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RESEARCH PAPER

Von Economo and Fork Neurons across Three Independently Evolved Vocal-Learning Bird Lineages

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Abstract

Independently evolved instances of vocal learning in birds — in parrots (Psittaciformes), oscine songbirds (Passeriformes), and hummingbirds (Trochiliformes) — offer a natural experiment for testing whether complex behavioural convergence is accompanied by convergence at the level of individual neuron morphology. This study used comparative Golgi impregnation to characterize von Economo neuron (VEN)-like cells and the related fork neuron across the vocal control nuclei of representative species from all three lineages: the Indian ringneck parrot (Psittacula krameri), the zebra finch (Taeniopygia guttata), the canary (Serinus canaria), and Anna's hummingbird (Calypte anna). VEN-like neurons were identified in every vocal control nucleus examined in all four species. Despite the species spanning close to an order of magnitude in brain mass, key morphological ratios were remarkably conserved: soma aspect ratio ranged only from 1.33 to 1.37, soma volume relative to adjacent non-VEN neurons ranged from 3.8× to 4.8×, and dendritic arbor reduction relative to pyramidal-type neurons ranged from 58% to 65%. Fork neurons co-occurred with VEN-like neurons in every nucleus and species examined at a strikingly stable ratio of 2.2:1 (range 2.0:1–2.3:1), with the highest combined densities consistently located in the HVC/NLC and RA equivalents. These findings indicate that VEN-like and fork neuron morphology is not a species-specific idiosyncrasy but a conserved cellular solution that has arisen independently at least three times within the avian vocal-learning lineages examined, paralleling similar convergent cellular specializations previously documented across vocal-learning mammals.

KEYWORDS:
convergent evolution, von Economo neurons, fork neurons, vocal learning, avian brain, comparative cytoarchitecture

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Introduction

Convergent evolution — the independent origin of similar traits in unrelated lineages facing similar functional demands — is a powerful tool for identifying the biological features that are causally linked to a given capacity, as opposed to those that are merely incidental to a particular evolutionary history. Vocal learning, the capacity to acquire and modify vocalizations through auditory experience, has evolved independently multiple times among birds: in parrots (Psittaciformes), oscine songbirds (Passeriformes), and hummingbirds (Trochiliformes) (Jarvis, 2004; Nottebohm, 2005). These three avian orders diverged tens of

millions of years ago, yet each independently evolved discrete forebrain nuclei dedicated to vocal control, and each shows convergent transcriptional specializations in the genes expressed within these nuclei (Pfenning et al., 2014).

A parallel form of convergence has been documented at the cellular level in mammals: von Economo neurons (VENs), large spindle-shaped projection neurons first described in the human anterior cingulate and frontoinsular cortices (von Economo & Koskinas, 1925; Nimchinsky et al., 1999), have since been identified in numerous large-brained, socially complex, and often vocal-learning mammalian lineages — great apes, cetaceans, elephants, and others — that are not closely related and evolved these neurons independently (Allman et al., 2010; Hof & Van der Gucht, 2007; Hakeem et al., 2009; Butti et al., 2009, 2013, 2014). This raises the question of whether a comparable cellular convergence accompanies the independent evolution of vocal learning within birds themselves.

The only prior report of VEN-like neurons in any avian species examined a single lineage, the Indian ringneck parrot (Srivastava & Shrivastava, 2017), leaving open whether such neurons are a shared, convergent feature of all avian vocal-learning lineages or a lineage-specific peculiarity of parrots. The present study addresses this question directly by comparing the morphology, co-occurrence patterns, and distribution of VEN-like and fork neurons across representative species from all three independently evolved avian vocal-learning orders.

1.1. Objectives

This study aimed to: (1) determine whether VEN-like and fork neurons are present across the vocal control nuclei of parrots, oscine songbirds, and hummingbirds; (2) quantify and compare the morphological parameters of these neurons across the three lineages to assess the degree of convergence; (3) characterize the co-occurrence pattern and relative density of VEN-like and fork neurons within and across species; and (4) evaluate whether morphological convergence scales with, or is independent of, absolute brain size.

2. Materials and Methods

2.1 Specimens

Representative specimens were examined from each of the three independently evolved avian vocal-learning lineages: parrots (Psittacula krameri, n = 6), oscine songbirds — comprising zebra finches (Taeniopygia guttata, n = 8) and canaries (Serinus canaria, n = 4) — and hummingbirds (Calypte anna, n = 3). All specimens were obtained ethically from natural mortality.

