PLOS ONE

RESEARCH ARTICLE

Unilateral vocal nerve resection alters neurogenesis in the avian song system in a region-specific manner

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Citation: Aronowitz JV, Perez A, O'Brien C, Aziz S, Rodriguez E, Wasner K, et al. (2021) Unilateral vocal nerve resection alters neurogenesis in the avian song system in a region-specific manner. PLoS ONE 16(8): e0256709. https://doi.org/10.1371/journal.pone.0256709

Editor: Brenton G. Cooper, Texas Christian University, UNITED STATES

Received: March 20, 2021

Accepted: August 12, 2021

Published: August 31, 2021

Data Availability Statement: All data have been made public on Open Science Framework (OSF) data repository, link: osf.io/486ez.

Funding: CLP: TRADA42613, PSCREG401131, PSC-CUNY Research Award, https://www.rfcuny.org/gp/welcome.aspx CLP: NS063182, National Institute of Neurological Disorders and Stroke, National Institutes of Health, https://www.nih.gov/ DG: T34 GM070387, National Institute of General Medical Sciences, National Institutes of Health, https://www.nih.gov/ ER: T34 GM070387, National

Abstract

New neurons born in the adult brain undergo a critical period soon after migration to their site of incorporation. During this time, the behavior of the animal may influence the survival or culling of these cells. In the songbird song system, earlier work suggested that adult-born neurons may be retained in the song motor pathway nucleus HVC with respect to motor progression toward a target song during juvenile song learning, seasonal song restructuring, and experimentally manipulated song variability. However, it is not known whether the quality of song per se, without progressive improvement, may also influence new neuron survival. To test this idea, we experimentally altered song acoustic structure by unilateral denervation of the syrinx, causing a poor quality song. We found no effect of aberrant song on numbers of new neurons in HVC, suggesting that song quality does not influence new neuron culling in this region. However, aberrant song resulted in the loss of left-side dominance in new neurons in the auditory region caudomedial nidopallium (NCM), and a bilateral decrease in new neurons in the basal ganglia nucleus Area X. Thus new neuron culling may be influenced by behavioral feedback in accordance with the function of new neurons within that region. We propose that studying the effects of singing behaviors on new neurons across multiple brain regions that differentially subserve singing may give rise to general rules underlying the regulation of new neuron survival across taxa and brain regions more broadly.

Introduction

New cells that are produced postnatally generally have predetermined lifespans, varying by tissue type, and largely independent of the experience of the cell. In the healthy adult human, epithelial cells survive less than 2 weeks [1] white blood cells live for about 13–20 days, red blood

Institute of General Medical Sciences, National Institutes of Health, https://www.nih.gov/.

Competing interests: The authors have declared that no competing interests exist.

cells survive 100–120 days [2], and liver hepatocytes turnover about every 200–300 days [3,4]. On the other hand, across species and brain regions, lifespans of neurons born in the postnatal brain are highly variable. More interestingly, lifespans differ not only by neuron type, but are dramatically influenced by the cell's activity and experience, setting them apart in this way from other continually regenerating cell types [5–14].

In songbirds, new neurons are incorporated into regions of the song system that function in song learning, memory, perception, and song production (Fig 1A). These regions make well-defined and different contributions to song behaviors, therefore provide a system for comparing effects of behavioral feedback on new neuron survival across functionally distinct areas within the same individuals.

Behavioral factors affecting new neuron survival have been studied most systematically in HVC [15,16], a sensorimotor nucleus in which about half of the population of new neurons project to the premotor robust nucleus of the arcopallium (RA), becoming part of the motor pathway for song production (HVC_{RA}) [17–19]. In the canary, song production positively correlates with new neuron survival [20–22]. This association is mediated by BDNF and testosterone, both of which directly increase new neuron survival [16,23–27]. Housing conditions also impact the lifespan of both HVC_{RA} neurons and new neurons in the auditory region caudomedial nidopallium (NCM), with more new neurons seen in zebra finches or canaries housed in groups compared to singly housed birds [28–31]. Notably, Shevchouk et al., (2017) [29] showed that new neuron proliferation and the subsequent survival of new neurons in HVC can be independently affected by male- or female-paired housing. Numerous behaviors may underlie this effect, including increased singing and increased exposure to songs. NCM affects HVC activity indirectly [32] and perhaps is a source of auditory input into the song system. In addition to HVC and NCM, the basal ganglia nucleus Area X also continues to receive new neurons throughout adulthood and processes song-related sensory feedback [33]. Area X receives projections from a population of non-neurogenic HVC neurons (HVC_X) and is part of the anterior forebrain pathway necessary for juvenile song learning [34,35] and adult song plasticity [36–38]. Behavioral factors that may influence new neuron numbers in Area X have not yet been investigated. Given the functional and anatomical connections between HVC, NCM and Area X, we explored effects of behavioral feedback on new neurons across these regions.

During embryonic brain development, neuronal activity may contribute to cell survival, whereas lack of activity increases culling, putatively improving network efficiency. Perhaps a similar process continues in adulthood in regions that receive new neurons, such that pathway activity influences the survival of newly incorporated neurons, improving circuit function [39]. This idea is the basis for a model in which new HVC neurons audition for a spot in the song production pathway and get the part when their contribution improves song performance [15,40]. This model considers not only the amount of songs produced, but also the ongoing quality of the song. In this way, accurate progression in a goal-directed behavior (accurate song production) interacts with number of iterations of songs produced in promoting new neuron retention [15,41].

Consistent with this idea, experimentally altered song structure by reversible paralysis of the syringeal muscles resulted in a positive correlation between numbers of new neurons in HVC and song recovery [42]. However, in this work song recovery was confounded with song quality. Higher quality songs improved more, and more quickly, thus had higher values of recovery [42]. Therefore, either song quality *per se* (regardless of song improvement), or song recovery (requiring improvement) may have influenced new neuron survival.

To pull apart these confounded variables, here we tested whether song quality without recovery impacts new neuron survival. To do this, we produced a stable, irreversible, aberrant

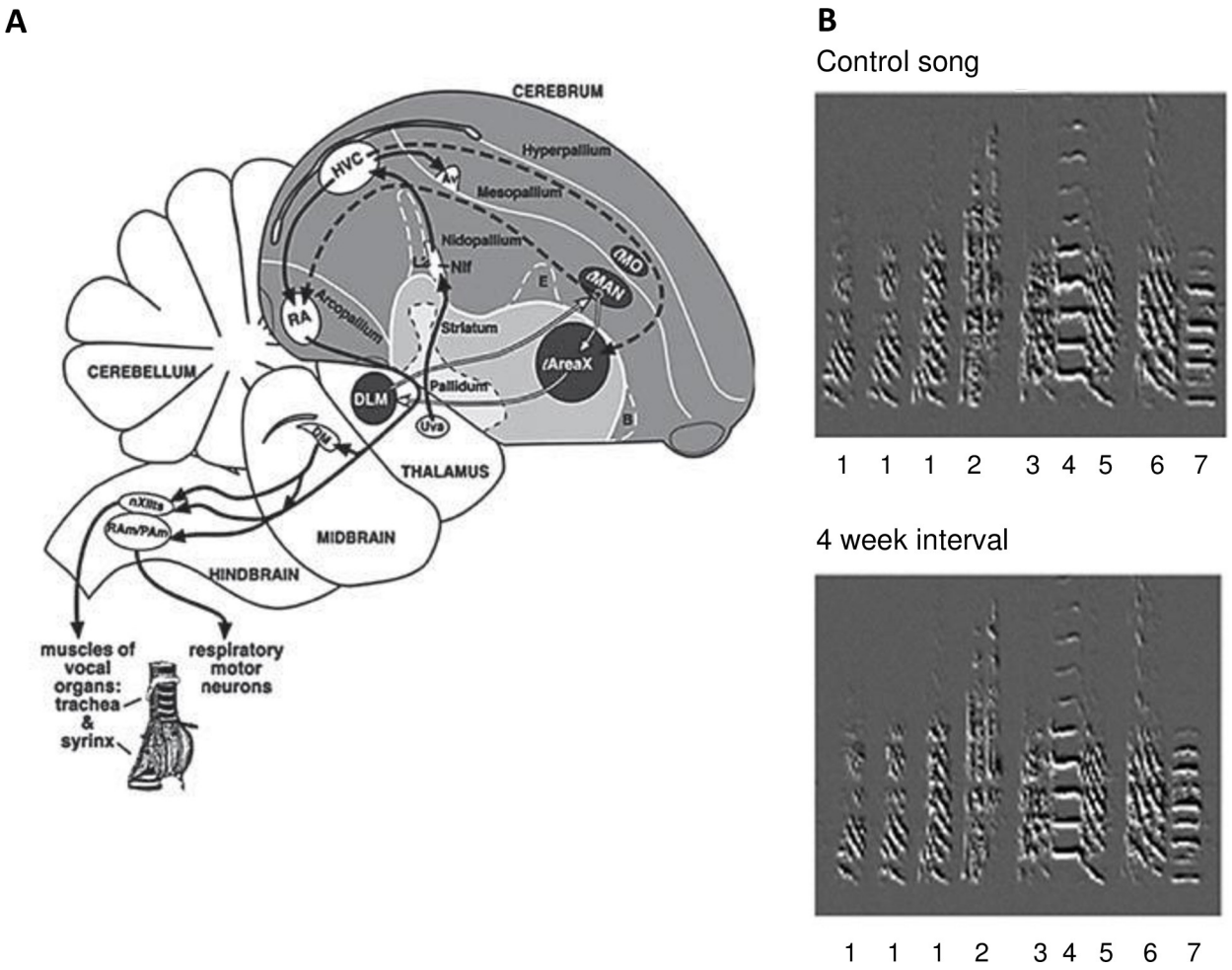

Fig 1. Song system and song stability. A. Diagram of the song system in a sagittal section, showing the vocal motor pathway (black solid lines) and the anterior forebrain pathway (dashed and white lines). White lines show the feedback loop between the striatum, thalamus, nidopallium, and projection back to the striatum. AV = Nucleus avalanche; B = nucleus basorostralis; DM = dorsal medial nucleus; DLM = dorsal lateral nucleus of the medial thalamus; E = entopallium; LMAN = lateral magnocellular nucleus of the anterior nidopallium; LMO = lateral oval nucleus of the mesopallium; Nif = interfacial nucleus of the nidopallium; PAm = para-ambiguus; Ram = nucleus retroambiguus; Uva, nucleus uvaeformis; nXIIts = tracheosyringeal portion of the hypoglossal nucleus. Reproduced from Pytte et al. (2011) [42]; syrinx modified from Goller and Suthers (1996) [62]. **B.** Spectral derivatives of a song motif generated with Sound Analysis Pro 2011 [56] showing example song stability over a 4 week interval in a young adult bird (5 mos old). Numbers identify different song elements based on qualitative assessment of acoustic structure, shown here to highlight similarity of structure in the two recordings; number "1" is a repeated introductory syllable. Modified from Pytte et al., (2012) [65].

https://doi.org/10.1371/journal.pone.0256709.g001

song by unilaterally denervating the syrinx in adult male zebra finches by sectioning either the left or right tracheosyringeal nerve (nXIIts, called here "TS"). This allowed us to determine directly whether song quality impacts neuronal culling or survival, and also to broaden our investigation to song regions outside of HVC.

We also speculated that distinct effects of feedback on new neurons may occur not only across brain regions, but also perhaps between hemispheres in a given brain region. More new neurons are added to left NCM than right NCM of adult zebra finches [43]. Moreover, the degree of this asymmetry was positively correlated with the quality of song learning and the strength of neuronal memory for songs [43]. Inter-hemisphere comparisons between other brain regions have not yet been conducted. Improving our understanding of how new neuron survival is regulated across brain regions and between hemispheres may inform strategies that

consider interconnected effects of a whole brain system in enhancing the survival of new neurons post brain injury or disease as well as in the healthy brain [44–46].

Materials and methods

Animals

All methods were approved by the Queens College Institutional Animal Care and Use Committee (protocol #165). Only male zebra finches sing; therefore, we used adult males (14 controls, 17 left TS-cut, 18 right TS-cut). The study was conducted with multiple cohorts, differing in targeted brain regions. Therefore, sample sizes differ depending on the brain region and new neuron marker (DCX+ or BrdU+) and are reported in the figure captions.

Birds were hatched in either the Queens College or Wesleyan University breeding colony and kept with their parents until 90 days of age. Thereafter, birds were group housed, with experimental and control birds housed together in group cages, with auditory and visual interaction with colony birds of both sexes throughout the study. Siblings from the same clutch (within a few days of age) were distributed across the 3 treatment groups when there were 3 or 6 males within a clutch. When equal distribution into treatment groups was not possible within a clutch, individuals were matched across treatment groups across same-aged clutches. Birds were between 4–11 months of age at the time of BrdU injections. We also matched nest of origin across groups, using sequential clutches matched across experimental cohorts. Birds were maintained on a 12:12 h light:dark schedule with food and water available *ad libitum*.

Song recording and analysis

Song changes after unilateral syringeal denervation have been well documented [47–49]. It is also known that the fine structure of adult song is highly, although not exactly, stereotyped over months, and more so over a 1 week interval as was used in the present study [Fig 1B, 50–53]. TS-cuts can trigger central changes over longer durations of denervation (> 1 week) [47,48,54,55] and we sought to avoid this effect by using a 1 week survival post TS-cut. Because the focus of our study was on the effects of denervation on new neurons and not on documenting the changes in song structure, we only conducted minimal recordings (controls = 5, left TS-cut = 5, right TS-cut = 4).

