THE JOURNAL OF COMPARATIVE NEUROLOGY 487:119–126 (2005)

Adult Female and Male Zebra Finches Show Distinct Patterns of Spine Deficits in an Auditory Area and in the Song System When Reared without Exposure to Normal Adult Song

CHRISTINE LAUAY,* ROBERT W. KOMOROWSKI, ANNA E. BEAUDIN, AND TIMOTHY J. DEVOOGD
Department of Psychology, Cornell University, Ithaca, New York 14853

ABSTRACT

Male songbirds typically require exposure to normal adult conspecific song during development in order to learn a normal song of their own. Females require exposure to conspecific song during development in order to select high-quality, learned song over the incomplete song produced by males reared in isolation. Altering males' opportunity for song learning during development affects the neuroanatomy of brain regions involved in song production (the song system), but in females the neural effects of song learning are unknown. We raised male and female zebra finches *(Taeniopygia guttata)* with differing amounts of exposure to singing males during development. At 120 days, we Golgi-stained their brains and measured the frequency of dendritic spines in brain areas used in song perception or production. We found that females reared with little or no exposure to song have 31% fewer dendritic spines per unit length of dendrite in caudomedial nidopallium (NCM), a brain area activated by song perception, compared to control females. The deprived females had small deficits in the frequency of spines in HVC, a region activated by song production in males. Males with limited exposure to song had a 24% lower spine density in HVC than controls but only a 10% lower density in NCM. These data support the hypothesis that NCM is important in auditory learning, while HVC is involved in sensorimotor learning, and that these capacities are differentially emphasized in the two sexes. J. Comp. Neurol. 487:119–126, 2005.

Indexing terms: HVC; NCM; Golgi stain; hearing; synaptogenesis; song; vocalization

Male songbirds typically require exposure to adult male song as juveniles to sing normal song as adults (Marler, 1989) and most species will never produce a complex song if this does not occur. For example, zebra finch *(Taeniopygia guttata)* males raised without exposure to normal song produce a song with fewer notes and simpler frequency modulations than song produced by males reared with normal song (Price, 1979).

Males produce song using several brain nuclei ("the motor song system") (Fig. 1; reviewed by DeVoogd and Lauay, 2001). One of these, HVC, is essential to integrating and producing song. Damaging HVC permanently disrupts singing (Nottebohm et al., 1976), and the immediate early gene (IEG) ZENK is activated in HVC and other motor song system nuclei while producing song (Jarvis and Nottebohm, 1997; Kimpo and Doupe, 1997; Jin and Clayton, 1997; Bolhuis and Eda-Fujiwara, 2003). ZENK expression is closely related to neural activity, and expression of this IEG has been widely used to identify brain

Grant sponsor: National Science Foundation; Grant number: IBN 0090963 (to T.J.D.).

Robert W. Komorowski's present address is Department of Psychology, Boston University, Boston, MA 02215.

*Correspondence to: Christine Lauay, Department of Psychology, Uris Hall, Cornell University, Ithaca, NY 14853. E-mail: CHL8@cornell.edu

Received 8 October 2004; Revised 10 December 2004; Accepted 15 January 2005

DOI 10.1002/cne.20591

Published online in Wiley InterScience (www.interscience.wiley.com).

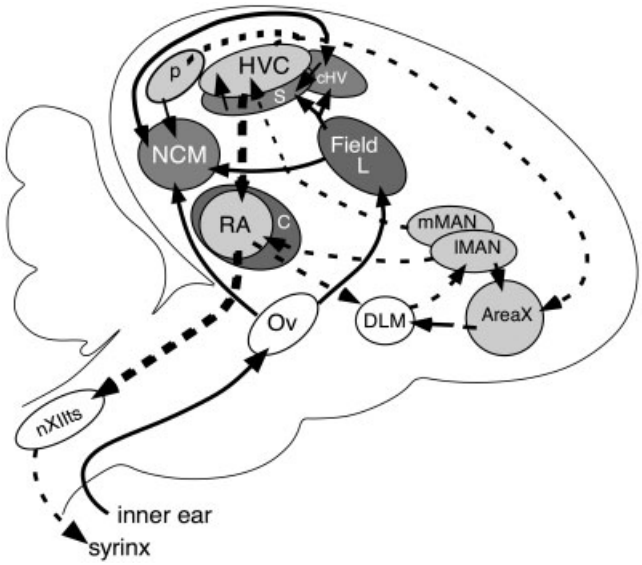

Fig. 1. The avian song system. Dashed lines are primarily motor or integrative projections; solid lines are primarily auditory projections. Lightly shaded nuclei show immediate early gene activation in birds that are producing song (the motor song system); darkly shaded nuclei show immediate early gene activation in birds that are hearing song (the auditory song system) (adapted from DeVoogd and Lauay 2001; nomenclature from Reiner et al., 2004). S-HVC shelf, p-paraHVC, c-RAcup.

areas involved in perceiving or producing song (reviewed by Clayton, 2000).

Adult females of many species assess song quality and/or distinguish conspecifics based on song, often choosing to mate with males having the most complex songs (Catchpole, 2000). Female zebra finches prefer the songs of their mates over the songs of strangers (Miller, 1979a), the song of their male tutor to an unfamiliar male (Miller, 1979b; Riebel et al., 2002), and the song of a tutored male (singing a complex song) to the song of an untutored male (Nowicki et al., 2002; Lauay et al., 2004). Females raised without exposure to complete adult song no longer prefer tutored male song over untutored song (Lauay et al., 2004), suggesting that aspects of these abilities are learned during development (Riebel, 2003).

