The Relationship Between Rates of HVc Neuron Addition and Vocal Plasticity in Adult Songbirds

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ABSTRACT: In adulthood, songbird species vary considerably in the extent to which they rely on auditory feedback to maintain a stable song structure. The continued recruitment of new neurons into vocal motor circuitry may contribute to this lack of resiliency in song behavior insofar as new neurons that are not privy to auditory instruction could eventually corrupt established neural function. In a first step to explore this possibility, we used a comparative approach to determine if species differences in the rate of vocal change after deafening in adulthood correlate positively with the extent of HVc neuron addition. We confirmed previous reports that deafening in adulthood changes syllable phonology much more rapidly in bengalese finches than in zebra finches. Using [^{3}H]thymidine autoradiography to identify neurons generated in adulthood, we found that the proportion of new neurons in the HVc one month after labeling was nearly twice as great in bengalese than in zebra finches. Moreover, among the subset of HVc vocal motor neurons that project to the robust nucleus of the archistriatum, the incidence of [^{3}H]thymidine-labeled neurons was nearly three times as great in bengalese than in zebra finches. This correlation between the proportion of newly added neurons and the rate of song deterioration supports the hypothesis that HVc neuron addition may disrupt stable adult song production if new neurons cannot be "trained" via auditory feedback. J Neurobiol 43: 79–88, 2000

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Passerine songbirds vary markedly in the extent to which auditory input is required to maintain normal song behavior in adulthood. Adult canaries (*Series canarius*) and bengalese finches (*Lonchura domestica*) cannot maintain stable song structure without auditory feedback. In both species, deafening produces significant changes within 1 week, with continued deterioration occurring over the ensuing weeks (Nottebohm et al., 1976; Okanoya and Yamaguchi, 1997; Woolley and Rubel, 1997). In contrast, adult white-crowned sparrows (*Zonotrichia leucophrys*), chaffinches (*Fringilla coelebs*), and zebra finches (*Peophila guttata*) are much more resistant to the effects of deafening in adulthood. Gradual changes in song behavior are not apparent until 1–2 months after deafening for adult zebra finches (Nordeen and Nordeen, 1992), while deafened white-crowned sparrows (Konishi, 1965a) and chaffinches (Nottebohm, 1968) do not exhibit any song degradation after many months of observation. We have suggested that the deterioration of stable song patterns after adult deafening may arise in part from the continued incorporation of new neurons into the vocal motor pathway (Alvarez-Buylla et al., 1988; Nordeen and Nordeen, 1988). In the present report we provide evidence supporting one strong prediction of this hypothesis; species differences in song degradation after adult deafening correlate with differences in the extent of neuronal incorporation.

The recruitment of new neurons into circuits con-

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trolling song has been studied most extensively in the context of development and seasonal remodeling of song behavior. Throughout life, neurons are produced and inserted into the vocal motor pathway. Specifically, both interneurons and RA (robust nucleus of the archistriatum)-projecting neurons (but not Area X–projecting neurons) are added to the HVc (acronym used as proper name), a premotor nucleus essential for song production (Nottebohm et al., 1976; McCasland, 1987; Simpson and Vicario, 1990; Vu et al., 1994). While these neurons are added at a modest pace during the production of stable adult song, the insertion of neurons is greatest during periods of vocal plasticity (Kirn et al., 1994; Alvarez-Buylla et al., 1988; Nordeen and Nordeen, 1988). It has been suggested that the synaptic plasticity afforded by these new neurons may be essential to vocal learning (Alvarez-Buylla et al., 1988; Nordeen and Nordeen, 1988); however, there is not yet direct evidence that vocal learning requires coincident HVc neuron addition or replacement.

Normal avian song learning requires auditory input (Konishi, 1965a,b; Nottebohm, 1968; Price, 1979), which functions, in part to modify the activity of the vocal motor pathway. Since HVc neuron addition is particularly robust during periods of song learning and HVc also receives highly processed auditory input (Kelley and Nottebohm, 1979; Fortune and Margoliash, 1995; Vates et al., 1996; Mello et al., 1998), it is likely that auditory experience organizes immature HVc circuitry during song development. Indeed, many HVc neurons respond preferentially to the bird's own song pattern (Margoliash, 1983, 1986; Margoliash and Fortune, 1992; Janata and Margoliash, 1999), and this selectivity emerges as birds use auditory feedback to match their own vocal behavior to song templates memorized earlier in life (Volman, 1993). Given that HVc neuron addition continues even during stable song production, we further speculate that auditory feedback from stereotyped song normally shapes and integrates new circuitry (in addition to reinforcing preexisting connections) to ensure continued stability in song structure. After removing auditory feedback's instructional signals from newly recruited neurons, naïve circuitry may not be organized to support production of the previously learned song pattern, and the accumulation of this circuitry may lead to song deterioration. If so, we hypothesize that song deterioration after deafening will relate positively to the extent of HVc neuronal addition.

In the present study, we compared the relationship between rates of adult neurogenesis and song degradation after deafening in two species that normally do not modify their songs in adulthood. After cochlear removal, adult bengalese finch song changes markedly by 4 weeks (Okanoya and Yamaguchi, 1997; Woolley and Rubel, 1997), but adult zebra finch song changes only slightly (Nordeen and Nordeen, 1992). Using thymidine autoradiography in these two species of finches, we established that the faster deterioration of song in adult deafened bengalese finches is accompanied by proportionally more HVc neuron incorporation than in zebra finches.