Adult male zebra finches were temporarily housed individually in sound attenuated chambers in order to record songs pre- and post-surgery. They were recorded 2–4 days prior to surgery, and again within post-operative days 2–4. After recording, the birds were returned to their home cages. Recordings were made using cardioid microphones (Earthworks SR20) and sound-activated Avisoft Recorder (Avisoft Bioacoustics). Zebra finches have a repertoire of a single song type that consists of a variable number of repetitions of an introductory note followed by repeating sequences of generally about 4 to 10 acoustic elements in a consistent order called a "motif." Recordings of songs were edited to a single motif using Raven sound analysis software (Cornell Lab of Ornithology).

We used two measures to document post-operative changes to the motif: (1) accuracy and (2) percentage of similarity (termed here "similarity") using Sound Analysis Pro 2011 [56]. The accuracy score indicates the fidelity of song elements by comparing 1 ms song segments between pre- and post-operative motifs. Similarity indicates sameness of song structure at longer intervals (~50 ms) (http://soundanalysispro.com/manual-1). We compared 10 pre- and post-operative motif pairs for each bird and used the means of each of these scores as a measure of postoperative change in song acoustic structure. Pre- and post-operative motif pairs were selected to be identical in song element number and sequence. We used a 10 x 10 comparison matrix such that every motif exemplar was compared with each of the 9 others and

redundant comparisons removed from the analysis. We then compared pre-to-post motif accuracy and similarity scores with numbers of new neurons. We also conducted linear regressions between the following measures of pre- to post-operative song change and new neurons counts: accuracy, similarity, difference in pitch, difference in frequency modulation (FM), difference in entropy, difference in goodness of pitch (a measure of periodicity) and difference in amplitude modulation (AM) (see definitions in http://soundanalysispro.com/manual-1). Singing rates were not calculated because the length of recordings were not sufficient in the already small sample size of birds that were recorded to accurately gauge individual singing rates or effects of treatment on song output.

Bromodeoxyuridine (BrdU) injections and tracheosyringeal denervation

All birds received intramuscular injections of 5-Bromo-2'-deoxyuridine (BrdU; 74 μg/g, pH 7.4, Sigma) 3x/day for three days to label mitotically active cells. Unilateral tracheosyringeal denervation surgeries were performed over 2 days, 21–22 days after the last BrdU injection, such that new neurons were 21–24 days old when song feedback was altered. This time frame was selected in order to ensure new neurons had migrated into the three regions of interest prior to nerve resection.

Birds were anesthetized with either a mixture of ketamine and xylazine (0.03–0.05 mg/g and 0.06 mg/g, respectively), or isoflurane in air (3%, Henry Schein). There were no differences in mean densities of new neurons in HVC, Area X, or NCM (hemispheres combined) between animals anesthetized with either mechanism (2 factor ANOVA, repeated measure on brain region, controls only: $F = 0.12$, $p = 0.733$, all treatments combined: $F = 0.08$, $p = 0.778$).

Using a surgical microscope (Zeiss Universal S3B), a 3–4 mm rostro-caudal incision was made < 1 mm lateral to the ventral midline to expose the tracheosyringeal nerve. A 2–4 mm section of either the left or right tracheosyringeal nerve was resected to prevent regrowth following surgery. Sham birds received all surgical manipulations except nerve cuts.

Histology

Birds experienced altered song for seven days and then were overdosed with sodium pentobarbital (Euthasol) and transcardially perfused with 0.1 M phosphate buffered saline (PBS, pH 7.4) followed by 4% paraformaldehyde (PFA; Sigma-Aldrich; pH 7.4). TS nerve sections were confirmed following the perfusion. Brains were post-fixed for 1 h in 4% PFA, rinsed in PBS for 3 h and embedded in polyethylene glycol (MW = 1500; Polysciences). Six-μm sagittal sections were cut on a rotary microtome. The brain was oriented with the midline parallel to the blade edge. The first complete section through the telencephalon was saved and subsequently every sixth section of tissue was mounted onto Superfrost Plus + slides (VWR). All series were stored at -20˚C prior to processing using immunohistochemistry (IHC).

DCX immunohistochemistry

Sections were brought to room temperature in tris buffered saline (TBS), followed by a 10-min wash in fresh TBS. Sections were then incubated for 30-min in a H_2O_2 solution (97% TBS, 1% methanol, 2% of 3% retail H_2O_2) to eliminate endogenous peroxidases. After three 5-min TBS rinses, non-specific binding was blocked with 5% normal horse serum (Jackson ImmunoResearch Laboratories Inc.) and 0.5% Triton X-100 (Sigma-Aldrich) in TBS for 30-min at room temperature. This was followed by exposure to anti-DCX antibody (goat polyclonal IgG, Santa Cruz Biotechnology; sc-8066, 1:150; or rabbit polyclonal IgG, Abcam; ab18723, 1:1000) in the same blocking buffer overnight at 4˚C. After three 5-min TBS rinses, sections were incubated for 3 h in biotinylated horse anti-goat (1:200, Vector Labs) or biotinylated goat anti-rabbit

secondary antibody (1:200, Vector Labs) in TBS, rinsed again, and exposed for 1 h to an avidin-biotin complex (Vector Labs). Sections were rinsed in TBS and then reacted in a solution of 0.04% of 3,3'-diaminobenzidine tetrahydrochloride (DAB, Vector Labs) with nickel until the tissue changed color (~3–10 min). Following three 5-min TBS rinses, sections were dehydrated in ethanols, delipidized in xylenes, and cover slipped with Krystalon (Millipore-Sigma).

BrdU/Hu immunohistochemistry

Sections were brought to room temperature in 0.1 M phosphate buffer (PB; pH 7.4), then exposed to citrate buffer (pH 5.6–6.0) at 90–95˚C for 10 min, followed by a 5-min PB wash (37˚C), 3 min in a solution of 0.28% pepsin in 400 ml 0.1 M HCl at 37˚C, and three 5-min washes in PB at room temperature. Non-specific binding was blocked with 3% normal donkey serum (Jackson Labs) and 0.5% Triton X-100 in PB ("block") for 1 h at room temperature, followed by 24–48 h exposure to sheep anti-BrdU (1:239, Capralogics) in block at 4˚C. Sections were again rinsed in PB and then incubated overnight in biotinylated donkey anti-sheep IgG in PB (1:200, Vector Labs), followed by overnight incubation in streptavidin-conjugated Alexa 488 in PB (1:800, ThermoFisher Scientific) for visualization of BrdU. The next day, sections were washed with PB, blocked for one hour, and incubated overnight in mouse anti-Hu primary antibody at 4˚C (1:200 in 3% block; Invitrogen). After three 5-min PB rinses at room temperature, tissue was exposed to donkey anti-mouse IgG conjugated to Cy-3 in PB (1:80; EMD Millipore) for 1 h for visualization of Hu. Finally, sections were washed, dehydrated with ethanols, delipidized with xylenes, and cover slipped with Krystalon (Millipore-Sigma).

Microscopy

Data were collected without knowledge of bird identity, treatment, or brain hemisphere. Area measurements and cell counts for all regions were performed using a computer-yoked microscope and mapping software (Olympus BX51; Lucivid LED microprojection, Neurolucida, Microbrightfield, Inc.). The boundaries of regions of interest were traced in 6–12 sections per hemisphere per bird. Boundaries for HVC were established with dark-field optics based on neuropil density and contrast. Area X is found ventral and anterior to the densely reflective oval shaped lateral magnocellular nucleus of anterior nidopallium (lMAN) and is demarcated by a dense haze of terminals and small cells. Area X and lMAN are separated by lamina pallio-subpallialis (LPS) which is visible in darkfield as less reflective than the nuclei.

NCM was identified as in [57]. DCX^+ cells were visualized with bright field light microscopy (Fig 2A and 2B). $BrdU^+/Hu^+$ cells were visualized using fluorescein isothiocyanate (FITC) and rhodamine filters and a dual FITC/rhodamine filter (Fig 2C–2E). New neurons per square millimeter was calculated by dividing the numbers of labeled cells by the total area sampled.

Statistical analysis

Data are presented as means and SEMs unless otherwise specified. Analyses were performed using one-way ANOVAs, two-factor ANOVAs with repeated measures on hemisphere, and two-tailed *t*-tests for independent samples. Tukey's HSD post-hoc tests were used following significant effects determined by ANOVA. Following significant mixed ANOVAs, we performed one-way ANOVAs between groups within individual hemispheres and then Holm's procedure with adjusted p values for multiple tests. Pearson correlations were computed between cell counts across brain regions or hemispheres. The lateralization index (LI) indicates the relative number of new neurons between hemispheres, normalized for the bird's mean number of new neurons per area sampled in both hemispheres (as in Tsoi et al., 2014) [24].

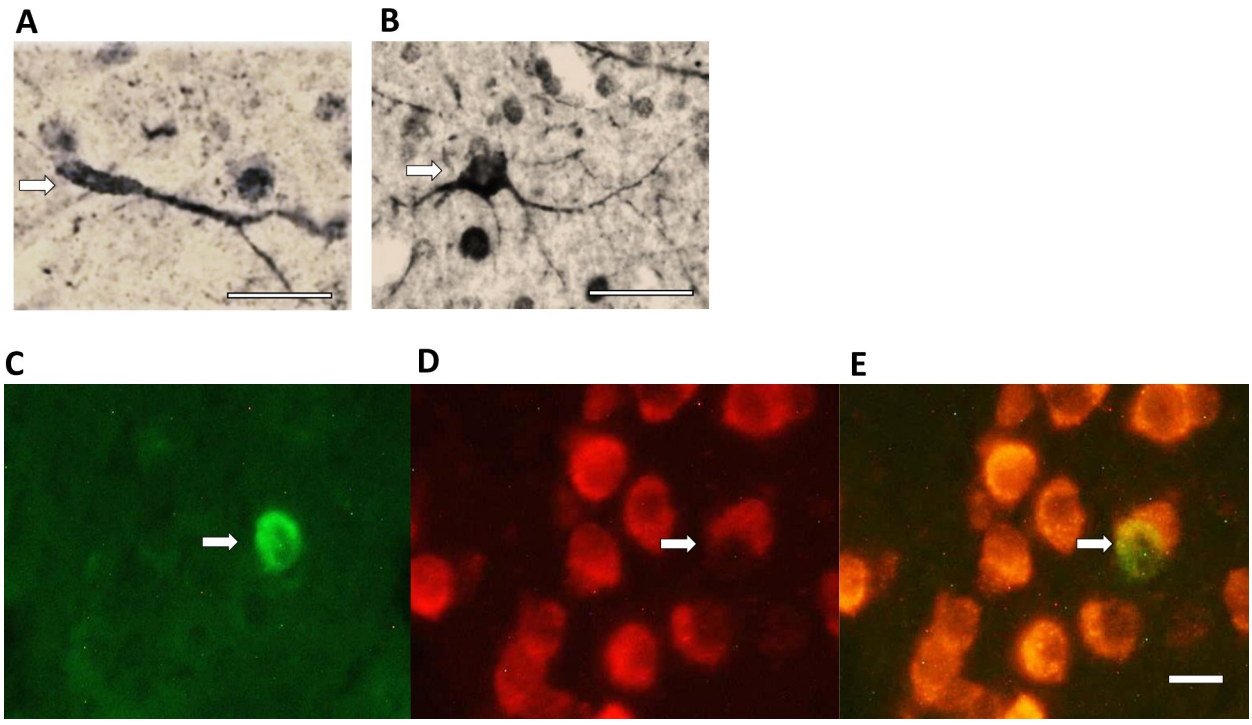

Fig 2. Photomicrographs of doublecortin-positive neurons in HVC. A. Fusiform, approximately 1–2 weeks old as indicated by its thin, elongated soma and unipolarity and **B.** Multipolar, approximately 2–3 weeks old as indicated by large, round soma and multiple processes (cell age estimates follow Balthazart, et al., 2008) [59]. **C.** Fluorescent markers used to identify 28–30 d old neurons in HVC, $BrdU^+$ nucleus visualized with FITC filter. **D.** Hu^+ neurons seen under rhodamine filter. **E.** $BrdU^+/Hu^+$ neuron showing colocalization of the two markers (rhodamine/FITC filter) in the same field of view. Scale bars A, B = 25 μm; C, D = 10 μm.

https://doi.org/10.1371/journal.pone.0256709.g002

Higher positive values indicate more new neurons in the left hemisphere relative to the right, an outcome of arbitrarily subtracting right from left in the equation:

$$\frac{\text{Left neurons/mm}^2 - \text{Right neurons/mm}^2}{(\text{Left neurons} + \text{Right neurons})/(\text{Left area sampled} + \text{Right area sampled})}$$

Results

Song analysis

Birds that underwent either a left or right TS cut (Fig 3A) had significantly lower accuracy scores, a measure of song change post-denervation, than did controls measured over the same time interval (One-way ANOVA, df = 2, F = 35.28, $p < 0.0001$, Fig 3B). Both left TS-cut and right TS-cut scores were lower than those of controls (Tukey's post-Hoc test, $p < 0.01$) and there was no difference between the two denervated groups (Tukey's post-Hoc test $p > 0.05$). There was also a significant difference in pre-to-postoperative similarity scores among groups (one way ANOVA, df = 2, F = 4.24, p = 0.043, Fig 3C). Similarity scores of the right TS-cut birds were significantly lower than those of control birds (Tukey's post-Hoc test $p < 0.05$). Scores of the left TS-cut group did not differ significantly from either the right TS-cut or control birds. Likewise, changes in individual song features were not significantly different between right or left TS-cut groups ($p > 0.05$ for all: pitch, FM, entropy, goodness of pitch, AM; all data are available at: osf.io/486ez).

