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Comparative Golgi-Nissl Evidence for Von Economo-like Neurons in Vocal-Learning Birds

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Abstract
Von Economo neurons (VENs) are large, spindle-shaped projection neurons originally described in the human anterior cingulate and frontoinsular cortices and subsequently identified across a broad range of large-brained, socially complex mammals, most of which are capable of vocal learning. To date, VEN-like cells have been reported in only a single avian species, the Indian ringneck parrot (*Psittacula krameri*), and no study has systematically compared their occurrence across multiple vocal-learning and non-vocal-learning bird taxa. This study examined 32 avian specimens spanning three independently evolved vocal-learning lineages — parrots (n = 6), oscine songbirds (zebra finch and canary, n = 12), and hummingbirds (n = 3) — alongside two vocal non-learning species, domestic chickens (n = 6) and rock pigeons (n = 5). Golgi-Colonnier impregnation and Nissl staining were used to characterize neuronal morphology and cytoarchitecture in the principal vocal control nuclei (or their homologues) and equivalent brain regions. VEN-like neurons meeting six established morphological criteria were identified in 100% of vocal-learning specimens (21/21) and were completely absent in all vocal non-learning specimens (0/11; Fisher's exact test, $p < 0.001$). Vocal learners additionally showed markedly higher cell density, larger soma size, greater proportions of large projection neurons, and sharper nuclear boundary definition in their vocal control regions than non-learners (all $p < 0.001$). These findings provide the first multi-species comparative evidence that VEN-like neurons constitute a convergent cellular specialization tightly linked to the capacity for vocal production learning in birds, mirroring their distribution across vocal-learning mammals.

Keywords: Von Economo neurons, vocal learning, avian brain, Golgi staining, comparative neuroanatomy, convergent evolution

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1. Introduction

The neural basis of vocal learning — the capacity to acquire and modify vocalizations through auditory experience rather than through fixed genetic programming — remains one of the most compelling problems in comparative neuroscience. This capacity is rare among vertebrates, documented with confidence only in humans, cetaceans, elephants, pinnipeds, some bats, and, among birds, the oscine songbirds, parrots, and hummingbirds (Jarvis, 2004; Janik & Slater, 1997).

A striking parallel exists between the distribution of vocal learning and the distribution of von Economo neurons (VENs), also called spindle cells. First described by Theodor Kaes (1907) and characterized systematically by von Economo and Koskinas (1925) in the human anterior cingulate and frontoinsular cortices, VENs were long thought unique to humans. Nimchinsky et al. (1999) subsequently identified them in great apes, and later work extended their known range to cetaceans

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(Hof & Van der Gucht, 2007), elephants (Hakeem et al., 2009), and a range of other large-brained, socially complex mammals (Butti et al., 2009, 2013, 2014). Notably, nearly all mammalian species in which VENs have been documented are either confirmed or suspected vocal learners.

In birds, VEN-like neurons have been reported in only one species: the Indian ringneck parrot (Srivastava & Shrivastava, 2017). Whether such neurons are a general feature of avian vocal-learning circuitry, present across independently evolved vocal-learning lineages and absent in vocal non-learners, has never been tested. This gap motivated the present investigation, which used comparative Golgi impregnation and Nissl staining to examine vocal control regions across three vocal-learning avian orders and two vocal non-learning species.

1.1 Objectives

This study aimed to: (1) determine whether VEN-like neurons are present in the vocal control nuclei of vocal-learning birds and absent in vocal non-learners; (2) characterize the cytoarchitecture and morphologically distinct neuronal classes of vocal control regions in vocal learners; and (3) quantitatively compare neuronal density, soma morphology, and nuclear organization between vocal-learning and non-learning taxa.

2. Materials and Methods

2.1 Specimens

Thirty-two naturally deceased avian specimens were examined, sourced ethically without harm to living animals. The vocal-learning group (n = 21) comprised Indian ringneck parrots (*Psittacula krameri*, n = 6), zebra finches (*Taeniopygia guttata*, n = 8), canaries (Serinus canaria, n = 4), and Anna's hummingbirds (*Calypte anna*, n = 3). The vocal non-learning group (n = 11) comprised domestic chickens (*Gallus gallus domesticus*, n = 6) and rock pigeons (*Columba livia*, n = 5). Chicken brains were obtained commercially; all other specimens were recovered from natural mortality.