Several studies have looked for sites in the brain that are responsible for song discrimination. Lesioning HVC in females causes deficits in discrimination (Brenowitz, 1991; Del Negro et al., 1998), although it is difficult to distinguish effects of damage to HVC from effects of damage to adjacent areas (MacDougall-Shackleton, 1998). Hearing song causes robust IEG expression in both sexes within several areas afferent to the motor song system, here termed the "auditory song system" (Fig. 1) (Mello et al., 1992; Mello and Clayton, 1994; Jarvis and Nottebohm, 1997). The responses are especially acute within the caudomedial portion of the nidopallium (NCM) (Chew et al., 1996; Mello et al., 1995; Bailey et al., 2002). In female canaries, IEG responses to categorically distinct features of song are differentially distributed in NCM (Ribeiro et al., 1998). Also, songs that are more attractive to female European starlings induce higher IEG responses in NCM (Gentner et al., 2001). In addition, neurons within NCM show physiological responses to song presentation (Chew et al., 1996; Stripling et al., 1997; Kruse et al., 2004). Together, these data suggest a substantial role for NCM in auditory discrimination.

We tested whether developmental differences in the opportunity to hear song has neuroanatomical effects that persist into adulthood. We measured the density of dendritic spines, a feature that varies with learning in many paradigms (Yuste and Bonhoeffer, 2001). We hypothesized that, since producing song in deafened birds induces IEG expression in HVC but not NCM (Jarvis and Nottebohm, 1997), and hearing song induces IEG expression in NCM but not HVC, males deprived of exposure to adult song would show deficits in the number of spines on neurons found in HVC and NCM, while females would only show deficits in the number of spines on neurons found in NCM.

MATERIALS AND METHODS

Animals

Subjects consisted of 44 zebra finches hatched and raised at Cornell University (Ithaca, NY). At the beginning of the experiment, 8–10 breeding pairs of zebra finches were housed in each of several wire mesh, free-flight aviaries (1.8 × 0.8 × 1.0 m) with a light period of 14L:10D. Eight nest boxes were added to each aviary and nesting material was made available for the duration of the experiment. A standard lab diet of dry seed, water, grit, and shell was available ad libitum, supplemented daily with fresh greens and hard-boiled eggs crushed with shell. All animal procedures were approved by the Cornell University Institutional Animal Care and Use Committee (IACUC) and met all applicable state and federal guidelines.

Experimental manipulations

The day that the first chick hatched was designated Aviary Day 1 for each aviary. The hatch dates of all offspring were recorded and leg bands color-coded for hatch date applied when individuals were 10–14 days of age. Experimental manipulations of all birds in an aviary were performed on Aviary Days 18 and 50, as described below (note that many of the individuals in the aviary were younger than 18 or 50 days of age when these manipulations were done).

Male and female zebra finches were reared under three conditions, as described previously (Lauay et. al. 2004). Group names designate categories of birds present in the aviary in which subjects were raised, with uppercase letters representing adult birds (M = adult male, F = adult female) and lowercase letters representing offspring (m = male offspring, f = female offspring). Chicks in the MFmf (control) group were raised in multiple nest aviaries by both parents along with male and female siblings until perfusion at 120 days of age. Chicks in the Fmf group were raised in an aviary in a different room, acoustically isolated from the other aviaries. Adult males were removed on Aviary Day 18 (i.e., when the oldest juvenile was 18 days of age), and well before the sensitive period for song model acquisition (Immelmann, 1969; Jones et al., 1996). Male and female juveniles then continued living together with the adult females until 120 days of age. Chicks in the Ff and Fm groups also had all adult males removed on Aviary Day 18. Female and male juveniles continued liv-

