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High associative neuron numbers could drive cognitive performance in corvid species

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Abstract

Corvids possess cognitive skills, matching those of nonhuman primates. However, how these species with their small brains achieve such feats remains elusive. Recent studies suggest that cognitive capabilities could be based on the total numbers of telencephalic neurons. Here we extend this hypothesis further and posit that especially high neuron counts in associative pallial areas drive flexible, complex cognition. If true, avian species like corvids should specifically accumulate neurons in the avian associative areas meso- and nidopallium. To test the hypothesis, we analyzed the neuronal composition of telencephalic areas in corvids and noncorvids (chicken, pigeons, and ostriches—the species with the largest bird brain). The overall number of pallial neurons in corvids was much higher than in chicken and pigeons and comparable to those of ostriches. However, neuron numbers in the associative mesopallium and nidopallium were twice as high in corvids and, in correlation with these associative areas, the corvid subpallium also contained high neuron numbers. These findings support our hypothesis that large absolute numbers of associative pallial neurons contribute to cognitive flexibility and complexity and are key to explain why crows are smart. Since meso-/nidopallial and subpallial areas scale jointly, it is conceivable that associative pallio-striatal loops play a similar role in executive decision making as described in primates.

KEYWORDS
birds, cognition, isotropic fractionator, mesopallium, nidopallium, subpallium

1 | INTRODUCTION

The last two decades have shown that birds of the corvid family possess extraordinary cognitive skills that match those of nonhuman primates (Emery & Clayton, 2004; Güntürkün & Bugnyar, 2016; Güntürkün, Ströckens et al., 2017). Crow species use tools and meta-tools (Bird & Emery, 2009a; Taylor et al., 2007), reason about causality (Jelbert et al., 2014), self-regulate their behavior (Kabadayi et al., 2016), plan the future (Kabadayi & Osvath, 2017), possess a theory of mind (Bugnyar et al., 2016), recognize themselves in the mirror (Prior et al., 2008), and show signatures of consciousness (Nieder et al., 2020). However, little is known about how these species, with their small absolute brain sizes in comparison to primates, can achieve such impressive cognitive skills. Although avian and mammalian forebrains are overall differently organized (Güntürkün, 2012; Güntürkün & Bugnyar, 2016; Güntürkün, Stacho et al., 2017), avian and mammalian pallia are homologous (Jarvis et al., 2005; Reiner et al., 2004), share network connectivity patterns, (Shanahan et al., 2013), and cortex-like canonical circuits in their sensory pallia (Stacho et al., 2020). In primates, higher cognitive capabilities are thought to correlate with cortical neuron numbers

(Dicke & Roth, 2016; Herculano-Houzel, 2012a; Herculano-Houzel, 2017) and large pallial neuron numbers could also underlie corvid cognition (Herculano-Houzel, 2017). Indeed, due to high neuron densities, the pallia of songbirds (including corvids) and parrots reach twice the amount of neurons than of primate species with similarly sized brains (Olkowicz et al., 2016). Additionally, the pallial mass of songbirds and parrots scales as a power function of its neuron numbers with an exponent close to 1, that is, linearly, which implies that average neuronal cell size does not increase as brains are made of more neurons (Herculano-Houzel et al., 2015). This is a rare trait, shared with primates, while in other species the pallium expands faster in volume than in neuron numbers. Songbird and parrot brains thus remain fairly small as they gain neurons, and as a consequence, corvids with their larger brains probably accumulate more pallial neurons than other avian species with similar or even larger absolute brain sizes (Herculano-Houzel, 2009, 2012b; Olkowicz et al., 2016), possibly providing corvids with pronounced cognitive abilities (Cnotka et al., 2008; Dicke & Roth, 2016).

Complex cognition might especially rely on neuron numbers in associative pallial areas. Although the associative prefrontal region of the human cortex contains a similar 8% of cortical neurons as in other primates, the absolute number of prefrontal neurons is much larger in humans than in other primates (Gabi et al., 2016). In corvid species, the associative pallial brain areas meso- and nidopallium are enlarged and their relative volume is correlated with innovation rate and tool use (Lefebvre, 2013; Lefebvre et al., 2002; Mehlhorn, Hunt et al., 2010; Sayol et al., 2016; Timmermans et al., 2000). The mesopallium is a classic associative forebrain entity that receives no direct sensory input and does not project out of the telencephalon (Atoji & Wild, 2012). This is similar for the nidopallium, with the sole exception of a small thalamopallial sensory input zone at the nidopallial base (Atoji & Wild, 2009). We therefore hypothesized that corvid cognition rests, at least in part, on larger neuron numbers in these two pallial associative areas. To test our prediction, we analyzed in six avian species the number as well as the relative and absolute distribution of pallial neurons among five pallial areas (hyperpallium, arcopallium/amygdala complex, hippocampal formation, mesopallium, and nidopallium) and the subpallium. We used three corvid species (hooded crow, carrion crow, and rook), as well as pigeons, chicken, and ostriches. Pigeon and chicken are widely used in behavioral studies and have cognitive skills below corvids (Güntürkün, Ströckens et al., 2017; Wright et al., 2017). The ostrich has the largest absolute brain size in birds but is not known to show remarkable cognitive skills (Overington et al., 2009). It thus constitutes a critical case for neuron number comparisons with our crows. We used the isotropic fractionator to determine neuron numbers and densities in each forebrain structure independently of structure size (Herculano-Houzel & Lent, 2005).

