Motor pathway convergence predicts syllable repertoire size in oscine birds

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Behavioral specializations are frequently associated with expansions of the brain regions controlling them. This principle of proper mass spans sensory, motor, and cognitive abilities and has been observed in a wide variety of vertebrate species. Yet, it is unknown if this concept extrapolates to entire neural pathways or how selection on a behavioral capacity might otherwise shape circuit structure. We investigate these questions by comparing the songs and neuroanatomy of 49 species from 17 families of songbirds, which vary immensely in the number of unique song components they produce and possess a conserved neural network dedicated to this behavior. We find that syllable repertoire size is strongly related to the degree of song motor pathway convergence. Repertoire size is more accurately predicted by the number of neurons in higher motor areas relative to that in their downstream targets than by the overall number of neurons in the song motor pathway. Additionally, the convergence values along serial premotor and primary motor projections account for distinct portions of the behavioral variation. These findings suggest that selection on song has independently shaped different components of this hierarchical pathway, and they elucidate how changes in pathway structure could have underlain elaborations of this learned motor behavior.

birdsong | brain evolution | HVC | neocorticalization

Many behavioral abilities are related to the size of their underlying brain regions, a phenomenon referred to as the principle of proper mass (1, 2). These associations are found throughout the vertebrate lineage and include brain areas involved in sensory processing [e.g., visual (3, 4), auditory (5, 6), olfactory (5, 7), somatosensory (8), and electrosensory (9)]; sensorimotor integration and motor coordination (10, 11); and cognitive tasks such as spatial memory (12, 13), procedural learning (14), and possibly human language (15). Such relations are generally thought to exist because larger brain regions possess greater computational power and/or exert greater influence over other areas (2), but little is known about their actual underlying causes because they have not been explored in detail. In particular, it is unknown if this principle extends to entire neural pathways or whether behavioral capacities relate to features of circuit structure other than overall size. The former is predicted by positive correlations between the sizes of functionally connected brain areas (16), but neither has been directly assessed. It is also unknown how these associations fit with more general studies of encephalization or neocorticalization, wherein behaviors are linked to the size of the whole brain relative to body size or the isocortex relative to the rest of the brain, respectively (17). We investigate these issues in songbirds, for which the singing behavior of many species has been documented and the underlying neural system has been studied in detail.

Birdsong is a learned vocal communication signal characterized by tremendous interspecific diversity. Oscine species vary especially in the number of song components that they produce, ranging from those that learn a single note or note cluster, often termed a syllable (18), to others that possess repertoires of thousands (19). The neural basis for this variation is most likely within the song system, a discrete and conserved sensorimotor network dedicated to song learning and production (Fig. 1) (20, 21). Two parallel pathways that arise from premotor nucleus HVC constitute the majority of this circuit; a caudal motor pathway controls song production (22, 23), and a rostral, basal ganglia loop mediates song learning and plasticity but is not required for song production in adults (24, 25).

Three lines of evidence suggest that the size of nucleus HVC is a principal determinant of repertoire size. First, repertoire size and HVC volume are positively correlated within species (26–28), between species (29, 30), and between sexes (31, 32). Second, they covary after experimental treatments that enhance (33) or constrain (34) song learning. HVC can be large in individuals with small repertoires and its volume is not affected by early auditory experience, however, suggesting that large HVC volumes permit the acquisition of large repertoires rather than result from it (26, 35). Third, the potential physiological basis for this association was identified in the zebra finch (*Taeniopygia guttata*). HVC sparsely encodes temporal features of song whereby individual premotor neurons fire a single, precisely timed burst of action potentials during each song rendition (23). If this specificity between single neurons and short segments of individual syllables is general across species (36), then evolutionary increases in repertoire size would seem to require corresponding increases in HVC neuron number.

The significance of the HVC volume–repertoire association remains contentious, however, because HVC size usually explains only a small proportion of the behavioral variation and large outliers can skew the correlations (37). Moreover, conspecific males and females that sing similarly complex songs can have dimorphic HVC volumes (38, 39), age-related increases in repertoire size can occur without increases in HVC volume (40), and seasonal fluctuations in HVC volume are not necessarily accompanied by changes in repertoire size (41, 42). These observations seem to contradict notions of a strict correspondence between the two. Here, we investigate whether consideration of the entire song system across many diverse species improves this brain–behavior association.