2.2 Tissue Processing

Brains were dissected within 2–4 hours post-mortem and fixed in 4% paraformaldehyde (0.1 M phosphate buffer, pH 7.4) for four days at 4°C, then bisected sagittally. The left hemisphere of each brain underwent Golgi-Colonnier impregnation (potassium dichromate/glutaraldehyde chromation followed by silver nitrate impregnation, repeated across two cycles) to reveal complete dendritic architecture. The right hemisphere underwent Nissl staining to delineate nuclear boundaries and cytoarchitecture. Tissue quality was assessed across six parameters (staining clarity, impregnation density, preservation index, section completeness); all specimens exceeded minimum acceptability thresholds (overall quality scores 7.8–9.1/10), and none were excluded from analysis.

2.3 Regions Examined

In vocal learners, the principal song-system nuclei were examined: the HVC (or its parrot homologue, the nucleus of the lateral neostriatum, NLC), the robust nucleus of the arcopallium (RA), Area X, and the lateral magnocellular nucleus of the anterior nidopallium (LMAN). In non-learners, the cytoarchitecturally equivalent nidopallial, arcopallial, striatal, and anterior nidopallial regions were examined in the absence of discrete song nuclei.

2.4 VEN-like Neuron Identification

Neurons were classified as VEN-like if they satisfied six criteria adapted from the mammalian VEN literature: (1) a large, elongated soma exceeding four times the mean volume of neighboring neurons; (2) a bipolar dendritic arrangement with a single apical and single basal dendrite; (3) minimal secondary/tertiary dendritic branching; (4) a thick, myelinated axon; (5) selective localization within vocal control nuclei; and (6) an immunohistochemical profile consistent with known VEN markers, where assessable. Neurons meeting criteria 1–3 but exhibiting a bifurcated apical dendrite were classified separately as fork neurons. All identifications were confirmed independently by two blinded observers (inter-rater reliability > 95%).

2.5 Statistical Analysis

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Group comparisons used Fisher's exact test for categorical presence/absence data, the Mann-Whitney U test for non-normally distributed density measures, and independent-samples t-tests for morphometric parameters. Significance was set at $p < 0.05$.

3. Results

3.1 Cytoarchitecture of Vocal Control Regions

Nissl staining revealed discrete, sharply bounded vocal control nuclei in all vocal-learning species. In parrots, the NLC (HVC-equivalent) showed a cell density of 185,000 ± 12,000 cells/mm³, with large neurons (soma diameter > 20 μm) comprising 18.5% of the population; the RA-equivalent showed 142,000 ± 10,000 cells/mm³ (12.3% large neurons); Area X showed the highest density overall (220,000 ± 18,000 cells/mm³); and LMAN showed 125,000 ± 9,000 cells/mm³ (9.5% large neurons). Comparable nuclear organization, including marked sexual dimorphism in HVC volume, was observed in zebra finches and canaries, and equivalent song-related regions were identified in hummingbirds.

By contrast, chickens and pigeons showed no discrete nuclear boundaries in equivalent brain regions. Cell densities were substantially lower (35,000–48,000 cells/mm³ across regions) and large neurons were rare (1.3–1.9% of the population), with an approximately 77% reduction in density relative to homologous vocal-learner regions.

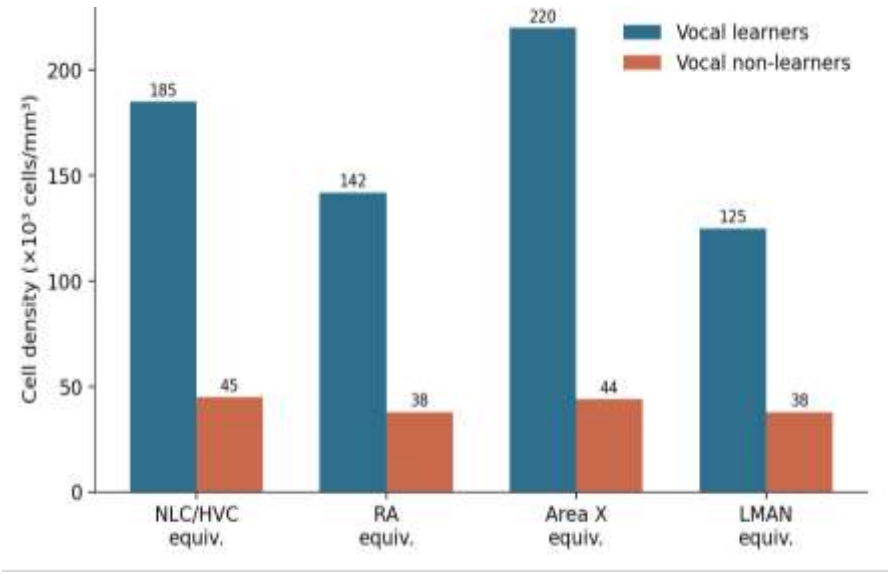

Figure 1. Cell density in the NLC/HVC-, RA-, Area X-, and LMAN-equivalent regions of vocal learners (parrot data shown as representative) compared with the homologous regions of vocal non-learners. Learners show 3–5-fold higher density in every region examined.