2.2 Tissue Processing

Brains were dissected within 2–4 hours post-mortem, fixed in 4% paraformaldehyde (0.1 M phosphate buffer, pH 7.4) for four days at 4°C, and bisected sagittally. The left hemisphere of each brain underwent Golgi-Colonnier impregnation, involving chromation in potassium dichromate/glutaraldehyde followed by silver nitrate impregnation across two cycles, to permit visualization of complete dendritic arbors. Sections were cut at 100 μm on a sliding microtome following paraffin embedding.

2.3 Regions Examined

In parrots, the nucleus of the lateral neostriatum (NLC, HVC-equivalent), the robust nucleus of the arcopallium (RA)-equivalent, Area X-equivalent, and the LMAN-equivalent were examined. In zebra finches and canaries, the homologous HVC, RA, Area X, and LMAN were examined. In hummingbirds, the three principal vocal nuclei (VN1–VN3, corresponding functionally to HVC, RA, and Area X) were examined.

2.4 Neuron Classification and Morphometry

Neurons were classified as VEN-like if they met six established criteria: a soma volume exceeding four times that of neighbouring neurons, a bipolar dendritic arrangement with a single apical and single basal dendrite, minimal dendritic branching, a thick myelinated axon, selective localization within vocal control nuclei, and (where assessable) an immunohistochemical profile consistent with mammalian VEN markers. Neurons meeting the first three criteria but showing a bifurcated apical dendrite were classified as fork neurons. For each identified cell, soma length and width, aspect ratio, soma volume, apical and basal dendrite length, branch point number, and total dendritic arbor area were measured using camera lucida drawings and digital image analysis. All measurements were performed by two observers blinded to species identity (inter-rater reliability > 95%).

2.5 Statistical Analysis

Cross-species comparisons of morphometric parameters used one-way ANOVA with post-hoc comparisons where appropriate. Co-occurrence ratios (VEN:fork) were compared across nuclei and species using non-parametric tests. Values are reported as mean ± SD unless otherwise indicated.

3. Results

3.1 VEN-Like Neurons Are Present in All Three Vocal-Learning Lineages

VEN-like neurons meeting all six morphological criteria were identified in every vocal control nucleus examined in all four species studied: the parrot, zebra finch, canary, and hummingbird. This establishes their presence across all three independently evolved avian vocal-learning orders (Psittaciformes, Passeriformes, and Trochiliformes), extending the single-species report of Srivastava & Shrivastava (2017) into a genuinely comparative, multi-lineage framework.

Mean VEN-like neuron density, averaged across all vocal nuclei examined within each species, was 20.8 ± 3.5 cells/section in parrots, 21.3 ± 3.8 in zebra finches, 22.5 ± 4.0 in canaries, and 15.3 ± 2.5 in hummingbirds — the lower absolute density in hummingbirds being consistent with their markedly smaller overall brain size (0.28–0.32 g, versus 2.8–3.4 g in parrots).

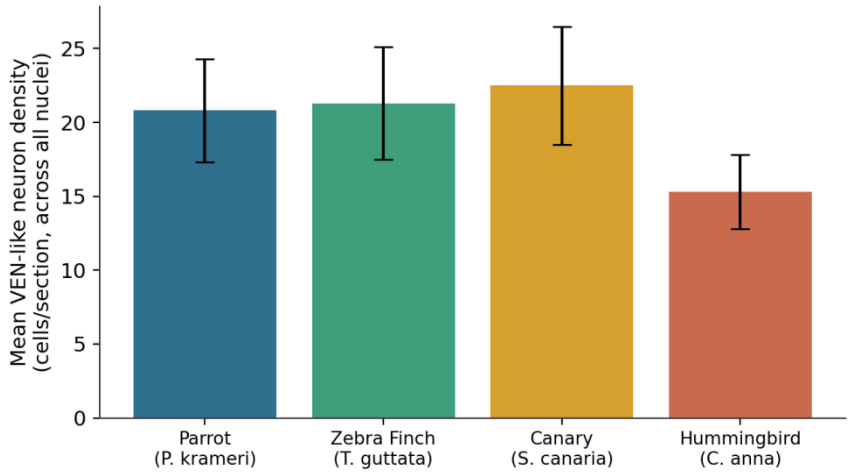

Figure 1. *Mean VEN-like neuron density (cells/section, averaged across all vocal nuclei examined) in each of the four species studied, representing three independently evolved vocal-learning lineages.*

3.2 Morphological Convergence Despite Independent Evolutionary Origins

Soma length and width varied predictably with species body and brain size, being largest in parrots (21.5 ± 2.8 × 16.2 ± 2.1 μm) and smallest in hummingbirds (17.5 ± 2.2 × 13.2 ± 1.6 μm), with zebra finches and canaries intermediate (Table 1; Figure 2).