If total pallial neuron numbers predict cognitive abilities across bird species, we would expect corvids to possess more pallial neurons than the other three species. If, however, only higher associative neuron numbers are relevant, corvids should specifically have more meso- and nidopallial neurons. For comparison, we chose the nonassociative hyperpallium as a primary sensory and motor area, the hippocampal formation (relevant for avian spatial navigation), arcopallium/amygdala complex (constituted by the pre/motor arcopallium and adjacent amygdaloid areas), and the subpallium (constituted by striatum, pallidum, septum, and nucleus accumbens) (Atoji & Wild, 2009, 2012; Ditz & Nieder, 2016; Gentner & Margoliash, 2003; Güntürkün & Bugnyar, 2016; Lengersdorf et al., 2014; Moll & Nieder, 2015).

2 | MATERIALS AND METHODS

2.1 | Specimen

All procedures were carried out in accordance with the guidelines for care and use of animals provided by a National Ethics Committee of the State of North Rhine-Westphalia, Germany. Four pigeons (*Columba livia domestica*, racing homer breed) and four chicken (*Gallus gallus domesticus*, brown warren breed) were obtained from local breeders, were killed by an overdose of pentobarbital, and perfused with 4% paraformaldehyde in phosphate buffer (0.1 M, pH 7.4, PFA). Brains were removed and postfixed for 1–2 weeks in 4% PFA. Four Carrion crows (*Corvus corone*), four hooded crows (*Corvus cornix*) and four rooks (*Corvus frugilegus*) were killed by hunters in Austria and Germany during regular pest control. Directly afterwards, brains were removed, and immersion fixed in 4% PFA for 2–4 weeks. For each species, body weights were taken before the head was removed. Four ostriches (*Struthio camelus*) bred for consumption purpose were killed by a local butcher and brains were removed and treated as described for the crow species. For long term storage, brains were transferred to an anti-freeze solution (30% glycerol, 30% ethylene glycol, 40% phosphate buffer, and stored at −20°C in a freezer; Herculano-Houzel et al., 2014). Before processing, brains were transferred to a 30% sucrose in 0.1 M phosphate buffer (PBS) for cryoprotection until brains until brains sank to the bottom of the solution. One hemisphere of each brain was then cut in 80 μm thick frontal sections using a cryostat (Leica, Wetzlar), while the other hemisphere was stored in anti-freeze solution for future studies. Brain slices were stored in 0.1 M PBS + 0.1% sodium azide until further processing.

2.2 | Dissection

Telencephalic subdivisions hyper-, meso-, nido-, and subpallium, as well as hippocampal formation and arcopallium/amygdala complex, were dissected in the sliced hemisphere with the aid of a microscope and using micro blades. Separation of hyperpallium and mesopallium was performed along the lateral pallial lamina, which was easily visible in all species. To separate hyperpallium from hippocampal formation, we cut along the dorsal pallial lamina. However, this lamina, separating parahippocampal area and hyperpallium, was not always identifiable in anterior aspects of the telencephalon. To cope for this problem, a straight cut from the upper tip of the lateral ventricle to the dorsal border of the brain was made as soon as the lateral ventricle showed up. In most cases, this was only necessary for 2–3 slices since afterwards the dorsal pallial lamina became visible. Separation of mesopallium from hyper- and nidopallium was done along the lateral and the ventral pallial lamina, respectively. Both borders were well

visible in all investigated species. As nidopallial borders, we used the ventral pallial lamina to separate nido- from mesopallium and the pallial-subpallial boundary to separate nidopallium from subpallium. Again, these borders were easy to identify in all animals. Delineation of the subpallium from the rest of the telencephalon was done along the pallial-subpallial boundary. To separate subpallium from diencephalon, we cut directly below the anterior commissure. When we reached the rostral extent of the commissure, we continued slicing on this level until the lateral ventricle reached the outer liquor space, forming a natural border between telencephalon and diencephalon. Thus, the area we call subpallium contains major subareas medial and lateral striatum, septal nuclei, pallidal nuclei, and the nucleus accumbens. Delineation of the arcopallium/amygdala complex posed no problem at all, since the dorsal amygdaloid lamina, representing the border between arcopallium/amygdala complex and nidopallium was always visible. To delineate the hippocampal formation (comprising the hippocampus proper and parahippocampal areas), we used the borders described above. In addition, we included all tissue on the outer side of the lateral ventricle (mainly dorso-lateral corticoid area). Due to the lack of clear borders between some parts of the delineated areas (e.g., anterior aspects of the hyperpallium—hippocampal formation border, subpallium—diencephalon border posterior to the anterior commissure) we cannot exclude a slight intermingling between areas. However, based on the relatively small extent of these uncertain border regions we assume this effect is negligible. In pigeons and chickens, the olfactory bulb could be delineated as well. However, since we could not delineate this area in the other species, in which the olfactory bulb is barely present, we did not include these samples into our analysis. To verify our delineations, we used brain atlases when applicable. For pigeons, we used the brain atlas by Karten and Hodos (1967), for chickens, the atlas by Puelles et al. (Puelles, 2007) and for corvid species the atlas of the Japanese jungle crow by Izawa & Watanabe (2007). For the ostrich, no atlas was available. However, the majority of borders (especially the pallial laminae) were very well visible in the ostrich, making delineations easy. Figure 1 depicts delineation examples for pigeons, chicken, ostriches, and carrion crows.