Results

We tested for evolutionary relations between traits using phylogenetic generalized least squares (PGLS) models, which explain the variation in a dependent variable as a function of one or more independent variables while accounting for the statistical nonindependence of comparative data. Species relatedness was represented by a fully resolved phylogeny generated from published molecular studies (Fig. 2). All anatomical and be-

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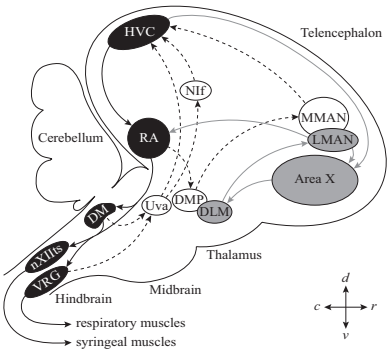

Fig. 1. The oscine song system. The song motor pathway (SMP; black) underlies song production, the anterior forebrain pathway (gray) is required for song learning and plasticity, and feedback projections (dashed lines) coordinate activity between nuclei and hemispheres. VRG, ventral respiratory group is a collection of brainstem respiratory nuclei.

havioral data are provided in *SI Materials and Methods* (Fig. S1 and Datasets S1 and S2).

Relations Between Song Nuclei. The volume of each song nucleus scaled directly with the rest of the brain, but the strength of these allometric relations varied widely across nuclei ($0.29 < R^2 < 0.75$) (Table S1). HVC and the medial magnocellular nucleus of the anterior nidopallium (MMAN) were especially variable (both $R^2 < 0.33$) and differed markedly in some similarly sized species. For example, their respective volumes were 15- and 9-fold larger in the spotted flycatcher (*Muscicapa striata*) than the common yellowthroat (*Geothlypis trichas*), whereas the robust nucleus of the arcopallium (RA), Area X of the striatum, lateral magnocellular nucleus of the anterior nidopallium (LMAN), and tracheosyringeal portion of the hypoglossal nucleus (nXIIts) were only 1.5 to 4 times as large (Fig. S2). Despite this heterogeneity, strong positive correlations linked the relative volumes of most nuclei (Table S2). Thus, the song system evolved as a cohesive yet nonuniform network in that the relative sizes of most nuclei changed in parallel but some nuclei are much more variable than others.

Relations Between Repertoire Size and the Song System. We constructed PGLS models to explain species variation in syllable repertoire size as a function of various song nucleus volume combinations and a size covariate [brain–song system (B–SS)], some of which are detailed in Table S3. The full model contained all song nucleus volumes as independent variables and was strongly related to the pattern of behavioral variation ($R^2 = 0.73$). Three of the reduced models tested were comparable with the full model, and each was comprised of nuclei along the song motor pathway (SMP): HVC, RA, and nXIIts [$R^2 = 0.72$; likelihood ratio test (LRT) relative to the full model, $P = 0.64$]; HVC and RA ($R^2 = 0.68$; $P = 0.18$); and HVC and nXIIts ($R^2 = 0.68$; $P = 0.14$). In other words, after accounting for the SMP, the remaining nuclei did not explain any additional behavioral variation. Inclusion of interaction terms did not significantly improve the predictive value of these reduced models (all LRT $P > 0.51$).

We were interested in whether syllable repertoire size related more strongly to the relative size of the entire song system (akin to encephalization) or to relative size differences between nuclei (neocorticalization). Three aspects of the statistical models favored the latter. First, the full model above [Akaike Information Criterion (AIC) = 56.7] was significantly better than one based on the sum of all nucleus volumes ($R^2 = 0.22$, AIC = 95.5). Second, repertoire size was only correlated with relative HVC and MMAN volumes (both $P < 3.92 \times 10^{-5}$) when nuclei were considered individually, but it was related to several others (RA, Area X, LMAN, and nXIIts) after its covariation with another nucleus had been factored out (Table S3). Third, the partial coefficients in reduced models had consistently opposing signs; the slope associated with the upstream nucleus (relative to the syrinx) was positive whereas the slope with the downstream nucleus was usually negative. This indicated that evolutionary changes in repertoire size were more closely related to changes in the size of higher motor areas relative to lower than to their collective size relative to the rest of the brain.

