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Dissociation between extension of the sensitive period for avian vocal learning and dendritic spine loss in the song nucleus lMAN☆

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Abstract

Several instances of early learning coincide with significant rearrangements of neural connections in regions contributing to these behaviors. In fact developmentally restricted learning may be constrained temporally by the opportunity for experience to selectively maintain appropriate synapses amidst the elimination of exuberant connections. Consistent with this notion, during the normal sensitive period for vocal learning in zebra finches (*Taenopygia guttata*), there is a decline in the density of dendritic spines within a region essential for song development, the lateral magnocellular nucleus of the anterior nidopallium (lMAN). Moreover, in birds isolated from conspecific song shortly after hatching, both the closure of the sensitive period for vocal learning and the pruning of spines from lMAN neurons is delayed. Here, we employed a more subtle form of deprivation to delay the close of the sensitive period for song learning, and found that late song learning occurred without obvious alterations in the pruning of dendritic spines on lMAN neurons. At posthatch day (PHD) 65 (beyond the end of the normal sensitive period for song memorization in zebra finches), birds isolated from song beginning on PHD30 did not differ from normally reared birds in measures of dendritic spine density on Golgi-Cox stained lMAN neurons. Moreover, tutor exposure from PHD65 to 90 did not increase spine elimination in these isolates (who memorized new song material) relative to controls (who did not). Thus, we conclude that the extent of normally occurring lMAN spine loss is not sufficient to account for the timing of the sensitive period for zebra finch song learning.

Keywords: Birdsong; Development; lMAN; Synapse selection; Zebra finch; Plasticity

1. Introduction

The capacity for behavioral and synaptic plasticity frequently is exaggerated during development, but the neural mechanisms that contribute to closure of these "sensitive periods" are not well characterized. "Synapse selection" theories postulate that plasticity is reduced as an initially exuberant set of connections is reduced through activity-dependent synaptic rearrangement (Changeux, 1997; Changeux & Dehaene, 1989; Dehaene, Changeux, & Nadal, 1987; Edelman, 1993; Lichtman & Colman, 2000; Tononi, Sporns, & Edelman, 1996). Consistent with this idea, both filial and sexual imprinting in birds are associated with large-scale, experience-dependent pruning of dendritic spines in forebrain regions associated with these behaviors (Bischof, Geissler, & Rollenhagen, 2002; Bock & Braun, 1999a, 1999b; Rollenhagen & Bischof, 1998). Moreover, early deprivation typically extends sensitive periods, and work on sexual imprinting suggests that the timecourse of synaptic elimination limits such extensions of learning (Bischof et al., 2002). In the present study, we test this hypothesis in the

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context of avian song learning, where the sensitive period for vocal imitation coincides with the loss of dendritic spines within a region implicated specifically in vocal learning.

Avian vocal learning begins with 'sensory acquisition,' when birds normally memorize the song of an adult conspecific male. Next, during 'sensorimotor learning,' juveniles practice the complex motor commands required for song production and use auditory feedback to alter vocalizations to match the stored song template (Immelmann, 1969). In Australian zebra finches, only males sing, and sensory acquisition normally occurs between posthatch day (PHD) 25 and 65. Sensorimotor learning in this species begins about PHD35 and culminates with the production of a stereotyped song pattern by about PHD120. As with other sensitive periods, the timing of vocal learning is experience-dependent; depriving young finches of conspecific song exposure extends the period during which they are capable of copying a new song model (Aamodt, Nordeen, & Nordeen, 1995; Eales, 1987; Jones, Ten-Cate, & Slater, 1996; Price, 1979).

