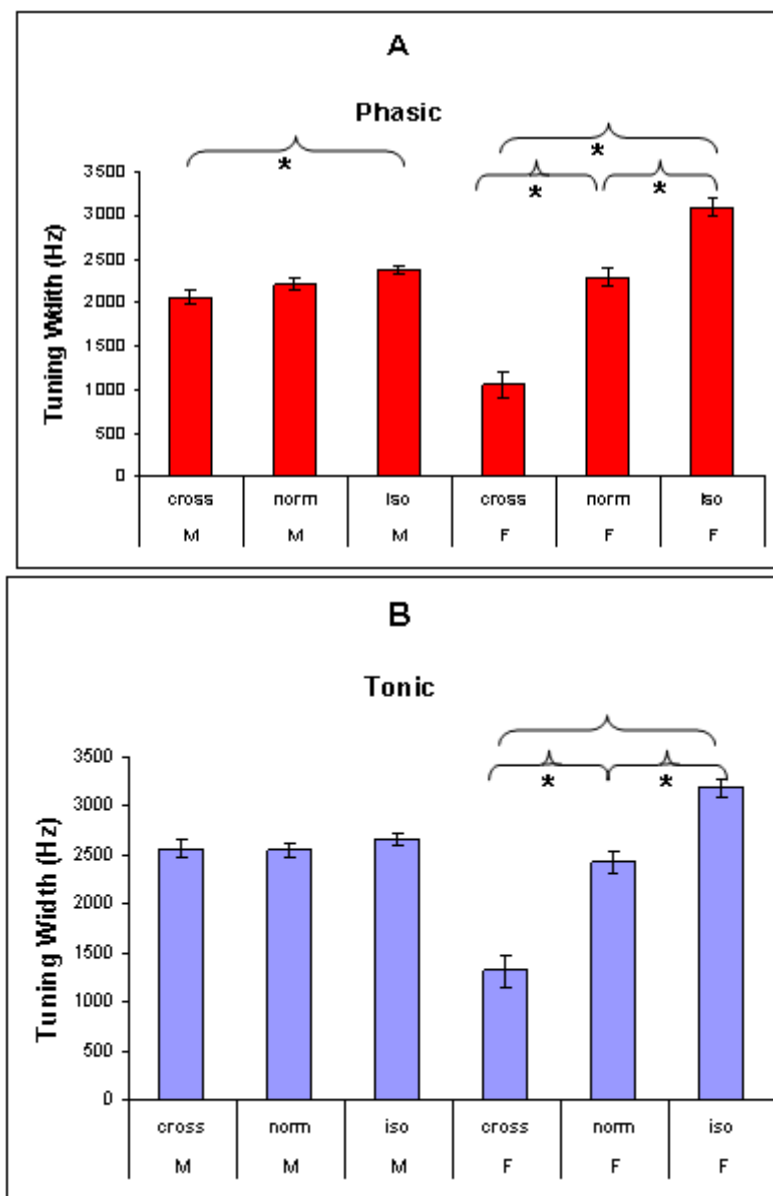
Figure 8. Effect of auditory environment (isolation vs. normal vs. cross) on phasic (A)and tonic (B) tuning width of canaries. The effect on female canaries (F) is stronger than on male canaries (M).

