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Neuronal satellitosis is a common finding in the avian brain

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ABSTRACT
Perineuronal or neuronal satellitosis is the term describing the presence of glial cells in the satellite space surrounding the neuronal perikaryon. Confusingly, this finding has been described both as a physiologic and pathologic condition in humans and animals. In animals, neuronal satellitosis has been described in mammals, as well as in avian species. For the latter, the authors wondered whether neuronal satellitosis is expressed in the normal telencephalon of different avian orders and families and whether this pattern in different species shows a specific brain-region association. For these aims, this study explored the presence of neuronal satellitosis in the major areas of the healthy telencephalon in wild and domestic avian species of different orders and families, evaluating its grade in different brain regions. Neuronal satellitosis was seen in the hyperpallium and mesopallium as areas with the highest grade. Passeriformes showed the highest grade of neuronal satellitosis compared to diurnal or nocturnal raptors, and Charadriiformes. To clarify the exact role of neuronal satellitosis in animals without neurological disease, further studies are needed.

RESEARCH HIGHLIGHTS

- Neuronal satellitosis is a common finding in the healthy avian telencephalon.
- Neuronal satellitosis is a species- and brain-region-associated finding in birds.
- Passeriformes have the highest grade of neuronal satellitosis.

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Introduction

Perineuronal or neuronal satellitosis is the term describing the presence of glial cells in the satellite space, a 6-µm width space surrounding the neuronal perikaryon (Wohlsein *et al.*, 2013). The presence of perineuronal glial cells as a normal finding in the central nervous system (CNS) was first described during the late 19th century and at the beginning of the 20th century by eminent researchers like Santiago Ramón y Cajal and Wilder Penfield (Brownson, 1956; Civita *et al.*, 2020; Vijayan *et al.*, 1993). In these years, the term "satellitosis" or "neuronal satellitosis" (NS) was specifically attributed to the increased number of perineuronal glial cells in the satellite space; first described as a normal and reactive "vitalizing-like phenomenon" (Brownson, 1956). It soon also gained a pathologic connotation as a reactive or neoplastic feature of the disease (Brain & Greenfield, 1950; Scherer, 1940). Innes & Saunders (1962) described the NS as a clear pathologic reaction of oligodendrocytes to neuronal damage, with or without associated neuronal changes (e.g. cellular swelling and chromatolysis). From 1990 onwards, NS again gained the definition of a finding with "no pathological significance" (Poirier *et al.*, 1990), and in 1993, Vijayan and colleagues described NS in the human hippocampus as a non-pathologic region-related finding (Vijayan *et al.*, 1993). Concurrent studies have confirmed oligodendrocytes as more common perineuronal glial cells, although astrocytes or microglial cells have also been reported (Garman, 2011; Vijayan *et al.*, 1993), all contributing to the maintenance of neuronal homeostatic conditions (Wohlsein *et al.*, 2013). Since NS has been reported in the literature as both a physiologic and a pathologic condition, creating confusion in its regard, this term should be used cautiously. Currently, the term NS seems to be accepted in both physiologic and pathologic conditions (Garman, 2011; Wohlsein *et al.*, 2013). In animals, NS has been reported in different species, from mammals (Wohlsein *et al.*, 2013) to birds (Medina *et al.*, 2013). For birds, neuronal satellitosis has been reported as a feature of avian neuroinvasive viral diseases, such as avian influenza, West Nile fever and Usutu (Austin *et al.*, 2004; Bröjer *et al.*, 2012; Rijks *et al.*, 2016; Benzarti *et al.*, 2020). Only a single study reports this finding

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in Passeriformes as a brain-area-associated normal pattern, characterized by high numbers of glial cells surrounding a neuronal soma, namely "perineuronal glial clusters" (PGCs) (Medina *et al.*, 2013). Unfortunately, more studies on the association between satellitosis as a normal feature and specific brain regions in different avian orders are lacking. The possible role of satellitosis in disease is important in the diagnosis of zoonotic viral neuroinvasive diseases of wild birds, such as those caused by West Nile virus (WNV), Usutu virus (USUV), and highly pathogenic avian influenza virus.