Song learning normally overlaps with an extensive pruning of connections within the lateral magnocellular nucleus of the anterior nidopallium (lMAN), a region implicated specifically in vocal plasticity. In male zebra finches, the density of spines on proximal portions of lMAN dendrites declines between ~PHD35 and 50. On more distal portions of the dendrite this pruning continues through sensorimotor learning, and even into adulthood (Nixdorf-Bergweiler, Wallhausser-Franke, & DeVoogd, 1995). Importantly, if the sensitive period for sensory acquisition is extended in young zebra finches by isolating them from conspecific song soon after hatching, the pruning of lMAN dendritic spines is attenuated or delayed. At PHD55, such isolated males have approximately 40% more spines along lMAN dendrites than do socially reared controls (Wallhausser-Franke, Nixdorf-Bergweiler, & DeVoogd, 1995). The lMAN is part of a specialized cortical–basal ganglia-thalamo-cortical loop, the anterior forebrain pathway (AFP), that is essential for vocal plasticity (Bottjer, Miesner, & Arnold, 1984; Nordeen & Nordeen, 1992; Scharff & Nottebohm, 1991; Sohrabji, Nordeen, & Nordeen, 1990). The lMAN occupies a central position in this loop, receiving its thalamic output, projecting back to the basal ganglia, and providing direct input to the descending motor pathway mediating song production. Thus, it is interesting that the developmental reduction in lMAN spines is mirrored by a generalized focusing of thalamic afferents (Johnson & Bottjer, 1992) and pruning of individual thalamocortical axon branches (Iyengar & Bottjer, 2002a).

The lMAN may be critically involved in encoding the song template and/or translating that auditory memory into vocal motor patterns. Thus, it is possible that the extensive spine loss that accompanies sensory acquisition and the early stages of song production contributes to these processes, by selectively retaining connections representing tutors song and/or those enabling appropriate vocal motor behaviors. If so, the end of the sensitive period for vocal plasticity may be linked to a reduced capacity for synaptic pruning within the lMAN. To test this hypothesis, we used the Golgi-Cox staining method to examine changes in the density of spines on lMAN dendrites when the timing of song learning was displaced with respect to age. Unlike socially reared controls, animals isolated from conspecific song at PHD30 (late isolates) maintain the capacity to imitate songs first heard after PHD65 (Heinrich, Singh, Nordeen, & Nordeen, 2003). Here, we report that this extension of the sensitive period is not accompanied by enhanced pruning of lMAN dendritic spines after PHD65; both before and after new song learning, late isolates exhibit lMAN dendritic spine densities equivalent to age-matched controls that do not imitate songs heard after PHD65. Thus, we conclude that the sensitive period for song learning is not defined by the normal time course of pruning of exuberant dendritic spines in lMAN.

2. Methods

2.1. Animals and experimental design

Four groups of male zebra finches (*Taeniopygia guttata*) were raised to measure lMAN spine density both before and after exposure to a tutor during late adolescence. "Controls" were raised normally in free flight aviaries containing six breeding pairs and their offspring. "Late isolates" were raised in these same free flight aviaries only until PHD30, and then they were placed into individual cages that were visually isolated from one another and acoustically isolated from adult male song. At PHD65, a subset of each group was sacrificed for analysis of lMAN dendritic spine density. The remaining birds then were individually housed with an unfamiliar adult male tutor until PHD90, when they were visually isolated from each other until sacrifice at PHD120. During tutoring, each cage containing a pupil and tutor was placed adjacent to a stimulus female, and was visually separated from other pupil–tutor pairs. Each tutor was used for at least one pair of control and late isolate birds. Assignments for tutoring were made to maximize dissimilarity between tutor song and the pupil's song at PHD65.

To confirm our ability to detect the previously reported developmental decline in lMAN spine density (Nixdorf-Bergweiler et al., 1995), a fifth group of normally reared control birds was sacrificed at PHD35. These birds were processed along with PHD65 controls.

All animals were maintained on a 14:10 h light/dark schedule, with food and water ad libitum. Each of the groups consisted of 5–7 male zebra finches.