A residual analysis was used to illustrate that SMP convergence, defined as the sizes of HVC relative to RA [$HVC_{(RA)}$] and RA relative to nXIIts [$RA_{(nXIIts)}$], was a better predictor of repertoire size than were their volumes relative to more general size references (Table S4). Syllable repertoire size was more strongly related to $HVC_{(RA)}$ ($R^2 = 0.62$; AIC = 58.2) than HVC volume relative to a midbrain vocal–respiratory motor nucleus [the dorsomedial nucleus of the intercollicular complex (DM)], hindbrain trigeminal motor nuclei [motor nucleus of the trigeminal nerve (MV), motor nucleus of the facial nerve (nVII), and lingual portion of the hypoglossal nucleus (nXIIling)], the hippocampus (Hp), B–SS, or body mass (all AIC > 69.4). Similarly, repertoire size was significantly related to $RA_{(nXIIts)}$ ($R^2 = 0.22$; AIC = 93.3; $P = 0.001$) but not to RA or nXIIts volumes relative to the other size references (all AIC > 102.7; all $P > 0.09$). Interestingly, $HVC_{(RA)}$ and $RA_{(nXIIts)}$ were not related to each other ($P = 0.08$), and both retained positive coefficients [2.6 ($P = 4.53 \times 10^{-11}$) and 1.4 ($P = 0.001$), respectively] in a two-variable model explaining species variation in repertoire size ($R^2 = 0.70$; AIC = 48.7). The latter model was significantly better than the one using total SMP volume relative to brain size [HVC + RA + $nXIIts_{(B–SS)}$] as an explanatory variable ($R^2 = 0.35$; AIC = 84.3).

Next, we estimated neuron densities and numbers (#) in some nuclei to examine the likelihood that $HVC_{(RA)}$ and $RA_{(nXIIts)}$ volumes reflected true neuronal convergence. First, the relative volumes of all three nuclei closely paralleled their relative neuron numbers. HVC volume was strongly related to HVC# ($r = 0.98$, $P < 1.0 \times 10^{-16}$) but not to neuron density ($P = 0.44$) after controlling for B–SS, whereas RA and nXIIts volumes were directly related to their respective neuron numbers (both $r > 0.88$, $P < 1.0 \times 10^{-16}$) and also inversely related to their respective neuron densities (both $r < -0.43$, $P < 0.001$). Second, species differences in pathway convergence imply divergent numbers of descending axons; this was expected to create inverse relations between relative neuron number in an upstream nucleus and relative neuron density in its downstream target. Consistent with this, neuron density in RA was inversely related to HVC# after controlling for B–SS, LMAN#, and RA# ($r = -0.54$; $P = 2.11 \times 10^{-5}$), and neuron density in nXIIts was inversely related to RA# after accounting for B–SS and nXIIts# ($r = -0.41$; $P = 0.002$). Third, repeating the residual analyses above using neuron number estimates yielded similar results (Fig. 3 and Table S5). $HVC\#_{(RA\#)}$ and $RA\#_{(nXIIts\#)}$ together were strongly associated with repertoire size ($R^2 = 0.64$; AIC = 58.2), were a significantly better predictor of it than was $SMP\#_{(B–SS)}$ ($R^2 = 0.43$; AIC = 77.9), and were not related to each other ($P = 0.57$).