2.3 | Acquisition of neuron numbers

Neuron numbers for each sample were determined using the isotropic fractionator method (Herculano-Houzel & Lent, 2005), which has been independently demonstrated by at least three groups to be more efficient and at least as accurate and precise as standard stereology, with the advantage that estimated cell numbers are completely independent of tissue volume and sampling strategy (Bahney & Bartheld, 2014; Miller et al., 2014; Ngwenya et al., 2017).

Following delineation, tissue was carefully rinsed in phosphate buffered saline (PBS) and then briefly dried using Whatman cellulose filter paper (Merck, Darmstadt) to remove excess amount of PBS. Afterwards, the tissue was weighed and then homogenized in 40 mM sodium citrate with 1% Triton X-100 (Merck, Darmstadt) using a Tenbroeck tissue grinder (Wheaton, Millville). The DNA marker 4′,6-diamidino-2-phenylindole (DAPI) was added to the suspension at a final concentration of 0.5 μg/ml to stain cell nuclei. To determine the total amount of nuclei in the suspension, 10 μl of the suspension were filled in a Neubauer improved counting chamber (Merck, Darmstadt). Using a fluorescence microscope (AxioImager M1, Zeiss, Göttingen, 200×–400× magnification, numerical aperture 1.0 [200×] and 0.75 [400×], mercury short arc lamp light source with Zeiss filter set 49) stained nuclei (and thus cells) within the suspension were counted. Counting for each area was performed in at least four 10 μl samples. Additional aliquots were counted when the coefficient of variance between the samples was higher than 0.15. Since, in contrast to mammals, avian erythrocytes contain a nucleus, care was taken to not include nuclei of these blood cells into the counts. This was relatively easy since avian erythrocytes possess an oval, elongated nucleus which is distinctively different from the rather roundish nuclei of neurons and glial cells. Furthermore, erythrocytes showed a strong auto fluorescence in the red and green spectrum that could be uses as a further exclusion criterion (see Figure 2 for an example). Based on the average counts of all samples for an individual area and the volume of sample, the total amount of cells for each area could be calculated. To determine the proportion of neurons, an immunocytochemistry against the neuronal nuclear marker NeuN was performed. A fraction of each sample was labeled with a mouse monoclonal anti-NeuN antibody, coupled to either a red or green fluorophore (MAB377X, clone A60, either Alexa Fluor®488 or 594 conjugated, Merck, Darmstadt; dilution: 1:300), which has been previously used to label neurons in birds (Olkowicz et al., 2016). For each sample, 500 randomly selected DAPI positive cells were checked for a double labeling against NeuN (see Figure 2), using a fluorescent microscope (AxioImager M1, Zeiss, Göttingen, 400× magnification, numerical aperture 0.75, mercury short arc lamp light source with Zeiss filter set 49 for DAPI labeling, Zeiss filter set 38 for Alexa Fluor®488 labeling, Zeiss filter set 45 for Alexa Fluor®594 labelling). Again, erythrocytes were discarded from the analysis. Based on the ratio between NeuN positive and negative cells, the ratio of neurons/non-neurons could be calculated for each sample and total neuron numbers as well as the amount of total nonneuronal cells could be determined. We than calculated neuron densities for each of the six areas as well as the percentage of total telencephalic neurons situated in each area.