Based on these premises, this study explored the presence of neuronal satellitosis in the form of single cells or perineuronal glial clusters in the telencephalon of healthy wild and domestic birds of different orders. Satellitosis was graded, and the association with specific brain regions was assessed.

Table 1. List of the examined avian species (and number of birds) divided into categories.

Category	Latin name	Common name	No. birds
Diurnal raptors ($n = 11$)	*Falcus tinnunculus*	Kestrel	$n = 6$
	Buteo Buteo	Common buzzard	$n = 2$
	Accipiter nisus	Sparrowhawk	$n = 1$
	Falco subbuteo	Eurasian hobby	$n = 1$
	Buteo lagopus	Buzzard	$n = 1$
Nocturnal raptors ($n = 14$)	*Athene noctua*	Little owl	$n = 6$
	Strix aluco	Tawny owl	$n = 5$
	Long-eared owl	Common owl	$n = 2$
	Tyto alba	Barn owl	$n = 1$
Passeriformes ($n = 14$)	*Serinus canaria*	Canary	$n = 7$
	Turdus merula	Blackbird	$n = 4$
	Pica pica	Magpie	$n = 2$
	Garrulus glandarius	Jay	$n = 1$
Anseriformes ($n = 2$)	*Cygnus olor*	Mute swan	$n = 1$
	Anser anser	Goose	$n = 1$
Charadriiformes ($n = 4$)	*Larus michahellis*	Herring gull	$n = 4$
Pelecaniformes ($n = 1$)	*Ardea cinerea*	Gray heron	$n = 1$
Columbiformes ($n = 2$)	*Columba livia*	Wild pigeon	$n = 1$
	Streptopelia decaocto	Collared turtle dove	$n = 1$
Piciformes ($n = 1$)	*Picus viridis*	Green woodpecker	$n = 1$

Materials and methods

Bird collection and data

Forty-nine ($n = 49$) adult wild and domestic birds submitted to the Veterinary Pathology Service of the Department of Veterinary Medicine in Perugia (Italy) for *post-mortem* investigation, and included in a wider monitoring study on emerging zoonotic arboviruses (WNV and USUV) were examined for the current study. Carcasses of all birds were in good general condition (Code 1 or 2) with absent or very mild *post-mortem* artefacts (McAloose *et al.*, 2018). To humanely euthanize birds, euthanasia was performed for each bird according to AVMA recommendations (Underwood & Anthony, 2020) and an exotic animal formulary (Carpenter & Marion, 2017). Specifically, inhalant anaesthesia was performed with isoflurane (2%), followed by IV injection of sodium pentobarbital overdose (2 ml/kg). No ethical approval was needed for the study as birds were euthanized for critical medical conditions and submitted after death for routine *post-mortem* examination including the evaluation of the CNS. As inclusion criteria for the study, the birds must have no reported history of neurological signs. In cases of traumatic injuries, birds were included in the study only if lesions were located at extremities (wings and legs) and did not involve the skull. The birds were divided into eight categories based on their taxonomic tribe: diurnal raptors ($n = 11$), nocturnal raptors ($n = 14$), Passeriformes ($n = 14$), Anseriformes ($n = 2$), Charadriiformes ($n = 4$), Pelecaniformes ($n = 1$), Columbiformes ($n = 2$), Piciformes ($n = 1$). Data regarding categories and species of birds are listed in Table 1. Complete necropsies were performed, and organs were sampled for subsequent histopathology and molecular testing. Data regarding the final diagnosis and type of death are present as supplementary material (Supplementary Table 1).

Brain evaluation and neuronal satellitosis scoring

For the aim of this research, details of tissue processing are given only for the brain. Removed brains were entirely fixed in 10% neutral-buffered formalin. Gross examination was performed on 0.3-cm thick transverse brain sections. Tissues were processed routinely, and subsequent histological evaluation was performed on 3-µm sections with haematoxylin and eosin (H&E) stain. The presence of perineuronal glial cells and NS grade were assessed in major areas of the avian telencephalon as the main representative part of the brain: hyperpallium (h. apicale, h. intercalatum, h. densocellulare), mesopallium (m. dorsale, m. ventrale), nidopallium, entopallium, basal ganglia and olfactory cortex, according to the most recent avian brain nomenclature (Reiner *et al.*, 2004) and recognized similarities with mammalian neocortex (Bolhuis & Gahr, 2006; Bolhuis *et al.*, 2010).