Finally, we assessed whether intraspecific sample sizes or variability among reported repertoire sizes signficantly affected the results. Most of the variation in log-transformed and relative song nucleus volumes was attributable to between-species differences (one-way ANOVA: $RA_{(nXIIts)}$, $R^2 = 0.75$; all other measures, $0.82 < R^2 < 0.99$). Relations this strong prevented the inflation of Type I error rates in simulations with comparably small sample sizes (43). The pattern described above was also preserved in statistical models that incorporated measurement error, which can reduce bias in the coefficient estimates (Table S6) (44). Lastly, we found more than one repertoire size estimate for 28 of the 43 species with documented repertoires (Dataset S2). Most species-

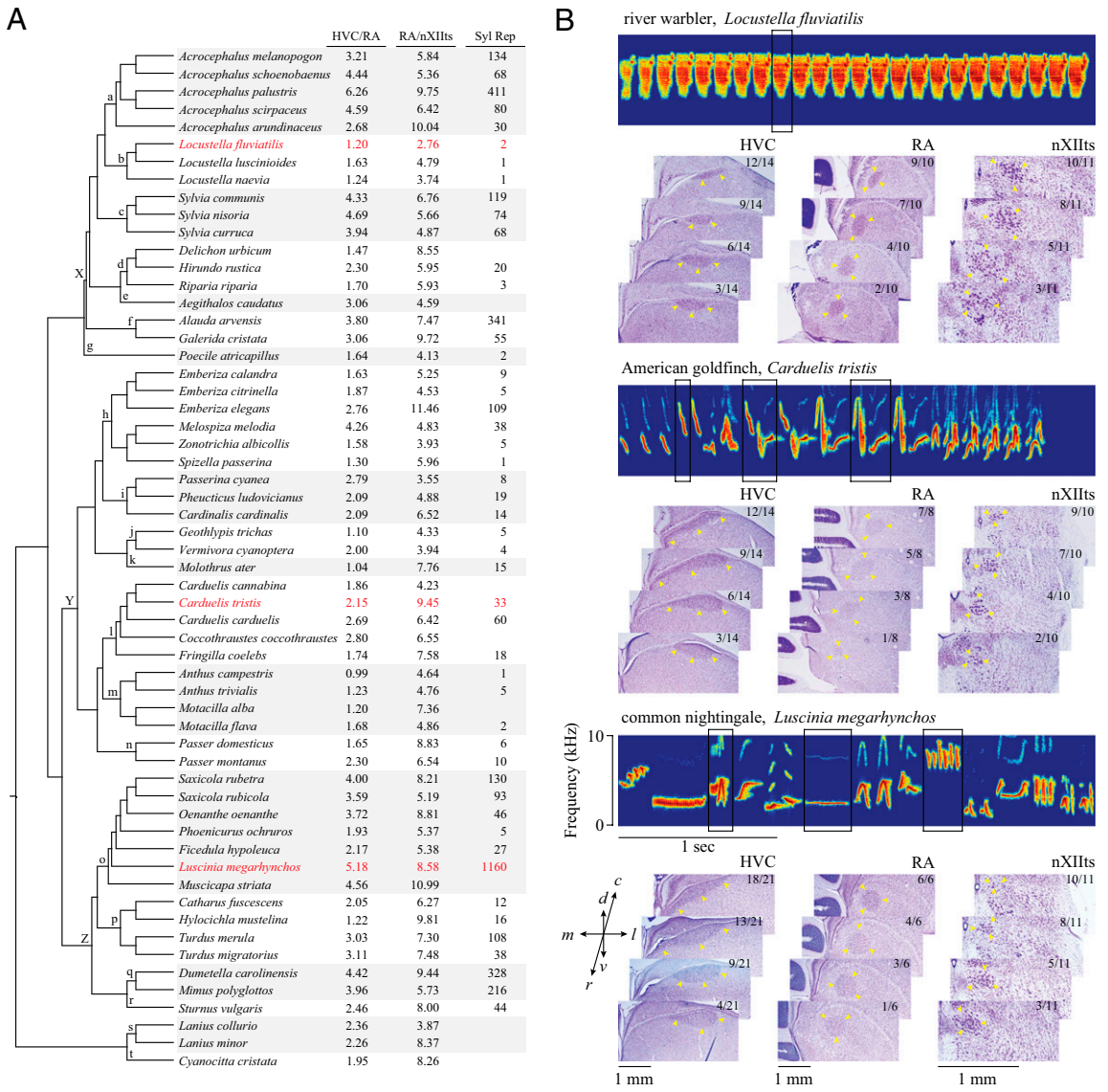

Fig. 2. (*A*) Phylogenetic relationships between the 58 species studied here. HVC-to-RA and RA-to-nXIIts ratios are listed for illustrative purposes only and were not used in the statistical analyses (details in *Materials and Methods*). Syl Rep size, syllable repertoire size. The 20 families sampled were: a, Acrocephalidae; b, Locustellidae; c, Sylviidae; d, Hirundinidae; e, Aegithalidae; f, Alaudidae; g, Paridae; h, Emberizidae; i, Cardinalidae; j, Parulidae; k, Icteridae; l, Fringillidae; m, Motacillidae; n, Passeridae; o, Muscicapidae; p, Turdidae; q, Mimidae; r, Sturnidae; s, Laniidae; t, Corvidae. The three superfamilies within the Passerida parvorder are X, Sylvioidea; Y, Passeroidea; and Z, Muscicapoidea. (*B*) Song spectrograms and Nissl-stained brain sections showing the ranges of observed behavioral and neural variation. Examples of single- and multi-note syllables are outlined. Each bird's left HVC, RA, and nXIIts are demarcated by yellow arrows; labels indicate the section number out of the total number of stained sections containing that nucleus (only alternate sections were stained).

typical estimates were consistent, and pathway convergence values remained the superior predictors of repertoire size regardless of which behavioral values were used (Table S7).