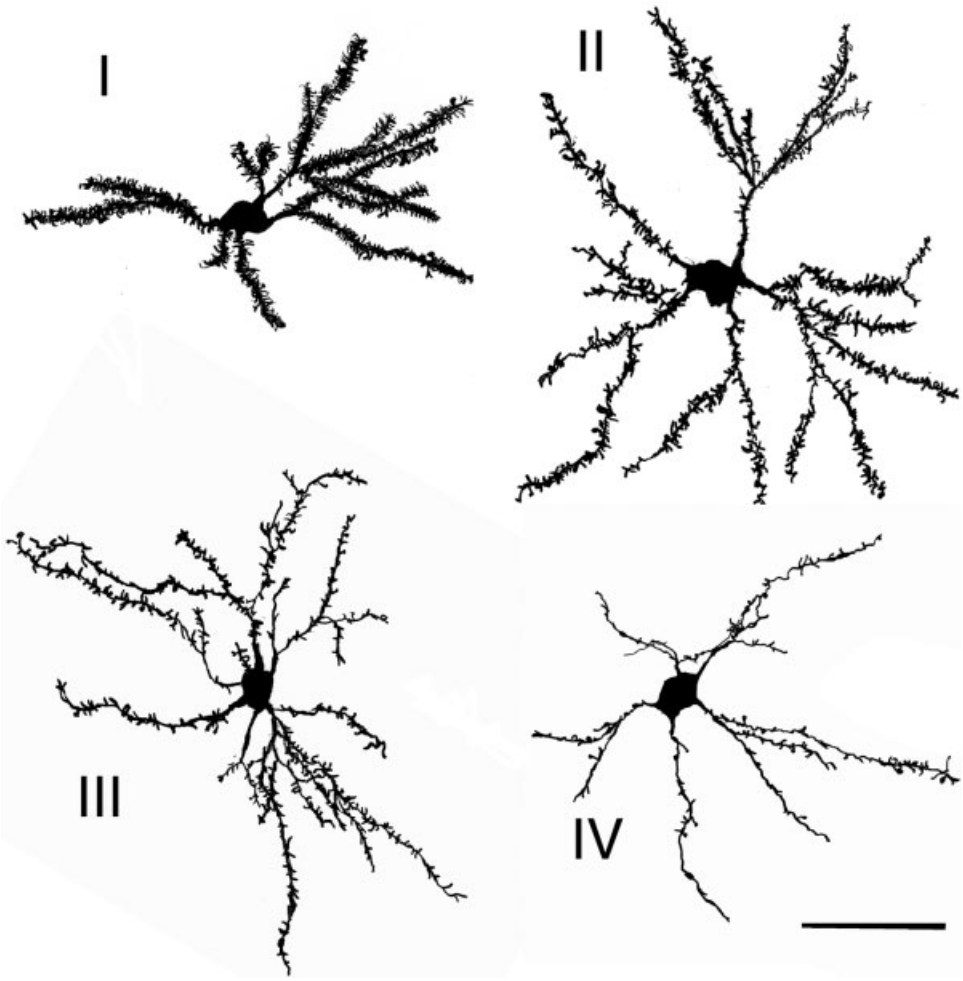

Fig. 2. Golgi-stained neurons in NCM were classified into four categories based on thickness and radius of dendrites and density of spines. Spine density was measured at proximal, middle and terminal segments of dendrites from neuron classes II and III. Scale bar = 50 μm

ing together until Aviary Day 50, when the emergence of sexually dimorphic plumage made sexing of the juveniles possible. At that time, juveniles were removed from the rearing aviary and housed in same-sex (Fm = all male; Ff = all female) aviaries (1.8 × 1.8 × 1 m) that were acoustically isolated from all other aviaries until 120 days of age. Juveniles that hatched later, and therefore were too young to show sexually dimorphic plumage when the oldest bird was 50 days old, remained in the rearing aviary with the adult females until they could be sexed, at which time they were removed to the appropriate same-sex aviary.

Thus, females and males in the MFmf group were able to interact with their fathers (and thus were exposed to normal adult song) throughout development. Females in the Fmf group heard other juvenile males acquire isolate song, while females in the Ff group did not hear any song other than subsong from males in their hatching cohort. Males in both the Fmf and Fm groups formed isolate song and heard other juvenile males doing so.

Tissue processing

At 120 days of age, males and females from each of the three groups were anesthetized (Chloropent) and perfused transcardially with heparinized saline followed by 10% formalin. The brains were immersed in Golgi-Cox solution (protocol based on Glaser and Van der Loos, 1981) for ~5 weeks. The tissue was dehydrated and embedded in celloidin, sectioned at 90 μm in the sagittal plane, reacted with ammonia, mounted on slides, and coverslipped.

Quantification

We selected neurons for quantification in HVC, NCM, and nucleus rotundus (a visual relay nucleus chosen as a control site). Because neurons are categorized based on spine density as well as dendritic length and thickness, it is possible that large differences in response to treatment could cause a neuron to shift its morphology such that it appears to be a member of a different class. To minimize the impact of such changes, we selected the most spiny neurons in HVC for measurement These usually resembled TD or thick dendrite cells described in canary HVC (Nixdorf et al., 1989).

Neurons within NCM have not previously been classified. We identified at least four types of cells in NCM. Neurons in the first (class I, Fig. 2) have relatively short dendrites extending from their cell bodies that branch extensively. The dendrites contain a high density of spines, making them virtually impossible to count. Further, the spines of class I cells are longer than the spines

seen in the other cell types. These cells occur regularly in NCM in these brains, but less frequently than other cell types.

Class II (Fig. 2) neurons have longer dendrites than those in class I, with extensive dendritic branching, such that the diameter of arborization of these cells is generally larger than class I neurons. These neurons have spines that are branched, pedunculated (thin, mushroom-shaped, and gemmule), sessile (but longer than stubby spines) as well as varicosities, as described by Fiala and Harris (1999). These cells, while not as dense with spines as cells of type I, are moderately to heavily spinous and occur frequently within NCM.

Neurons in cell class III are quite similar to cell class II in size, dendritic branching, spine shape, and spine density (Fig. 2). However, the dendrites of these cells are noticeably thinner than the dendrites observed on class II neurons. These cells also occur frequently within NCM.

The neurons in cell class IV in NCM are small relative to those in classes II and III, with less extensive dendritic arborization and shorter dendrites (Fig. 2). These neurons have very delicate dendrites and very few spines. Further, the spines on these class IV cells are very small and often take the form of bumps, rather than the longer spines found in the other three cell classes.

Spine density was quantified for cell classes II and III due to the similarity of spine features and because well-stained instances of both cell classes were found throughout NCM. Neurons were sampled randomly throughout NCM, the primary criterion for selection being the clarity of the segment. As the lateral boundary of NCM is not distinct in Golgi-stained tissue, measurements were restricted to neurons in the three most medial tissue sections (270 μm), well within the zone characterized as NCM in connectivity studies (Vates et al., 1996). A few of the most dorsal measures may have been within the area medial to HVC that some researchers have named paraHVC (Kirn et al., 1989; Foster and Bottjer, 1998)

Within each of the three brain sites, spine density was counted using a 100× oil objective for three 12-μm dendrite samples that lay parallel to the plane of the section; a proximal sample (20–32 μm from the soma), a middle sample (~60 μm from the soma), and a terminal sample (the last 12 μm, typically ~120 μm from the soma). Whenever possible, all three samples were taken from the same dendrite; when this was not possible, additional measures were taken from other dendrites to increase the number of samples from each cell. Extensive glial staining about neuronal somata prevented us from obtaining a complete set of spine counts on proximal dendritic segments in NCM.