Phasic

Tonic
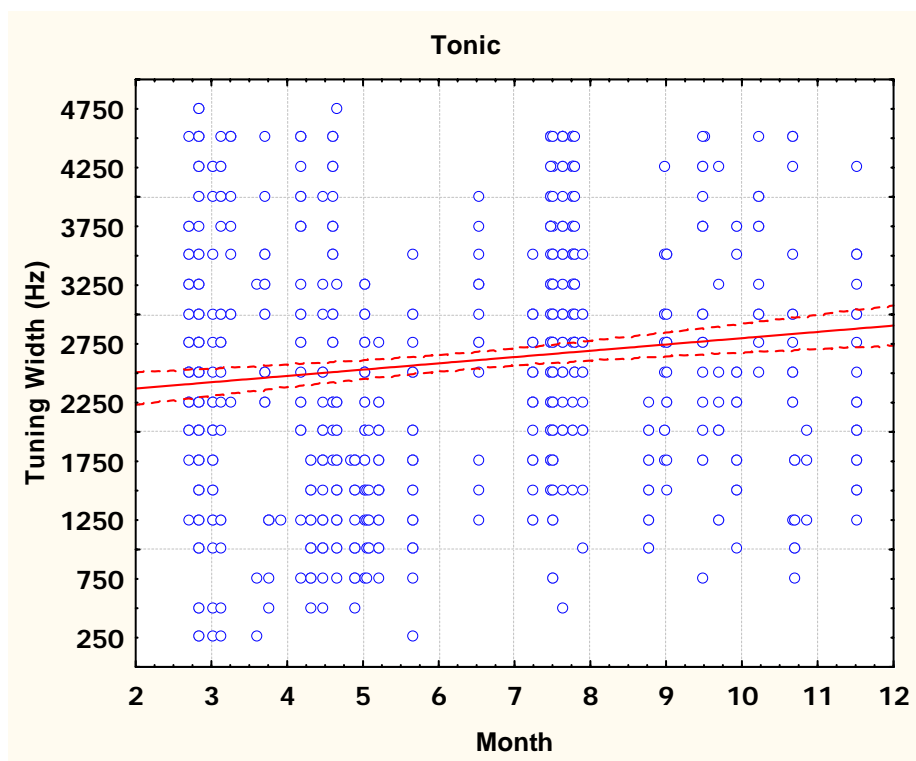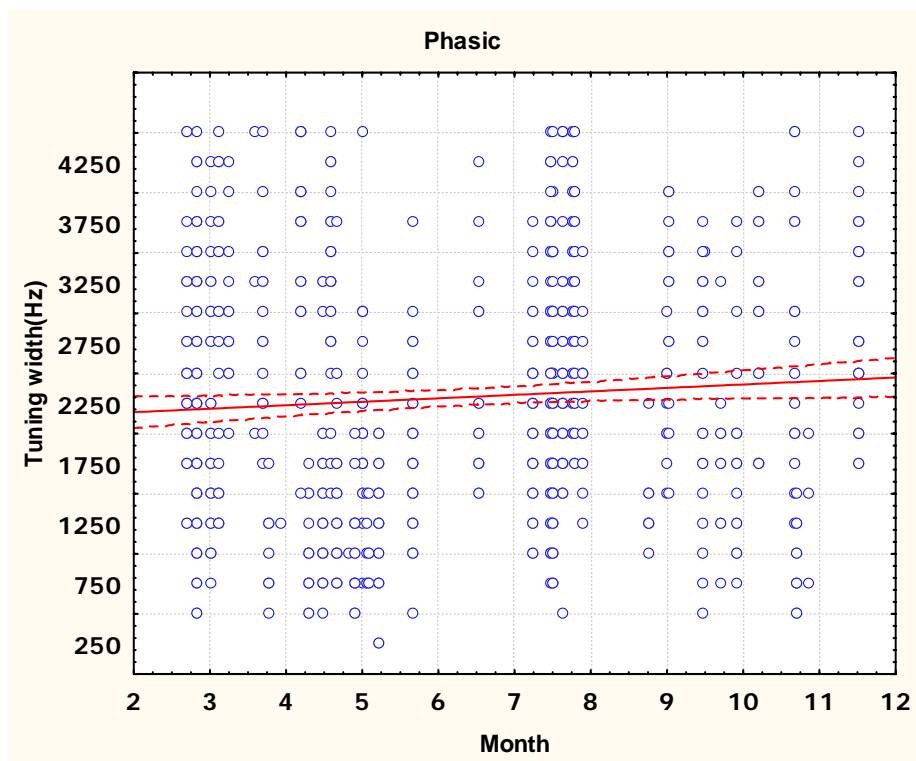
Figure 9. Effect of season on tuning widths. Open circles represent tuning width recorded at each site. Red lines represent the relationship between independent variables and dependent variables.