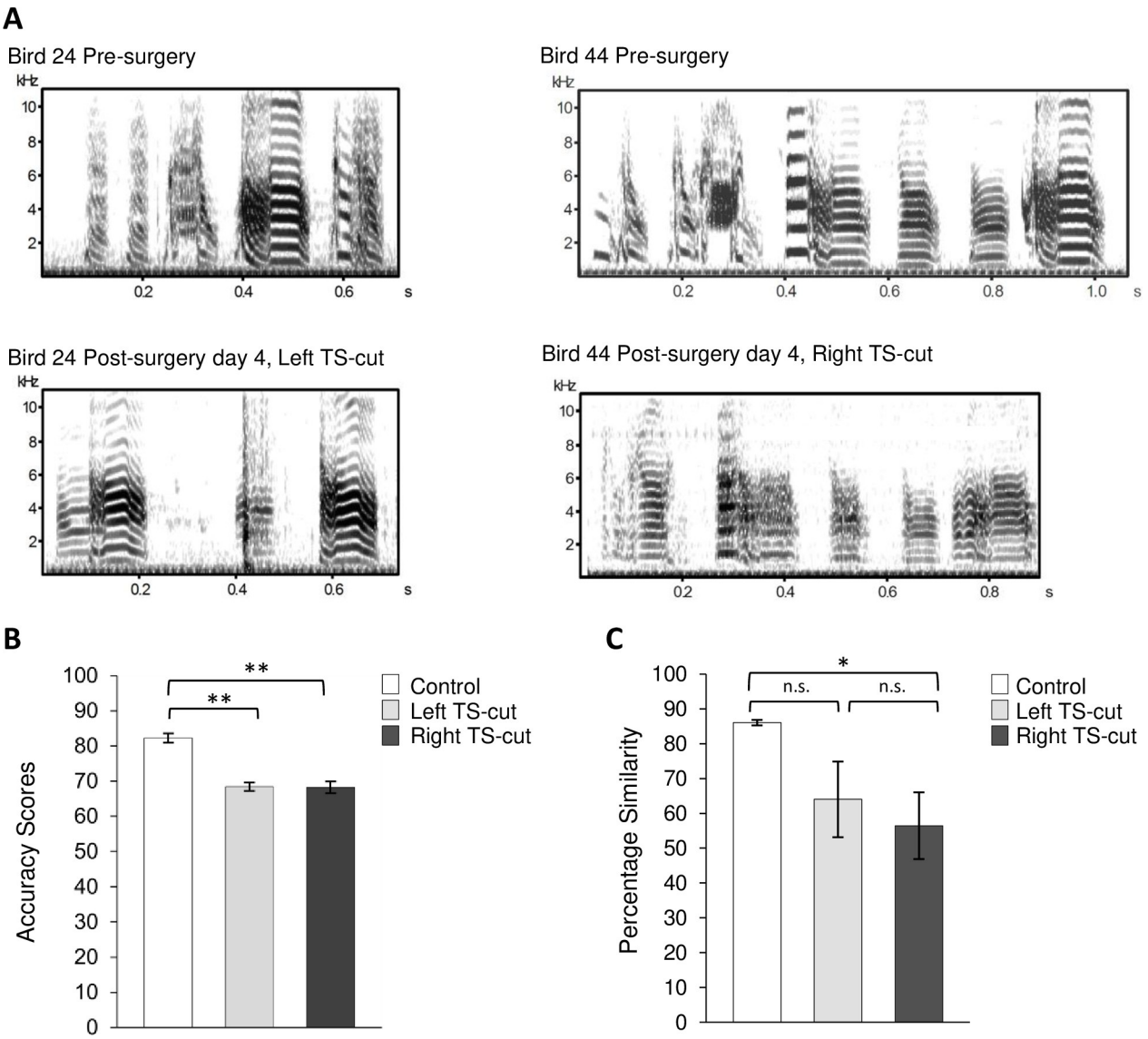

Fig 3. Effects of TS-cuts on song structure. A. Pre-operative spectrograms (top row) and post-operative spectrograms of left TS-cut (left) and right TS-cut (right) showing song changes after unilateral TS-cuts, recorded 4 days post-surgery. Changes in acoustic structure were highly varied across individuals. Spectrograms were generated with Sound Analysis Pro 2011 [56]. **B.** Birds that underwent left (n = 5) or right (n = 4) TS-cut had accuracy scores that were significantly lower than those of controls (n = 5), demonstrating that unilateral tracheosyringeal nerve cut impacted the quality of song production. **C.** There was a significant overall difference in the pre- to post- similarity scores among groups, and right TS-cut birds differed from controls. Control (n = 5), left TS-cut (n = 5), and right TS-cut birds (n = 4). Shown are means ± standard errors, * = $p < 0.05$, ** = $p < 0.01$, n.s. = $p > 0.05$.

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TS-cuts did not impact new neurons in HVC

There was no effect of treatment (df = 2, F = 0.75, p = 0.49), hemisphere (F = 0.04, p = 0.84), or interaction (F = 0.29, p = 0.75) on numbers of $BrdU^+/Hu^+$ cells in HVC that were 28–30 days old (two-factor ANOVA, repeated measures on hemisphere, Fig 4A). We also quantified DCX+ cells, a marker for immature neurons that is expressed for 2–3 weeks after mitosis in mammals [58] and was calculated to be expressed in newborn neurons for a similar time, approximately 20 days, in the canary [59]. The morphology of DCX+ neurons can also be used to estimate neuronal age in this time frame with fusiform, bipolar cells presumably

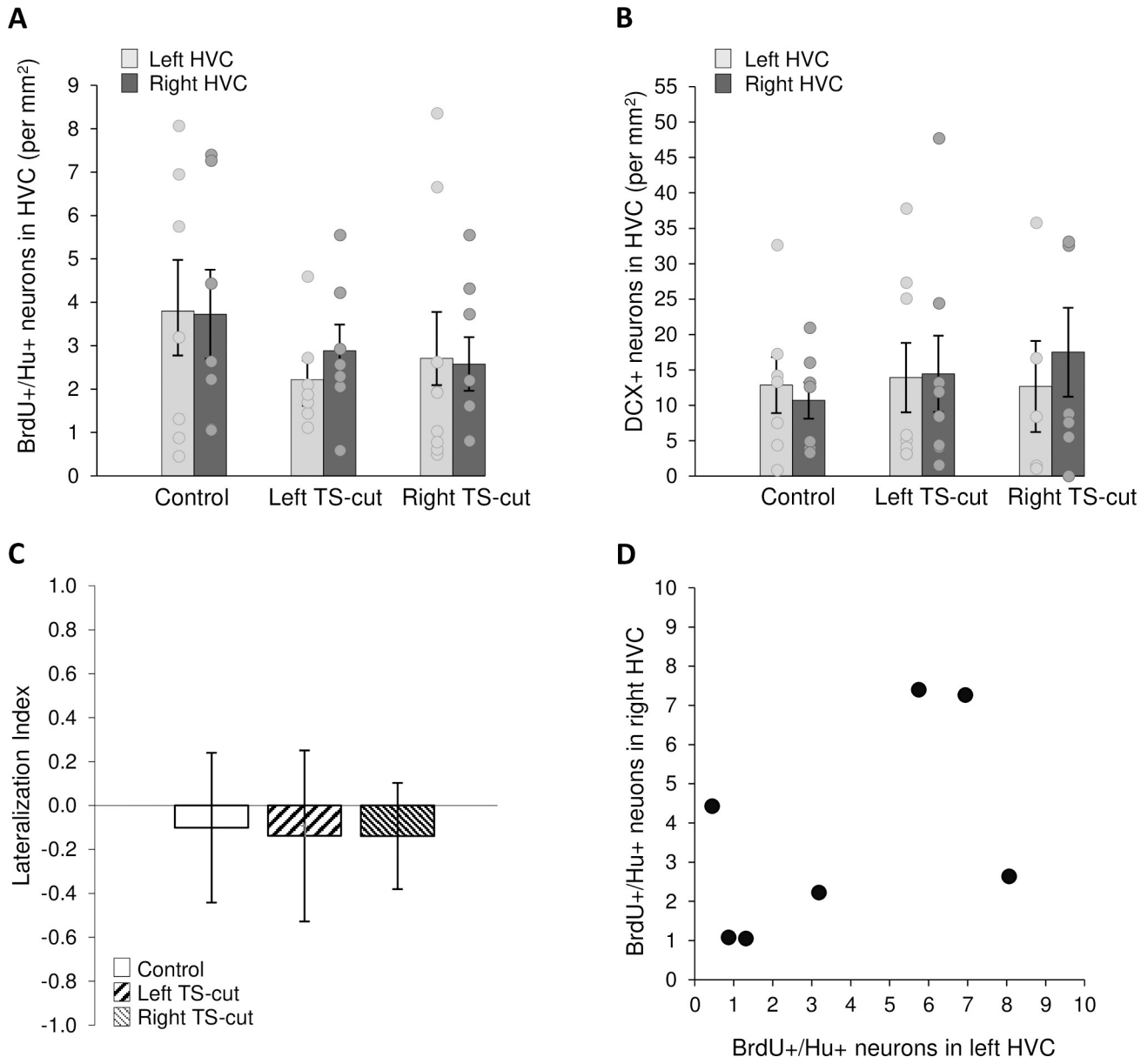

Fig 4. Numbers of new neurons in HVC as a function of hemisphere and experimental condition. A. Neither left (n = 7) nor right (n = 8) TS-cut affected the number of 28–30 d old BrdU+/Hu+ neurons in HVC compared to controls (n = 7). **B.** Neither left (n = 8) nor right (n = 5) TS-cut affected the number of ~1–3 week old DCX+ neurons in HVC compared with controls (n = 7). **C.** There were no differences in the Lateralization Index of BrdU +/Hu+ cells among treatment groups. **D.** There was no correlation between numbers of BrdU+/Hu+ cells between the left and right HVC in control birds.

https://doi.org/10.1371/journal.pone.0256709.g004

with a migratory morphology being younger, and round, multipolar cells being older (Fig 2A and 2B; Balthazart et al., 2008 [59]). We did not find treatment or hemisphere differences when fusiform and multipolar cell counts were analyzed independently ($p > 0.05$ for all); therefore, we combined DCX+ cells. We found no effect of treatment ($df = 2$, $F = 0.16$, $p = 0.85$), hemisphere ($df = 1$, $F = 0.05$, $p = 0.83$), or treatment by hemisphere interaction ($df = 2$, $F = 0.39$, $p = 0.68$) on numbers of DCX+ cells in HVC (two-factor ANOVA, repeated measures on hemisphere, Fig 4B). We also found no difference in new neuron

numbers (neither BrdU+/Hu+, nor DCX+) between hemispheres ipsilateral and contralateral to the TS nerve cut.

Unilateral TS-cuts had no effect on the lateralization index of BrdU+/Hu+ or DCX+ neurons in HVC (one-way ANOVA, df = 2, F < 0.01, p = 1.0; df = 2, F = 1.19, p = 0.33, respectively, Fig 4C). We also found no relationship between the numbers BrdU+/Hu+ neurons (Fig 4D) or DCX+ cells (not shown) in left HVC and right HVC of control animals (r^2 = 0.53, t = 1.38, p = 0.23; r^2 = 0.66, t = 1.1, p = 0.32, respectively).

Because TS-cuts did not affect numbers of new neurons in HVC, we were not surprised that there were no significant correlations between numbers of new neurons and postoperative changes in song acoustic structure. We examined both young neurons expressing DCX and ~1 month old neurons labeled with BrdU expressing Hu. There were no correlations between new neurons in HVC either ipsilateral or contralateral to the TS-cut and any of our acoustic difference scores measured between the pre- and post-operative songs (similarity, accuracy, pitch, FM, entropy, pitch goodness, or AM; left TS cut n = 5 and right TS cut n = 4, groups combined for regression). There were also no correlations between new neurons in left, right, or combined hemispheres of HVC and these acoustic difference scores.

TS-cuts altered hemispheric lateralization of new neurons in NCM

There was not a main effect of treatment (F = 0.22, p = 0.80), hemisphere (F = 1.29, p = 0.27), nor treatment by hemisphere interaction (F = 0.83, p = 0.45) on numbers of BrdU+/Hu+ neurons in NCM (Fig 5A). As in HVC, we found no difference among treatments or hemispheres when DCX+ fusiform and multipolar cells were analyzed separately; therefore, we combined these categories. There was also no main effect of treatment (F = 0.48, p = 0.63), hemisphere (F = 2.28, p = 0.15), or interaction on DCX+ cells in NCM (F = 0.14, p = 0.87, two-factor ANOVA, repeated measures on hemisphere, Fig 5B).

Earlier work showed that in unmanipulated controls, there were more new neurons in the left NCM than the right, and that this asymmetry was lost after a unilateral tracheosyringeal nerve cut [43]. Here we also found more $BrdU^+/Hu^+$ neurons in the left than the right NCM in controls (paired sample t-test, t (11) = 2.26, p = 0.045; Fig 5A). As in Tsoi et al., (2014) [43] hemispheric asymmetry was not seen in either left TS-cut or right TS-cut groups (paired t-tests between hemispheres within groups, p = 0.67, p = 0.74, respectively). Consistent with this pattern, we found a significant difference in lateralization indices of BrdU+/Hu+ among the three treatment groups (df = 2, F = 3.92, p = 0.03). Interestingly, there was a greater effect of the right TS-cut than the left: the lateralization index of the right TS-cut group differed from controls (HSD, p <0.05) whereas the lateralization index of the left TS-cut group did not differ from that of controls or the right TS-cut group (Fig 5C).

Unlike in HVC, the numbers of BrdU+/Hu+ cells in left and right NCM of control birds were positively correlated (r^2 = 0.69, t = 3.06, p = 0.01, Fig 5D) as in Tsoi et al. (2014) [43]. There were no correlations in numbers of new neurons between NCM hemispheres in either TS-cut group (r^2 = 0.058, p = 0.45, left TS cut; r^2 = 0.026, p = 0.63, right TS-cut. There were no correlations in numbers of DCX+ cells between NCM hemispheres in any group (p > 0.05 for all).