MATERIALS AND METHODS

The songs of five adult male zebra finches (ZFs) raised in our aviaries and six adult male bengalese finches (BFs) purchased from a commercial breeder were recorded on a Marantz PMD 210 tape recorder. Birds were housed in individual cages and maintained on a 14:10 light–dark cycle throughout the course of the experiment. Songs were recorded in the presence of a stimulus female using a unidirectional condenser microphone (Fender P2) and sonograms were produced using a Kay Digital Sonograph (DSP 5000) set to the following input parameters: 8 kHz DC bandpass, flat input shaping, Hamming window, and transform size = 100 points. For each bird, comparison of sonograms from two preoperative recordings separated by an interval of at least 3 months confirmed that songs were stable.

The birds' cochleae were removed bilaterally immediately after the second preoperative recording (day 0). At this time, BFs were at least 6 months old and ZFs were 6–7 months old. The birds were anesthetized with Equithesin, and an incision was made over the ear canal so that the tympanic membrane could be cut and the columella (middle ear bones) removed. A fine wire hook was inserted through the oval window and maneuvered to extract the cochlea; the cochlea was then examined with a microscope to confirm that it was intact. Upon recovery from surgery, all birds exhibited some vestibular disturbances, especially opisothotonos (holding the head up and back); a few displayed erratic flight and instability. These symptoms decreased with time, although some birds continued to display mild signs of vestibular disruption throughout the experiment.

Song behavior was monitored for at least 5 hours daily for the first week after deafening and then weekly through postdeafening day 28 (d28). One BF sang the day after deafening, but died prior to the end of the experiment. None of the other birds sang before d3, but all sang by d6. All quantitative data is based upon the five ZFs and five BFs for which there is both behavioral and anatomical data. Recorded song was obtained weekly for all of these birds from d7 to d28. The d28 recordings consisted of at least 2 min of song.

We focused our behavioral analysis on changes in the acoustic structure (phonology) of song syllables because these occur gradually in both ZFs and BFs and may therefore reflect disruptions produced by the incorporation of

new vocal motor circuitry. We did not measure changes in the temporal structure of song because these occur only in BFs and their rapid onset after deafening (Okanoya and Yamaguchi, 1997; Woolley and Rubel, 1997) makes a causal link to neuronal incorporation unlikely. Song syllables were defined as acoustic energy surrounded by intervals of baseline energy lasting at least 5 ms. Low-amplitude, indistinct noises that are common in BF song were omitted from analysis because they do not occur in ZF song, and they lack distinct phonological features (frequency structure over time; see Figs. 1 and 2) necessary to perform the behavioral analysis.

Syllables produced at d28 were compared to those produced preoperatively (d0). The samples of BF and ZF preoperative song used for quantitative analysis included at least four examples of each introductory note and song syllable and were selected to represent preexisting, within-syllable variation in phonology. The sample of d28 song used to measure song degradation consisted of the first 50 syllables recorded. In both species, this sample represented the full spectrum of syllables produced in d28 song. The phonological similarity between a specific syllable in d28 song and the preoperative syllable it most resembled in contour, frequency, and duration was scored on a 0–3 scale. Frequency assessed the average frequency of the fundamental and harmonic suppression; contour assessed the rate, degree, and direction of frequency modulation (including any "noisiness/waveriness"); duration assessed the length of time over which acoustic energy was above baseline. These parameters were chosen because they are germane to the structure of both species' syllables. Postoperative syllables scored 0 if they had a major discrepancy in one or more acoustic parameters (contour, frequency, or duration) or moderate differences in each parameter; such syllables were not obviously similar to any single preoperative syllable and were operationally defined as unmatched. Other postoperative syllables were similar in at least some respects to a preoperative syllable, and their degree of similarity was scored on a scale of 1–3. A score of 1 was assigned to postoperative syllables if there were minor differences in two of the three acoustic parameters. A score of 2 was assigned if there was a minor difference in only one of the three acoustic parameters, and a score of 3 was assigned if a note was virtually identical to a preoperative syllable. Because syllable sequencing 4 weeks after deafening was highly variable in BF song but remained intact in ZF song, the location (context) of a syllable was not considered in the scoring.

Two measures were calculated using the d28 sample of 50 syllables: the percentage of postoperative syllables that were unmatched to preoperative syllables (number that scored 0/50 syllables) and the average score given to syllables similar to preoperative syllables (those scoring ≥ 1). In 3 BFs, scores generated from the 50 syllable sample did not significantly differ from those generated from a larger sample consisting of up to 92 syllables for the percentage of postoperative syllables scored as unmatched [$p = .23$, $F(2, 5) = 3.0$] or for the average score assigned to syllables similar to preoperative syllables [$p = .32$, $F(2, 5) = 1.7$]. For one ZF, the two sample sizes yielded identical results. A third measure, the percentage of preoperative syllables that were retained in song recorded at d28, was defined as the percentage of preoperative syllables to which at least one syllable in d28 song was scored as similar (scored a 1, 2, or 3). For this final measure, the entire d28 recording was analyzed. All data are presented as means ± S.E.M. One-tailed Mann-Whitney *U* tests were used for statistical comparison because previous behavioral studies document that adult BF song deteriorates faster after deafening than does adult ZF song (Nordeen and Nordeen, 1992; Okanoya and Yamaguchi, 1997; Woolley and Rubel, 1997).