Regarding the NS scoring, a semiquantitative scoring system was established following literature recommendations (Gibson-Corley *et al.*, 2013). Based on the number of glial cells surrounding the neuronal perikaryon, evaluated on 10 FN22/40× randomly selected fields, neuronal satellitosis was scored as follows: Grade 0 - absent (when > 60% of neurons had 0–1 glial cells per neuron); Grade 1 - mild (when > 60% of neurons had 2–3 glial cells per neuron); Grade 2 - moderate (when > 60% of neurons had 4–6 glial cells per neuron); Grade 3 - marked (when > 60% of neurons had > 6 glial cells per neuron).

Additional tests and statistical analysis

The brains of all the selected birds were tested by RT-qPCR as part of a monitoring study for WNV and USUV at the Istituto Zooprofilattico Sperimentale "G. Caporale" in Teramo, Italy. The main criteria of inclusion for the selected birds consisted of a negative RT-qPCR test for WNV and USUV and a lack of histological findings referable to neurological diseases. Additional tests for viral infections like avian influenza viruses, paramyxoviruses, polyomaviruses, and bornaviruses were not performed due to the lack of histological lesions suggestive of a viral infection, such as mononuclear perivascular cuffs, acidophilic neuronal necrosis, and neuronophagia.

Additional statistical analyses were performed using SPSS 26.0 software (IBM, SPSS Inc., Chicago, IL, USA), and statistical significance was set at $P \leq 0.05$. Graphics were used to test assumptions. A Kruskal–Wallis test was performed to evaluate different cerebrum cortex distribution of "satellitosis grade" in different categories with at least four examined birds (e.g. diurnal raptors, nocturnal raptors, Passeriformes, Charadriiformes). A pairwise comparison was performed to identify which specific categories differed from each other on the satellitosis grade. Bonferroni's index was used to adjust the *P*-value in the pairwise comparisons.

Results and discussion

The term perineuronal or neuronal satellitosis (NS) describes the presence of glial cells around neurons. Over the years, NS has been considered both a pathologic and a physiologic finding. Information about the presence of NS in the normal telencephalon of more avian orders and families, and its distribution in different areas of the brain, are not available.

In this study, histological evaluation of the healthy avian brain was performed on the telencephalon as the most consistent part of the cerebrum. It showed variable presence and grade of NS in different telencephalic areas and in different species.

Neuronal satellitosis in different telencephalic areas

In the hyperpallium apicale (HA), neuronal satellitosis was commonly observed ($n = 41/49$; 83.7% birds), with grade 1 as the most representative. Similarly to the HA, the hyperpallium intercalatum (HI) also frequently showed neuronal satellitosis ($n = 46/49$; 93.9%), but compared to HA, grade 1 ($n = 29$; 63%) and grade 2 ($n = 17$; 37%) were less and more frequent, respectively. Compared to HA neuronal satellitosis, hyperpallium densocellulare (HD) neuronal satellitosis was observed in more birds ($n = 48/49$; 97.9%) ranging from grade 1 ($n = 23/48$; 48%) to grade 3 ($n = 14/48$; 29%) as reported previously (Medina *et al.*, 2013) (Table 2). The hyperpallium (formerly known as the Wulst), and in particular the HD, is generally considered the avian equivalent of the cortex of mammals, playing a major role in the somatosensory system and in the action and control of movement (Reiner *et al.*, 2005; Medina, 2007; Stacho *et al.*, 2020). For this reason, the high number of glial cells in NS may suggest a diffuse high neuronal metabolic rate of this cerebral region (hyperpallium) with HD as the most active.