2.4 | Statistical analysis

Statistical analyses were performed using SPSS (version 20, SPSS Inc., Chicago, IL, United States of America). For all analyses, we used linear parametric methods with an α-level of.05. To test if neuron numbers, neuron densities, and distribution of total telencephalic neurons differed between species in one area, we conducted univariate analyses of variance for each area with "species" (pigeon, chicken, ostrich, carrion crow, hooded crow, rook) as within-subject factor for each variable, with subsequent Bonferroni corrected post hoc tests. Since an increasing amount of studies suggest an interaction of striatal with associative cortical structures during processing of higher cognitive functions in mammals (Antzoulatos & Miller, 2011; Boot et al., 2017), we also calculated linear regression analyses between subpallial neurons and neurons in each of

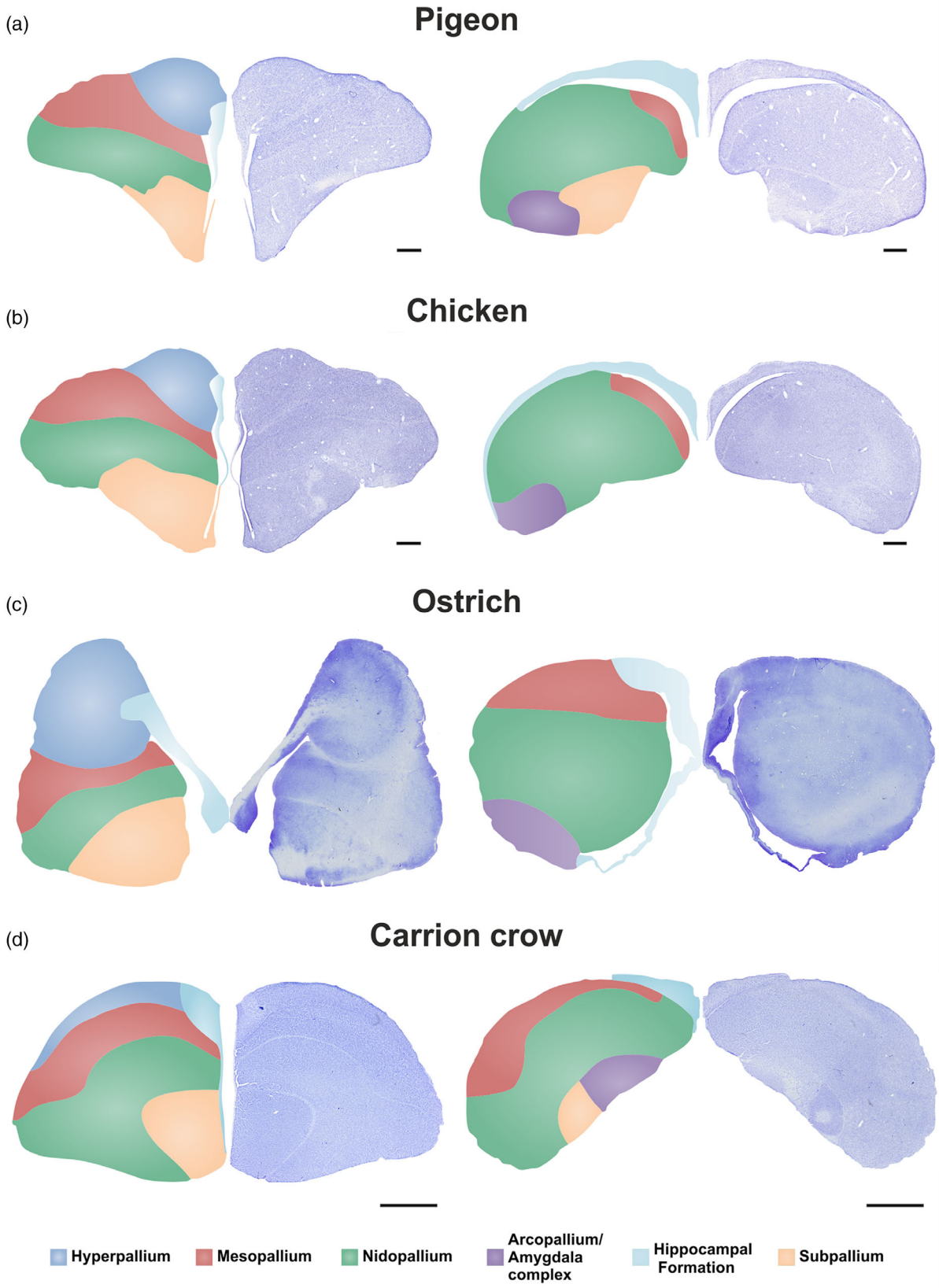


FIGURE 1 Schematic outlines of the areas dissected in this study (left side) and corresponding brain slices stained with a Nissl stain (right side). Depicted are anterior and posterior examples for pigeons (a), chicken (b), ostriches (c), and carrion crows (d). Outlines for hooded crows and rooks were almost identical to carrion crows and are thus not shown. Note that most of the borders between the areas are clearly visible due to the pallial laminae, which were also visible in the unstained slices used in this study. Scale bars indicate 1000 μm in (a) and (b), and 5000 μm in (c) and (d). We would like to thank Christina Herold and Noemi Rook for supplying pictures of Nissl-stained sections