Because TS cuts decreased left-NCM dominance in ~30-day old neurons, we sought to identify acoustic parameters that correlated with the lateralization index. Side of TS cut did not affect the degree of change in acoustic feature (p > 0.05, comparing effects per feature between left- and right-TS groups). Therefore, we combined left and right TS cut groups for comparisons between degree of feature change and numbers of new neurons. The greater the post-operative change in pitch, the fewer ~30 day old neurons (BrdU/Hu+) relative to the

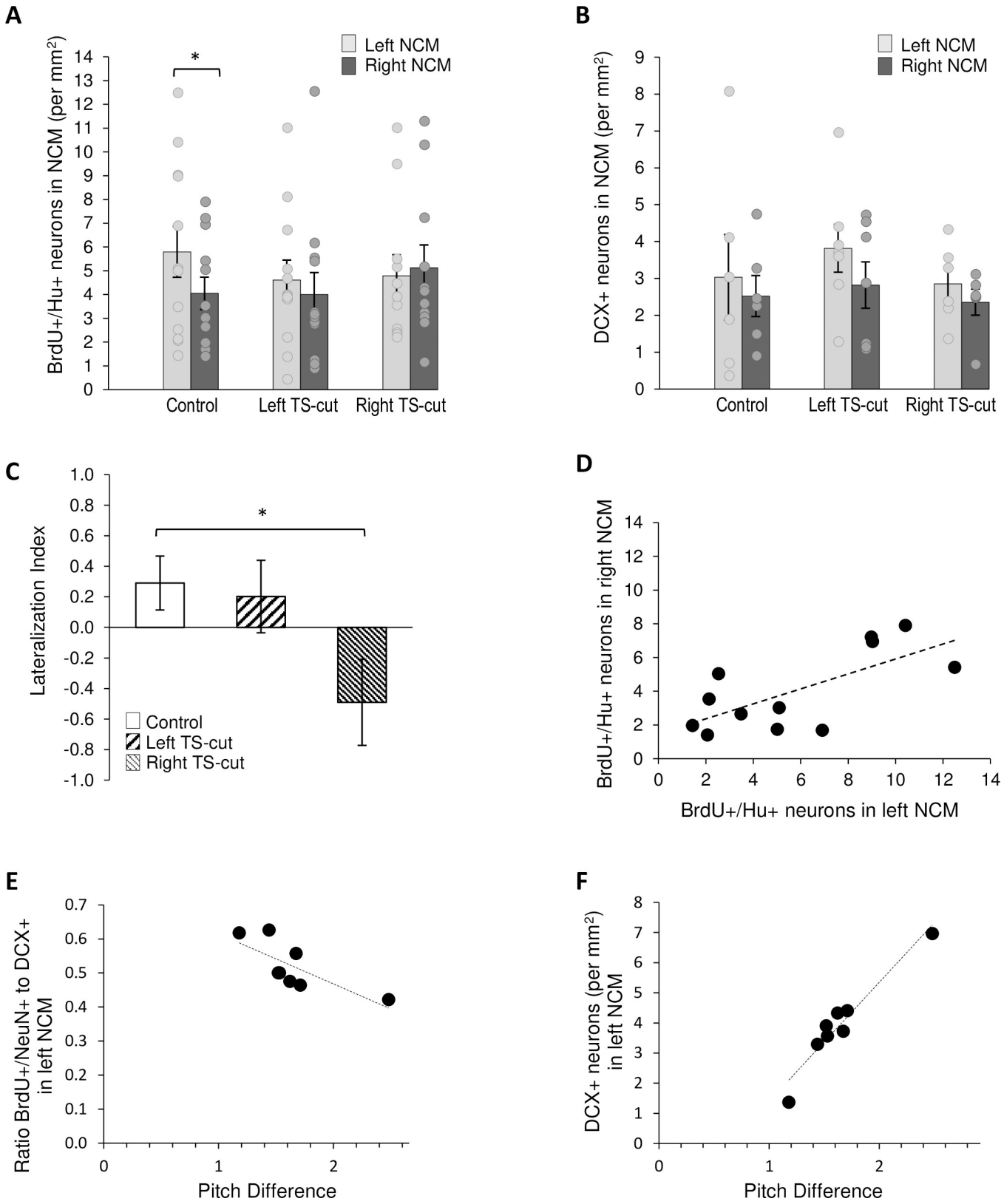

Fig 5. Numbers of new neurons in NCM as a function of hemisphere and experimental condition. A. Left (n = 12) and right (n = 11) TS-cuts had no effect on the overall number of 28–30 d old BrdU+/Hu+ neurons in NCM. Control birds (n = 12) had more BrdU+/Hu+ neurons in left NCM than the right and this

asymmetry was not seen in the left or right TS-cut group. **B.** Neither left (n = 7) nor right (n = 6) TS-cut differed from controls (n = 7) in numbers of DCX + neurons in NCM. **C.** The lateralization index of the right TS-cut group was significantly different from that of the controls; that of the left-TS cut group did not differ from controls or the right TS-cut group. **D.** There was a positive correlation between numbers of 28–30 d old neurons in left NCM and right NCM of control birds. **E.** We found a significant inverse correlation between the ratio of ~30 day old neurons (BrdU+/Hu+) to younger neurons (DCX+) and postoperative change in pitch (n = 4 left TS-cut and n = 4 right TS-cut with vocal recordings). The greater the change in pitch, the lower proportion of older neurons maintained out of the pool of younger neurons. Two data points are indistinguishable in the graph. **F.** There was a significant positive correlation between DCX+ neurons and the change in motif pitch ($r^2 = 0.925$, F = 73.926, p = 0.0001; n = 4 left TS-cut, n = 4 right TS-cut birds with vocal recordings) This correlation was still significant without the highest value DCX+ data point ($r^2 = 0.878$, F = 36.072, p = 0.002). * = $p < 0.05$.

https://doi.org/10.1371/journal.pone.0256709.g005

numbers of younger neurons (DCX+) in left NCM ($r^2 = 0.568$, F = 7.899, p = 0.031, Fig 5E; right NCM > 0.05, not shown). This ratio may be more intuitively thought of as the numbers of young (DCX+) cells that survive to day ~30 (BrdU+/Hu+).

We then looked at correlations between changes in acoustic features and DCX+ and BrdU +/Hu+ neurons individually. Change in pitch was positively correlated with numbers of young DCX+ neurons in the left NCM ($r^2 = 0.925$, F = 73.926, p = 0.0001, Fig 5F; right NCM > 0.05, not shown). Change in pitch did not correlate with numbers of BrdU+/Hu+ cells in either hemisphere ($p > 0.05$). Together, this suggests that the postoperative change in song pitch increased recruitment of new neurons in the left hemisphere, but not their survival. No one particular acoustic feature that we measured accounted for the postoperative loss of left hemisphere dominance in new neurons of the BrdU+/Hu+ cohort. No other measure of change in song was correlated with numbers of new neurons in left or right NCM.

TS-cuts decreased new neurons bilaterally in Area X

We found a significant difference in the number of BrdU+/Hu+ neurons in Area X among the three treatment groups (two factor repeated measures ANOVA, F = 7.47, p = 0.002, Fig 6A). There was no difference between hemispheres (F = 0.25, p = 0.62) and no interaction between treatment and hemisphere (F = 1.16, p = 0.33). Within the left hemispheres (one way ANOVA, df = 2, F = 3.31, p = 0.048), there were fewer BrdU+/Hu+ cells in both the left TS-cut and right TS-cut groups compared to controls (t = 2.05, p = 0.048; t = 2.39, p = 0.045, respectively). The same pattern was found within the right hemispheres of Area X (one way ANOVA, df = 2, F = 6.84, p = 0.003). Compared to the right Area X of controls, there were fewer BrdU+/Hu + cells in the right Area X of both the left TS-cut and right TS-cut groups (t = 3.58, p = 0.002; t = 2.65, p = 0.012, respectively).

As in the other regions, we combined all DCX+ cells after finding no difference between fusiform and round DCX+ cell types assessed separately across treatments. There was no main effect of treatment (2 factor mixed ANOVA, F = 0.07, p = 0.93), hemisphere (F = 1.48, p = 0.24), or interaction (F = 1.22, p = 0.32; Fig 6B) on the numbers of DCX+ neurons in Area X.

There was no significant difference among groups in the Lateralization Index of BrdU+/Hu + or DCX+ cells (one-way ANOVA, F = 0.2, p = 0.82; F = 0.20, p = 0.82, respectively, Fig 6C). There were no correlations between the numbers of BrdU+/Hu+ neurons in the left and right Area X of control animals ($r^2 = 0.30$, p = 0.35; Fig 6D). However, DCX+ cells were positively correlated between hemispheres in control birds ($r^2 = 0.92$, p = 0.016, Fig 6E) and in birds with a left side TS-cut ($r^2 = 0.85$, p = 0.03), but not in birds with a right side TS-cut ($r^2 = 0.67$, p = 0.27).

We found an inverse correlation between the degree of change in FM and the numbers of 28–30 day old neurons in Area X in the left hemisphere ($r^2 = 0.565$, F = 7.808, p = 0.031, Fig 6F) and both hemispheres combined ($r^2 = 0.581$, F = 8.329, p = 0.028, n = 4 left TS-cut and 4 right TS-cut). Despite the decrease in new neurons of TS-cut birds in each hemisphere, no other measure of change in song was correlated with neuron numbers in left or right Area X.

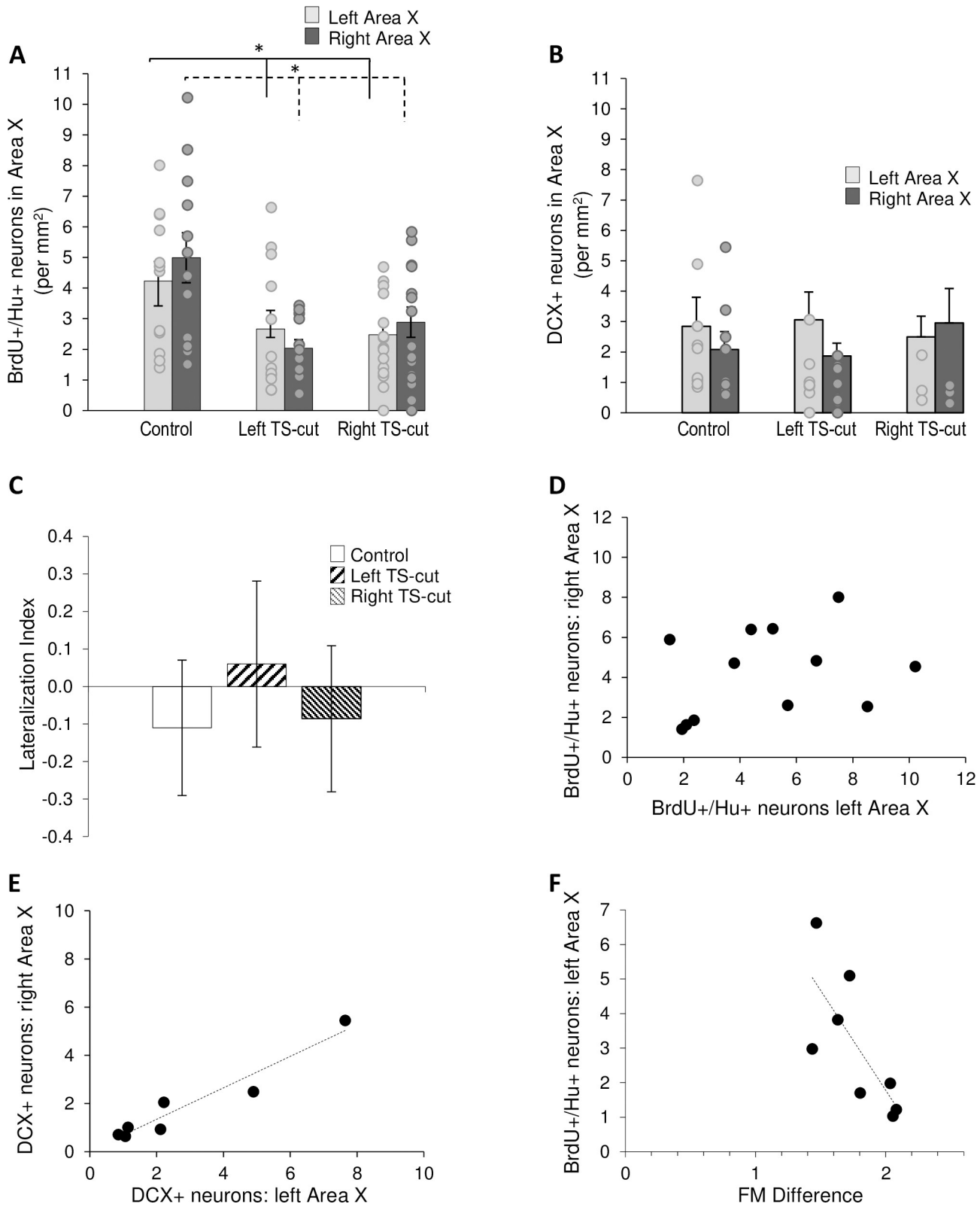

Fig 6. Numbers of new neurons in Area X as a function of hemisphere and experimental condition. A. Left (n = 12) and right (n = 14) TS-cuts significantly decreased the numbers of 28–30 day old neurons compared to controls (n = 12). Control birds had significantly more new neurons in the left hemisphere compared to the left hemispheres of the left and right TS-cut birds (solid lines). Similarly, control birds had significantly more new

neurons in the right hemisphere than did left TS-cut and right TS-cut birds (dashed lines). **B.** Neither left (n = 9) nor right (n = 8) TS-cut groups differed from controls (n = 7) in numbers of DCX+ neurons in Area X. **C.** Lateralization indices for BrdU+/Hu+ neurons in Area X. LIs did not differ between controls, left TS-cut, and right TS-cut conditions. **D.** There was no relationship between the numbers of BrdU+/Hu+ neurons in left versus right Area X in controls. **E.** We found a significant correlation between left and right Area X in numbers of DCX+ neurons in controls. **F.** The degree of change in song frequency modulation post-TS cut was significantly inversely correlated with numbers of BrdU/Hu+ neurons in left Area X (n = 4 left TS-cut and n = 4 right TS-cut birds with vocal recordings). * = $p < 0.05$.

https://doi.org/10.1371/journal.pone.0256709.g006

No correlations in numbers of new neurons between regions within hemispheres

We were also interested in whether new neuron numbers may be linked between functionally associated regions in control birds. However, we found no correlations in numbers of BrdU +/Hu+ cells between NCM and HVC or between HVC and Area X in either the left ($r^2 = 0.69$, $p = 0.34$; $r^2 = 0.89$, $p = 0.063$, respectively) or right ($r^2 = 0.69$, $p = 0.35$; $df = 5$, $r^2 = 0.49$, $p = 0.61$, respectively) hemispheres. There were no correlations between DCX+ cells within either hemisphere between NCM and HVC (left: $r^2 = 0.48$, $p = 0.340$; right: $r^2 = 0.47$, $p = 0.35$) or between HVC and Area X (left: $r^2 = 0.79$, $p = .063$; right: $r^2 = 0.23$, $p = 0.612$).