In the mesopallium dorsale (MD), NS was commonly observed in the examined birds ($n = 48/49$; 97.9%). It ranged from grade 1 ($n = 19/48$; 39.5%) to grade 2 ($n = 15/48$; 31%) or grade 3 ($n = 14/48$; 29%) (Table 2). The MD expressed grades similar to those observed in the HD. The high grade of NS was characterized by "perineuronal glial clusters" in Passeriformes (Medina *et al.*, 2013) (Figure 1). As for the mesopallium ventrale (MV), NS was observed in 47/49 birds, much more commonly with grade 1 or grade 2 (Table 2). The role of the mesopallium is still controversial. It seems to be involved in the mechanism of auditory perception (Prather *et al.*, 2017) and vision (Reiner *et al.*, 2005; Stacho *et al.*, 2020) as well as in social behaviour (Nomura & Izawa, 2017) of birds. This can explain why the highest grade of NS was observed in the mesopallium (M) of nocturnal birds of prey and passerines, which include avian categories with a well-known highly-developed visual and auditory perception (Nelson & Suthers, 2004; Potier *et al.*, 2020). From a functional point of view, no differences have been seen in the gene expression profiling comparing the MD to the MV (Jarvis *et al.*, 2013). What really drives different distributions of NS in these areas, and if they have a functional role, still remains to be determined.

In the remaining examined areas of the brain, NS was found in almost all of the birds, generally of grade 1. As for the nidopallium (N), NS was frequently observed ($n = 45/49$; 91.8%) in birds, with grade 1 as the most common and only rarely with grade 2. The nidopallium is mainly involved in visual perception (Reiner *et al.*, 2005; Stacho *et al.*, 2020) and social behaviour (Nomura & Izawa, 2017) but, contrarily to the mesopallium, which is involved in similar neurofunctions, it showed grade 1 NS more frequently, suggesting a lower metabolic rate of the N region compared to the M. The entopallium (E) is the avian counterpart of the mammalian superior colliculi for vision (Butler & Cotterill, 2006). In this study, NS of the E was observed in 46/49 (93.9%) birds, including 37 with grade 1 (80.4%) and only eight of grade 2 (17.3%). Similarly to the N and E, in the basal ganglia, NS was a common finding generally of grade 1 ($n = 45/57$ 95.7%), more rarely of grade 2 ($n = 2/47$; 4%).

Table 2. Summary of grade distribution for neuronal satellitosis for each examined telencephalic area.

	HA		HI		HD		MD		MV		N		E		BG		OC	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Grade 1	34/41	83	29/46	63	23/48	48	19/48	39.5	25/47	53	42/45	93	37/46	80.4	45/47	95.7	42/45	93.3
Grade 2	7/41	17	17/46	37	11/48	23	15/48	31.2	21/47	44.6	3/45	6.6	8/46	17.3	2/47	4.2	3/45	6.6
Grade 3	ND	–	ND	–	14/48	29	14/48	29	1/47	2	ND	–	ND	–	ND	–	ND	–

Note: HA = Hyperpallum apicale; HI = Hyperpallium intercalatum; HD = Hyperpallium densocellulare; MD = Mesopallium densocellulare; MV = Mesopallium ventrale; N = Nidopallium; E = Entopallium; BG = Basal ganglia; OC = Olfactory cortex; ND = Not detected.

Similar results were found in the olfactory cortex, with grade 1 ($n = 42/45$; 93%) being much more frequent than grade 2 ($n = 3/45$; 6.6%). Details on the grade distribution and frequencies for each examined area are listed in Table 2, while data regarding the specific localization and grade of distribution for each examined species are reported in Table 3. These results suggest a variable expression of neuronal satellitosis in different areas of the healthy avian telencephalon for different orders. The exact role of this difference remains to be clarified. However, a close association with function-related high metabolic rate might be suspected.

Intra-species and avian categories differences

As for intra-species differences in NS grades for each area, they were mild for species of some categories (e.g. Passeriformes). For this reason, mild variability in the grade of neuronal satellitosis should always be considered when examining avian brain tissue. In addition, no differences were seen comparing grade of NS in captive and wild Passeriformes ($P > 0.05$), for this reason, domestication might have little or no effect on these specific features of the avian brain.