2.2. Histological preparation

Brains were processed for Golgi-Cox staining as previously described for zebra finch tissue (Nixdorf-Bergweiler et al., 1995; Wallhausser-Franke et al., 1995), using a slightly modified version of the Glaser and Van der Loos protocol (Glaser & Van der Loos, 1981). Briefly, birds were anesthetized and transcardially perfused with buffered 0.9% saline followed by buffered 4% paraformaldehyde. Whole brains were kept in Golgi-Cox solution (5% potassium dichromate, 1% mercuric chloride, and 1% potassium chromate) for 30 days. They then were embedded in celloidin (5.3% followed by 10.6%), and sectioned on a sliding microtome at 90 μm. The Golgi precipitate was reduced in ammonia and then processed further in Kodak developer and fixer. Sections then were dehydrated and mounted on glass slides. Tissue was stained and processed in multiple batches, however, each of the treatment groups was represented in each batch.

2.3. Spine density quantification

lMAN could be identified in the Golgi-Cox stained tissue by the clustering of cells with large radial dendritic trees and a darker background stain. In preliminary work, alternate Golgi-Cox processed sections were Nissl counter-stained, and we verified that the location of lMAN could be correctly identified in the non-counter-stained tissue. The boundary between $lMAN_{core}$ and $lMAN_{shell}$ could not be distinguished reliably in Golgi-stained material, however, analysis of spine density was restricted to neurons near the center of lMAN and therefore likely reflect attributes of $lMAN_{core}$ neurons. Other lMAN neuronal parameters (number of primary dendrites, dendritic field size, probability of branching, number of distal endings, and dendritic thickness) are not affected significantly by age or song experience (Nixdorf-Bergweiler et al., 1995; Wallhausser-Franke et al., 1995), and thus were not measured in this study.

To determine lMAN spine density, sections were examined at a magnification of 1000X with an Olympus AX70 connected to a motorized stage. Dendritic segments of spiny neurons (see Section 3) that remained largely within the plane of focus were photographed to obtain measurements of segment length (ImagePro Plus 4.5.1, Media Cybernetics). All visible dendritic spines along consecutive 10 μm segments, starting 20 μm from the cell body, were counted through the microscope by an investigator blind to the experimental group. Analysis was restricted to fully impregnated spiny lMAN neurons, and concentrated on the dendritic region beginning 20 μm from the cell body and continuing out to 80 μm, as previous work has demonstrated that song experience affects spine density within this region (Wallhausser-Franke et al., 1995).

The quality of Golgi-staining was satisfactory for analysis in at least five animals in each group (PHD35 controls ($n=5$), PHD65 controls ($n=6$) PHD65 late isolates ($n=7$) PHD120 controls ($n=6$), PHD120 late isolates ($n=5$)). Spine densities (# spines/10 μm) were calculated for a minimum of 26 dendrites per animal (mean: 37 ± 5 dendrites/animal), from an average of 25 ± 4 neurons/animal. Neurons were sampled from both hemispheres. For each animal, an overall average spine density was derived from the mean spine density per dendrite (this value did not differ significantly from that calculated on the basis of average dendritic spine density *per neuron*).

Two-way ANOVAs were used to assess group differences in overall spine density as well as average spine density within specific dendritic intervals (20–40, 40–60, and 60–80 μm).