Beginning 30 days after deafening, we labeled neurons generated in adult deafened birds by injecting [^{3}H]thymidine (THY) twice daily for 10 days. This injection regime labels a small but consistent proportion of HVc neurons in adult intact ZFs (personal observation). Although behavioral change was not assessed during this latter part of the experiment, previous studies have shown that BF and ZF songs continue to gradually degrade after deafening (Nordeen and Nordeen, 1992; Okanoya and Yamaguchi, 1997; Woolley and Rubel, 1997). To identify HVc neurons that project to RA, the retrograde tracer fluorogold (FLG; 2% in saline, 0.04 μL) was injected unilaterally into the left RA 26 days after the last THY injection. Five days later, birds were overdosed with Equithesin and perfused, and the brains were embedded in paraffin. Coronal sections (10 μm) were cut through HVc and RA and processed for THY autoradiography. Slides were dipped in emulsion (NTB3) and stored at 0°C for 23–25 days, developed in D19, and fixed (Rapid Fix). Every third slide was stained with thionin.

Using UV illumination, we confirmed in the unstained sections that our FLG injection filled the RA. We then scanned fields ($\times$100) in the left HVc for FLG-labeled neurons and recorded the number of silver grains over each FLG-labeled cell profile ($>$60 cells/bird). Neurons were considered THY-labeled if the number of silver grains over the cell body exceeded 5, which was greater than 10 times background grain density. For each bird we calculated the percentage of HVc-RA neuron profiles that were THY-labeled. Species differences were compared using a two-tailed *t* test.

To estimate the overall density of neurons and the density of THY-labeled neurons in HVc, sections were stained with thionin, and neuron-counting fields were chosen on the left side by systematic random selection. Cell profiles were considered neurons if they had a clear nucleus, densely stained cytoplasm, and large, darkly stained nucleoli. Neurons with more than one nucleolus were counted as a single cell. The number of neurons and number of THY-labeled ($>$5 silver grains) neurons falling within a 1.21×10^{-4} mm^3 field were counted at $\times$100, and at least 20 fields were averaged per bird. Counting fields in every fourth section throughout the rostrocaudal extent of HVc were chosen via systematic random sampling. For each THY-labeled neuron, the number of silver grains over the soma was recorded so that frequency distributions of labeling intensity could be

constructed. While sampling neuron density, we also estimated the percentage of neurons with multiple nucleoli since such cells appeared particularly common in BFs and their incidence affects the magnitude of errors in estimating cell number. For each bird, the number of nucleoli was recorded for each neuron counted in 2–3 sample fields, and in each THY-labeled neuron found throughout the HVc of 1–3 randomly selected sections. The proportion of multinucleolated neurons was significantly greater in BFs than in ZFs [two-tailed $p < .05$, $t(8) \geq 2.70$] both among HVc neurons overall (40.1 ± 2.79% vs. 23.9 ± 5.27%) and among THY-labeled HVc neurons (42.8 ± 10.9% vs. 23.7 ± 3.57%). To our knowledge, no stereological method corrects for the incidence of multinucleolated profiles, and we were therefore unable to accurately compare the number of HVc neurons or THY-labeled neurons in BFs and ZFs. However, measures of the percentage of THY-labeled neurons should not be affected by this species difference in multinucleolation since, in both species, the incidence of multinucleolated neurons in HVc overall and THY-labeled HVc neurons was similar. We therefore restricted our analysis to this proportional measurement of neuronal incorporation. Species differences in the percentage of HVc and HVc-RA neurons that were THY-labeled were compared using a two-tailed *t* test.

To evaluate our subjective impression that THY-labeled neurons were more abundant in BFs than ZFs throughout much of the telencephalon, we analyzed a portion of the ventromedial hippocampal formation (HP), the "V." This region of densely packed neurons is thought to be homologous to the hippocampus proper in mammals (Lee et al., 1998), specifically Ammon's horn (Szekely, 1999), and was chosen because it has distinct architectonic boundaries (see Fig. 6) and is ontogenetically different from the HVc. THY-labeling in this region appeared to be regionally heterogeneous, and so the V was divided into three subdivisions: apex, medial, and lateral. Apical measurements were obtained from the first complete field at the tip of the V, and fields in the medial and lateral portions of the V were chosen by moving 3 to 4 fields away from the apex. Neurons were counted (×100) in the left HP in sections that also contained HVc. One field in each of the V subdivisions was measured per section, and an average of 14 sections per bird was sampled. Species differences in the incidence of HP-labeled neurons within each subdivision were evaluated using two-way *t* tests, and regional differences within each species were compared with analysis of variance.