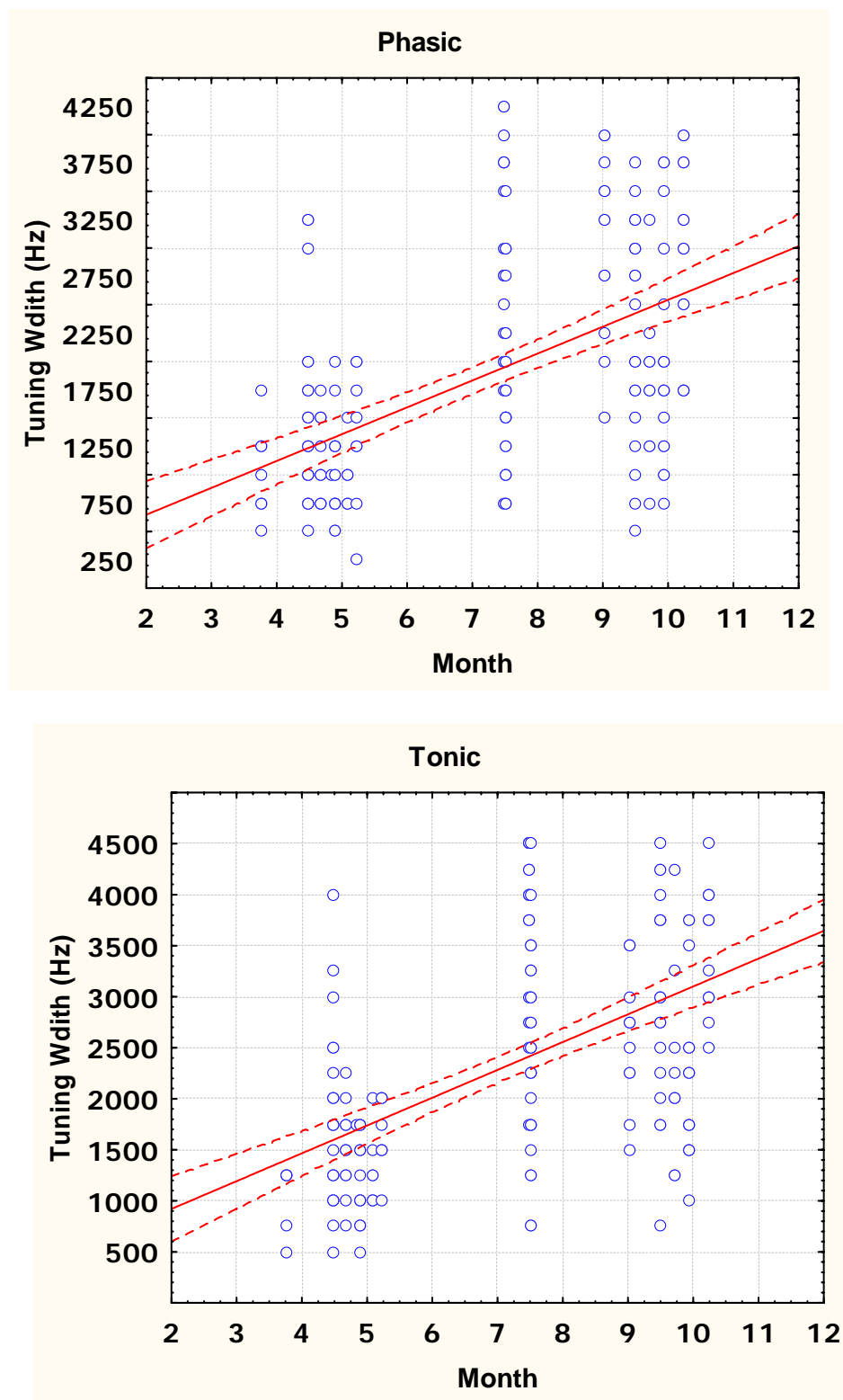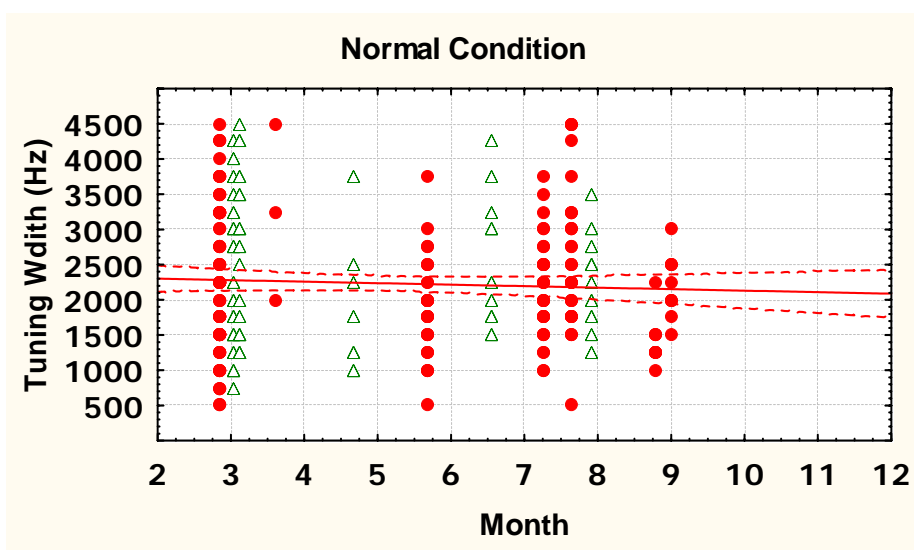