2.4. Behavior

In animals sacrificed for measures of lMAN spine densities at PHD120, female-directed song was recorded prior to tutoring (~PHD63), after tutor exposure (~PHD90), and before sacrifice (~PHD120) using Avisoft Recorder software (sampling rate of 22,050 Hz). An observer blind to the experimental conditions analyzed song spectrograms to assess song similarity using a visual method of comparison routinely used in our laboratory (Aamodt, Nordeen, & Nordeen, 1996; Basham, Nordeen, & Nordeen, 1996; Heinrich et al., 2003; Sohrabji et al., 1990). Spectrograms created from song bouts of each pupil recorded at PHD120 were compared to spectrograms of the tutor's song. Individual syllables were defined as acoustic units ($\geqslant$20 ms) surrounded by intervals of baseline energy lasting at least 10 ms except in cases of abrupt frequency transitions (>1 kHz) where the intersyllable interval could be as short as 5 ms. Each syllable from the pupil's song was matched to the syllable in the tutor's song that it most closely resembled. The phonological similarity of the pair then was scored on a 0–3 scale (0 = no similarity, 1 = slight similarity, 2 = highly similar, and 3 = matched). Pupil syllables that received a score of a 2 or a 3 were operationally defined as learned (or copied). The percent of tutor's song imitated was defined as the number of tutor syllables scored as learned in pupil's song divided by the total number of tutor syllables available to the pupil. Two-tailed Mann–Whitney U tests were used to evaluate group differences in the average percent of tutor's song imitated.

Sound Analysis software (v3.19) also was used to generate a percent similarity score for song samples based on pitch, frequency modulation, spectral continuity, and Wiener entropy within each sample (Tchernichovski, Nottebohm, Ching, Pesaran, & Mitra, 2000). Song samples consisted of one complete song motif (one example of each syllable plus an introductory note) which ranged

from .9 to 1.4 s long. Tutor motifs always served as the reference sample, and scoring was done in the overall mode with the similarity threshold set at 92%, interval at 70 ms, and a similarity section of 30 ms (the software default parameters optimized for zebra finch song). The product of the percent of significant similarity (the proportion of tutor's song that has a similar version in the pupil's song) and accuracy (the average similarity between time windows of the tutor and pupil's song) was calculated for a measure of accurate imitation. Percent of accurate imitation scores were averaged from the results of three different phrase example files for each bird. Two-tailed Mann–Whitney U tests were used to evaluate group differences in average percent of accurate imitation (see Tchernichovski et al., 2000).

3. Results

Golgi-impregnated lMAN neurons, which have somata larger than neurons of the surrounding nidopallium, could be broadly classified into two classes as described by Nixdorf-Bergweiler et al. (1995) and Wallhausser-Franke et al. (1995). The majority of cells had extensive, spiny dendritic arbors, while a second class had simpler, aspinous dendrites. On spiny neurons, the size and shape of spine heads and necks were quite heterogeneous, even along a single dendrite of the same animal. Average soma size was not significantly different between animals of different age or rearing condition.

Consistent with previous findings (Nixdorf-Bergweiler et al., 1995), normally reared zebra finches exhibited a significant developmental decline in dendritic spine density on proximal portions of lMAN neurons. Between PHD35 and 65, spine density on the dendritic segment 20–40 μm from the soma decreased by 28% ($t = 4.57$, $p = .01$, see Fig. 1). Only this proximal portion of the dendrite (where the previously reported developmental decline was particularly robust) was measured in PHD35 animals.

When exposed to a novel song between PHD65 and 90, late isolates demonstrated the capacity to learn new song while control birds did not. At PHD120 both groups produced a similar number of song syllables (controls = 6.5 ± .93, late isolates = 7.4 ± .84; mean ± SEM), yet late-isolate animals imitated a larger proportion of the tutor's song than did controls ($z = 2.92$; $p < .005$). Scoring based on visual comparison of sonograms indicated that late isolates imitated an average of 71% of the tutors' songs, while control birds copied only 12% (Fig. 2). Comparisons using Sound Analysis software (Tchernichovski et al., 2000) also confirmed that late isolates' songs were significantly more similar to their tutor's song, than were controls ($z = 2.92$; $p < .005$). The percent of accurate imitation averaged 49% for late-isolate animals, and 27% for control animals. These findings are in agreement with previously reported learning differences in similarly raised animals (Heinrich et al., 2003).