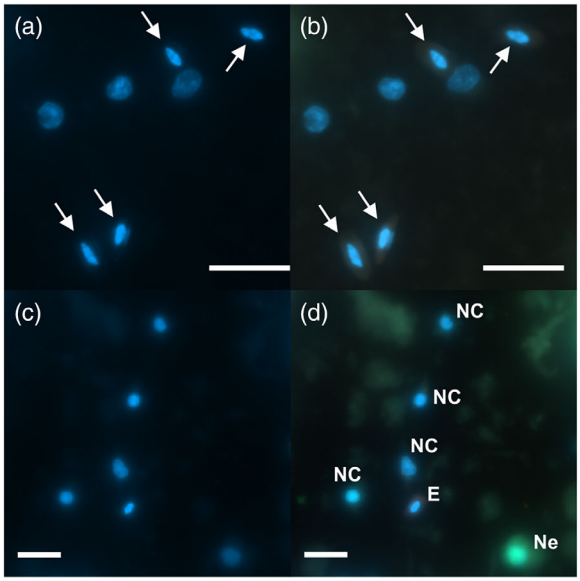


FIGURE 2 Example picture of a DAPI stained cell solution of an ostrich nidopallium sample (a). In contrast to mammalian species, avian erythrocytes possess a nucleus and are thus stained by the DNA marker DAPI (white arrows). To not inflate the number of nonneuronal cells, erythrocytes numbers were not included in the analysis. Erythrocytes nuclei could be easily differentiated from other nonneuronal and neurons by their unique elongated shape and a strong autofluorescent corona surrounding the DAPI stain (b), not present in other cell types. After acquisition of total cell numbers, samples were immunocytochemically stained against the neuronal nuclei marker NeuN. (c) depicts an example picture of a such stained sample of the ostrich nidopallium showing only the DAPI signal, while (d) depicts the same sample showing also the NeuN signal in green. E, Erythrocyte; NC, nonneuronal cell; Ne, Neuron. Scale bars indicate 20 μm

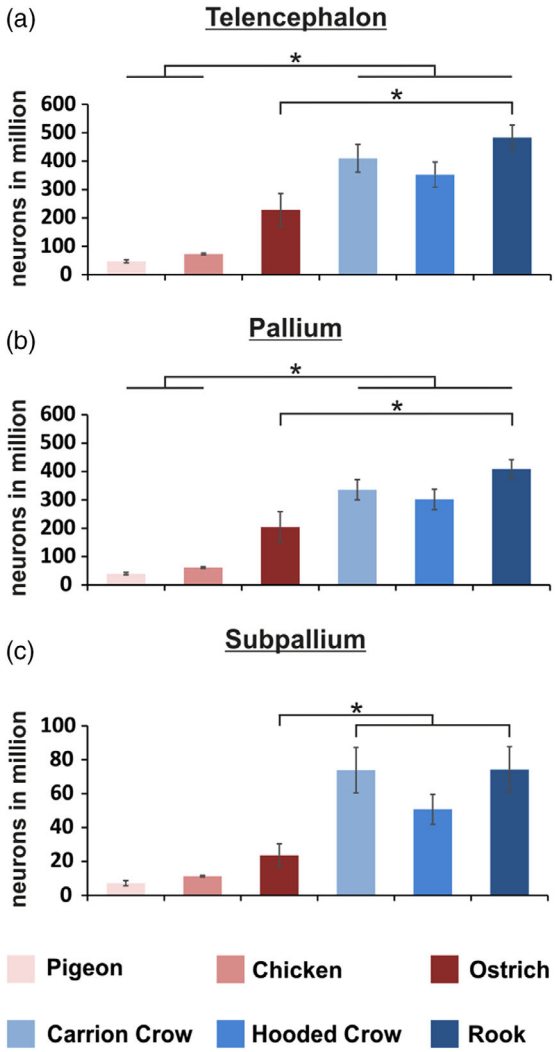


FIGURE 3 Absolute neuron numbers within one hemisphere of the telencephalon (a), pallium (b), and subpallium (c) in three noncorvid (red tones) and three corvid species (blue tones). While corvids showed significant higher pallial neuron numbers than pigeons and chickens for telencephalon and pallium, only one corvid species (rook) had significantly more telencephalic/pallial neurons than ostriches. Telencephalic and pallial neuron numbers in carrion crows and hood crows were statistically on par with ostriches. For the subpallium, carrion crow, and rook neuron numbers were significantly higher than in ostriches, while hooded crow numbers were comparable to ostriches. Further significance values can be found in Table 1. Error bars indicate standard error of the mean

the pallial areas with a Bonferroni corrected significance level at $p < .001$.