In each animal, ~10 counts were made from middle segments and 15 from terminal segments in HVC. Approximately 15 were made from middle and 30 from terminal segments in NCM. All spines were counted, including spines that resemble a bump on the dendrite and long thin projections. The experimenters were blind to sex and to treatment throughout the quantification.

Statistical analyses

Spine counts at each location on the dendrite were averaged for each neuron. The values at each locus were then averaged to obtain a proximal, middle, and terminal mean spine density for each bird. These values were compared across groups using Multivariate General Linear Model (GLM), with experimental group, sex, and dendritic segment as factors, using the SPSS statistical package (Chicago, IL, v. 10.0.7a). Significant effects in the GLM were assessed with planned contrasts between the control group and the two experimental groups, and between the two experimental groups. About 90% of the NCM measurements were on neurons clearly located in the dorsal or ventral portions of the region. As some data indicate differences in the functions of these areas (Vates et al., 1996; Ribeiro et al., 1998; Gentner et al., 2001; Terpstra et al., 2004), follow-up analyses compared values obtained in the two subdivisions. Finally, the number of days from the hatch date of each juvenile to the day that the adult males were removed from the aviary was recorded for the two experimental groups. For each group, correlations between the number of days that the chicks spent with adult males and spine density were tested for significance using Pearson's r correlation coefficients.

RESULTS

Analyses of the counts made at the proximal segment of the dendrite in HVC revealed no significant differences due to rearing environment ($F_{1,20} = 0.01$, $P = 0.94$) or sex ($F_{1,20} = 0.84$, $P = 0.37$) and the interaction between the two was not significant ($F_{1,20} = 0.12$, $P = 0.74$). Given this lack of variation and previous data showing that learning and hormonal manipulations only affect more distal portions of the dendrite (Airey et al., 2000; Wallhausser-Franke et al., 1995; DeVoogd and Nottebohm, 1981), we focused our analyses on the middle and terminal segments of dendrite.

Dendritic spine frequency in HVC differed among rearing conditions ($F_{2,27} = 3.80$, $P = 0.04$). There were no significant main effects of the segment of dendrite ($F_{1,27} = 1.61$, $P = 0.22$) or of sex ($F_{1,27} = 0.19$, $P = 0.67$) on spine frequency (Table 1, Fig. 3). However, the interaction between rearing condition and segment of dendrite from which spine counts were taken was significant ($F_{2,27} = 4.22$, $P = 0.03$). This was due to large deficits in spine densities in the Fmf groups of both sexes relative to controls at the middle, but not the terminal, dendritic segment. An analysis contrasting spine density (mean of middle and terminal values) between control males and the two groups of experimental males (with the loss of statistical power that this entails), showed an effect of the experimental treatments ($t_{8.61} = 2.51$, $P < 0.04$), whereas the equivalent analysis in females only approached significance ($t_{11.13} = 2.00$, $P < 0.08$). When averaged across both dendritic segments, spine density was 24% lower in males from the two experimental groups than in controls, and was 14% lower in females from the two experimental groups than in controls. The correlations between spine density in HVC and the number of days raised with adult males were not significant for either sex in any rearing condition.

Dendritic spine frequencies in NCM differed between rearing conditions ($F_{2,33} = 18.38$, $P = < 0.0001$), sexes ($F_{1,33} = 7.32$, $P = 0.011$), and dendrite segments ($F_{1,33} = 218.01$, $P < 0.001$). The interaction between rearing condition and sex was also significant ($F_{2,33} = 6.145$, $P = 0.005$), due to spine deficits in females from both experimental groups relative to female controls, but in only one of the male experimental groups relative to male controls. The interaction between segment and sex approached sig-

TABLE 1. Dendritic Spines per 12 μm Sample

Rearing (N)	Middle dendrite	Terminal dendrite	Means	C vs. E1,E2	
HVC					
		Males			
MFmf (5) (control)	26.60 ± 2.42	22.74 ± 2.10	24.67 ± 1.82	$P < 0.04$	24.67
Fmf (8)	20.25 ± 2.00	21.11 ± 2.73	20.68 ± 2.13		18.82
Fm (6)	17.33 ± 1.50	16.60 ± 1.81	16.97 ± 1.61		24%
		Females			
MFmf (5) (control)	23.00 ± 1.68	21.05 ± 0.78	22.03 ± 1.18	$P < 0.08$	22.03
Fmf (4)	16.95 ± 1.89	19.01 ± 1.81	17.98 ± 1.65		18.91
Ff (6)	21.01 ± 1.61	18.67 ± 2.32	19.84 ± 1.82		14%
NCM					
		Males			
MFmf (5) (control)	47.20 ± 1.54	38.82 ± 2.70	43.01 ± 2.04	$P < 0.10$	43.01
Fmf (9)	38.63 ± 1.21	32.11 ± 1.54	35.37 ± 1.31		38.59
Fm (7)	46.32 ± 1.52	37.31 ± 0.97	41.82 ± 1.17		10%
		Females			
MFmf (7) (control)	48.34 ± 2.90	42.63 ± 2.25	45.48 ± 2.52	$P < 0.001$	45.49
Fmf (6)	34.84 ± 1.92	28.29 ± 1.60	31.57 ± 1.73		31.26
Ff (5)	33.89 ± 2.37	28.00 ± 1.74	30.95 ± 1.96		31%

[1]Mean spine density values (±SEM) at middle and terminal sites on dendrites for male and female zebra finches that had been raised in normal colonies (MFmf), colonies from which adult males were removed in the third week after hatching (Fmf), or colonies in which adult males were removed in the third week after hatching and the opposite sex was removed in the seventh week after hatching. *P* values are from ANOVAs contrasting the mean spine density values for control animals with the two experimental groups.