Despite clear differences in sensory acquisition after PHD65, spine elimination between PHD65 and 120 did not differ between late isolates and controls. As shown in Fig. 3, average lMAN spine density declined significantly across both groups (main effect of age: $F[1,19] = 12.79$; $p < .005$), but there was neither a main effect of rearing (song exposure), nor an interaction between age and

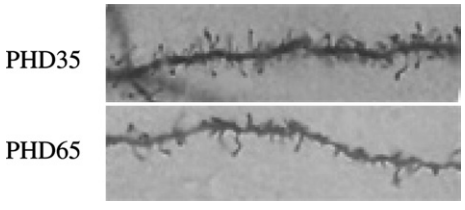

Fig. 1. Photomicrographs of Golgi-Cox stained lMAN dendrites illustrating the developmental decrease in spine density that occurs in normally reared males. The segment shown on top is from a PHD35 male, and that shown on the bottom is from a PHD65 male.

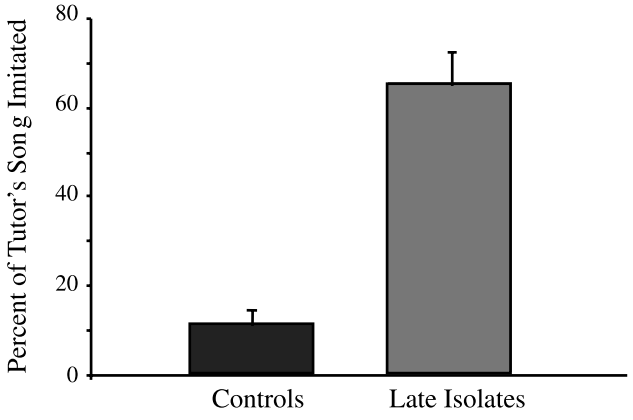

Fig. 2. At PHD120, late isolates imitated significantly more of a tutor's song heard between PHD65 and 90 than did socially reared males ($p < .005$). Data shown are group means ± SEM (visual method of scoring).

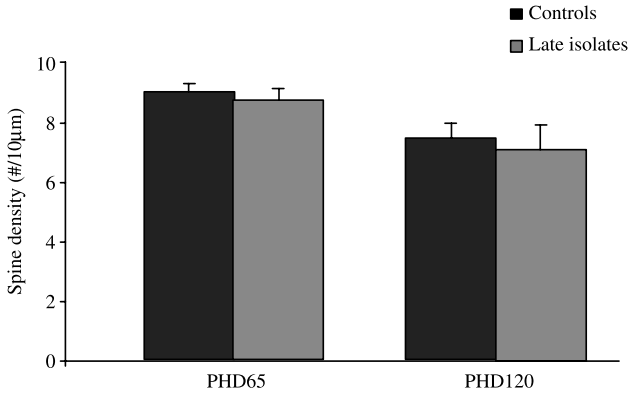

Fig. 3. At PHD65 and 120, both control (black) and late-isolate (gray) groups exhibit similar average spine densities along the 20–80 μm dendritic segment of lMAN neurons. Analysis of variance revealed a significant overall main effect of age ($p < .005$), but no effect of rearing, and no interaction between age and rearing. Data are group means ± SEM.

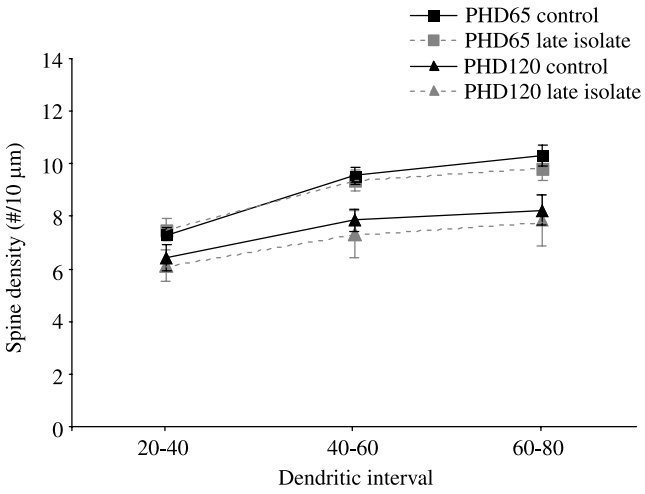

Fig. 4. Within each lMAN dendritic segment measured, the age-related decline in spine density was similar in control and late-isolate birds. For each segment, there was a significant main effect of age (see text), but no effect of rearing and no interaction. Data shown are group means ± SEM.