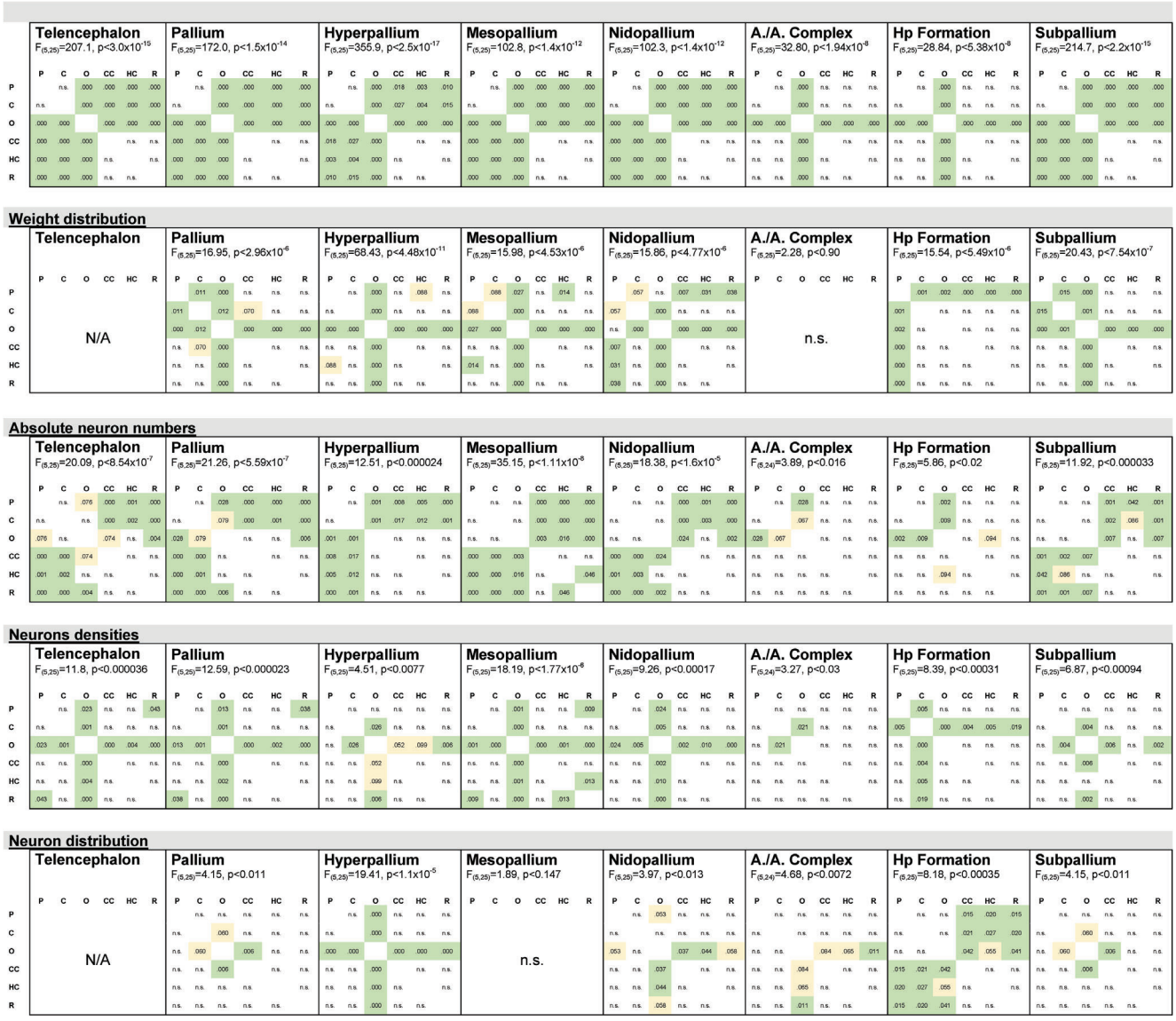
3 | RESULTS

3.1 | Corvid brains have high pallial and subpallial neuron numbers

The ostrich telencephalon weighs 8.2 g per hemisphere and is thus about three times larger than those of our three corvid species (ca. 2.5 g) and over 16 times larger than those of pigeons and chicken (all $p < .05$, Tables 1 and 2). Still, all three corvid species had over 350 million neurons in each telencephalic hemisphere, comparable and, in case of rooks, even higher neuron numbers than the 282 million telencephalic neurons of ostriches. With fewer than 100 million neurons, pigeons and chickens trailed far behind corvids ($p < .05$ for all corvid vs. pigeon/chicken comparisons and ostrich–rook comparison, all other comparisons: not significant (n.s.), Figure 3a, Tables 1 and 2). This pattern was due to major differences in neuron densities between corvids, pigeon, and chicken on one side (100,000–200,000 neurons/mg telencephalon) and ostriches on the other (<30,000 neurons/mg; $p < .05$, Tables 1 and 2).

Within the telencephalon, the ostrich pallium alone, with its 7.5 g per hemisphere, was over three times as large as those of corvids (ca. 2 g), and more than 17 times as large as the pallium of pigeons and chicken (all $p < .05$, Tables 1 and 2). Despite these size differences, corvid species had over 300 million pallial neurons, compared to 200 million in ostriches and fewer than 100 million in pigeon and chicken (Figure 3b, Table 2). At the statistical level, corvids had more pallial neurons than pigeons and chicken (all $p < .05$), rooks more than ostriches ($p < .05$), and ostriches more than pigeons ($p < .05$; Figure 3b and Table 2). Thus, corvid species have overall more (rook vs. ostrich) or at least as many pallial neurons as the ostrich (carrion and hooded crow vs. ostrich), although the ostrich pallium is by far the largest.