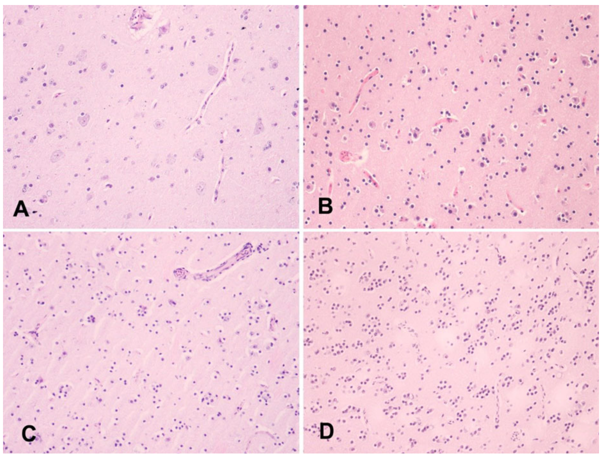

Figure 1. Neuronal satellitosis in the avian telencephalon (A–D); A. *Accipiter nisus*. Mesopallium; absence of neuronal satellitosis; rarely single glial cells are in the perineural space (40×); B. *Picus viridis*. Mesopallium; neuronal satellitosis of low grade (Grade 1); 2–3 cells in the satellite space) (40×); C. *Serinus canaria*. Mesopallium; neuronal satellitosis of moderate grade (Grade 2); (4–6 cells in the satellite space) (40×) D. *Turdus merula*. Mesopallium; neuronal satellitosis of high grade (Grade 3) with evidence of perineuronal glial clusters (40×).

Regarding the statistical analysis, multiple comparisons to unravel differences in the distribution of NS grade of HD in the different examined avian categories was significant (H (7) = 33.408, $P < 0.00$) and subsequent pairwise comparisons with adjusted *P*-value (Bonferroni's correction) was applied to specifically identify differing categories.

The pairwise comparisons on the NS grade in HD identified the diurnal raptors (*Accipiter*, *Falcus* and *Buteo* species) as significantly different from Passeriformes (*Serinus canaria*, *Turdus merula*, *Pica pica* and *Garrulus glandarius*) in the distribution of the NS grade ($P < 0.01$); significant differences were also observed for nocturnal raptors (*Athene noctua* and *Strix* species) compared to Passeriformes ($P = 0.001$). For the HD, the remaining categories did not show a statistically significant difference in the NS grade distribution ($P > 0.05$). Due to the strong interplay between neurons and satellite glia, and the role of the latter in neuronal metabolism (Jah & Morrison, 2018), the difference in NS could be explained by a possible higher metabolic rate of neurons of the avian hyperpallium in the examined passerine species (canary, blackbird, magpie and blue jay) compared to the examined prey birds, suggesting higher metabolic support from glial cells and perhaps a higher somatosensitive and motor development in this avian order.

Similarly to the HD, the MD also showed a significantly different distribution of the NS grade among different bird categories (H (7) = 33.930, $P < 0.01$). The pairwise comparisons in the MD revealed that diurnal raptors were significantly different from Passeriformes ($P < 0.01$) as in the HD. The remaining categories did not show statistically significant differences ($P > 0.05$).

In the MV, the NS grade distribution was found to be significantly different among bird categories (H (7) = 28.937, $P < 0.01$). Pairwise comparisons identified diurnal raptors as significantly different from Passeriformes ($P < 0.01$); a similar result was found for Charadriiformes, showing a significant difference compared to Passeriformes ($P < 0.001$). The remaining categories did not show statistically significant differences ($P > 0.05$) in the MV. We can suppose differences in the role of the mesopallium in auditory and visual perception, and its effects on the biology and abilities of these avian species are related to the neuronal activity and different grades of neuronal satellitosis.

Table 3. Localization and grade distribution of neuronal satellitosis in the examined avian species.