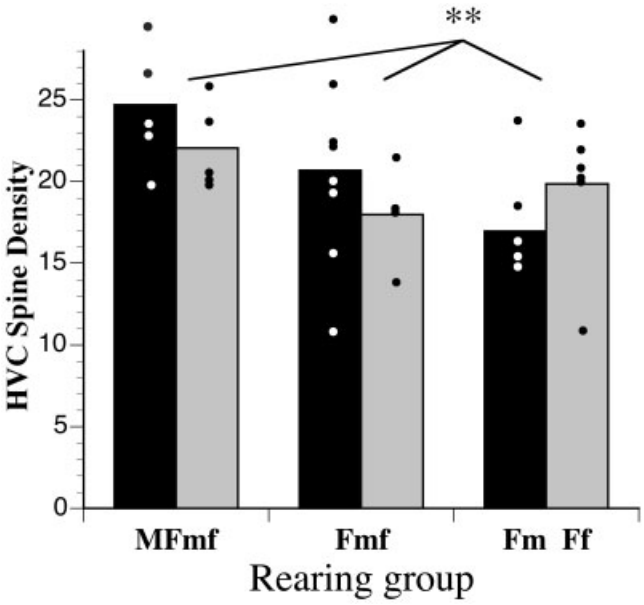

Fig. 3. In HVC, experimental males (black) (Ffm, Fm) and females (gray) (Ffm, Ff) have significantly fewer spines per 12 μm of dendrite (± SEM) (middle and terminal counts averaged) than do normally reared birds (MFmf) (** $P < 0.01$), although the magnitude of the difference is greater in males. In single sex contrasts, values for the two experimental groups are significantly less than controls in males and approach significance in females. Circles indicate individual animal values.

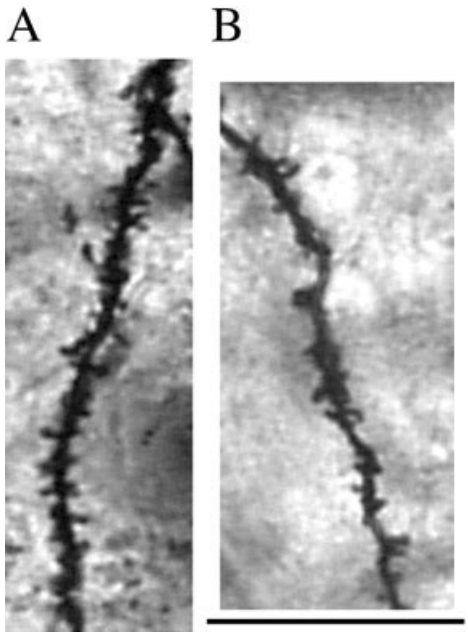

Fig. 4. Dendrite segments in NCM typical of control females (**A**) and females raised without exposure to adult or juvenile males (**B**). Scale bar = 10 μm.

nificance ($F_{1,33} = 4.07$, $P < 0.06$) (Table 1, Figs. 4, 5). Within sexes, an analysis contrasting spine density (mean of middle and terminal values) in the control males with the two groups of experimental males (with the loss of statistical power that this entails) approached significance ($t_{5.55} = 1.99$, $P < 0.10$). A contrast between the two experimental groups indicates that Fmf males have a lower spine density than Fm males ($t_{3.7} = 14.57$, $P < 0.002$). In females, a contrast of spine density between controls and the two experimental groups indicates that dendrites from experimental animals have lower spine densities than dendrites from controls ($t_{9.19} = 5.01$, $P < 0.001$). Across both dendritic segments and both experimental groups, males from the two experimental groups had 10% fewer spines than controls, and females from the two experimental groups had 31% fewer spines than controls.

Values from neurons in dorsal and ventral NCM were not differentially related to sex or to rearing group. In both

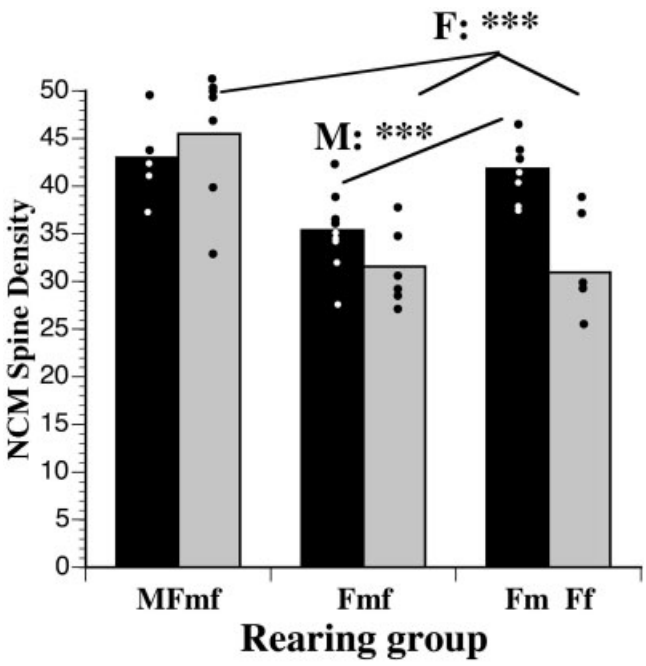

Fig. 5. In NCM, experimental females (gray) (Fmf, Ff) have significantly fewer spines per 12 μm of dendrite (± SEM) (middle and terminal counts averaged) than do normally reared females (MFmf) (single sex statistical contrasts). The same comparison only approaches significance in males. Males reared with juvenile males and both adult and juvenile females (Fmf) have significantly lower spine densities than males reared with other juvenile males only (Fm). ***$P < 0.002$

locations, animal means for samples from the middle of dendrites were highly correlated with animal means for samples from ends of dendrites ($r = 0.92$ and 0.80, respectively). Measures from the middle of dendrites taken in dorsal NCM were significantly correlated with measures from the middle of dendrites taken in ventral NCM ($r = 0.69$, $P < 0.001$), as were measures from the ends of dendrites ($r = 0.66$, $P < 0.001$).