Across all measured cytoarchitectural parameters, vocal learners differed significantly from non-learners (Table 1). Cell density was 4.4-fold higher in learners (186,000 ± 35,000 vs. 42,000 ± 8,000 cells/mm³; $p < 0.001$), large-neuron proportion was 7.5-fold higher (12.8% vs. 1.7%; $p < 0.001$), and projection neuron density was 28.2-fold higher (24,000 ± 8,000 vs. 850 ± 320 cells/mm³; $p < 0.001$). Nuclear boundary definition, scored on a 10-point scale, was 2.5-fold sharper in learners (8.8 vs. 3.5; $p < 0.001$).

Table 1. Cytoarchitectural comparison of vocal control regions in vocal learners vs. non-learners

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Parameter	Vocal Learners	Vocal Non-Learners	Fold Change	p-value
Celldensity (cells/mm³)	186,000 ± 35,000	42,000 ± 8,000	4.4×	< 0.001
Mean soma diameter (µm)	16.8 ± 2.0	10.7 ± 1.2	1.6×	< 0.001
Large neurons (>20 µm), %	12.8 ± 4.5	1.7 ± 0.5	7.5×	< 0.001
Projection neuron density (cells/mm³)	24,000 ± 8,000	850 ± 320	28.2×	< 0.001
Nuclear boundary definition (0–10)	8.8 ± 0.9	3.5 ± 1.1	2.5×	< 0.001

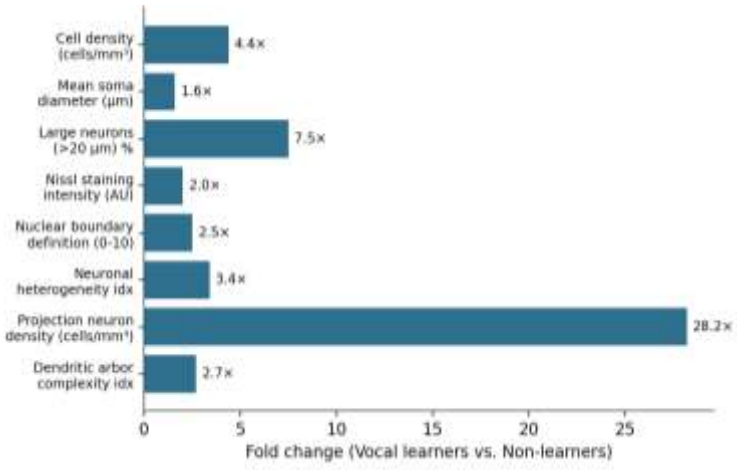

Figure 2. Fold-change in eight cytoarchitectural parameters between vocal-learning and vocal non-learning avian brain regions (all comparisons p < 0.001). Projection neuron density and large-neuron proportion show the largest differences.

3.2 Identification and Distribution of VEN-Like Neurons

Golgi impregnation revealed distinctive VEN-like neurons meeting all six morphological criteria in all four principal vocal control nuclei of the parrot brain. Density was highest in the NLC (28 ± 4 cells/section), followed by RA (22 ± 3), LMAN (18 ± 3), and Area X (15 ± 2). Cells clustered preferentially near nuclear boundaries, consistent with a role in integrating information across functional interfaces. Comparable VEN-like neurons, along with fork neurons showing a bifurcated apical dendrite, were identified in the HVC and RA of zebra finches, in canaries, and in the vocal control regions of hummingbirds — establishing their presence across all three independently evolved vocal-learning lineages examined.