	HA	HI	HD	MD	MV	N	E	BG	OC
Falcus tinnunculus	+	+	+	+	+	+	+	+	+
Buteo Buteo	–	+	+	+	+	+	+	+	+
Accipiter nisus	–	–	+	–	–	–	–	–	–
Falco subbuteo	+	++	++	++	++	+	+	+	+
Buteo lagopus	+	+	+	+	+	+	+	+	+
Athene noctua	+ to ++	+ to ++	+to+++	+ to ++	+to++	+	+	+	+
Strix aluco	+	+ to ++	+ to ++	+ to ++	+ to ++	+ to ++	+ to ++	+ to ++	+ to ++
Long-eared owl	+ to ++	+ to ++	++to+++	++to+++	+ to ++	+	+	+	+
Tyto alba	+	+	+	+	+	+	+	+	+
Serinus canaria	– to +	+ to ++	+++	+++	++	+	+	+	+
Turdus Merula	+ to ++	++	+++	+++	++	+	+	+	+
Pica pica	+	+	++	++	++	+ to ++	++	+	+ to ++
Garrulus glandarius	+	++	+++	+++	++	++	++	++	++
Cygnus olor	–	–	–	+	+	–	–	+	–
Anser anser	–	+	+	+	+	–	–	+	–
Larus michahellis	+	+	++	++	+	+	++	+	+
Ardea cinerea	+	+	+	+	+	+	+	+	+
Columba livia	–	–	+	+	–	–	–	–	–
Picus viridis	+	++	++	++	+	+	+	+	+
Streptopelia decaocto	+	+	+	+	+	+	+	+	+

Note: –, absence of neuronal satellitosis; +, grade 1; ++, grade 2; +++, grade 3.

In the entopallium, the ND grade distribution among the avian categories was statistically significant (H (6) = 22.021, $P = 0.001$) with pairwise comparisons identifying diurnal raptors differing from Charadriiformes ($P < 0.01$), and nocturnal raptors differing from Charadriiformes ($P = 0.001$), and Passeriformes differing from Charadriiformes ($P < 0.05$). The remaining categories did not show statistically significant differences ($P > 0.05$). Data regarding the scoring of neuronal satellitosis in the different examined species are available as supplementary material (Supplementary Table 2).

Final remarks

From a diagnostic point of view, as for mammals (Bradley *et al.*, 2020), the term neuronal satellitosis with a pathologic connotation should be carefully used. In the absence of recognized data on different brain areas or species, comparison of foci of neuronal satellitosis with neighbouring neurons is recommended. It is generally unlikely that NS occurs as a diffuse process. In birds, pathologic neuronal satellitosis should be considered when neurons show signs of damage (e.g. neuronal swelling, cytoplasmic rarefaction, chromatolysis, and acidophilic neuronal necrosis) or when the number of glial cells in the "satellite space" exceeds that of the surrounding neurons, supporting the idea of ongoing cellular damage.

Results reported in this study regarding neuronal satellitosis in avian categories must be interpreted with caution for categories with a low number of examined birds. Moreover, the selected cohort of adult birds did not allow a comparison with young birds. Regarding the final diagnoses, some of the birds showed systemic conditions or lesions that, even if limitedly, could have possibly caused a change in the systemic metabolism (e.g. hypoxia due to airsacculitis) that might have contributed to the development and/or an increase in grade of the neuronal satellitosis. For these reasons, further large-scale studies, on healthy euthanized birds, are needed to try to better understand the role and the mechanisms involved in neuronal satellitosis in the absence of proven brain disease.

Conclusions

This study demonstrates that different areas of the adult avian telencephalon in different species of wild and domestic birds can show neuronal satellitosis of various grades as a normal finding. As previously reported, hyperpallium and mesopallium are the areas with the highest grade of neuronal satellitosis. Moreover, Passeriformes showed the highest grade of neuronal satellitosis compared to diurnal and nocturnal raptors and Charadriiformes. The exact role of neuronal satellitosis is still unclear. To support the interpretation of satellitosis as a pathologic feature, it is strongly recommended to compare the suspected neuron to the adjacent neurons and to carefully identify early neuronal changes commonly associated with pathologic forms of neuronal satellitosis.

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Disclosure statement

No potential conflict of interest was reported by the authors.