RESULTS

Our behavioral analysis confirmed previous reports that deafening produces much more rapid changes in song behavior in adult BFs than in ZFs (Figs. 1 and 2). By 28 days after deafening, all BFs exhibited phonological degradation of song syllables, all but one produced unmatched syllables, and several omitted one or more preoperative syllables from their songs. In contrast, no ZFs omitted preoperative syllables 4 weeks after deafening, and most changes in syllable phonology were minor. Only one ZF produced any unmatched syllables, which were infrequently produced in place of one preoperative syllable that was at other times produced with morphology identical to that found preoperatively. Thus, unmatched syllables (score = 0) made up 19.6 ± 9.17% of d28 songs in BFs but only 2.8 ± 2.8% of d28 songs in ZFs ($z = 1.98$, one-tailed $p < .025$). Also, the average score of the remaining syllables in d28 song was significantly lower in BFs (2.2 ± 0.16) than in ZFs (2.7 ± 0.37; $z = 2.61$; one-tailed $p < .009$). Finally, while the majority of preoperative syllables in both species could still be identified 4 weeks after deafening, BFs retained only 86.5 ± 6.32% of their preoperative syllables, whereas ZFs retained 100 ± 0% of their syllables (one-tail $p < .05$, $z = 1.78$).

Although syllable structure changed relatively rapidly in BFs, the change was not immediate. Only one BF sang the day after surgery, but he produced all preoperative syllables in a form that was not detectably different from those produced prior to deafening. Similarly, there was little phonological change from preoperative song in another BF that sang 3 days after deafening, suggesting that the accurate production of crystallized syllable structure does not require immediate auditory feedback. However by d7, syllable deterioration was more apparent, with most BFs producing one or more syllables differently from that produced preoperatively (Fig. 1).

Qualitatively, we confirmed previous reports that deafening produces rapid changes in syllable ordering in adult BFs but not ZFs. By d5, BF song phrases were notably less stereotyped in their syllable ordering, and by d28 no phrase structure or stereotyped syllable order was apparent (see Fig. 1). Also, the number of consecutive repetitions of syllables decreased after deafening in BFs, and the ratio of noise sounds to syllables increased. None of these changes were evident in ZFs d28 after deafening.

The faster rate of song degradation in adult deafened BFs corresponded to a significantly larger percentage of newly incorporated HVc neurons in BFs compared to ZFs (Fig. 3). As shown in Figure 4, the overall incidence of THY-labeled neurons in HVc was over two times greater in BFs than in ZFs [3.84 ± 0.52% vs. 1.9 ± 0.52%; $t(8) = 2.61$; two-tailed $p < .05$]. When we focused our analysis on just that subpopulation of HVc neurons that project to the RA, we found that the proportion of THY-labeled HVc-RA neurons was more than three times greater in BFs (2.94 ± 0.51%) than in ZFs [(0.878 ± 0.43%;

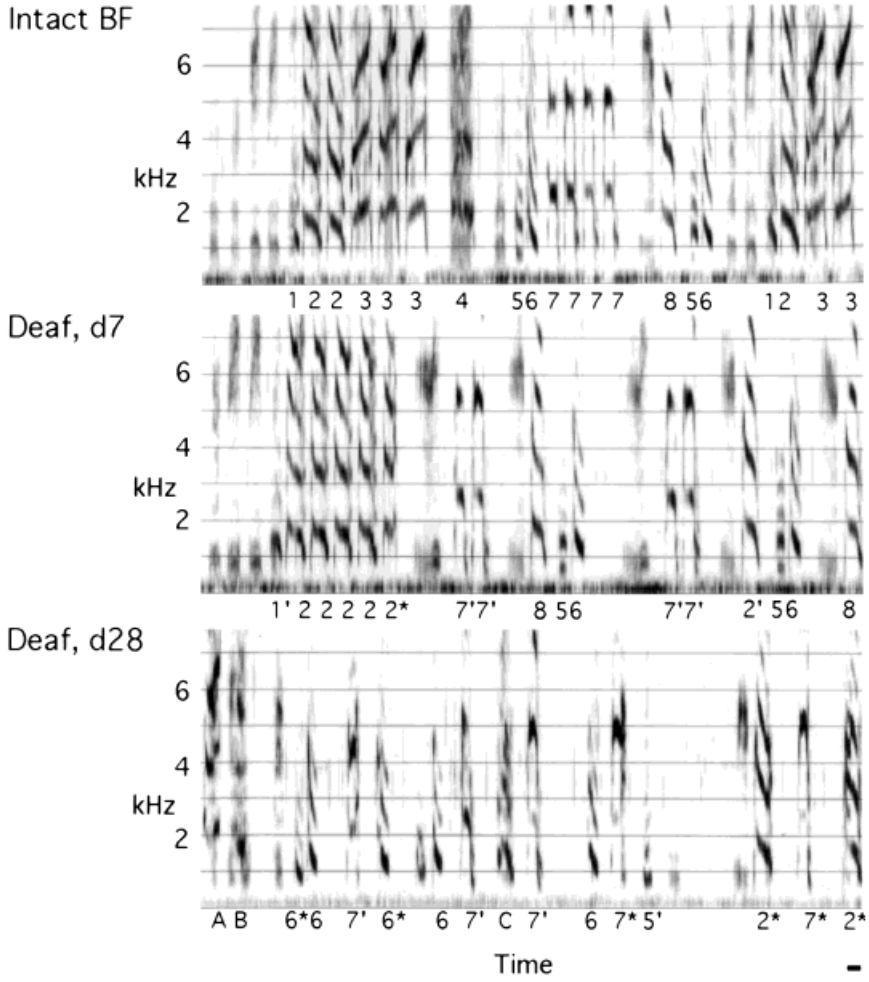

Figure 1 Sonograms of bengalese finch (BF) song recorded preoperatively and 7 and 28 days after deafening. In preoperative song, all syllables are identified by numbers below the time axis. In postoperative song, syllables are identified by the number corresponding to the preoperative syllable to which they were most closely matched. Asterisk indicates score of 1; prime indicates score of 2; and unmarked indicates score of 3. Unmatched syllables (scored 0) are identified with a syllable letter. Changes in temporal ordering and slight changes in phonology were seen on day 7. By day 28 phonological degradation was marked. Calibration bar, 75 ms.

two-tailed $p < .05$, $t(7) = 3.0$]. Despite these significant between species differences in THY-labeling, individual differences within species in the degree of behavioral degradation did not correlate significantly with individual differences in the incidence of THY-labeled neurons overall or the incidence of THY-labeled HVc-RA neurons.