Among males from the Fm group (fathers removed at Aviary Day 18 and in all-male aviaries after Day 50), we observed a significant positive correlation between the number of days spent with adult males and dendritic spine density at the middle ($r = 0.969$, $P < 0.001$, n = 7) and terminal ($r = 0.757$, $P < 0.05$, n = 7) segments of NCM dendrites. The correlations between spine density in NCM and the number of days raised with adult males were not significant for males in the Fmf (fathers removed at Aviary Day 18 and in mixed sex aviaries until adulthood). In females, the correlations between spine density in NCM and the number of days raised with adult males were not significant in either experimental rearing condition.

Spine density in the visual relay nucleus rotundus did not differ significantly by sex ($F_{1,12} = 0.43$, $P = 0.52$) or by rearing condition ($F_{1,12} = 0.81$, $P = 0.39$) and the interaction between the two was not significant ($F_{1,12} = 0.11$, $P = 0.74$). This observation in the thalamus does not rule out the possibility that our rearing manipulations had some effects that were general in the telencephalon. However, this observation, the overall similarity in behavioral maturation across rearing groups, and the male–female difference in the location of major anatomical effects indicate that the groups did not differ substantially in brain structure and function more generally.

DISCUSSION

The present data show that exposure to song during development affects the adult neuroanatomy of female zebra finches. Adult females that have been reared without access to adult males and adult song have only about two-thirds the density of dendritic spines on neurons found within the auditory region NCM compared with females reared with adult males and adult song. Lack of exposure to song has a smaller effect on dendritic spine densities on neurons in HVC, an integrative area that in males is important for learning and monitoring song. Females deprived in this way are also impaired as adults in selecting learned song over isolate song (Lauay et al., 2004). These results are the first to show a relation between opportunity for song learning, adult behavior, and song system neuroanatomy in female zebra finches.

Female zebra finches undergo auditory but not sensorimotor learning (Riebel, 2000, 2003; Lauay et al., 2004). As adults, normally reared females are able to make precise discriminations based on song (Miller, 1979a,b; Wiley et al., 1991; Cynx and Nottebohm, 1992; Williams et al., 1993), sometimes more accurately than do males (Searcy and Brenowitz, 1988). The present data indicating that the anatomy of NCM varies with varying exposure to song during development are consistent with the hypothesis that NCM is involved in auditory song learning as well as song perception. IEG expression in NCM in response to song develops at the time that auditory learning begins in young male zebra finches (Stripling et al., 2001). IEG expression within NCM habituates with repeated presentation of a song, increasing once again with presentation of a novel song (Mello et al., 1995). Amount of IEG expression within NCM of males is significantly correlated with the number of sound elements copied from a tutor's song, although in portions of NCM more lateral than those assessed in the present research (Bolhuis et al., 2000; Terpstra et al., 2004).

Given that varying the females' rearing environment has much larger effects on dendritic spines in NCM than in HVC in the present data, it seems likely that NCM plays a more significant role in auditory learning in females than does HVC. These results indicate that the development of neural pathways within NCM is dependent on environmental input in juvenile females. Perhaps circuitry in NCM that is critical for the processing of song stimuli during adulthood is fine-tuned during song learning in a normal rearing environment. Thus, a female denied the opportunity to learn song may have underdeveloped or missing neural circuitry, leading to poor processing of song stimuli and poor discrimination in adulthood. Further work must be done to assess whether preventing early learning impairs a female's ability to learn to recognize new songs during adulthood and/or impairs the capacity of NCM to respond to novel songs immediately (with IEG activation) or with changes in synaptic morphology. In addition, neural pathways in NCM may allow females to learn the songs of their father, siblings, and other male members of the flock and to maintain the ability to discriminate these familiar songs

from those of unfamiliar males throughout life. Thus, the deficits in spine density seen in the song-deprived groups in our study may reflect either a lower number of songs learned by the females or reduced capacity for further auditory learning.

In male zebra finches, limiting exposure to song during development has somewhat different consequences for dendritic spine density. Adult males that had been reared without access to an adult tutor have 24% fewer dendritic spines on neurons found within HVC compared with males raised with adult tutors. This is similar to earlier observations in male marsh wrens *(Cistothorus palustris)*. Presenting a limited number of song types to male marsh wrens during rearing results in their learning a song repertoire that contains a similarly limited number of song types. These birds also have fewer dendritic spines on neurons in HVC than males reared hearing (and learning) a normal number of song types (Airey et al., 2000). Together, these two sets of results support the hypothesis that plastic changes in the neurons of HVC are involved in sensorimotor learning.

Males reared without exposure to song and to song tutors have much smaller deficits in spine density on neurons within NCM than do deprived females. Males in the group reared with adult (mothers) and juvenile females (Fmf) have significantly lower spine densities in NCM than males in the group without any contact with females after Aviary Day 50 (Fm). These somewhat different anatomical effects in the two experimental groups suggest that these two forms of deprivation have different consequences for the development of the male song system. Perhaps negative reactions by the adult females to the isolate songs practiced by juvenile males affects NCM connectivity. Or, perhaps the loss of juvenile and adult females at day 50 in the Fm group led to enhanced song practice and improvisation, reflected in enhanced numbers of spines. Spine density in NCM in this group does not differ significantly from controls. However, within the group more contact with fathers prior to day 18 is correlated with greater spine density. If this observation is replicated, it would suggest that social factors begin to act on connectivity within this brain area well before the beginning of the sensitive period for auditory acquisition. In females, spine density in both NCM and HVC does not differ between the two experimental groups, suggesting that these effects of song deprivation on females are dependent solely on the presence (or absence) of adult males and are not modified by contact with juvenile males.