Relations Between Repertoire Size and Other Neural Traits. Syllable repertoire size was weakly related to the volume of the telencephalon-song system (T-SS) relative to the rest of the brain ($R^2 = 0.10$; $P = 0.04$), but it was not related to any of the other traits measured (all $P > 0.10$) (Table S8). These included B–SS and T-SS volumes relative to body mass and relative volumes of the mesopallial subdivision of the telencephalon (M), limbic nuclei (Hp, septum, and nucleus taeniae of the amygdala), auditory nuclei (nucleus ovoidalis and the dorsal portion of the lateral mesencephalic nucleus), and trigeminal sensorimotor nuclei [nucleus basorostralis, MV, nVII, and nXIIling].

Discussion

Studies on the correlated evolution of brain and behavior have long been used to generate hypotheses about the causes of species differences in behavior. Most common among their findings is that species with relatively greater behavioral capacities possess larger brain regions controlling them. These relations with behavior can involve various levels of specific neural pathways [forebrain (4, 8, 12), midbrain (9, 11), and hindbrain (6)] and extend to heterogeneous brain subdivisions (45) and the entire brain (17). Yet, despite their ubiquity, some of these associations' most basic facets remain ambiguous. For example, functional correlates of mammalian brain size can depend critically on the size reference used, but it can be unclear whether indices of encephalization or neocorticalization are most appropriate (2, 46, 47). This issue also pertains to func-

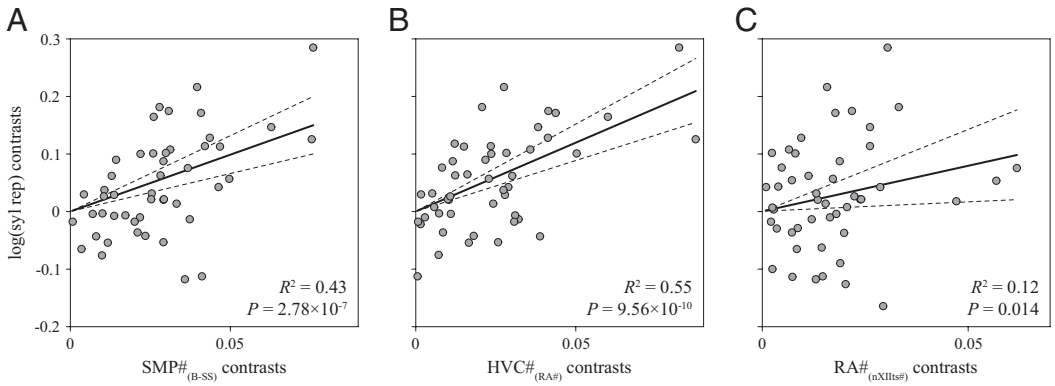

Fig. 3. Least squares regressions (±95% confidence intervals) between standardized independent contrasts of $\log_{10}$(syllable repertoire) and (*A*) song motor pathway neuron number (SMP#) relative to B–SS, (*B*) HVC# relative to RA#, and (*C*) RA# relative to nXIIts#. Results from independent contrasts are equivalent to those from PGLS models when assuming a Brownian motion model of evolution, which was done here (n = 48).