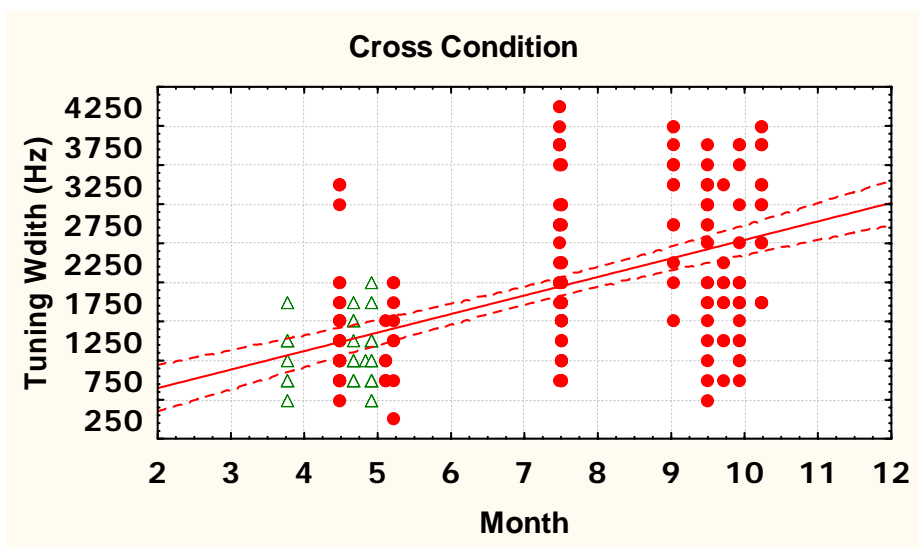
Figure 10. Seasonal effect on phasic and tonic response in cross groups.