Spine density at middle and terminal dendrite segments do not always covary. In HVC, birds of both sexes raised without fathers but with the other sex had fewer spines than controls for the middle but not the terminal segments. In NCM, terminal segments generally had lower spine densities than middle segments. Further work would be needed to determine if such differences reflect differences in the connectivity of different dendritic domains on the same neurons.

Our data do not assess total spine number in males and females. While density values for HVC are similar in the two sexes, this does not indicate that the nucleus functions similarly in the two sexes. HVC has perhaps eight times as many neurons in adult males as in adult females (Kirn and DeVoogd, 1989) and the neurons that comprise the structure have much more extensive dendritic fields in males than in females (Nixdorf et al., 1989).

The opportunity to learn song during development has major consequences for zebra finches. Directly or indirectly, it shapes adult behaviors in both sexes. We find that it also has a major impact on the neuroanatomy of telencephalic nuclei important in song-related behaviors. Rearing without exposure to song leads to deficits in dendritic spines both in NCM, which is normally activated by hearing song, and in HVC, which is activated by singing. The effects of deprivation during rearing appear to be greater in NCM in females, and in HVC in males, perhaps indicating differential emphases across the sexes on accurate song perception versus production. Until now, relatively little has been known of song-related neural plasticity in females. These results, showing environmentally mediated modifications in NCM and HVC in female zebra finches, may indicate that females experience juvenile learning that confers the elaborate song discriminations of which they are capable.

ACKNOWLEDGMENTS

We thank Dr. Richard Darlington for statistical advice, Dr. Claudio Mello for helpful discussions regarding NCM, Jasmin Garcia and Emily Sluzas for assistance with the breeding colonies, and Zach Buchan for technical assistance.

REFERENCES

Airey DC, Kroodsma DE, DeVoogd TJ. 2000. Increased song repertoire size is associated with increased spine density in HVC but not in RA in tape-tutored eastern marsh wrens. Neurobiol Learn Mem 73:274–281.

Bailey DJ, Rosebush JC, Wade J. 2002. The hippocampus and caudomedial neostriatum show selective responsiveness to conspecific song in the female zebra finch. J Neurobiol 52:43–51.

Bolhuis JJ, Eda-Fujiwara H. 2003. Bird brains and songs: neural mechanisms of birdsong perception and memory. Anim Biol 53:129–145.

Bolhuis JJ, Zijlstra GGO, den Boer-Visser AM, Van der Zee EA. 2000. Localized neuronal activation in the zebra finch brain is related to the strength of song learning. Proc Natl Acad Sci U S A 97:2282–2285.

Brenowitz E. 1991. Altered perception of species-specific song by female birds after lesions of a forebrain nucleus. Science 251:303–305.

Catchpole CK. 2000. Sexual selection and the evolution of song and brain structure in *Acrocephalus* warblers. Adv Stud Behav 29:45–97.

Chew SJ, Vicario DS, Nottebohm F. 1996. A large capacity memory system that recognizes the calls and songs of individual birds. Proc Natl Acad Sci U S A 93:1950–1955.

Clayton DF. 2000. The genomic action potential. Neurobiol Learn Mem 74:185–216.

Cynx J, Nottebohm F. 1992. Role of gender, season and familiarity in discrimination of con-specific song by zebra finches *(Taeniopygia guttata)*. Proc Natl Acad Sci U S A 89:1368–1371.

Del Negro C, Gahr M, Leboucher G, Kreuzer M. 1998. The selectivity of sexual responses to song displays: effects of partial lesion of the HVc in female canaries. Brain Behav Res 96:151–161.

DeVoogd TJ, Lauay C. 2001. Emerging biopsychology of the avian song system. In: Blass E, editor. Handbook of behavioral neurobiology, vol. 13. New York: Kluwer/Plenum. p 357–392.

DeVoogd TJ, Nottebohm F. 1981. Gonadal hormones induce dendritic growth in the adult brain. Science 214:202–204.

Fiala JC, Harris KM. 1999. Dendritic structure. In: Stuart G, Spruston N, Hausser M, editors. Dendrites. Oxford: Oxford University Press. p 1–34.

Foster EF, Bottjer SW. 1998. Axonal connections of the high vocal center and surrounding cortical regions in juvenile and adult male zebra finches. J Comp Neurol 397:118–138.

Gentner TQ, Hulse SH, Duffy D, Ball GF. 2001. Response biases in auditory forebrain regions of female songbirds following exposure to sexually relevant variation in male song. J Neurobiol 46:48–58.

Glaser EM, Van der Loos H. 1981. Analysis of thick brain sections by obverse-reverse microscopy: application of a new, high clarity Golgi-Nissl stain. J Neurosci Methods 4:117–125.

Immelmann K. 1969. Song development in the zebra finch and other estrildid finches. In: Hinde RA, editor. Bird vocalizations. Cambridge, UK: Cambridge University Press. p 61–74.

Jarvis ED, Nottebohm F. 1997. Motor-driven gene expression. Proc Natl Acad Sci U S A 94:4097–4102.

Jin H, Clayton DF. 1997. Localized changes in immediate-early gene regulation during sensory and motor learning in zebra finches. Neuron 19:1049–1059.

Jones AE, Ten-Cate C, Slater PJB. 1996. Early experience and plasticity of song in adult male zebra finches *(Taeniopygia guttata)*. J Comp Psychol 110:354–369.