No correlations in numbers of new neurons and bird age

There were no correlations between bird age at the time of BrdU injections and numbers of new neurons in either the left, right, or combined HVC ($F = 0.009$, $r^2 = 0.001$, $p = 0.926$; $F = 0.002$, $r^2 < 0.001$, $p = 0.965$; $F = 0.191$, $r^2 = 0.027$, $p = 0.675$; respectively). Similarly, there were no correlations between bird age at the time of BrdU injections and numbers of new neurons in either the left, right, or combined NCM or Area X, ($p > 0.6$ for all).

Discussion

This is a broad-strokes study, serving as a first pass in understanding whether the quality of song structure affects new neuron survival and whether regulation of new neuron survival is independent across brain regions and hemispheres. We found that irreversible disruption to song structure by unilateral denervation of the syrinx in adult male zebra finches impacted the numbers of 28–30 day old neurons in a region and hemisphere-specific manner. Altered song feedback had no effect on numbers of one month old neurons in either hemisphere HVC, resulted in a loss of left-sided dominance of new neurons in NCM consistent with Tsoi et al. (2014) [43], and decreased neuron survival in both hemispheres of Area X. These effects were not present in younger neurons expressing DCX, perhaps due to a lack of functional connectivity in this cohort at the time of treatment. In earlier work, we posited that either song quality or song recovery influenced new neuron survival in HVC [42]. Here we suggest that song quality in itself does not influence new neuron recruitment (of DCX+ cells) or new neuron survival (of 28–30 day old BrdU+/Hu+ cells) in HVC. These negative results therefore lend support to the idea that either song recovery promotes new neuron survival in HVC or vice versa [42]. Larson et al. (2013) [14] showed that RA activity promotes new neuron survival in HVC in adult male Gambel's white crowned sparrows, using unilateral muscimol infusion into RA. Interestingly, the treatment also altered song structure, allowing an assessment of sensory factors that may impact new neuron survival. Consistent with our results, they found no correlation between the degree of song degradation and the number of new neurons in HVC either ipsi- or contralateral to the side of infusion. Together, these findings increase support for the idea that new neuron addition to HVC may be insensitive to song-related sensory feedback.

What does the TS nerve section do?

The aspects of the TS nerve cut that affected new neuron survival in Area X and lateralization in NCM are not known. In addition to changing the acoustic structure of vocalizations, TS-nerve sectioning also would alter putative somatosensory feedback from syringeal muscles [60,61] and potentially somatosensory feedback from the air sac receptors if bronchial resistance was changed by syringeal denervation [62]. It is not known whether somatosensory information from the syrinx feeds back into NCM or the song system thereby providing an opportunity to impact the brain regions examined. But it has been proposed that this may occur via syringeal afferents to the caudolateral part of the interpolaris subdivision sensory trigeminal nuclei (nTTDi) via nucleus uvaeformis [61,63]. TS nerve cuts may also have altered the rate of song production, as did botox injections within the first week after treatment [42]. The quantity of song output corresponds to numbers of new neurons in canary HVC [20,21,24,64] but this has not yet been found in zebra finches [42,65]. To our knowledge, there is no information about whether singing rate impacts numbers of new neurons in song regions other than HVC in any species.

More associations between measures of acoustic change and numbers of new neurons in NCM and Area X would have better supported the idea that mismatched acoustic feedback influenced the survival of new neurons in these regions. Regardless, it is intriguing that the postoperative change in pitch was inversely correlated with numbers of new neurons in left NCM and the postoperative change in FM was inversely correlated with numbers of new neurons in Area X. In sum, we cannot rule out the possibility that the TS nerve cuts brought about the change in new neurons in Area X by decreasing singing rate, or through an interaction between singing rate and altered acoustic structure.

New neurons in NCM are sensitive to TS nerve cuts

We speculate that the change in new neuron lateralization in the experimental birds may be due to a mismatch between expected and received acoustic feedback. Neurons in NCM respond to playback of the bird's own song as well as the song of the bird's tutor and other conspecifics [66,67]. Moreover, NCM is active while a bird is singing, suggesting it is not gated during singing and may process auditory feedback from song in real time [36]. In particular, motor recovery from experimental distortion of song pitch requires an intact NCM, identifying a role for NCM in storage and/or recall of at least this feature of a bird's own song [68]. As such, mismatched pitch may lead to fewer new neurons being maintained during early culling of the new neurons recently arriving in NCM.

NCM receives a robust influx of new neurons throughout adulthood, although very little is known about the function of these new neurons. We do know that new neurons are upregulated in birds housed in groups [30,31,69,70] and are diminished in birds that have been deafened [57]. Together, this suggests that activity in NCM promotes the maintenance of new neurons; however, does not shed light on their function. Generally, the continual addition of new neurons may preserve and/or improve the resolution of stored song memories.

A comparison between expected and received feedback requires a memory of the bird's own song. Because the effect of the TS cut on new neurons was lateralized, perhaps NCM is functionally lateralized in performing this comparison. We found that numbers of new neurons were greater in the left than right hemisphere in control birds, and that this asymmetry was lost in TS cut bird. Both findings are consistent with Tsoi et al., (2014) [43]. The loss of asymmetry was due more to a decrease in numbers of new neurons in the left NCM than an increase in new neurons in the right NCM, although the degree of difference was not statistically significant. One of several explanations for this finding is that the new neurons in the left

hemisphere may be more sensitive to feedback mismatch, and may be so if a memory of expected feedback is lateralized to the left, rather than right, NCM. Although a comparison need not take place in the same hemisphere as the new neuron effect, it is a parsimonious explanation.

Evidence for a lateralized response in NCM to the bird's own song is mixed. It is suggestive that estradiol synthesis in the left NCM but not the right NCM is necessary for a bird's preference for his own song [71]. In a behavioral study, adult male zebra finches showed deficits in discriminating their own song from that of a cage-mate when the left side ovoidalis, part of the ascending auditory pathway, was lesioned and not when the right side was lesioned [72]. However, selectivity for playback of the bird's own song was found to be lateralized toward the right side in the midbrain dorsal lateral mesencephalic nucleus (MLd), the analog of the mammalian inferior colliculus [73]. Like ovoidalis, MLd is part of the unilateral ascending auditory pathway that eventually projects to NCM. Moreover, both left and right NCM responded to playbacks of the bird's song, although not selectively over conspecific song, and there was no evidence of hemispheric asymmetry [74].

In a different model, hemispheric asymmetry may be based on specializations for sound acoustic features rather than categories such as own song versus those of others [75]. Playback of conspecific songs with filtered spectral structure increased blood-oxygen level dependent (BOLD) fMRI responses in the left NCM and slightly decreased BOLD responses in the right NCM compared to nonmanipulated songs [75]. Because spectral filtering removed frequency information from the song leaving primarily temporal content, the authors interpreted this finding as indicating that the left NCM is specialized for processing temporal information. They suggested that this asymmetry is normally masked by the spectral content of whole songs [75]. On the other hand, we suggest that perhaps the increased response in the left NCM reflects a left-dominant sensitivity to aberrant information, resonating with our findings. The left hemisphere of NCM has been shown to be sensitive to novel auditory experience more broadly [76,77] consistent with the idea that NCM (in these studies, bilaterally) is sensitive to expectation [78,79]. Thus perhaps it is the novelty of the distorted song, and not feedback about the bird's own song *per se*, that underlies the observed decrease in new neurons in the left NCM.

It is a consistent finding across studies that the magnitude of asymmetry in different metrics of NCM corresponds to performance in learning [80]. For instance, the degree of left side dominance in new neurons [43], and left side dominance in activity during sleep [77] predict accuracy of song learning of the bird's own song. Left side dominance in NCM activity in response to song playback is also correlated with rate of learning in a conspecific song discrimination task [81]. The results of the current study and that of Tsoi et al., (2014) [43] add that lateralization of new neurons can be influenced by the bird's experience.

Is the decrease in new neurons in Area X mediated by dopamine?

Dopamine is a likely candidate for mediating new neuron survival in Area X in response to altered song acoustic structure. Area X plays a role in juvenile song learning [34,35,82], and in modifying the adult song motor pattern in response to social context, deafening, and perturbations in feedback [37,83–89]. New neurons are continually added to Area X, increasing cell packing density with increasing bird age [90], as occurs in HVC [28]. Approximately 80% of new neurons become medium spiny neurons (MSN), express D1 and D2 receptors, and fire during singing [90], although the contribution of new neurons in particular is not known.

Dopamine promotes new neuron survival in the mammalian hippocampus and in the subventricular zone via D1 and D2 receptor activation [91,92] and is a known modulator of

activity in Area X. The substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA) send direct dopaminergic projections to Area X [93–95]. Through evaluation of predicted and actual auditory feedback, dopaminergic signals modulate the output of the anterior forebrain pathway [96–102].

HVC neurons that project to Area X encode own-song acoustic information and respond to delayed auditory feedback with aberrant firing, suggesting dynamic input of own-song acoustic information into Area X [103]. This is consistent with earlier work showing that dopaminergic neurons in Area X are active in response to playback of the bird's own song [104] and thus are poised to evaluate own song feedback and respond with dopamine release. More precisely, Gadagkar et al., (2016) recorded dopaminergic VTA neurons that project to Area X while birds were singing normally, and also while singing as experimenters played back distorted song syllables timed to be interpreted as own-song errors [105]. They found that VTA firing was suppressed after distorted syllables, and concluded that error signals can be encoded in the dopamine response. This is important for many reasons, but relevant to our findings it shows that DA modulation is a read out of the bird's perceived vocal success based on an internally encoded, or expected, goal. Similarly, decreasing VTA activity in Area X via 6-OHDA-induced retrograde lesions of dopaminergic cells in the VTA resulted in loss of the vocal learning that is normally driven by disruptive auditory feedback in a negative reinforcement task [106,107]. The association between DA and encoding of altered auditory feedback was further tested via optogenetic inhibition and excitation of VTA axon terminals during singing, modulating DA in Area X. DA modulation predictably guided changes in targeted song syllable production consistent with positive and negative reinforcement of performance [101]. Moreover, externally reinforced vocal pitch learning relies on the same VTA-Area X pathway as internally guided vocal copying in juvenile zebra finches [108]. Thus it is suggestive that we found an inverse correlation between the degree of song change specifically in frequency modulation and numbers of new neurons in Area X. Area X is also known to modulate the production of song spectral features but not temporal features, and to do so via activation of D1 receptors [85,108,109]. However, we cannot account for the effect being limited to the left, and not in both hemispheres, as there are no reports of asymmetries in Area X function.

Interestingly, the influence of the VTA on Area X is reciprocal. Area X makes an indirect connection to SNc/VTA via a projection to ventral pallium (VP) [94]. Through this pathway, Area X output drives changes in the firing rate of dopaminergic neurons in the SNc and VTA. VP neurons are spontaneously active and, notably, are preferentially inhibited by BOS, suggesting that Area X disinhibits dopaminergic neurons by inhibiting VP [104]. This relationship suggests that Area X plays a role in feedback evaluation of song with respect to an internal model of the bird's own song, and transmits this information to dopaminergic neurons. Perhaps this pathway results in decreased dopamine signaling in the TS-cut birds with mismatched feedback, and fewer new neurons, in Area X.

There were no correlations in numbers of new neurons between regions

Few studies in any species have made inter-regional comparisons of adult neurogenesis with an aim toward identifying region-specific regulatory mechanisms. Notably, prenatal stress has been shown to decrease neurogenesis in the mouse hippocampus in adulthood, with no effect on neurogenesis in the olfactory bulb [8]. In the aging pigeon, the extent of new neuron decline is greater in the olfactory bulb than in the hippocampus, again differentiating regulatory mechanisms between neurogenic regions [110]. Rates of decline in new neurons during aging also differ between HVC and Area X [53]. Consistent with these findings, our results

also suggest that regulatory mechanisms may have independent components across brain regions, even those linked functionally and anatomically.