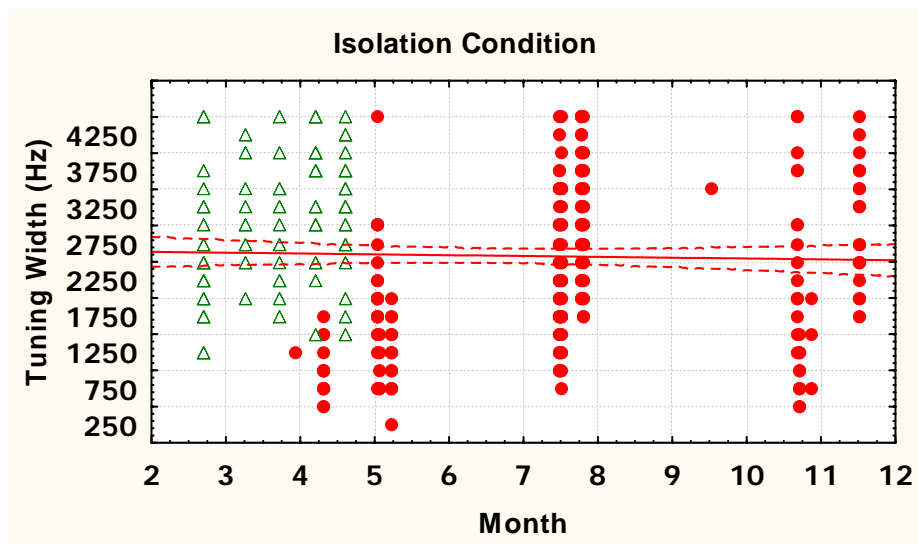
A

B

C

Figure 11. Seasonal effect in cross condition (A), normal condition (B) and isolation condition (C). Red dots represent sites recorded from male canaries. Green triangles represent sites recorded from female canaries.
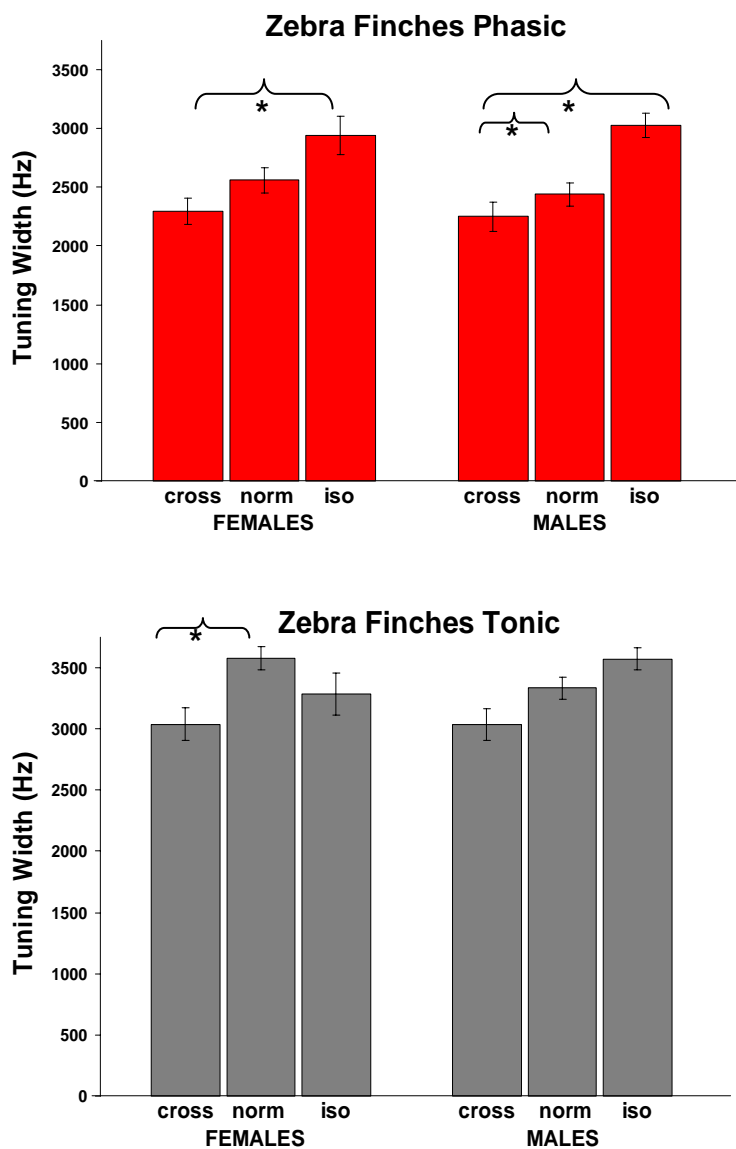
Figure 12. Effect of housing condition on tuning width of zebra finches.

(Results of preliminary experiments by Terleph AT)

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