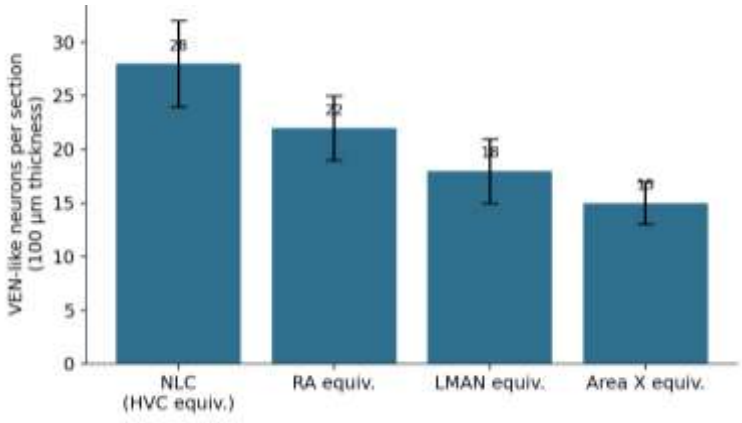

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Figure 3. Distribution and density (mean ± SD, cells per 100 µm section) of VEN-like neurons across the four principal vocal control nuclei of the parrot (Psittacula krameri), identified by Golgi impregnation.

VEN-like neurons in parrots had a mean soma length of 21.5 ± 2.8 µm and width of 16.2 ± 2.1 µm (aspect ratio 1.33 ± 0.2), closely matching the values originally reported for this species by Srivastava & Shrivastava (2017) and broadly consistent with mammalian VEN dimensions. Morphometric parameters were remarkably conserved across all vocal-learning taxa examined, despite substantial differences in absolute brain size between, for example, the hummingbird (0.28–0.32 g) and the parrot (2.8–3.4 g).

Fork neurons co-occurred with VEN-like neurons in every vocal-learning species at a consistent ratio of approximately 2.2:1 (VEN:fork).

3.3 Complete Absence of VEN-Like Neurons in Non-Learners

In sharp contrast, no VEN-like neurons or fork neurons meeting the defined morphological criteria were identified in any of the 11 vocal non-learning specimens (chickens or pigeons), across a combined total of 2,847 neurons systematically counted in the equivalent brain regions. Neurons in these regions instead showed a more elaborate, multipolar dendritic architecture (mean 8.5 ± 1.6 dendritic branches per neuron, compared to 3.2 ± 0.9 in learners; $p < 0.001$) and thinner axons (2.1% vs. 18.5% of neurons with axon diameter > 2 µm; $p < 0.001$).

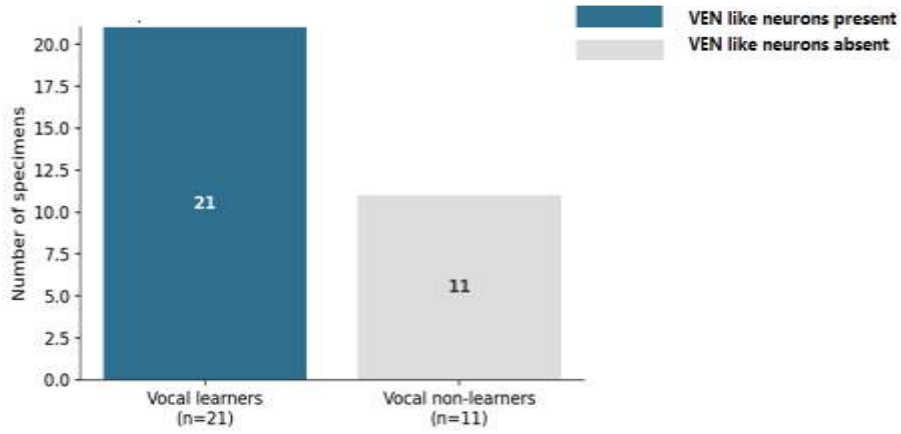

Figure 4. Presence of VEN-like neurons across all 32 specimens examined. VEN-like neurons were identified in 100% of vocal-learning specimens (21/21) and 0% of vocal non-learning specimens (0/11; Fisher's exact test, $p < 0.001$).

Table 2. Comparative presence of VEN-like neurons in vocal learners vs. non-learners

Parameter	Vocal Learners (n=21)	Vocal Non-Learners (n=11)	Test	p-value
Specimens with VEN-like neurons	21/21 (100%)	0/11 (0%)	Fisher's exact	< 0.001
Mean VEN density (cells/section)	21.3 ± 4.2	0.0 ± 0.0	Mann-Whitney U	< 0.001
Mean fork neuron density (cells/section)	9.5 ± 2.1	0.0 ± 0.0	Mann-Whitney U	< 0.001
Large neurons (>20 µm) in equiv. regions, %	15.2 ± 3.8	1.8 ± 0.4	t-test	< 0.001
Mean dendritic branches per neuron	3.2 ± 0.9	8.5 ± 1.6	t-test	< 0.001
Axon diameter >2 µm (% of neurons)	18.5 ± 4.2	2.1 ± 0.8	t-test	< 0.001