Ethical statement

No ethical approval was needed. Birds were submitted after death for routine *post-mortem* examination and monitoring projects on West Nile virus and Usutu virus in Umbria, Central Italy.

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References

Austin, R.J., Whiting, T.L., Anderson, R.A. & Drebot, M.A. (2004). An outbreak of West Nile virus-associated disease in domestic geese (*Anser anser domesticus*) upon initial introduction to a geographic region, with evidence of bird to bird transmission. *Canadian Veterinary Journal*, 45, 117–123.

Benzarti, E., Rivas, J., Sarlet, M., Franssen, M., Desmecht, D., Schmidt-Chanasit, J., Savini, G., Lorusso, A., Van Laere, A.S. & Garigliany, M.M. (2020). Experimental Usutu virus infection in domestic canaries *Serinus canaria*. *Viruses*, *12*(2), 164.

Bolhuis, J.J. & Gahr, M. (2006). Neural mechanisms of birdsong memory. *Nature Reviews Neuroscience*, 7, 347–357.

Bolhuis, J.J., Okanoya, K., & Scharff, C. (2010). Twitter evolution: converging mechanisms in birdsong and human speech. *Nature Reviews Neuroscience*, 11(11), 747–759.

Bradley, A.E., Bolon, B., Butt, M.T., Cramer, S.D., Czasch, S., Garman, R.H., George, C., Gröters, S., Kaufmann, W., Kovi, R.C., Krinke, G., Little, P.B., Narama, I., Rao, D.B., Sharma, A.K., Shibutani, M. & Sills, R. (2020). Proliferative and nonproliferative lesions of the rat and mouse central and peripheral nervous systems: new and revised INHAND terms. *Toxicologic Pathology*, 48, 827–844.

Brain, W.R. & Greenfield, J.G. (1950). Late infantile metachromatic leuco-encephalopathy, with primary degeneration of the interfascicular oligodendroglia. *Brain*, 73, 291–317.

Bröjer, C., Ågren, E.O., Uhlhorn, H., Bernodt, K., Jansson, D.S. & Gavier-Widén, D. (2012). Characterization of encephalitis in wild birds naturally infected by highly pathogenic avian influenza H5N1. *Avian Diseases*, 56, 144–152.

Brownson, R.H. (1956). Perineuronal satellite cells in the motor cortex of aging brains. *Journal of Neuropathology & Experimental Neurology*, 15, 190–195.

Butler, A.B. & Cotterill, R.M.J. (2006). Mammalian and avian neuroanatomy and the question of consciousness in birds. *Biological Bulletin*, 211, 106–127.

Carpenter, J.W. & Marion, C. (2017). *Exotic animal formulary*. St. Louis: Elsevier Health Sciences.

Civita, P., Valerio, O., Naccarato, A.G., Gumbleton, M. & Pilkington, G.J. (2020). Satellitosis, a crosstalk between neurons, vascular structures and neoplastic cells in brain tumours; early manifestation of invasive behaviour. *Cancers*, 12, 3720.

Garman, R.H. (2011). Histology of the central nervous system. *Toxicologic Pathology*, 39, 22–35.

Gibson-Corley, K.N., Olivier, A.K. & Meyerholz, D.K. (2013). Principles for valid histopathologic scoring in research. *Veterinary Pathology*, 50, 1007–1015.

Innes, J.R.M. & Saunders, L.Z. (1962) *Comparative neuropathology*. New York: Academic Press.

Jarvis, E.D., Yu, J., Rivas, M.V., Horita, H., Feenders, G., Whitney, O., Jarvis, S.C., Jarvis, E.R., Kubikova, L., Puck, A.E.P., Siang-Bakshi, C., Martin, S., McElroy, M., Hara, E., Howard, J., Pfenning, A., Mouritsen, H., Chen, C. & Wada, K. (2013). Global view of the functional molecular organization of the avian cerebrum: mirror images and functional columns. *Journal of Comparative Neurology*, 521, 3614–3665.