rearing. The lack of effect of early song exposure or interaction between song exposure and age on spine density suggests that the late isolates' extended capacity to learn song is not accompanied by a retention of elevated spine density. Also, when each dendritic interval was evaluated separately (Fig. 4), the pattern of spine loss between PHD65 and 120 was similar across groups. For each segment, 2-way ANOVA revealed a main effect of age (20–40 µm: $F[1,19]=7.55$, $p<.05$; 40–60 µm; $F[1,19]=16.18$, $p<.001$; 60–80 µm: $F[1,19]=14.53$, $p<.005$), but no effect of rearing ($p>.40$ for all segments), or interaction of age and rearing ($p>.50$ for all segments). Thus, the group differences in learning capacity after PHD65 were not accompanied by group differences in spine pruning.

4. Discussion

Selectionist models of learning during development postulate that initially over-abundant neural connections serve as a substrate for experience guided synaptic refinement. A potentially important corollary of this view is that sensitive periods for developmental plasticity may be constrained by the gradual paring of connections. Developmental patterns of synapse elimination in the avian song system are consistent with this hypothesis. Dendritic spine loss within the lMAN normally coincides with song learning, and is attenuated or delayed when early acoustic isolation extends the sensitive period for learning (Nixdorf-Bergweiler et al., 1995; Wallhausser-Franke et al., 1995). However, here we report that zebra finches allowed limited early exposure to song exhibit an apparently normal pattern of spine loss among lMAN neurons, yet still display an extended sensitive period for memorizing new songs. Specifically, if earlier exposure to song is restricted to the period before PHD30, birds are able to successfully imitate syllables from a novel tutor heard between PHD65 and 90, beyond the normal close of the sensitive period (Heinrich et al., 2003). Yet, the density of dendritic spines on lMAN neurons in these birds does not differ from that measured in normally reared birds at either PHD65 or 120. Evidently, extended learning does not require that earlier, elevated levels of spine density be retained, and thus a normal extent of lMAN spine loss between PHD25 and 65 is not sufficient to close the sensitive period for song learning.

Another important aspect of the present study is the finding that memorization of an external song model need not entail increased lMAN spine loss. That is, even during the period of extended learning, net spine loss in the lMAN was no greater in birds that were memorizing new song material (i.e., late isolates) than in control birds not memorizing new songs. We conclude from this that retaining an exuberance of synapses within the lMAN is not necessary for extended sensory acquisition. However, it could still be that in normally reared birds lMAN spine loss is a critical aspect of acquisition, and that extended learning exploits alternative mechanisms not involving net changes in spine number (e.g., changes in synaptic efficacy). Additionally, our measures do not capture possible group differences in the dynamics of synaptic redistribution during the period of learning. Thus, late sensory acquisition may still depend on retaining a capacity for dynamic synapse loss and stabilization. Recent work in the visual and somatosensory systems suggests that there are developmental and experience-driven changes in the rate of synapse turnover (Grutzendler, Kasthuri, & Gan, 2002; Trachtenberg et al., 2002). Thus, new spines may be continually added and lost even when static anatomical approaches do not detect a net change in spine number. The fact that our analysis of spine density does not distinguish between the extrinsic and intrinsic synapses on lMAN neurons also may be important in this context. The recurrent collaterals of lMAN neurons appear to selectively amplify/modulate responses presumably generated from afferent DLM projections (Rosen & Mooney, 2000). These two classes of synapses differ in their physiological response properties and glutamatergic receptor profiles (Boettiger & Doupe, 1998; Livingston & Mooney, 1997). Furthermore, the developmentally regulated ability to induce LTP at recurrent collaterals is accompanied by LTD of the thalamic inputs to lMAN (Boettiger & Doupe, 2001). Consequently, these two synaptic populations may be differentially affected by song exposure, and our ability to detect a correlation between spine density and the capacity for late learning within a single synaptic population could be masked by an opposite trend in the remaining synapses. It will be important to examine