Species differences in the incidence of THY-labeled neurons could result from species differences in various parameters that affect the probability of detecting labeled neurons (e.g., nuclear size, cell cycle kinetics). Because differences in the probability of detection should be reflected in differences in the intensity of labeling, we computed frequency distributions of the number of silver grains over THY-labeled neurons in each species. As shown in Figure 5, the frequency distribution of silver grain counts for labeled neurons was remarkably similar across the two species. The average grain density for labeled neurons in BFs (32.4 ± 3.3) was not significantly different from that in ZFs [26.12 ± 2.7; two-tailed $p = .21$, $t(8) = 1.5$].

Quantitative analysis of THY-labeling in the hippocampal V (Figure 6) confirmed our impression that the species difference in neuronal incorporation was not specific to the HVc. As shown in Figure 7, BFs had a higher incidence of THY-labeled cells than ZFs in both the medial and lateral regions of the hippocampal V. The proportion of THY-labeled neurons in BFs compared to ZFs was 10 times greater in the

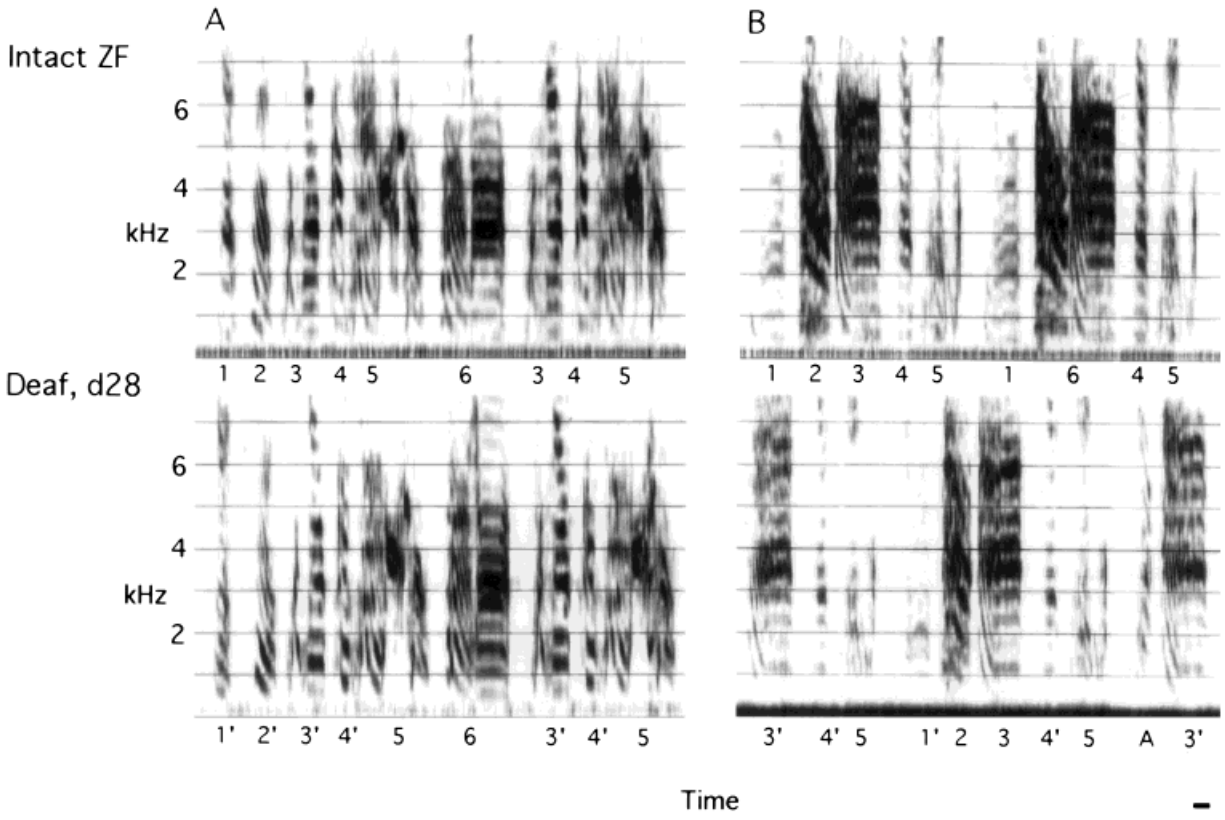

Figure 2 Sonograms of zebra finch (ZF) song recorded preoperatively, and 28 days after deafening show no change in temporal ordering and very little change in syllable phonology. Syllables are identified and designated as in Figure 1. By postdeafening day 28, most ZFs exhibited only very slight changes in syllable phonology (representative shown in A). Only one bird occasionally produced syllables that did not resemble any preoperative song syllable (B). Calibration bar, 75 ms.

medial portion [1.1 ± 0.48% vs. 0.11 ± 0.12%; two-tailed $p < .05$, $t(8) = 2.31$] and almost 4 times greater in the lateral portion [2.4 ± 0.73% vs. 0.57 ± 0.32%; two-tailed $p < .05$, $t(8) = 2.62$; Fig. 7]. In the apex of the V, the incidence of THY-labeling was not significantly different between BFs and ZFs (1.6 ± 0.27% and 1.5 ± 0.36%, respectively). The regional variation in the percent of THY-labeled HP cells was significant in ZFs [$p < 0.01$, $F(2, 12) = 7.94$], but was not significant in BFs [$p = .19$, $F(2, 12) = 3.59$].