There were no correlations in numbers of new neurons and bird age

Although our birds spanned the ages of 4–11 months, the majority of animals clustered at 4, 5, and 7 months of age at the time of the brdu injections, equally represented in the 3 groups, and this likely contributed to our finding that age was not a factor in our cell counts. Earlier work found no effect of age on new neuron numbers in HVC in zebra finches between the ages of 4–8 months (Wang et al., 2002) [14]. A decrease in new neurons was evident only when comparing birds younger than a year with those 12 mos or older, and an age-related correlation in new neurons required comparing birds across ages that spanned the period of 8–14 mos (Wang et al., 2002) [14] or between groups aged 3–5 months old and 14–21 month (Pytte et al., 2007) [53]. Therefore, the upper age range of our birds was also likely not high enough for age to be a correlate. Our finding of no effect of age in Area X and NCM is also consistent with the few reports in the literature. There was no difference in numbers of new neurons added to Area X in a grouped comparison between zebra finches 3–5 months old and those 14–21 months old (Pytte et al., 2007) [53]. Likewise, it was reported in starlings that there was no difference in new neurons in NCM in birds grouped as yearlings, 2nd year, and 3 years and older [111]. Ring doves, on the other hand, demonstrated an age-related decline in new neurons added to the caudal nidopallium at about 3 months and 1 year of age [112]. Given the well-documented age-related decline in hippocampal neurogenesis in mammals [113], this is likely a common principal; however, not one captured by our study.

Taken together. . .

Song learning by juveniles requires incremental improvement toward a target motor behavior and requires both Area X and HVC, in concert with neuronal tuning to the tutor and the bird's own song in NCM. Moreover, song learning occurs at a time when new neuron incorporation is high. In one model of song learning, akin to procedural motor learning more broadly, Area X and the anterior forebrain pathway is thought to evaluate feedback and influence subsequent motor commands produced by HVC, and the output of feedback evaluation is thought to influence subsequent motor commands. Our findings suggest that the accumulation of new neurons in Area X may correspond directly to the quality of song feedback in matching to the target own-song template. As in bilateral Area X, new neurons in left NCM may likewise be culled when song feedback no longer matches expected feedback of the bird's own song. On the other hand, new neurons in HVC seem to not be culled or maintained based on the output of a match between expected and received feedback. New neurons in HVC may instead 1) be influenced via feedback with respect to progress toward a goal of an ideal song or 2) not be affected by song feedback at all, and instead new neurons may drive progress toward a goal song in a feedforward direction only (both ideas consistent with Pytte et al., 2011) [42]. Here we eliminate the hypothesis that the quality of song per se influences HVC new neuron survival. In sum, perhaps the continual addition of new neurons in the song system, and perhaps in NCM also, increases the resolution of these neuronal substrates which in turn achieves increased song stereotypy both by better precision of the evaluation of song feedback (NCM and Area X) and by fine tuning motor output (HVC).

Conclusions

This study demonstrates that altered feedback affects new neuron recruitment and survival in brain regions that subserve and monitor those behaviors. We found that altering song

production via unilateral tracheosyringeal denervation resulted in decreased 28–30 day old neurons in Area X, loss of lateralization of neurons in NCM, and had no effect in HVC. This indicates that the effects of syringeal denervation on neurogenesis vary depending both on the region and hemisphere that receives new neurons.

Acknowledgments

John Kirn generously gifted birds for the study. Ben Koo contributed to tissue processing and cell quantification. In memory of John R. Kirn (1952–2019) who contributed to the conceptualization of this study.

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Writing – original draft: Jake V. Aronowitz, Alice Perez, Carolyn L. Pytte.

Writing – review & editing: Carolyn L. Pytte.

References

1. Spalding KL, Bhardwaj RD, Buchholz BA, Druid H, Frisen J. Retrospective birth dating of cells in humans. Cell. 2005; 122(1):133–43. Epub 2005/07/13. https://doi.org/10.1016/j.cell.2005.04.028 PMID: 16009139.
2. Krzyzanski W, Brier ME, Creed TM, Gaweda AE. Reticulocyte-based estimation of red blood cell lifespan. Exp Hematol. 2013; 41(9):817–22. Epub 2013/05/29. https://doi.org/10.1016/j.exphem.2013.05.001 PMID: 23711405; PubMed Central PMCID: PMC4061324.
3. Macdonald RA. "Lifespan" of liver cells. Autoradio-graphic study using tritiated thymidine in normal, cirrhotic, and partially hepatectomized rats. Arch Intern Med. 1961; 107:335–43. Epub 1961/03/01. https://doi.org/10.1001/archinte.1961.03620030023003 PMID: 13764742.
4. Stocker E, Heine WD. [Proliferation and regeneration in liver and kidney of juvenile rats. Autoradiographic studies after continuous infusion of 3H-thymidine (author's transl)]. Verh Dtsch Ges Pathol. 1971; 55:483–8. Epub 1971/01/01. PMID: 4130752.
5. Larson TA, Thatra NM, Hou D, Hu RA, Brenowitz EA. Seasonal changes in neuronal turnover in a forebrain nucleus in adult songbirds. J Comp Neurol. 2019; 527(4):767–79. Epub 2018/10/07. https://doi.org/10.1002/cne.24552 PMID: 30291632; PubMed Central PMCID: PMC6333494.
6. Kirn J, O'Loughlin B, Kasparian S, Nottebohm F. Cell death and neuronal recruitment in the high vocal center of adult male canaries are temporally related to changes in song. Proc Natl Acad Sci U S A. 1994; 91(17):7844–8. Epub 1994/08/16. https://doi.org/10.1073/pnas.91.17.7844 PMID: 8058721; PubMed Central PMCID: PMC44500.
7. Kirn JR, Schwabl H. Photoperiod regulation of neuron death in the adult canary. J Neurobiol. 1997; 33(3):223–31. Epub 1997/09/23. https://doi.org/10.1002/(sici)1097-4695(199709)33:3<223::aid-neu2>3.0.co;2-3 PMID: 9298761.

8. Belnoue L, Grosjean N, Ladeveze E, Abrous DN, Koehl M. Prenatal stress inhibits hippocampal neurogenesis but spares olfactory bulb neurogenesis. PLoS One. 2013; 8(8):e72972. Epub 2013/09/07. https://doi.org/10.1371/journal.pone.0072972 PMID: 24009723; PubMed Central PMCID: PMC3756947.

9. Gheusi G, Cremer H, McLean H, Chazal G, Vincent JD, Lledo PM. Importance of newly generated neurons in the adult olfactory bulb for odor discrimination. Proc Natl Acad Sci U S A. 2000; 97 (4):1823–8. Epub 2000/03/04. https://doi.org/10.1073/pnas.97.4.1823 PMID: 10677540; PubMed Central PMCID: PMC26520.

10. Rochefort C, Gheusi G, Vincent JD, Lledo PM. Enriched odor exposure increases the number of newborn neurons in the adult olfactory bulb and improves odor memory. J Neurosci. 2002; 22(7):2679–89. Epub 2002/03/30. doi: 20026260. PMID: 11923433; PubMed Central PMCID: PMC6758329.

11. Shors TJ, Townsend DA, Zhao M, Kozorovitskiy Y, Gould E. Neurogenesis may relate to some but not all types of hippocampal-dependent learning. Hippocampus. 2002; 12(5):578–84. Epub 2002/11/21. https://doi.org/10.1002/hipo.10103 PMID: 12440573; PubMed Central PMCID: PMC3289536.

12. Liu PZ, Nusslock R. Exercise-Mediated Neurogenesis in the Hippocampus via BDNF. Front Neurosci. 2018; 12:52. Epub 2018/02/23. https://doi.org/10.3389/fnins.2018.00052 PMID: 29467613; PubMed Central PMCID: PMC5808288.

13. Dupret D, Revest JM, Koehl M, Ichas F, De Giorgi F, Costet P, et al. Spatial relational memory requires hippocampal adult neurogenesis. PLoS One. 2008; 3(4):e1959. Epub 2008/05/30. https://doi.org/10.1371/journal.pone.0001959 PMID: 18509506; PubMed Central PMCID: PMC2396793.

14. Larson TA, Wang TW, Gale SD, Miller KE, Thatra NM, Caras ML, et al. Postsynaptic neural activity regulates neuronal addition in the adult avian song control system. Proc Natl Acad Sci U S A. 2013; 110(41):16640–4. Epub 2013/09/26. https://doi.org/10.1073/pnas.1310237110 PMID: 24062453; PubMed Central PMCID: PMC3799304.

15. Wilbrecht L, Kirn JR. Neuron addition and loss in the song system: regulation and function. Ann N Y Acad Sci. 2004; 1016:659–83. Epub 2004/08/18. https://doi.org/10.1196/annals.1298.024 PMID: 15313799.

16. Brenowitz EA, Larson TA. Neurogenesis in the adult avian song-control system. Cold Spring Harb Perspect Biol. 2015; 7(6). Epub 2015/06/03. https://doi.org/10.1101/cshperspect.a019000 PMID: 26032719; PubMed Central PMCID: PMC4448602.

17. Alvarez-Buylla A, Theelen M, Nottebohm F. Birth of projection neurons in the higher vocal center of the canary forebrain before, during, and after song learning. Proc Natl Acad Sci U S A. 1988; 85 (22):8722–6. Epub 1988/11/01. https://doi.org/10.1073/pnas.85.22.8722 PMID: 3186755; PubMed Central PMCID: PMC282533.

18. Alvarez-Buylla A, Kirn JR, Nottebohm F. Birth of projection neurons in adult avian brain may be related to perceptual or motor learning. Science. 1990; 249(4975):1444–6. Epub 1990/09/21. https://doi.org/10.1126/science.1698312 PMID: 1698312.

19. Kirn JR, Alvarez-Buylla A, Nottebohm F. Production and survival of projection neurons in a forebrain vocal center of adult male canaries. J Neurosci. 1991; 11(6):1756–62. Epub 1991/06/01. https://doi.org/10.1523/JNEUROSCI.11-06-01756.1991 PMID: 2045885; PubMed Central PMCID: PMC6575395.

20. Li XC, Jarvis ED, Alvarez-Borda B, Lim DA, Nottebohm F. A relationship between behavior, neurotrophin expression, and new neuron survival. Proc Natl Acad Sci U S A. 2000; 97(15):8584–9. Epub 2000/07/13. https://doi.org/10.1073/pnas.140222497 PMID: 10890902; PubMed Central PMCID: PMC26991.

21. Alvarez-Borda B, Nottebohm F. Gonads and singing play separate, additive roles in new neuron recruitment in adult canary brain. J Neurosci. 2002; 22(19):8684–90. Epub 2002/09/28. https://doi.org/10.1523/JNEUROSCI.22-19-08684.2002 PMID: 12351743; PubMed Central PMCID: PMC6757767.

22. Ball GF, Auger CJ, Bernard DJ, Charlier TD, Sartor JJ, Riters LV, et al. Seasonal plasticity in the song control system: multiple brain sites of steroid hormone action and the importance of variation in song behavior. Ann N Y Acad Sci. 2004; 1016:586–610. Epub 2004/08/18. https://doi.org/10.1196/annals.1298.043 PMID: 15313796.

23. Ball GF, Madison FN, Balthazart J, Alward BA. How does testosterone act to regulate a multifaceted adaptive response? Lessons from studies of the avian song system. J Neuroendocrinol. 2020; 32(1): e12793. Epub 2019/09/13. https://doi.org/10.1111/jne.12793 PMID: 31514252.

24. Alward BA, Madison FN, Parker SE, Balthazart J, Ball GF. Pleiotropic Control by Testosterone of a Learned Vocal Behavior and Its Underlying Neuroplasticity(1,2,3). eNeuro. 2016; 3(1). Epub 2016/02/03. https://doi.org/10.1523/eneuro.0145-15.2016 PMID: 26835510; PubMed Central PMCID: PMC4724066.

25. Alvarez-Borda B, Haripal B, Nottebohm F. Timing of brain-derived neurotrophic factor exposure affects life expectancy of new neurons. Proc Natl Acad Sci U S A. 2004; 101(11):3957–61. Epub 2004/03/09. https://doi.org/10.1073/pnas.0308118101 PMID: 15004273; PubMed Central PMCID: PMC374351.

26. Rasika S, Nottebohm F, Alvarez-Buylla A. Testosterone increases the recruitment and/or survival of new high vocal center neurons in adult female canaries. Proc Natl Acad Sci U S A. 1994;

91(17):7854–8. Epub 1994/08/16. https://doi.org/10.1073/pnas.91.17.7854 PMID: 8058723; PubMed Central PMCID: PMC44502.

27. Rasika S, Alvarez-Buylla A, Nottebohm F. BDNF mediates the effects of testosterone on the survival of new neurons in an adult brain. Neuron. 1999; 22(1):53–62. Epub 1999/02/23. https://doi.org/10.1016/s0896-6273(00)80678-9 PMID: 10027289.

28. Walton C, Pariser E, Nottebohm F. The zebra finch paradox: song is little changed, but number of neurons doubles. J Neurosci. 2012; 32(3):761–74. Epub 2012/01/21. https://doi.org/10.1523/JNEUROSCI.3434-11.2012 PMID: 22262875; PubMed Central PMCID: PMC6621147.

29. Shevchouk OT, Ball GF, Cornil CA, Balthazart J. Studies of HVC Plasticity in Adult Canaries Reveal Social Effects and Sex Differences as Well as Limitations of Multiple Markers Available to Assess Adult Neurogenesis. PLoS One. 2017; 12(1):e0170938. Epub 2017/02/01. https://doi.org/10.1371/journal.pone.0170938 PMID: 28141859; PubMed Central PMCID: PMC5283688.

30. Lipkind D, Nottebohm F, Rado R, Barnea A. Social change affects the survival of new neurons in the forebrain of adult songbirds. Behav Brain Res. 2002; 133(1):31–43. Epub 2002/06/06. https://doi.org/10.1016/s0166-4328(01)00416-8 PMID: 12048172.