TABLE 1 Significance values for all comparisons made in this study



Note: Significance values for all comparisons made in this study. For all analyses, we used linear parametric methods with an α-level of .05. For each variable, we calculated univariate ANOVAs for each area with species as within-subject factor, followed by subsequent post hoc tests (Bonferroni corrected for multiple comparisons) in case of a significant main effect. Significant differences are highlighted in green ($p < .05$) while nonsignificant trends are highlighted in yellow ($p < .1$).

Abbreviations: A./A. complex, arcopallium/amygdala complex; C, chicken; CC, carrion crow; HC, hooded crow; O, ostrich; P, pigeon; R, rook.

The ostrich subpallium with its 0.67 g per hemisphere, is about twice as large as that of the three corvid species and has over 10 times the mass of those of pigeons and chicken (Table 2). However, ostriches have only 24 million subpallial neurons, while hooded crows (51 million), carrion crows, and rooks (both 74 million) have many more (ostrich vs. carrion crow/rook, each $p < .05$; Figure 3c, Table 2). Despite their much smaller brains, pigeons, and chicken were still statistically on par with ostriches. These effects resulted from the much lower subpallial neuron densities in ostriches (35,000 neurons/mg), compared to the other species (100,000–200,000 neurons/mg, compare Figure 5b). Finally, we also analyzed the proportions of mass and neuron numbers in the different compartments of the telencephalon (Figure 4, Tables 1 and 2).

We found that the relative distribution of mass or numbers of neurons in the telencephalon does not systematically distinguish corvids from the smaller pigeons and chickens. However, the distributions do differ in ostriches in comparison to the other species, especially for the hyperpallium.

3.2 | Corvids have more associative pallial neurons

We hypothesized that corvids have high associative pallial neuron numbers even when compared with the large brained ostrich. Indeed, the ostrich mesopallium weighs 1.2 g while corvids stand at 0.5 g (all

TABLE 2 Data overview

Whole Telencephalon													
	Weight			All cells				Neurons			Nonneuronal cells		
Species	Structure (mg)	% telenc.	Body weight (g)	Total	Cells / mg	% telenc.	% neurons	Total	Neurons / mg	% telenc.	Total	Non Neuronal cells / mg	% telenc.
Pigeon Mean	408	100	469	83,250,000	205,000	100	59.91	47,260,000	117,800	100	35,990,000	87,250	100
Pigeon *SEM*	16		8	11,480,000	29,250			5,330,000	17,570		12,420,000	29,480	
Chicken Mean	487	100	1557	112,200,000	233,200	100	65.39	73,170,000	151,300	100	39,010,000	81,900	100
Chicken *SEM*	25		83	2,884,000	18,000			3,185,000	9120		4,577,894	13,430	
Ostrich Mean	8168	100	100,000	561,400,000	68,320	100	40.78	228,300,000	27,700	100	333,200,000	40,620	100
Ostrich *SEM*	452		0	52,200,000	2730			57,800,000	6190		67,380,000	7000	
Carrion Crow Mean	2512	100	463	600,100,000	238,000	100	68.12	409,900,000	162,500	100	190,200,000	75,490	100
Carrion Crow *SEM*	38		16	64,970,000	22,750			48,900,000	17,410		21,210,000	7590	
Hooded Crow Mean	2666	100	406	562,430,000	233,200	100	62.89	352,700,000	136,800	100	209,800,000	79,520	100
Hooded Crow *SEM*	156		26	51,600,000	40,860			44,100,000	26,640		35,200,000	13,700	
Rook Mean	2401	100	463	674,900,000	281,400	100	72.06	483,000,000	201,000	100	191,900,000	80,380	100
Rook *SEM*	37		33	69,200,000	29,640			44,090,000	17,400		34,620,000	15,520	
Whole Pallium													
	Weight			All cells				Neurons			Nonneuronal cells		
Species	Structure (mg)	% telenc.	Body weight (g)	Total	Cells / mg	% telenc.	% neurons	Total	Neurons / mg	% telenc.	Total	Non Neuronal cells / mg	% telenc.
Pigeon Mean	346	84.81	469	69,370,000	202,000	83.54	61.10	40,090,000	117,900	85.31	29,280,000	84,070	81.47
Pigeon *SEM*	16		8	9,178,000	28,800			3,970,000	16,550		10,300,000	29,210	
Chicken Mean	431	88.39	1557	95,570,000	225,100	85.18	64.90	61,860,000	144,700	84.49	33,720,000	80,330	86.23
Chicken *SEM*	23		83	2,683,000	19,080			2,998,000	9080		4,218,000	14,010	
Ostrich Mean	7516	91.91	100,000	513,000,000	67,840	91.27	40.77	208,100,000	27,450	91.27	304,900,000	40,390	91.28
Ostrich *SEM*	464		0	50,100,000	2630			52,120,000	6020		63,260,000	6990	
Carrion Crow Mean	2148	85.55	463	500,600,000	232,300	83.68	67.07	336,100,000	155,900	82.42	164,500,000	76,360	86.55
Carrion Crow *SEM*	26		16	49,700,000	20,440			35,780,000	14,920		18,120,000	7660	
Hooded Crow Mean	2314	86.71	406	475,300,000	210,700	84.76	63.68	301,819,000	135,300	85.75	173,400,000	75,430	82.57
Hooded Crow *SEM*	148		26	38,140,000	31,040			35,850,000	26,170		29,870,000	12,610	
Rook Mean	2075	86.41	463	571,900,000	276,300	84.99	72.12	408,700,000	197,000	85.01	163,200,000	79,360	84.19
Rook *SEM*	33		33	54,630,000	28,160			32,760,000	15,220		31,960,000	16,800	