DISCUSSION

In adult bengalese and zebra finches, species differences in the rate of vocal change after deafening correlate positively with estimates of neuronal recruitment within the song nucleus HVc. By 1 month after elimination of auditory feedback, syllable phonology had deteriorated significantly more in BFs than in ZFs. Correspondingly, 1 month after labeling neurons with THY, deafened BFs had a higher incidence of THY-labeled HVc neurons overall and a higher incidence of THY-labeled HVc-RA projection neurons than did deafened ZFs. These data are consistent with the view that the continuous insertion of new HVc neurons in the absence of auditory input contributes to the degradation of syllable structure. We speculate that syllable structure in adult birds normally remains stable because auditory feedback reinforces preexisting circuitry and organizes new circuitry. When this instructional signal is removed, the rate at which naïve circuits are being constructed contributes to the rate at which stereotyped behavior becomes disorganized.

It is important to reiterate that the species difference in neuronal recruitment reported here is in the incidence of recently generated neurons within the HVc and the HVc-RA pathway, not in their total number. Significant species differences in multinucleolation precluded accurate estimates of

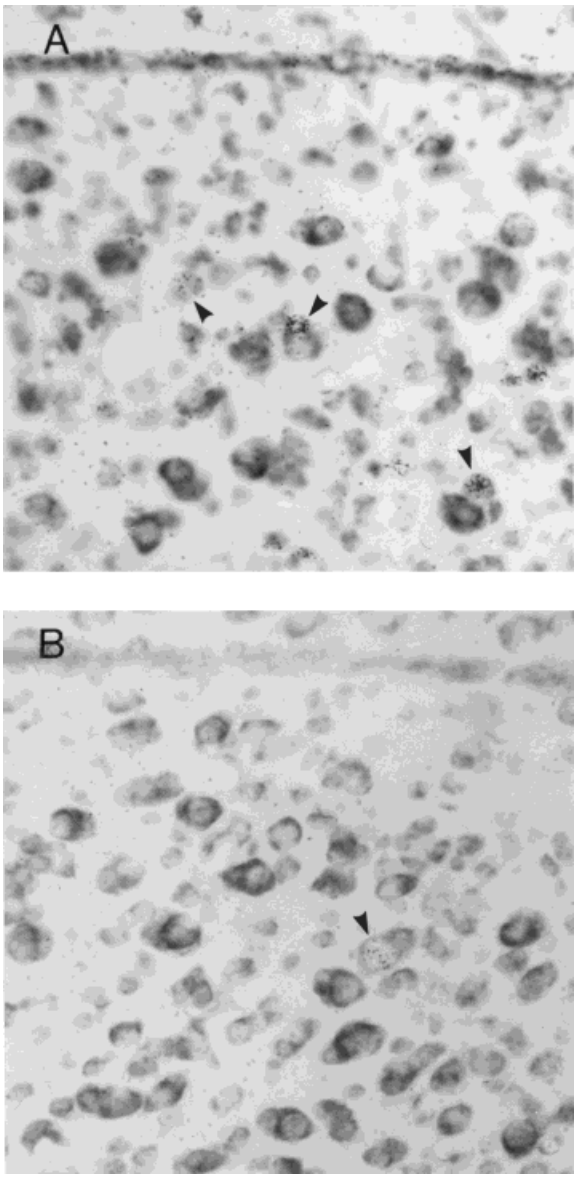

Figure 3 Photomicrographs show thymidine-labeled cells (arrows) in HVc of BF (A) and ZF (B). The incidence of labeled cells was noticeably higher in BFs than in ZFs.

neuron number. However, the relative rate of neuronal incorporation likely is most relevant for behavioral plasticity since the functional impact of a given number of new neurons will be determined largely by their relative contribution to the size of the circuit into which they are incorporated. Our results show clearly that in proportion to the HVc neuronal population, the incorporation of neurons surviving at least 1 month is significantly greater in adult deafened BFs than in ZFs.