Kimpo RR, Doupe AJ. 1997. FOS is induced by singing in distinct neuronal populations in a motor network. Neuron 18:315–325.

Kirn JR, DeVoogd TJ. 1989. The genesis and death of vocal control neurons during sexual differentiation in the zebra finch. J Neurosci 9:3176–3187.

Kirn JR, Clower RP, Kroodsma DE, DeVoogd TJ. 1989. Song-related brain regions in the red-winged blackbird are affected by sex and season but not repertoire size. J Neurobiol 20:139–164.

Kruse AA, Stripling R,, Clayton DF. 2004. Context-specific habituation of the zenk gene response to song in adult zebra finches. Neurobiol Learn Mem 82:99–108.

Lauay C, Gerlach N, Adkins-Regan E, DeVoogd TJ. 2004. Female zebra finches require early song exposure to prefer high quality song as adults. Anim Behav 68:1249–1255.

MacDougall-Shackleton SA, Hulse SH, Ball GF. 1998. Neural bases of song preferences in female zebra finches (*Taeniopygia guttata*). Neuroreport 9:3047–3052.

Marler P. 1989. Song learning: innate species differences in the learning process. In: Marler P, Terrace HS, editors. The biology of learning. Berlin: Springer. p 289–309.

Mello CV, Clayton DF. 1994. Song-induced ZENK gene expression in auditory pathways of songbird brain and its relation to the song control system. J Neurosci 14:6652–6666.

Mello CV, Vicario DS, Clayton DF. 1992. Song presentation induces gene expression in the songbird forebrain. Proc Natl Acad Sci U S A 89:6818–6822.

Mello CV, Nottebohm F, Clayton D. 1995. Repeated exposure to one song leads to a rapid and persistent decline in an immediate early gene's response to that song in zebra finch telencephalon. J Neurosci 15:6919–6925.

Miller DB. 1979a. The acoustic basis of mate recognition by female zebra finches (*Taeniopygia guttata*). Anim Behav 27:376–380.

Miller DB. 1979b. Long-term recognition of father's song by female zebra finches. Nature 280:389–391.

Nixdorf BE, Davis SS, DeVoogd TJ. 1989. Morphology of Golgi-impregnated neurons in hyperstriatum ventralis, pars caudalis in adult male and female canaries. J Comp Neurol 284:337–349.

Nottebohm F, Stokes TM, Leonard CM. 1976. Central control of song in the canary. J Comp Neurol 165:457–468.

Nowicki S, Searcy WA, Peters S. 2002. Brain development, song learning and mate choice in birds: a review and experimental test of the "nutritional stress hypothesis." J Comp Physiol A 188:1003–1014.

Price P. 1979. Developmental determinants of structure in zebra finch song. J Comp Physiol Psychol 93:260–277.

Reiner A, Bruce L, Butler A, Csillag A Kuenzel W, Medina L, Paxinos G, Perkel D, Shimizu T, Striedter G, Wild M, Ball G, Durand S, Gunturkun O, Lee D, Mello CV, Powers A, White S, Hough G, Kubikova L, Smulders TV, Wada K, Dugas-Ford J, Husband S, Yamamoto K, Yu J, Siang C, Jarvis ED. 2004. Revised nomenclature for avian telencephalon and some related brainstem nuclei. J Comp Neurol 473:377–414.

Ribeiro S, Cecchi GA, Magnasco MO, Mello CV. 1998. Toward a song code: evidence for a syllabic representation in the canary brain. Neuron 21:359–371.

Riebel K. 2000. Early exposure leads to repeatable preferences for male song in female zebra finches. Proc R Soc Lond B 267:2553–2558.

Riebel K. 2003. The "mute" sex revisited: vocal production and perception learning in female songbirds. Adv Study Behav 33:49–86.

Riebel K, Smallegange IM, Terpstra NJ. 2002. Sexual equality in zebra finch song preference: evidence for a dissociation between song recognition and production learning. Proc R Soc Lond B 269:729–733.

Searcy WA, Brenowitz EA. 1988. Sexual differences in species recognition of avian song. Nature 332:152–154.

Stripling R, Volman SF, Clayton DF. 1997. Response modulation in the zebra finch neostriatum: relationship to nuclear gene regulation. J Neurosci 17:3883–3893.

Stripling R, Kruse AA, Clayton DF. 2001. Development of song responses in the zebra finch caudomedial neostriatum: role of genomic and electrophysiological activities. J Neurobiol 48:163–180.

Terpstra NJ, Bolhuis JJ, den Boer-Visser AM. 2004. An analysis of the neural representation of birdsong memory. J Neurosci 24:4971–4977.

Vates GE, Broome BM, Mello CV, Nottebohm F. 1996. Auditory pathways of caudal telencephalon and their relation to the song system of adult male zebra finches (Taenopygia guttata). J Comp Neurol 366:613–642.

Wallhausser-Franke E, Nixdorf-Bergweiler BE, DeVoogd TJ. 1995. Song isolation prevents the early loss of spine synapses in song system nucleus l-MAN of zebra finches. Neurobiol Learn Mem 64:25–35.

Wiley RH, BJ Hatchwell, Davies N. 1991. Recognition of individual males songs by female dunnocks — a mechanism increasing the number of copulatory partners and reproductive success. Ethology 88:145–153.

Williams H, Kilander K, Sotanski ML. 1993. Untutored song, reproductive success and song learning. Anim Behav 45:695–705.

Yuste R, Bonhoeffer T. 2001. Morphological changes in dendritic spines associated with long-term synaptic plasticity. Annu Rev Neurosci 24:1071–1089.