whether more fine scale changes in lMAN synapse number or type, correlate with the sensitive period for song learning. And finally, another possible explanation for the similar patterns of spine loss observed in the lMAN is that this loss relates to sensorimotor learning. Both groups of birds substantially modified their songs between PHD65 and 120, culminating in reasonably complex song patterns. Thus, it could be that this aspect of motor learning entails spine loss regardless of whether birds are improvising, drawing on a stored song model, or actively imitating a social tutor.

While the present results effectively rule out lMAN spine loss as sufficient for defining the sensitive period for sensory acquisition, they leave unresolved important questions regarding the role of early experience in promoting this extensive synaptic pruning. It has been known for some time that rearing birds in isolation from conspecific song extends the sensitive period for acquisition (Aamodt et al., 1995; Eales, 1987; Price, 1979), and a previous report demonstrating that such isolation also retards the normal decline in lMAN spine density (Wallhausser-Franke et al., 1995) motivated our direct investigation of the relationship between spine loss and the timing of acquisition. At first glance, our results appear at odds with this previous report. That is, while we replicated the normal developmental decline in lMAN spine density reported by Nixdorf-Bergweiler et al. (1995), the magnitude of this decline was unaltered by the more modest isolation protocol employed here, and we thus were able to effectively separate the effects of deprivation on the extent of spine loss from those that allow extended learning. One could interpret this as a failure to replicate Wallhausser-Franke et al.'s earlier study which suggested that lMAN spine loss is dependent on early song exposure. But we do not believe that our results necessarily challenge this earlier conclusion, since there are important differences between the two studies. Our earliest estimate of spine density in isolated animals was taken at PHD65, while those of Wallhausser-Franke et al.'s were taken at PHD55. Thus, it is possible that lMAN spine density in isolates "catches up" to control values sometime between these two ages. Perhaps even more important are procedural differences in both the onset and duration of isolation. That is, our isolation protocol did not begin until PHD30, whereas Wallhausser-Franke et al. isolated birds by PHD4. Thus, an alternative interpretation of the current results is that the limited early social and/or song experience granted to our isolates may be sufficient to trigger processes that encourage a normal trajectory of lMAN spine loss, a possibility that may provide insight into the many different ways early song experience could impact neural development.

For instance, if lMAN spine loss is driven normally by the encoding and/or imitation of a song model, it may be that even brief early exposure to song is sufficient to initiate these processes. Sensory acquisition in zebra finches can begin as early as PHD25 (Eales, 1985). Furthermore, recent work suggests that exposure to father's song until only PHD30 is sufficient to selectively tune neurons within the lMAN, even though such tuning can be overwritten by later exposure to a novel tutor after PHD65 (Yazaki-Sugiyama & Mooney, 2002). It is possible that even beyond PHD30, patterns of activity initiated by earlier song exposure could replay intermittently, as is known to occur during sensorimotor learning (Dave & Margoliash, 2000; Dave, Yu, & Margoliash, 1998; Margoliash, 2001), leading to continued spine elimination. Additionally, if lMAN spine loss is associated with the sensorimotor phase of learning, any effect of brief early song exposure on this aspect of vocal learning may encourage normal patterns of pruning. In fact, unpublished observations from our lab suggest that by PHD65 the songs of late isolates lack the abnormalities associated with so called "isolate song" (Price, 1979) and both syllable morphology and song stereotypy appear similar to that of socially raised control birds.