tionally specific neural circuits. It is unknown if the principle of proper mass broadly applies to entire pathways, because detailed descriptions of both the behavioral variation across species and the brain regions responsible for it are seldom available.

Songbirds are an exception to this, and comparisons across oscines can provide valuable insights into the mechanisms governing behavioral and brain evolution more generally. We find here that species differences in syllable repertoire size closely parallel differences in the degree of song motor pathway convergence. The number of neurons in HVC and RA relative to that in their downstream targets is a superior predictor of repertoire size than is the number of SMP neurons relative to the rest of the brain. This does not follow from the principle of proper mass, because species with substantially fewer neurons in higher areas than expected from their repertoire size also had relatively few neurons in lower nuclei, and vice versa. Thus, despite strong positive correlations linking most relative nucleus volumes, the size of higher motor areas relative to lower evolved more closely with behavior than did their collective size relative to the rest of the brain. The direction of particular changes through time is unknown, because our sparse sampling of this large and diverse group, with complete omission of major constituent lineages, reduces confidence in ancestral character state estimates. Nevertheless, this association exists throughout the entire phylogeny sampled and includes multiple convergent increases and/or decreases in repertoire size.

Distinct portions of the behavioral variation are attributable to the convergence along serial premotor [$HVC\#_{(RA\#)}$] and primary motor [$RA\#_{(nXIIts\#)}$] projections. Repertoire sizes could be expanded in a variety of ways—by producing novel sounds, altering the temporal properties and/or sequence of notes already mastered, or any combination of the two. If changes in convergence along the two projections alter repertoire size in different ways, these pathway components could be targets for distinct selective forces acting on different features of song. The data needed to explore this possibility are lacking, but current knowledge of song system physiology can provide clues as to why the two relations exist.

In the zebra finch, $HVC_{\rightarrow RA}$ projection neurons sparsely encode song whereby each cell fires a short (~6 ms), temporally precise burst of action potentials that corresponds to one specific song segment (23). These cells are connected via axon collaterals and believed to form a feed-forward synfire chain, where the sequential firing of neurons along a chain (or parallel chains) specifies an entire syllable or song (48, 49). Neurons that are simultaneously active during these short periods excite unique neuron ensembles in RA, which represents the first myotopic map of syringeal and respiratory muscles in this pathway (50). Population-level activity patterns in RA also correspond to short song segments and are as temporally precise as the activity in HVC (51, 52). Because many distinct RA ensembles can be associated with the same sound, it has been suggested that the linear sum of RA inputs onto nXIIts neurons determines the strength of each muscular contraction (52, 53). These mechanisms are thereby thought to control the temporal patterning and extent of syringeal labial adduction and abduction and the airflow past them, which largely shape the spectral features of song (54).

If these song-encoding mechanisms are generally true across oscines, increases in $HVC\#_{(RA\#)}$ could provide the substrate for a greater number of synfire chains and enable the acquisition of larger repertoires. Such a change would increase the number of ways to connect $HVC_{\rightarrow RA}$ neurons with axon collaterals and create new potential sequences of RA ensemble excitation, even if many neurons had redundant RA-projection patterns. It would also be likely to increase the number of RA-projection patterns that could excite novel RA ensembles, thereby enabling the production of new syringeal conformations and sounds. Evolutionary increases in $RA\#_{(nXIIts\#)}$ could increase the total amount of excitatory input received by each nXIIts neuron, expand its range of attainable firing rates, and increase the potential contractile strength of that motor unit. Alternatively, if increases in $RA\#_{(nXIIts\#)}$ were accompanied by decreases in RA axonal branching and/or synaptic strengths, greater convergence could enable more finely graded changes in nXIIts firing rates. Either would provide more control over syringeal shape and could lead to the production of more spectrally varied sounds.