If HVc neuron addition in adult deafened birds contributes to the degradation of song, it would suggest that neuron insertion is guided normally by auditory input and lend support to the idea that new HVc neurons provide a neural substrate for auditory experience to structure the vocal motor pathway. Although a causal link between HVc neuronal recruitment and vocal plasticity has yet to be demonstrated, there is a wealth of circumstantial evidence that is consistent with this view. In juvenile canaries and zebra finches, many new HVc neurons are generated as birds memorize song material and practice song production, and most of these new neurons differentiate into vocal motor neurons that innervate the RA (Alvarez-Buylla et al., 1988; Nordeen and Nordeen, 1988). In zebra finches, HVc neuronal incorporation is far greater in juvenile males than in juvenile females (who never produce song), and much greater in males during the sensitive period for song learning than later in life

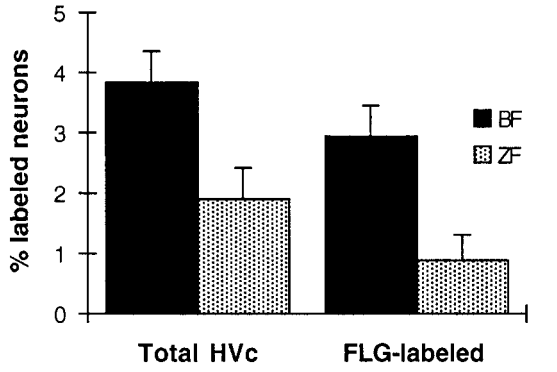

Figure 4 Histogram shows the percentage of THY-labeled cells among HVc neurons overall and among the subset of fluorogold-labeled (RA-projecting) HVc neurons. The overall incidence of THY-labeled HVc neurons was two times greater in BFs than ZFs. The incidence of THY-labeled RA-projecting HVc neurons was three times greater in BFs than ZFs. Data shown are means ± S.E.M.

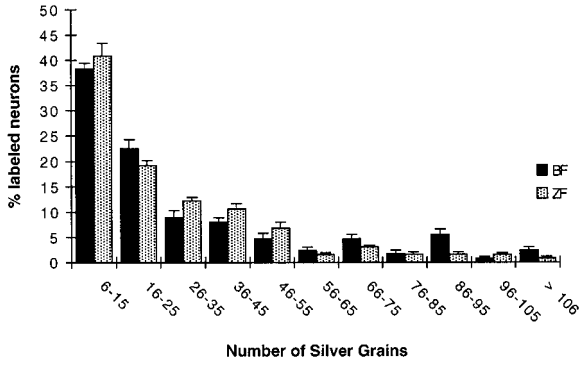

Figure 5 Histogram shows the similarity in the distribution of thymidine-labeling intensities in BFs and ZFs. Data shown are means ± S.E.M.

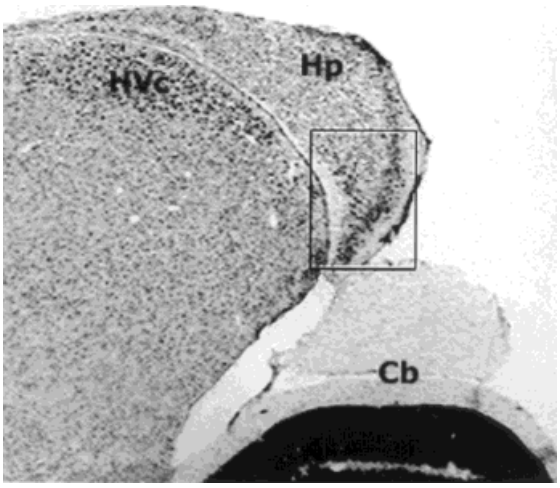

Figure 6 Photomicrograph of the avian hippocampal formation. The incidence of thymidine-labeled cells was measured within the "V" demarcated by the box.

after song patterns stabilize (Nordeen and Nordeen, 1988). In adult canaries, seasonal remodeling of song is accompanied by a pronounced increase in the addition of new HVc and HVc-RA projection neurons (Alvarez-Buylla et al., 1990; Kirn and Nottebohm, 1993; Kirn et al., 1994). Furthermore, the extent of HVc neuron addition in adult canaries during their seasonal periods of song learning is far greater than that which occurs in adult zebra finches, who do not normally exhibit vocal plasticity in adulthood (Alvarez-Buylla et al., 1990).

These observations support the hypothesis that an abundance of fresh synapses provided by HVc neuron addition presents a unique opportunity for auditory experience to shape the organization of the vocal motor circuitry. Thus, even though neuronal turnover in adult zebra and bengalese finches apparently never achieves a level sufficient to trigger a wholesale degradation and reconstruction of song in hearing birds, in the absence of feedback the influx of new cells may gradually disrupt the existing song pattern. This hypothesis could be tested directly by using antimitotic agents to slow or prevent neuron addition. If new HVc neurons promote plasticity, one would expect that reducing the insertion of new neurons would reduce the rate of song degradation after deafening.

Our estimates of the relative rate of HVc neuronal incorporation in deaf BFs and ZFs may not accurately reflect the rate of neuronal replacement in intact hearing birds. Wang et al. (1999) recently reported that fewer THY-labeled neurons are present in deaf than in intact ZFs 1 month after THY injections. These data are particularly interesting in light of the possibility that erroneous auditory feedback in intact birds may promote faster song degradation than does deafening. Using an elegant paradigm in which auditory feedback was perturbed in hearing zebra finches, Leonardo and Konishi (1999) report changes in song structure in one bird within 1 week of altered feedback, and significant changes in most birds within 6 weeks. These rates of behavioral change appear to be faster than what we have seen in deafened zebra finches (Nordeen and Nordeen, 1992). While a systematic study is needed to firmly establish if such a difference in the rate of vocal change exists between intact and deaf birds, it is interesting to speculate that such a difference might stem from the greater rate of HVc neuronal recruitment present in hearing birds as opposed to deafened birds. Of course, it may also be that incorrect auditory feedback provides a more powerful trigger for changing mature synapses than does the absence of feedback.