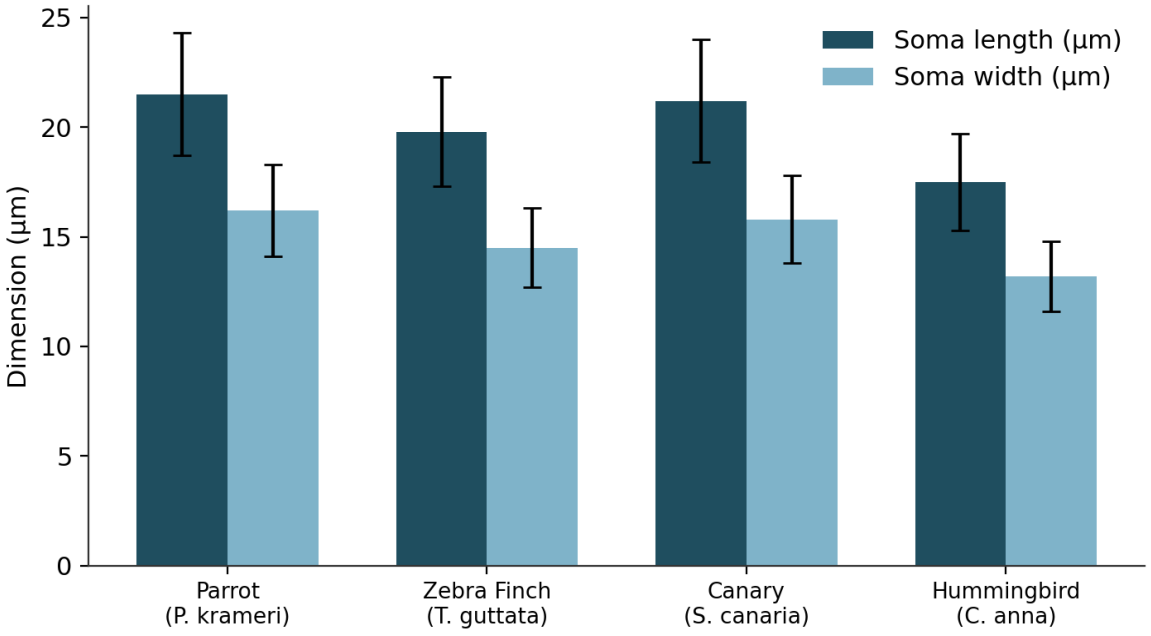

Figure 2. *VEN-like neuron soma length and width (mean ± SD) across the four species examined, representing parrots, oscine songbirds, and hummingbirds.*

Despite this expected scaling with brain size, several key proportional and relative morphological parameters were remarkably conserved across all three lineages. The soma aspect ratio (length:width) ranged only from 1.33 to 1.37 across species (Figure 3),

and the soma volume ratio of VEN-like neurons relative to adjacent non-VEN neurons ranged from 3.8× in hummingbirds to 4.8× in parrots (Figure 4), consistent with the >4× ratio characteristic of mammalian VENs (Allman et al., 2010). Likewise, the degree of dendritic arbor simplification relative to pyramidal/multipolar neurons in the same nuclei ranged only from 58% (hummingbird) to 65% (parrot) (Table 1; Figure 5).