31. Barnea A, Mishal A, Nottebohm F. Social and spatial changes induce multiple survival regimes for new neurons in two regions of the adult brain: An anatomical representation of time? Behav Brain Res. 2006; 167(1):63–74. Epub 2005/10/12. https://doi.org/10.1016/j.bbr.2005.08.018 PMID: 16216348.

32. Pawlisch BA, Remage-Healey L. Neuroestrogen signaling in the songbird auditory cortex propagates into a sensorimotor network via an 'interface' nucleus. Neuroscience. 2015; 284:522–35. Epub 2014/12/03. https://doi.org/10.1016/j.neuroscience.2014.10.023 PMID: 25453773; PubMed Central PMCID: PMC4268063.

33. Rochefort C, He X, Scotto-Lomassese S, Scharff C. Recruitment of FoxP2-expressing neurons to area X varies during song development. Dev Neurobiol. 2007; 67(6):809–17. Epub 2007/04/20. https://doi.org/10.1002/dneu.20393 PMID: 17443826.

34. Sohrabji F, Nordeen EJ, Nordeen KW. Selective impairment of song learning following lesions of a forebrain nucleus in the juvenile zebra finch. Behav Neural Biol. 1990; 53(1):51–63. Epub 1990/01/01. https://doi.org/10.1016/0163-1047(90)90797-a PMID: 2302141.

35. Scharff C, Nottebohm F. A comparative study of the behavioral deficits following lesions of various parts of the zebra finch song system: implications for vocal learning. J Neurosci. 1991; 11(9):2896–913. Epub 1991/09/01. https://doi.org/10.1523/JNEUROSCI.11-09-02896.1991 PMID: 1880555; PubMed Central PMCID: PMC6575264.

36. Jarvis ED, Scharff C, Grossman MR, Ramos JA, Nottebohm F. For whom the bird sings: context-dependent gene expression. Neuron. 1998; 21(4):775–88. Epub 1998/11/10. https://doi.org/10.1016/s0896-6273(00)80594-2 PMID: 9808464.

37. Woolley SC, Rajan R, Joshua M, Doupe AJ. Emergence of context-dependent variability across a basal ganglia network. Neuron. 2014; 82(1):208–23. Epub 2014/04/05. https://doi.org/10.1016/j.neuron.2014.01.039 PMID: 24698276; PubMed Central PMCID: PMC4132189.

38. Kubikova L, Bosikova E, Cvikova M, Lukacova K, Scharff C, Jarvis ED. Basal ganglia function, stuttering, sequencing, and repair in adult songbirds. Sci Rep. 2014; 4:6590. Epub 2014/10/14. https://doi.org/10.1038/srep06590 PMID: 25307086; PubMed Central PMCID: PMC4194444.

39. Barnea A, Pravosudov V. Birds as a model to study adult neurogenesis: bridging evolutionary, comparative and neuroethological approaches. Eur J Neurosci. 2011; 34(6):884–907. Epub 2011/09/21. https://doi.org/10.1111/j.1460-9568.2011.07851.x PMID: 21929623; PubMed Central PMCID: PMC3177424.

40. Wilbrecht L, Crionas A, Nottebohm F. Experience affects recruitment of new neurons but not adult neuron number. J Neurosci. 2002; 22(3):825–31. Epub 2002/02/05. https://doi.org/10.1523/JNEUROSCI.22-03-00825.2002 PMID: 11826112; PubMed Central PMCID: PMC6758520.

41. Pytte C, Wilbrecht L, Kirn JR. Regulation and function on neuronal replacement in the avian song system. Cambridge: Cambridge University Press; 2008 2008. 348–64 p.

42. Pytte C, Yu YL, Wildstein S, George S, Kirn JR. Adult neuron addition to the zebra finch song motor pathway correlates with the rate and extent of recovery from botox-induced paralysis of the vocal muscles. J Neurosci. 2011; 31(47):16958–68. Epub 2011/11/25. https://doi.org/10.1523/JNEUROSCI.2971-11.2011 PMID: 22114266; PubMed Central PMCID: PMC3247305.

43. Tsoi SC, Aiya UV, Wasner KD, Phan ML, Pytte CL, Vicario DS. Hemispheric asymmetry in new neurons in adulthood is associated with vocal learning and auditory memory. PLoS One. 2014; 9(9):e108929. Epub 2014/09/25. https://doi.org/10.1371/journal.pone.0108929 PMID: 25251077; PubMed Central PMCID: PMC4177556.

44. Winner B, Winkler J. Adult neurogenesis in neurodegenerative diseases. Cold Spring Harb Perspect Biol. 2015; 7(4):a021287. Epub 2015/04/03. https://doi.org/10.1101/cshperspect.a021287 PMID: 25833845; PubMed Central PMCID: PMC4382734.

45. Mu Y, Gage FH. Adult hippocampal neurogenesis and its role in Alzheimer's disease. Mol Neurodegener. 2011; 6:85. Epub 2011/12/24. https://doi.org/10.1186/1750-1326-6-85 PMID: 22192775; PubMed Central PMCID: PMC3261815.

46. Sun D. The potential of endogenous neurogenesis for brain repair and regeneration following traumatic brain injury. Neural Regen Res. 2014; 9(7):688–92. Epub 2014/09/11. https://doi.org/10.4103/1673-5374.131567 PMID: 25206873; PubMed Central PMCID: PMC4146269.

47. Williams H, McKibben JR. Changes in stereotyped central motor patterns controlling vocalization are induced by peripheral nerve injury. Behav Neural Biol. 1992; 57(1):67–78. Epub 1992/01/01. https://doi.org/10.1016/0163-1047(92)90768-y PMID: 1567335.

48. Williams H, Mehta N. Changes in adult zebra finch song require a forebrain nucleus that is not necessary for song production. J Neurobiol. 1999; 39(1):14–28. Epub 1999/04/23. PMID: 10213450.

49. Simpson HB, Vicario DS. Brain pathways for learned and unlearned vocalizations differ in zebra finches. J Neurosci. 1990; 10(5):1541–56. Epub 1990/05/01. https://doi.org/10.1523/JNEUROSCI.10-05-01541.1990 PMID: 2332796; PubMed Central PMCID: PMC6570078.

50. Lombardino AJ, Nottebohm F. Age at deafening affects the stability of learned song in adult male zebra finches. J Neurosci. 2000; 20(13):5054–64. Epub 2000/06/24. https://doi.org/10.1523/JNEUROSCI.20-13-05054.2000 PMID: 10864963; PubMed Central PMCID: PMC6772266.

51. Nordeen KW, Nordeen EJ. Deafening-induced vocal deterioration in adult songbirds is reversed by disrupting a basal ganglia-forebrain circuit. J Neurosci. 2010; 30(21):7392–400. Epub 2010/05/28. https://doi.org/10.1523/JNEUROSCI.6181-09.2010 PMID: 20505106; PubMed Central PMCID: PMC6632418.

52. Brainard MS, Doupe AJ. Postlearning consolidation of birdsong: stabilizing effects of age and anterior forebrain lesions. J Neurosci. 2001; 21(7):2501–17. Epub 2001/03/27. https://doi.org/10.1523/JNEUROSCI.21-07-02501.2001 PMID: 11264324; PubMed Central PMCID: PMC6762407.

53. Pytte CL, Gerson M, Miller J, Kirn JR. Increasing stereotypy in adult zebra finch song correlates with a declining rate of adult neurogenesis. Dev Neurobiol. 2007; 67(13):1699–720. Epub 2007/06/28. https://doi.org/10.1002/dneu.20520 PMID: 17595004.

54. Roy A, Mooney R. Song decrystallization in adult zebra finches does not require the song nucleus NIf. J Neurophysiol. 2009; 102(2):979–91. Epub 2009/06/12. https://doi.org/10.1152/jn.00293.2009 PMID: 19515953; PubMed Central PMCID: PMC2724348.

55. Roy A, Mooney R. Auditory plasticity in a basal ganglia-forebrain pathway during decrystallization of adult birdsong. J Neurosci. 2007; 27(24):6374–87. Epub 2007/06/15. https://doi.org/10.1523/JNEUROSCI.0894-07.2007 PMID: 17567798; PubMed Central PMCID: PMC6672454.

56. Tchernichovski O, Nottebohm F, Ho CE, Pesaran B, Mitra PP. A procedure for an automated measurement of song similarity. Anim Behav. 2000; 59(6):1167–76. Epub 2000/07/06. https://doi.org/10.1006/anbe.1999.1416 PMID: 10877896.

57. Pytte CL, Parent C, Wildstein S, Varghese C, Oberlander S. Deafening decreases neuronal incorporation in the zebra finch caudomedial nidopallium (NCM). Behav Brain Res. 2010; 211(2):141–7. https://doi.org/10.1016/j.bbr.2010.03.029 PMID: 20346987

58. Brown JP, Couillard-Despres S, Cooper-Kuhn CM, Winkler J, Aigner L, Kuhn HG. Transient expression of doublecortin during adult neurogenesis. J Comp Neurol. 2003; 467(1):1–10. Epub 2003/10/24. https://doi.org/10.1002/cne.10874 PMID: 14574675.

59. Balthazart J, Boseret G, Konkle AT, Hurley LL, Ball GF. Doublecortin as a marker of adult neuroplasticity in the canary song control nucleus HVC. Eur J Neurosci. 2008; 27(4):801–17. Epub 2008/03/13. https://doi.org/10.1111/j.1460-9568.2008.06059.x PMID: 18333960.

60. Bottjer SW, Arnold AP. Afferent neurons in the hypoglossal nerve of the zebra finch (Poephila guttata): localization with horseradish peroxidase. J Comp Neurol. 1982; 210(2):190–7. Epub 1982/09/10. https://doi.org/10.1002/cne.902100209 PMID: 7130479.

61. Faunes M, Botelho JF, Wild JM. Innervation of the syrinx of the zebra finch (Taeniopygia guttata). J Comp Neurol. 2017; 525(13):2847–60. Epub 2017/05/05. https://doi.org/10.1002/cne.24236 PMID: 28472866.

62. Suthers RA, Goller F, Wild JM. Somatosensory feedback modulates the respiratory motor program of crystallized birdsong. Proc Natl Acad Sci U S A. 2002; 99(8):5680–5. Epub 2002/04/12. https://doi.org/10.1073/pnas.042103199 PMID: 11943843; PubMed Central PMCID: PMC122831.

63. Faunes M, Wild JM. The sensory trigeminal complex and the organization of its primary afferents in the zebra finch (Taeniopygia guttata). J Comp Neurol. 2017; 525(13):2820–31. Epub 2017/05/26. https://doi.org/10.1002/cne.24249 PMID: 28542900.

64. Ball GF, Riters LV, Balthazart J. Neuroendocrinology of song behavior and avian brain plasticity: multiple sites of action of sex steroid hormones. Front Neuroendocrinol. 2002; 23(2):137–78. Epub 2002/04/13. https://doi.org/10.1006/frne.2002.0230 PMID: 11950243.

65. Pytte CL, George S, Korman S, David E, Bogdan D, Kirn JR. Adult neurogenesis is associated with the maintenance of a stereotyped, learned motor behavior. J Neurosci. 2012; 32(20):7052–7. Epub 2012/05/18. https://doi.org/10.1523/JNEUROSCI.5385-11.2012 PMID: 22593073; PubMed Central PMCID: PMC3407572.

66. Grace JA, Amin N, Singh NC, Theunissen FE. Selectivity for conspecific song in the zebra finch auditory forebrain. J Neurophysiol. 2003; 89(1):472–87. Epub 2003/01/11. https://doi.org/10.1152/jn.00088.2002 PMID: 12522195.

67. Hsu A, Woolley SM, Fremouw TE, Theunissen FE. Modulation power and phase spectrum of natural sounds enhance neural encoding performed by single auditory neurons. J Neurosci. 2004; 24(41):9201–11. Epub 2004/10/16. https://doi.org/10.1523/JNEUROSCI.2449-04.2004 PMID: 15483139; PubMed Central PMCID: PMC6730078.

68. Canopoli A, Herbst JA, Hahnloser RH. A higher sensory brain region is involved in reversing reinforcement-induced vocal changes in a songbird. J Neurosci. 2014; 34(20):7018–26. Epub 2014/05/16. https://doi.org/10.1523/JNEUROSCI.0266-14.2014 PMID: 24828654; PubMed Central PMCID: PMC6608103.

69. Adar E, Lotem A, Barnea A. The effect of social environment on singing behavior in the zebra finch (Taeniopygia guttata) and its implication for neuronal recruitment. Behav Brain Res. 2008; 187(1):178–84. Epub 2007/10/24. https://doi.org/10.1016/j.bbr.2007.09.011 PMID: 17950475.

70. Adar E, Nottebohm F, Barnea A. The relationship between nature of social change, age, and position of new neurons and their survival in adult zebra finch brain. J Neurosci. 2008; 28(20):5394–400. Epub 2008/05/16. https://doi.org/10.1523/JNEUROSCI.5706-07.2008 PMID: 18480295; PubMed Central PMCID: PMC6670650.

71. Remage-Healey L, Coleman MJ, Oyama RK, Schlinger BA. Brain estrogens rapidly strengthen auditory encoding and guide song preference in a songbird. Proc Natl Acad Sci U S A. 2010; 107(8):3852–7. Epub 2010/02/06. https://doi.org/10.1073/pnas.0906572107 PMID: 20133597; PubMed Central PMCID: PMC2840459.

72. Cynx J, Williams H, Nottebohm F. Hemispheric differences in avian song discrimination. Proc Natl Acad Sci U S A. 1992; 89(4):1372–5. Epub 1992/02/15. https://doi.org/10.1073/pnas.89.4.1372 PMID: 1741391; PubMed Central PMCID: PMC48452.