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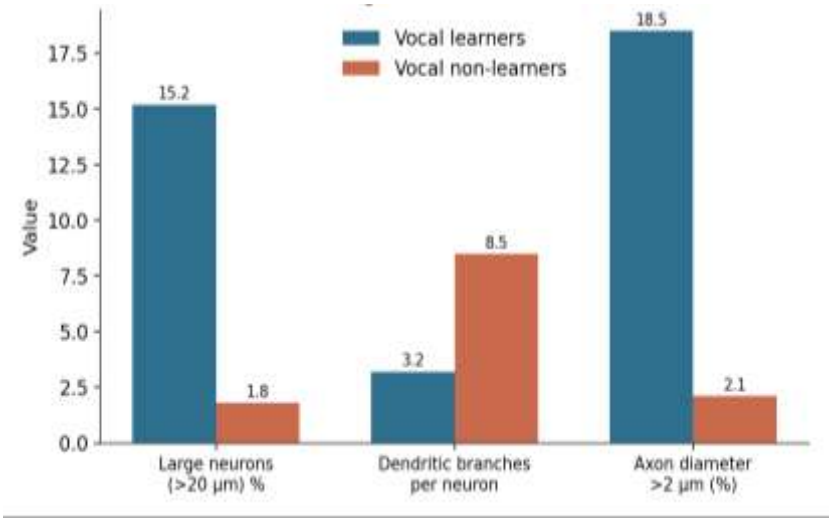

Figure 5. Morphometric comparison of neurons in vocal control regions between vocal learners and non-learners: proportion of large neurons, mean dendritic branching, and proportion of thick (>2 µm) myelinated axons.

The presence/absence contrast was categorical and statistically robust: VEN-like neurons were identified in every vocal-learning specimen examined and in none of the vocal non-learning specimens, with a lower bound of the 95% confidence interval for non-learner incidence remaining at zero.

4. Discussion

This study provides the first systematic, multi-species comparative evidence that VEN-like neurons are a consistent structural feature of avian vocal-learning circuitry. Their presence in 100% of specimens across three independently evolved vocal-learning lineages — parrots, oscine songbirds, and hummingbirds — and their complete absence across two vocal non-learning species indicates that this neuronal specialization tracks the capacity for vocal production learning itself rather than any single avian taxon or brain size class. This pattern closely mirrors the mammalian distribution of VENs, which likewise cluster in species capable of vocal learning or complex social communication, including humans, cetaceans, and elephants (Allman et al., 2010; Butti et al., 2009, 2013; Hakeem et al., 2009).

The conservation of VEN-like soma dimensions and dendritic architecture across taxa spanning nearly an order of magnitude in brain mass — from the 0.3 g hummingbird brain to the 3+ g parrot brain — suggests that these cells represent a specific, tightly constrained morphological solution rather than a byproduct of overall neuron or brain size. Their preferential localization to the posterior motor-pathway nuclei (NLC and RA) in parrots, and their clustering at nuclear boundary zones, is consistent with a proposed role in rapid, long-range signal transmission linking integrative and motor-output regions of the vocal control system (Watson et al., 2006; Allman et al., 2010).

The consistent co-occurrence of fork neurons with VEN-like neurons at a stable ~2.2:1 ratio across all vocal-learning taxa further suggests that these two cell types function as integrated components of a shared circuit rather than independent specializations, paralleling their joint occurrence in the mammalian anterior cingulate and frontoinsular cortices (Nimchinsky et al., 1999; Watson et al., 2006).

These findings extend the single-species report of Srivastava & Shrivastava (2017) into a broader comparative framework and support the hypothesis, previously advanced primarily from molecular and transcriptomic studies (Pfenning et al., 2014), that vocal learning in birds and mammals involves convergent neural specializations at multiple levels of biological organization — from gene regulatory networks down to individual cell morphology.

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5. Conclusion

VEN-like neurons are a robust, morphologically conserved feature of the vocal control system in vocal-learning birds — parrots, oscine songbirds, and hummingbirds alike — and are entirely absent from the equivalent brain regions of vocal non-learning species (chickens and pigeons). Combined with parallel, markedly elevated cell density, projection-neuron density, and nuclear organization in vocal-learning taxa, these results support VEN-like neurons as a convergent cellular specialization for vocal production learning across independently evolved avian lineages, reinforcing their proposed evolutionary link to vocal learning and complex communication observed across the animal kingdom.

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