It may also be that the developmental loss of spines on lMAN neurons represents a general, experience-sensitive feature of lMAN maturation, rather than a direct reflection of sensory acquisition or vocal practice. In this case, *complete* isolation from a conspecific tutor (as in Wallhausser-Franke et al., 1995) could impair or delay this developmental change in ways that are overcome by the more modest isolation paradigm used in the present study. In other words, early auditory, visual, and social contact with a tutor might be needed to trigger changes in neural and endocrine function that permit, rather than instruct synaptic loss within the lMAN. An example is illustrated by recent work indicating that song exposure until PHD30 is sufficient to trigger an important maturational change in the subunit composition of NMDA receptors within the lMAN, without terminating the sensitive period for acquisition (Heinrich et al., 2003). The change in receptor structure and function, which could be critical for promoting synaptic competition and elimination, *is* delayed when isolation is begun earlier (Livingston, White, & Mooney, 2000; Singh, Basham, Nordeen, & Nordeen, 2000). Thus, complete isolation from conspecific song could delay spine loss in the lMAN, not because learning has been prevented, but because maturation of a contributing receptor system is delayed. In fact, the effects of complete isolation on both spine loss and NMDAR maturation could be secondary to endocrine disruption. Early isolation reduces testosterone (T) levels (Livingston et al., 2000), and brief exposure to T, even without early song exposure, is sufficient to elicit the change in lMAN NMDAR composition and physiology (Heinrich et al., 2003; White & Mooney, 2000). These examples illustrate a danger of using standard early deprivation procedures to identify neural substrates of learning and/or sensitive period plasticity. That is, only by beginning isolation after some period of

normal social rearing are we able to dissociate the time-course of these synaptic changes from age-related changes in the capacity for vocal learning.

It is widely believed that sensitive periods for plasticity and learning are constrained by the developmental loss of molecular and/or anatomical features that favor experience-driven synaptic change. Since the timing of sensitive periods can be manipulated by early experience, a common strategy for identifying neural substrates has been to focus on developmental changes that can be influenced similarly by early experience (Crair & Malenka, 1995; Fagiolini & Hensch, 2000; Isaac, Crair, Nicoll, & Malenka, 1997; Kleinschmidt, Bear, & Singer, 1987; Lee & Nedivi, 2002; Quinlan, Philpot, Huganir, & Bear, 1999; Shi, Townsend, & Constantine-Paton, 2000). For instance, recent studies have similarly dissociated experience-driven developmental changes in NMDAR composition and/or physiology from sensitive periods for plasticity (Fagiolini et al., 2003; Heinrich et al., 2003; Livingston et al., 2000; Lu, Gonzalez, & Crair, 2001). There remain numerous molecular and anatomical changes that occur within the developing song system during the sensitive period, and several of them respond to rearing manipulations that also extend the sensitive period. For example, expression of protein kinase C (PKC), an enzyme associated with synaptic plasticity and early aspects of memory formation in mammals (Micheau & Riedel, 1999), transiently increases in the RA (lMAN's target nucleus in the vocal motor pathway) during the sensitive period for song learning. However, when young birds are deprived of song experience by either early deafening or isolation, PKC expression is retarded (Sakaguchi & Yamaguchi, 1997). Also, the projection from lMAN to RA becomes spatially segregated early during song development, unlike other anterior forebrain pathways that appear topographically organized by approximately PHD20 (Iyengar, Viswanathan, & Bottjer, 1999). Since this pathway is well situated to use auditory information to shape motor production, it has been hypothesized to be critical in template comparison/error generation. Moreover, the development of this topographical organization is sensitive to auditory input/feedback, as it is delayed in young deafened males (Iyengar & Bottjer, 2002b). Focusing on features of song system development that are song experience dependent will remain a powerful initial strategy for identifying events that represent or constrain learning. However, the present results point out the need to employ rearing manipulations that impact the time-course of learning without grossly compromising the system's general maturation.

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