73. Poirier C, Boumans T, Verhoye M, Balthazart J, Van der Linden A. Own-song recognition in the songbird auditory pathway: selectivity and lateralization. J Neurosci. 2009; 29(7):2252–8. Epub 2009/02/21. https://doi.org/10.1523/JNEUROSCI.4650-08.2009 PMID: 19228978; PubMed Central PMCID: PMC2677151.

74. Soyman E, Vicario DS. Principles of auditory processing differ between sensory and premotor structures of the songbird forebrain. J Neurophysiol. 2017; 117(3):1266–80. Epub 2016/12/30. https://doi.org/10.1152/jn.00462.2016 PMID: 28031398; PubMed Central PMCID: PMC5349330.

75. Van Ruijssevelt L, Washington SD, Hamaide J, Verhoye M, Keliris GA, Van der Linden A. Song Processing in the Zebra Finch Auditory Forebrain Reflects Asymmetric Sensitivity to Temporal and Spectral Structure. Front Neurosci. 2017; 11:549. Epub 2017/10/21. https://doi.org/10.3389/fnins.2017.00549 PMID: 29051725; PubMed Central PMCID: PMC5633600.

76. Yang LM, Vicario DS. Exposure to a novel stimulus environment alters patterns of lateralization in avian auditory cortex. Neuroscience. 2015; 285:107–18. Epub 2014/12/03. https://doi.org/10.1016/j.neuroscience.2014.10.022 PMID: 25453763.

77. Moorman S, Gobes SM, van de Kamp FC, Zandbergen MA, Bolhuis JJ. Learning-related brain hemispheric dominance in sleeping songbirds. Sci Rep. 2015; 5:9041. Epub 2015/03/13. https://doi.org/10.1038/srep09041 PMID: 25761654; PubMed Central PMCID: PMC4356971.

78. Lu K, Vicario DS. Familiar But Unexpected: Effects of Sound Context Statistics on Auditory Responses in the Songbird Forebrain. J Neurosci. 2017; 37(49):12006–17. Epub 2017/11/10. https://doi.org/10.1523/JNEUROSCI.5722-12.2017 PMID: 29118103; PubMed Central PMCID: PMC5719976.

79. Dong M, Vicario DS. Neural Correlate of Transition Violation and Deviance Detection in the Songbird Auditory Forebrain. Front Syst Neurosci. 2018; 12:46. Epub 2018/10/26. https://doi.org/10.3389/fnsys.2018.00046 PMID: 30356811; PubMed Central PMCID: PMC6190688.

80. Moorman S, Nicol AU. Memory-related brain lateralisation in birds and humans. Neurosci Biobehav Rev. 2015; 50:86–102. Epub 2014/07/19. https://doi.org/10.1016/j.neubiorev.2014.07.006 PMID: 25036892.

81. Bell BA, Phan ML, Vicario DS. Neural responses in songbird forebrain reflect learning rates, acquired salience, and stimulus novelty after auditory discrimination training. J Neurophysiol. 2015; 113(5):1480–92. Epub 2014/12/06. https://doi.org/10.1152/jn.00611.2014 PMID: 25475353; PubMed Central PMCID: PMC4346724.

82. Olveczky BP, Andalman AS, Fee MS. Vocal experimentation in the juvenile songbird requires a basal ganglia circuit. PLoS Biol. 2005; 3(5):e153. Epub 2005/04/14. https://doi.org/10.1371/journal.pbio.0030153 PMID: 15826219; PubMed Central PMCID: PMC1069649.

83. Hessler NA, Doupe AJ. Social context modulates singing-related neural activity in the songbird forebrain. Nat Neurosci. 1999; 2(3):209–11. Epub 1999/04/09. https://doi.org/10.1038/6306 PMID: 10195211.

84. Brainard MS, Doupe AJ. Auditory feedback in learning and maintenance of vocal behaviour. Nat Rev Neurosci. 2000; 1(1):31–40. Epub 2001/03/17. https://doi.org/10.1038/35036205 PMID: 11252766.

85. Ali F, Otchy TM, Pehlevan C, Fantana AL, Burak Y, Olveczky BP. The basal ganglia is necessary for learning spectral, but not temporal, features of birdsong. Neuron. 2013; 80(2):494–506. Epub 2013/10/01. https://doi.org/10.1016/j.neuron.2013.07.049 PMID: 24075977; PubMed Central PMCID: PMC3929499.

86. Woolley SC. Social context differentially modulates activity of two interneuron populations in an avian basal ganglia nucleus. J Neurophysiol. 2016; 116(6):2831–40. Epub 2016/09/16. https://doi.org/10.1152/jn.00622.2016 PMID: 27628208; PubMed Central PMCID: PMC5168002.

87. Woolley SC, Kao MH. Variability in action: Contributions of a songbird cortical-basal ganglia circuit to vocal motor learning and control. Neuroscience. 2015; 296:39–47. Epub 2014/12/03. https://doi.org/10.1016/j.neuroscience.2014.10.010 PMID: 25445191.

88. Kojima S, Kao MH, Doupe AJ, Brainard MS. The Avian Basal Ganglia Are a Source of Rapid Behavioral Variation That Enables Vocal Motor Exploration. J Neurosci. 2018; 38(45):9635–47. Epub 2018/09/27. https://doi.org/10.1523/JNEUROSCI.2915-17.2018 PMID: 30249800; PubMed Central PMCID: PMC6222063.

89. Sanchez-Valpuesta M, Suzuki Y, Shibata Y, Toji N, Ji Y, Afrin N, et al. Corticobasal ganglia projecting neurons are required for juvenile vocal learning but not for adult vocal plasticity in songbirds. Proc Natl Acad Sci U S A. 2019; 116(45):22833–43. Epub 2019/10/23. https://doi.org/10.1073/pnas.1913575116 PMID: 31636217; PubMed Central PMCID: PMC6842584.

90. Kosubek-Langer J, Schulze L, Scharff C. Maturation, Behavioral Activation, and Connectivity of Adult-Born Medium Spiny Neurons in a Striatal Song Nucleus. Front Neurosci. 2017; 11:323. Epub 2017/06/24. https://doi.org/10.3389/fnins.2017.00323 PMID: 28638318; PubMed Central PMCID: PMC5461290.

91. Borta A, Höglinger GU. Dopamine and adult neurogenesis. J Neurochem. 2007; 100(3):587–95. Epub 2006/11/15. https://doi.org/10.1111/j.1471-4159.2006.04241.x PMID: 17101030.

92. Mishra A, Singh S, Tiwari V, Parul, Shukla S. Dopamine D1 receptor activation improves adult hippocampal neurogenesis and exerts anxiolytic and antidepressant-like effect via activation of Wnt/β-catenin pathways in rat model of Parkinson's disease. Neurochem Int. 2019; 122:170–86. Epub 2018/12/01. https://doi.org/10.1016/j.neuint.2018.11.020 PMID: 30500462.

93. Sasaki A, Sotnikova TD, Gainetdinov RR, Jarvis ED. Social context-dependent singing-regulated dopamine. J Neurosci. 2006; 26(35):9010–4. Epub 2006/09/01. https://doi.org/10.1523/JNEUROSCI.1335-06.2006 PMID: 16943558; PubMed Central PMCID: PMC2474783.

94. Gale SD, Person AL, Perkel DJ. A novel basal ganglia pathway forms a loop linking a vocal learning circuit with its dopaminergic input. J Comp Neurol. 2008; 508(5):824–39. Epub 2008/04/10. https://doi.org/10.1002/cne.21700 PMID: 18398824.

95. Person AL, Gale SD, Farries MA, Perkel DJ. Organization of the songbird basal ganglia, including area X. J Comp Neurol. 2008; 508(5):840–66. Epub 2008/04/10. https://doi.org/10.1002/cne.21699 PMID: 18398825.

96. Ding L, Perkel DJ. Long-term potentiation in an avian basal ganglia nucleus essential for vocal learning. J Neurosci. 2004; 24(2):488–94. Epub 2004/01/16. https://doi.org/10.1523/JNEUROSCI.4358-03.2004 PMID: 14724247; PubMed Central PMCID: PMC6729982.

97. Alsehli AM, Olivo G, Clemensson LE, Williams MJ, Schiöth HB. The Cognitive Effects of Statins are Modified by Age. Sci Rep. 2020; 10(1):6187. Epub 2020/04/12. https://doi.org/10.1038/s41598-020-63035-2 PMID: 32277109; PubMed Central PMCID: PMC7148321.

98. Chen R, Goldberg JH. Actor-critic reinforcement learning in the songbird. Curr Opin Neurobiol. 2020; 65:1–9. Epub 2020/09/09. https://doi.org/10.1016/j.conb.2020.08.005 PMID: 32898752; PubMed Central PMCID: PMC7769887.

99. Woolley SC. Dopaminergic regulation of vocal-motor plasticity and performance. Curr Opin Neurobiol. 2019; 54:127–33. Epub 2018/10/26. https://doi.org/10.1016/j.conb.2018.10.008 PMID: 30359929.

100. Kearney MG, Warren TL, Hisey E, Qi J, Mooney R. Discrete Evaluative and Premotor Circuits Enable Vocal Learning in Songbirds. Neuron. 2019; 104(3):559-75.e6. Epub 2019/08/27. https://doi.org/10.1016/j.neuron.2019.07.025 PMID: 31447169; PubMed Central PMCID: PMC6842112.

101. Xiao L, Chattree G, Oscos FG, Cao M, Wanat MJ, Roberts TF. A Basal Ganglia Circuit Sufficient to Guide Birdsong Learning. Neuron. 2018; 98(1):208-21.e5. Epub 2018/03/20. https://doi.org/10.1016/j.neuron.2018.02.020 PMID: 29551492; PubMed Central PMCID: PMC5918681.

102. Fee MS, Goldberg JH. A hypothesis for basal ganglia-dependent reinforcement learning in the songbird. Neuroscience. 2011; 198:152–70. Epub 2011/10/22. https://doi.org/10.1016/j.neuroscience.2011.09.069 PMID: 22015923; PubMed Central PMCID: PMC3221789.

103. Daou A, Margoliash D. Intrinsic neuronal properties represent song and error in zebra finch vocal learning. Nat Commun. 2020; 11(1):952. Epub 2020/02/23. https://doi.org/10.1038/s41467-020-14738-7 PMID: 32075972; PubMed Central PMCID: PMC7031510.

104. Gale SD, Perkel DJ. A basal ganglia pathway drives selective auditory responses in songbird dopaminergic neurons via disinhibition. J Neurosci. 2010; 30(3):1027–37. Epub 2010/01/22. https://doi.org/10.1523/JNEUROSCI.3585-09.2010 PMID: 20089911; PubMed Central PMCID: PMC2824341.

105. Gadagkar V, Puzerey PA, Chen R, Baird-Daniel E, Farhang AR, Goldberg JH. Dopamine neurons encode performance error in singing birds. Science. 2016; 354(6317):1278–82. Epub 2016/12/13. https://doi.org/10.1126/science.aah6837 PMID: 27940871; PubMed Central PMCID: PMC5464363.

106. Hoffmann LA, Saravanan V, Wood AN, He L, Sober SJ. Dopaminergic Contributions to Vocal Learning. J Neurosci. 2016; 36(7):2176–89. Epub 2016/02/19. https://doi.org/10.1523/JNEUROSCI.3883-15.2016 PMID: 26888928; PubMed Central PMCID: PMC4756153.

107. Saravanan V, Hoffmann LA, Jacob AL, Berman GJ, Sober SJ. Dopamine Depletion Affects Vocal Acoustics and Disrupts Sensorimotor Adaptation in Songbirds. eNeuro. 2019; 6(3). Epub 2019/05/28. https://doi.org/10.1523/ENEURO.0190-19.2019 PMID: 31126913; PubMed Central PMCID: PMC6565373.

108. Hisey E, Kearney MG, Mooney R. A common neural circuit mechanism for internally guided and externally reinforced forms of motor learning. Nat Neurosci. 2018; 21(4):589–97. Epub 2018/02/28. https://doi.org/10.1038/s41593-018-0092-6 PMID: 29483664; PubMed Central PMCID: PMC5963939.

109. Leblois A, Perkel DJ. Striatal dopamine modulates song spectral but not temporal features through D1 receptors. Eur J Neurosci. 2012; 35(11):1771–81. Epub 2012/05/19. https://doi.org/10.1111/j.1460-9568.2012.08095.x PMID: 22594943; PubMed Central PMCID: PMC3370102.

110. Meskenaite V, Krackow S, Lipp HP. Age-Dependent Neurogenesis and Neuron Numbers within the Olfactory Bulb and Hippocampus of Homing Pigeons. Front Behav Neurosci. 2016; 10:126. Epub 2016/07/23. https://doi.org/10.3389/fnbeh.2016.00126 PMID: 27445724; PubMed Central PMCID: PMC4916210.

111. Absil P, Pinxten R, Balthazart J, Eens M. Effect of age and testosterone on autumnal neurogenesis in male European starlings (Sturnus vulgaris). Behav Brain Res. 2003; 143(1):15–30. Epub 2003/07/05. https://doi.org/10.1016/s0166-4328(03)00006-8 PMID: 12842292.

112. Ling C, Zuo M, Alvarez-Buylla A, Cheng MF. Neurogenesis in juvenile and adult ring doves. J Comp Neurol. 1997; 379(2):300–12. Epub 1997/03/10. PMID: 9050792.

113. Kuhn HG, Toda T, Gage FH. Adult Hippocampal Neurogenesis: A Coming-of-Age Story. J Neurosci. 2018; 38(49):10401–10. Epub 2018/11/02. https://doi.org/10.1523/JNEUROSCI.2144-18.2018 PMID: 30381404; PubMed Central PMCID: PMC6284110.