These speculations require future experiments to ascertain their merits. Generalizing physiological data from the zebra finch, a species whose song is unusual in several respects (55), is not ideal, but several observations are consistent with the scenario above. First, syringeal morphology and the peripheral mechanisms of sound production are similar across species (56, 57), making differences in neural control the most likely source of species differences in repertoire size. Second, distantly related species have similar physiological activity patterns in HVC (36), suggesting that basic song-encoding mechanisms have been conserved through the oscine lineage. Third, potential covariates of repertoire size, such as song duration, are not obviously related to SMP convergence. Species within our dataset that sing long songs (>1 s) comprised of a single, repeated syllable (e.g., the chipping sparrow, *Spizella passerina*) have small $HVC\#_{(RA\#)}$ values, whereas others that sing shorter songs but draw from larger repertoires have comparatively large $HVC\#_{(RA\#)}$ values (e.g., the American robin, *Turdus migratorius*). If HVC synfire chains encode song in these species, then the syllable repetitions seem more likely to be encoded by recurrent activation of a short

chain (or parallel chains) than by a single long chain encoding multiple syllables (36). More detailed comparisons of song nucleus composition, dendritic and axonal arborization, motor unit sizes, and physiological activity patterns are needed to elucidate the precise underpinnings of this brain–behavior association.

Comparing the behaviors of diverse species also requires care. Syllable repertoire size is a convenient metric because syllables are a basic song unit across oscines and it is clearly defined and quantifiable. It is only one of multiple song characteristics that varies between species, however, and features such as the range of spectrotemporal modulations or the rate and bandwidth of frequency modulations could relate to anatomical traits other than SMP convergence. Differences also exist in the use or valence of syllables within a repertoire (58, 59), and disparities in average syllable duration, spectrotemporal complexity, stereotypy (60), or number of unexpressed songs remembered (61) are likely to affect the relations reported here. Species also vary in singing style, and the neural demands for syllable repetitions vs. transitions are potentially different (62). Nonetheless, despite the many song qualities unaccounted for here, the immense interspecific variation in repertoire size is accurately predicted by robust differences in song system anatomy.

Large repertoires have independently evolved in multiple oscine lineages, but their communicative function is still unclear. Here, syllable repertoire size is weakly but positively related to telencephalon size relative to the rest of the brain, suggesting that this sexually selected signal could reflect the sender's degree of telencephalization and, potentially, indicate more general cognitive traits such as behavioral flexibility or learning proficiency (63). Such a link could be rooted in the mechanisms of brain development. The oscine telencephalon adds many neurons posthatching whereas the rest of the brain does not, and HVC recruits new neurons as juveniles learn to sing but RA does not (64, 65). Although more neurons are added to HVC than surrounding areas during this time, neuroblasts do continue to differentiate throughout the forebrain (65). Prolonged periods of neurogenesis and/or expanded pools of progenitors could thereby produce both increased SMP convergence and a greater degree of telencephalization (66).

It has long been thought that heightened behavioral abilities emerge from expansions of the neural circuits controlling them. Our results support this view on the whole but also reveal informative aspects of circuit architecture that are overlooked by this explanation. In particular, we show that the size of upstream areas relative to their downstream targets can be a superior indicator of behavioral abilities than the relative size of an entire neural pathway. These findings highlight specific mechanisms by which changes in pathway convergence could have underlain the evolution of a complex motor behavior.

Materials and Methods

Additional methodological details are provided in *SI Materials and Methods*.

Specimen Collection and Preparation. One to four adult male songbirds of 58 temperate zone species spanning 20 families were wild-caught with mist nets. Collections were restricted to spring months (April to June) when birds were reproductively active to minimize seasonal variation in song system anatomy. Brains were sectioned at 40 μm in the coronal plane, and alternate sections were Nissl stained with cresyl violet. All appropriate local, provincial, and/or national permits were held at the time of bird collection, and all procedures were approved by the Cornell University Institutional Animal Care and Use Committee.