Jha, M.K. & Morrison, B.M. (2018). Glia-neuron energy metabolism in health and diseases: new insights into the role of nervous system metabolic transporters. *Experimental Neurology*, 309, 23–31.

McAloose, D., Colegrove, K.M. & Newton, A.L. (2018). Wildlife necropsy. In K.A. Terio, D. McAloose, & J.S. Leger (Eds.), *Pathology of wildlife and zoo animals* (pp. 1–20). Cambridge: Academic Press.

Medina, F.S., Hunt, G.R., Gray, R.D., Wild, J.M. & Kubke, M.F. (2013). Perineuronal satellite neuroglia in the telencephalon of New Caledonian crows and other passeriformes: evidence of satellite glial cells in the central nervous system of healthy birds? *PeerJ*, 2013, 1–18.

Medina, L. (2007). Do birds and reptiles possess homologues of mammalian visual, somatosensory, and motor cortices? *Evolution of Nervous Systems*, 2, 163–194.

Nelson, B.S., & Suthers, R.A. (2004). Sound localization in a small passerine bird: discrimination of azimuth as a function of head orientation and sound frequency. *Journal of Experimental Biology*, 207(23), 4121–4133.

Nomura, T. & Izawa, E.I. (2017). Avian brains: insights from development, behaviors and evolution. *Development Growth and Differentiation*, 59, 244–257.

Poirier, J., Gray, F., Gray, F., & Escourolle, R. (1990). *Manual of basic neuropathology*. Saunders.

Prather, J.F., Okanoya, K. & Bolhuis, J.J. (2017). Brains for birds and babies: neural parallels between birdsong and speech acquisition. *Neuroscience and Biobehavioral Reviews*, 81, 225–237. Elsevier Ltd.

Potier, S., Mitkus, M., & Kelber, A. (2020). Visual adaptations of diurnal and nocturnal raptors. In *Seminars in cell & developmental biology*, 106, 116–126. Academic Press.

Reiner, A., Perkel, D.J., Bruce, L.L., Butler, A.B., Csillag, A., Kuenzel, W., Medina, L., Paxinos, G., Shimizu, T., Striedter, G., Wild, M., Ball, G.F., Durand, S., Gütürkün, O., Lee, D.W., Mello, C.V., Powers, A., White, S.A., Hough, G., Kubikova, L., Smulders, T.V., Wada, K., Dugas-Ford, J., Husband, S., Yamamoto, K., Yu, J., Siang, C. & Jarvis, E.D. (2004). Revised nomenclature for avian telencephalon and some related brainstem nuclei. *Journal of Comparative Neurology*, 473, 377–414.

Reiner, A., Yamamoto, K. & Karten, H.J. (2005). Organization and evolution of the avian forebrain. *Anatomical Record - Part A Discoveries in Molecular, Cellular, and Evolutionary Biology*, 287, 1080–1102.

Rijks, J.M., Kik, M., Slaterus, R., Foppen, R., Stroo, A., Ijzer, J., Stahl, J., Gröne, A., Koopmans, M., van der Jeugd, H. & Reusken, C. (2016). Widespread Usutu virus outbreak in birds in the Netherlands, 2016. *Eurosurveillance*, 21(45), 30391.

Scherer, H.J. (1940). The forms of growth in gliomas and their practical significance. *Brain*, 63, 1–35.

Stacho, M., Herold, C., Rook, N., Wagner, H., Axer, M., Amunts, K. & Güntürkün, O. (2020). A cortex-like canonical circuit in the avian forebrain. *Science*, 369.

Underwood, W. & Anthony, R. (2020). *AVMA guidelines for the euthanasia of animals: 2020 edition*. Retrieved on March, 2013 (30), 2020-2021.

Vijayan, V.K., Zhou, S-S, Russell, M.J., Geddes, J., Ellis, W. & Cotman, C.W. (1993). Perineuronal satellitosis in the human hippocampal formation. *Hippocampus*, 3, 239–250.

Wohlsein, P., Deschl, U. & Baumgärtner, W. (2013). Nonlesions, unusual cell types, and postmortem artifacts in the central nervous system of domestic animals. *Veterinary Pathology*, 50, 122–143.