Although rates of neuronal recruitment may differ between deaf and hearing birds, it is clear that in both cases some new neurons replace older neurons and survive for many months. Deafening young zebra finches does not retard the developmental increase in overall HVc neuron number, suggesting that long-term survival of new HVc neurons occurs in the absence of auditory input (Burek et al., 1991). Also, the number of THY-labeled neurons that survive for 4 months does not differ between deaf and intact adult ZFs, even though fewer new neurons are present in deaf than intact birds 30 days after THY injections (Wang et al., 1999). Because total HVc neuron number stabilizes during late adolescence in both intact and deaf ZFs (Kirn and DeVoogd, 1989; Burek et al., 1991) and in intact canaries (Alvarez-Buylla et al., 1992), the addition of new neurons in adulthood is

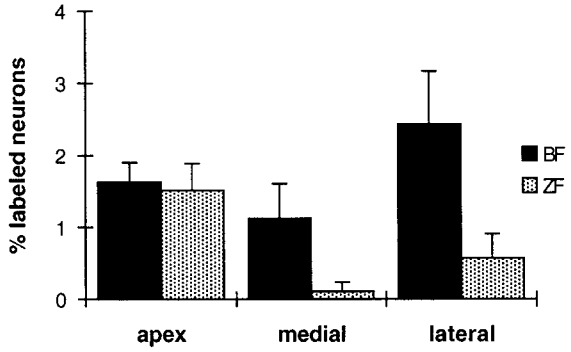

Figure 7 Histogram shows the percentage of THY-labeled cells in the apical, medial, and lateral portions of the hippocampal "V." The incidence of THY-labeled neurons in the medial and lateral portions was 10 and 4 times greater, respectively, in BFs than in ZFs. Data shown are means ± S.E.M.

part of a gradual turnover of HVc neurons (Kirn and Nottebohm, 1993). In adult canaries, high rates of neuronal recruitment (Alvarez-Buylla et al., 1990) coupled with their long-term survival (Kirn et al., 1991; Nottebohm et al., 1994) suggest that a significant proportion of HVc neurons is normally replaced in adulthood (rather than a small population turning over rapidly). This conclusion also is consistent with the observation that 33–50% of RA-projecting HVc neurons in adult male canaries are replaced between spring and fall (Kirn and Nottebohm, 1993).

The incorporation of new neurons into the vocal motor pathway is likely only one of several variables that affect the rate of vocal change after deafening. Within species, there are marked individual differences in the extent of song degradation after deafening, and in the present study these did not correlate with variation in the proportion of new neurons added to the HVc. These behavioral differences may reflect initial differences in syllable type, rate of song production, or number of syllable "chunks" produced (Williams and Staples, 1992) that could render songs more or less resilient to the absence of auditory feedback. Also, individual differences within the anterior forebrain pathway (AFP) may contribute to vocal change after deafening, as this circuit has been shown to play a role in adult plasticity. The AFP, which indirectly connects the HVc to the RA (via HVc → Area X → medial portion of the dorsolateral nucleus of the thalamus → lateral magnocellular nucleus of the anterior neostriatum (lMAN) → RA) contains auditory-sensitive neurons (Williams, 1989; Doupe and Konishi, 1991) and exhibits singing-related motor activity during song production (Hessler and Doupe, 1999). Importantly, its destruction arrests vocal change that normally accompanies deafening and syringeal denervation in adult birds (Brainard and Doupe, 1997; Williams and Mehta, 1999). Thus, the output of the AFP (from lMAN → RA) may provide an error signal that promotes change in synaptic weights among the vocal motor circuitry. Troyer et al. (1996) proposed that HVc sends an "expected auditory feedback" signal (or corollary discharge) to the anterior forebrain circuit, and comparison between this signal and the song template determines whether the lMAN provides a corrective signal to the RA. These authors propose that the relationship between the premotor command and the corollary discharge is formed in HVc circuitry during song development. If the preservation of this mapping requires auditory feedback, deafening (or altering feedback) could lead to the production of erroneous error signals in the lMAN-RA pathway, thus facilitating gradual modification of the vocal motor circuitry. In this case, lesions to lMAN might stabilize song behavior by eliminating such inappropriate signals.

The overall structure of song is markedly different in BFs and ZFs, and these differences could also affect rates of vocal deterioration after deafening if they demand encoding strategies that vary in their reliance on auditory feedback. One way to assess the contribution of song structure to the rate of song degradation after deafening would be to compare two species producing the same song structure. ZFs cross-fostered into BF clutches are able to learn many features of BF song structure (personal observation), and preliminary data from our laboratory suggest that cross-fostered ZFs deafened in adulthood exhibit little change in temporal ordering or syllable degradation within the first 2 postoperative weeks. However, more thorough studies are needed to evaluate the possibility that song structure influences the need for auditory feedback.

The idea that neuron incorporation disrupts stable song in deaf birds suggests that the maintenance of a learned behavior requires feedback if new neurons are being added into central pathways controlling that behavior. If neuron addition in an established neural pathway can disrupt circuitry, it is important to understand the degree of behavioral change we can expect to see and the factors that may modulate the behavioral disruption. Investigating these issues will elucidate the consequences of neuron addition in adult humans and medical treatments promoting neurogenesis and/or neuronal recruitment in adults.

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