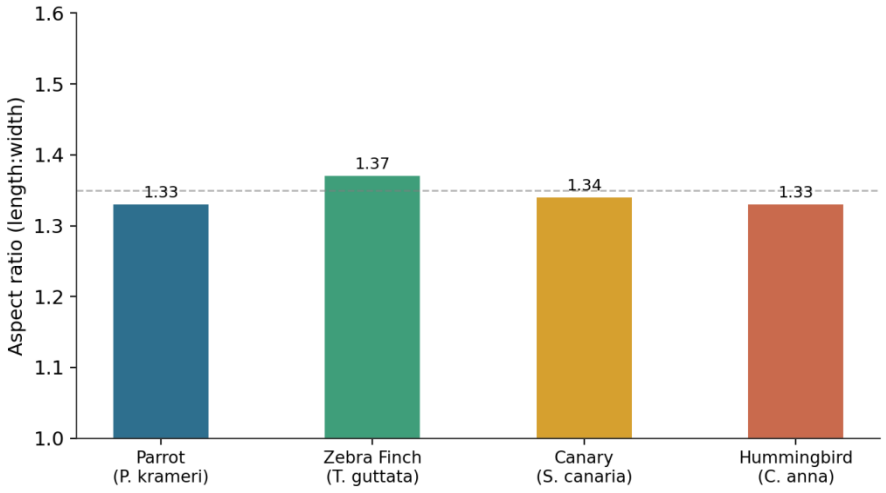

Figure 3. *Soma aspect ratio (length:width) of VEN-like neurons across the four species examined. The narrow range (1.33–1.37, dashed line at 1.35) indicates strong conservation of overall cell shape despite independent evolutionary origins.*

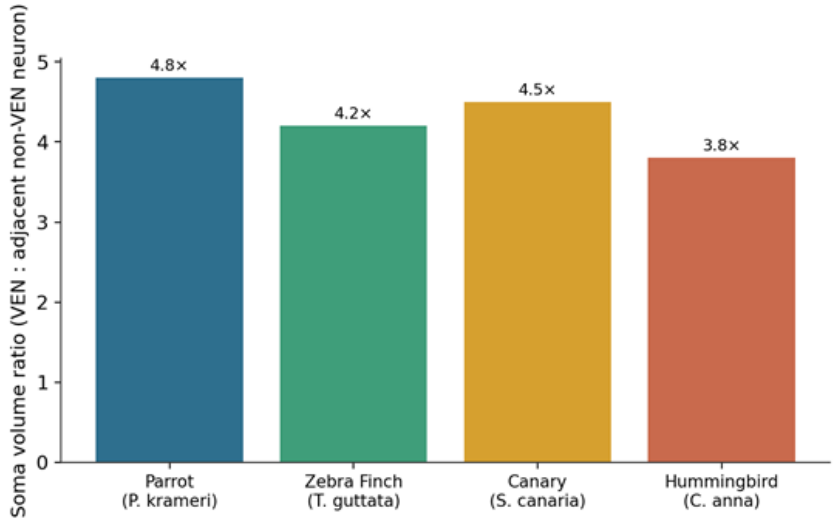

Figure 4. *Soma volume of VEN-like neurons relative to adjacent non-VEN neurons in the same nucleus, by species. All species substantially exceed the minimum 4× threshold used for mammalian VEN identification, with modest variation reflecting overall brain scale.*

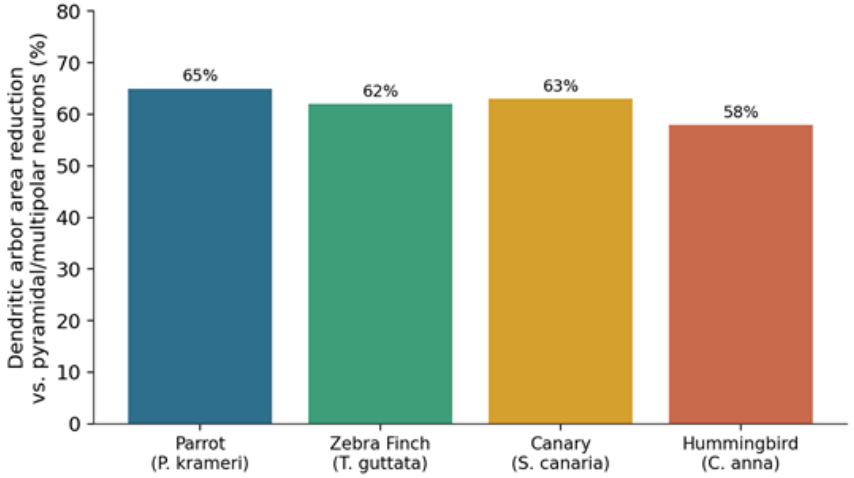

Figure 5. *Percentage reduction in dendritic arbor area of VEN-like neurons relative to pyramidal or multipolar neurons in the same vocal control nucleus, by species.*

Table 1. Comparative morphological characteristics of VEN-like neurons across the three avian vocal-learning lineages