Brain Measurements. Nucleus and brain region boundaries were traced using a camera lucida from alternate sections viewed with 40× magnification or from every fourth section with 20× magnification (M and Hp only). Brain and telencephalon boundaries were traced in every fourth section from unmagnified digital images. All reported values are from one side of the brain (typically the left except in cases where torn tissue or poor staining prevented measurements of that side). Cross-sectional areas of scanned boundary traces and images were measured using ImageJ software (67), and final volumes were computed by summing the areas and multiplying by the sampling interval (0.08 or 0.16 mm). Exemplar images of each nucleus are provided (Fig. S1).

Neuron densities in HVC, LMAN, RA, and nXIIts were measured in one brain of each species. Multiple cell counts were made throughout each nucleus in sampling windows with dimensions of 80 × 80 μm (600×; HVC, LMAN, and nXIIts) or 120 × 120 μm (400×; RA). On average, 12 tallies (range = 7–26) were made in each structure. Presumptive neurons were discriminated from glia primarily on the basis of their larger somata (greater than or equal to ∼10 μm diameter) and uniformly stained cytoplasm. Intermediate-sized cells were counted if they had a single darkly stained nucleolus because this was a characteristic feature of most large cells. Split cells that did not have a visible nucleolus were not counted. Nuclear neuron number estimates were computed by multiplying average volumes and neuron densities for each species.

Syllable Repertoires. Species-typical syllable repertoire sizes were obtained for 49 of 58 species, mostly from published sources (*SI Materials and Methods*). Repertoire sizes for six species were estimated from recordings held by the Macaulay Library at Cornell University. A song syllable was generally defined as a continuous trace on a spectrogram or a stereotyped sequence of notes separated by <25 ms (Fig. 2), while a repertoire was the number of unique syllables produced by an individual bird.

Phylogeny. Species names and taxonomy follow the suggestions of the International Ornithologists' Union (68). A fully resolved, composite phylogeny was constructed from published molecular phylogenies; sources are provided in *SI Materials and Methods*.

Comparative Analysis. Most analyses used PGLS models (44, 69), which measure the linear relations between two or more variables while accounting for their statistical nonindependence. Phylogenetic relatedness is incorporated in the form of a variance–covariance matrix, and regression parameters significantly different from zero indicate correlated evolutionary changes between the dependent and corresponding independent variables. Comprehensive reviews of phylogenetic comparative statistical methods can be found elsewhere (69, 70).

Using $\log_{10}$-transformed data, we constructed PGLS models to (*i*) explore the relations between relative song nucleus volumes, (*ii*) describe the relations between relative nucleus neuron numbers and relative nucleus neuron densities, and (*iii*) explain the variation in syllable repertoire size as a function of various neural measures. A body size covariate was usually included and was either body mass or the difference between brain volume and the respective subdivision/system volume of interest [i.e., telencephalon (B–T), mesopallium (B–M), sum of song nuclei (B–SS), sum of limbic structures, sum of auditory nuclei, or sum of trigeminal sensorimotor nuclei]. Nested models (i.e., those that contain a subset of the independent variables used in a full model) were compared with the full model using maximum LRTs, nonnested models were compared using the AIC (smaller values indicate a better fit and differences >2 are statistically significant), and partial *t*-tests were used to assess the significance of model parameters.

We conducted residual analyses to illustrate the concept of song motor pathway convergence. Here, using $\log_{10}$-transformed data from the 49 species with estimated repertoire sizes, we first calculated the regression slopes for HVC, RA, nXIIts, and SMP (HVC + RA + nXIIts) volumes and neuron numbers as a function of a size reference. This reference was either a general indicator of size [e.g., HVC vs. log(B–SS)] or the volume or neuron number of another nucleus [e.g., log(HVC#) vs. log(RA#)]. These slopes were then used to compute relative trait values with the formula $\log_{10}[\text{trait}/(\text{size}^b)]$, where b was the allometric regression slope and trait and size were the original data values. Residual analyses can yield biased parameter estimates when the independent variable and size reference covary, which was the case here; however, this bias is conservative and its impact was strongest on the models that performed best (71, 72).

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