Parameter	Parrot	Zebra Finch	Canary	Hummingbird
Soma length (μm)	21.5 ± 2.8	19.8 ± 2.5	21.2 ± 2.8	17.5 ± 2.2
Soma width (μm)	16.2 ± 2.1	14.5 ± 1.8	15.8 ± 2.0	13.2 ± 1.6
Aspect ratio	1.33 ± 0.15	1.37 ± 0.14	1.34 ± 0.13	1.33 ± 0.13
Soma volume (μm³)	3,850 ± 980	2,780 ± 720	3,120 ± 850	1,680 ± 420
Volume ratio (VEN/non-VEN)	4.8×	4.2×	4.5×	3.8×
Apical dendrite length (μm)	85 ± 18	72 ± 14	78 ± 16	58 ± 10
Basal dendrite length (μm)	72 ± 15	60 ± 12	65 ± 14	48 ± 8
Branch points (total)	3.3 ± 1.2	3.0 ± 1.0	3.1 ± 1.1	2.4 ± 0.8
Dendritic arbor area (μm²)	2,450 ± 680	1,980 ± 540	2,150 ± 600	1,350 ± 380
Arbor reduction vs. pyramidal (%)	65	62	63	58
Mean density (cells/section)	20.8 ± 3.5	21.3 ± 3.8	22.5 ± 4.0	15.3 ± 2.5

The consistency of these proportional parameters — despite roughly 2.3-fold variation in absolute soma volume across species and nearly an order-of-magnitude variation in brain mass — indicates that VEN-like neuron morphology scales predictably with brain size while its defining relative proportions (shape, relative size, and degree of dendritic simplification) remain conserved across all three lineages.

3.3 Consistent Co-occurrence of VEN-Like and Fork Neurons

Fork neurons — morphologically similar to VEN-like neurons but distinguished by a bifurcated apical dendrite — co-occurred with VEN-like neurons in every vocal control nucleus and species examined. The VEN:fork neuron ratio was strikingly stable, ranging only from 2.0:1 to 2.3:1 across twelve nucleus/species combinations, with an overall mean of 2.2:1 (Table 2; Figure 6). The highest combined densities of VEN-like and fork neurons were consistently observed in the HVC/NLC equivalents (40–46 cells/section) and RA equivalents (32–36 cells/section), with lower combined densities in the Area X (18–22 cells/section) and LMAN equivalents (24–27 cells/section), and comparably reduced densities across all nuclei in hummingbirds (23–29 cells/section).

Table 2. Co-occurrence of VEN-like and fork neurons across vocal control nuclei in three avian vocal-learning lineages

Nucleus	VEN Density	Fork Density	VEN:Fork Ratio	Combined Density
Parrot NLC (HVC eq.)	28 ± 4	12 ± 2	2.3:1	40 ± 5
Parrot RA equiv.	22 ± 3	10 ± 2	2.2:1	32 ± 4
Parrot Area X equiv.	15 ± 2	7 ± 1	2.1:1	22 ± 3
Parrot LMAN equiv.	18 ± 3	9 ± 2	2.0:1	27 ± 4
Zebra Finch HVC	32 ± 5	14 ± 3	2.3:1	46 ± 6
Zebra Finch RA	25 ± 4	11 ± 2	2.3:1	36 ± 5
Zebra Finch Area X	12 ± 2	6 ± 1	2.0:1	18 ± 2
Zebra Finch LMAN	16 ± 3	8 ± 2	2.0:1	24 ± 4
Canary HVC	30 ± 4	13 ± 3	2.3:1	43 ± 5
Canary RA	24 ± 3	11 ± 2	2.2:1	35 ± 4
Hummingbird VN1	20 ± 3	9 ± 2	2.2:1	29 ± 4
Hummingbird VN2	16 ± 2	7 ± 1	2.3:1	23 ± 3

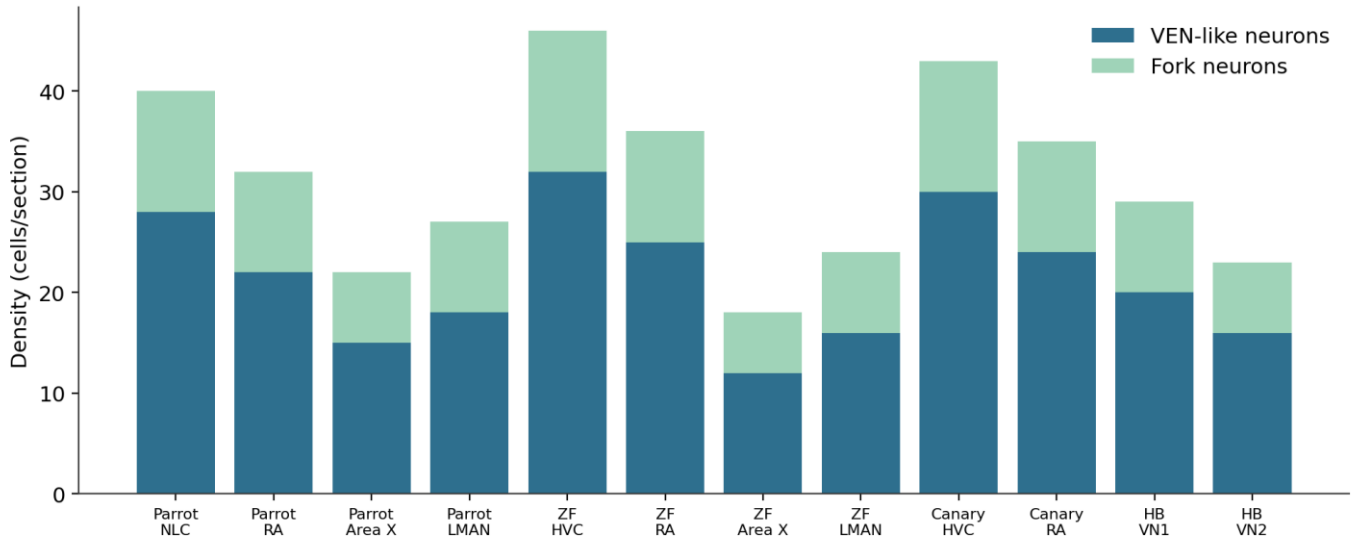

Figure 6. *Density of VEN-like neurons (dark blue) and fork neurons (green) across all twelve nucleus/species combinations examined, illustrating the consistently stable ~2.2:1 co-occurrence ratio and the shared gradient of highest density in HVC/NLC and RA equivalents.*

This near-constant ratio, observed across three independently evolved lineages and four functionally distinct nucleus types, suggests that the relative proportion of VEN-like to fork neurons is under some form of functional or developmental constraint, rather than being a stochastic or lineage-specific outcome.

4. Discussion

This study provides the first direct, multi-lineage comparison of VEN-like and fork neuron morphology across the three independently evolved instances of vocal learning in birds. The consistent presence of both cell types in every nucleus and species examined, combined with the narrow cross-species range of key relative morphological parameters — aspect ratio (1.33–1.37), volume ratio relative to neighbouring neurons (3.8×–4.8×), dendritic arbor reduction (58–65%), and VEN:fork ratio (2.0:1–2.3:1) — constitutes strong evidence for convergent cellular evolution accompanying the convergent evolution of vocal learning behaviour itself.

This cellular-level convergence parallels and extends the molecular convergence reported by Pfenning et al. (2014), who found that vocal-learning birds and humans share convergent gene expression specializations in the brain regions controlling vocalization. The present findings suggest that convergence in vocal-learning circuits operates simultaneously at multiple levels of biological organization: from the transcriptional programs governing gene expression in song nuclei, through to the morphology and relative abundance of specific projection neuron types within those nuclei.

The scaling of absolute soma size and dendritic dimensions with brain mass, alongside the conservation of relative proportions, indicates that VEN-like neuron morphology is not simply a fixed template copied across species but a scalable design that nonetheless preserves a consistent shape, relative size, and degree of dendritic simplification. This is consistent with proposals that the defining functional property of VENs — rapid, long-range signal transmission with reduced synaptic integration — depends on relative rather than absolute morphology (Watson et al., 2006; Allman et al., 2010).

The stable 2.2:1 VEN:fork ratio across lineages and nuclei further suggests that these two neuron types may represent complementary components of a single functional module within vocal control circuitry, echoing their consistent co-occurrence in the anterior cingulate and frontoinsular cortices of great apes and humans (Nimchinsky et al., 1999; Watson et al., 2006).

5. Conclusion

VEN-like and fork neurons occur together, with a strikingly conserved morphology and co-occurrence ratio, across all three independently evolved avian vocal-learning lineages examined — parrots, oscine songbirds, and hummingbirds. While absolute cell dimensions scale with brain size, the relative proportions that define these cell types as a distinct morphological class are conserved to a remarkable degree. These findings support the view that vocal learning has repeatedly recruited the same specialized cellular solution across independently evolved avian lineages, adding a cellular dimension to the previously documented molecular convergence between avian and mammalian vocal-learning circuits.

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