(Continues)

TABLE 2 (Continued)

Hyperpallium													
	Weight			All cells				Neurons			Nonneuronal cells		
Species	Structure (mg)	% telenc.	Body weight (g)	Total	Cells / mg	% telenc.	% neurons	Total	Neurons / mg	% telenc.	Total	Non Neuronal cells / mg	% telenc.
Pigeon Mean	38	9.17	469	8,276,000	238,200	10.03	58.10	4,681,000	145,900	9.66	3,595,000	92,300	9.66
Pigeon *SEM*	6		8	1,127,00	50,210			915,700	51,540		1,229,000	23,530	
Chicken Mean	50	10.30	1557	12,760,000	261,200	11.33	68.04	8,621,000	177,100	11.85	4,142,000	84,060	11.85
Chicken *SEM*	5		83	1,051,000	29,550			676,900	21,420		691,100	14,780	
Ostrich Mean	2335	28.66	100,000	149,900,000	64,110	26.98	40.92	60,890,000	25,900	27.56	89,000,000	38,210	27.10
Ostrich *SEM*	104		0	9,575,000	2,080			12,210,000	4740		15,360,000	6170	
Carrion Crow Mean	291	11.56	463	73,220,000	247,000	11.93	65.95	48,680,000	164,300	11.54	24,540,000	82,640	11.54
Carrion Crow *SEM*	22		16	13,320,000	29,910			10,150,000	25,900		5,133,000	12,020	
Hooded Crow Mean	349	13.02	406	75,330,000	224,600	13.40	66.59	50,470,000	152,500	14.38	24,860,000	72,100	14.38
Hooded Crow *SEM*	42		26	6,749,000	34,170			7,044,000	32,510		3,676,000	8930	
Rook Mean	309	12.85	463	87,990,000	285,000	13.16	72.42	62,970,000	204,200	13.39	25,020,000	80,760	13.39
Rook *SEM*	7		33	8,446,000	26,190			4,581,000	15,060		5,534,000	17,440	
Mesopallium													
	Weight			All cells				Neurons			Nonneuronal cells		
Species	Structure (mg)	% telenc.	Body weight (g)	Total	Cells / mg	% telenc.	% neurons	Total	Neurons / mg	% telenc.	Total	Non Neuronal cells / mg	% telenc.
Pigeon Mean	74	18.15	469	16,150,000	220,700	20.29	68.36	10,340,000	142,200	22.48	5,809,000	78,470	22.48
Pigeon *SEM*	6		8	1,958,000	28,920			832,200	16,750		2,770,000	34,390	
Chicken Mean	97	20.81	1557	23,920,000	249,900	21.32	68.22	16,170,000	168,900	22.19	7,747,000	80,980	22.19
Chicken *SEM*	7		83	1,018,000	16,130			759,100	11,330		1,613,000	18,160	
Ostrich Mean	1230	15.03	100,000	97,950,000	79,520	17.53	42.16	41,200,000	32,590	17.90	56,750,000	46,940	16.94
Ostrich *SEM*	95		0	9,375,000	4630			11,63,000	6840		12,230,000	10,050	
Carrion Crow Mean	501	19.94	463	130,310,000	258,300	21.61	70.91	89,410,000	178,100	22.40	40,890,000	80,210	22.40
Carrion Crow *SEM*	11		16	17,740,000	29,940			5,183,000	6640		12,570,000	23,330	
Hooded Crow Mean	573	21.52	406	122,900,000	220,600	21.73	66.51	81,140,000	145,900	23.01	41,750,000	74,690	23.01
Hooded Crow *SEM*	31		26	13,990,000	38,310			10,450,000	27,830	0.61	8,131,000	16,010	
Rook Mean	497	20.70	463	149,900,000	303,000	22.15	79.58	116,000,000	233,800	24.32	33,870,000	69,210	24.32
Rook *SEM*	9		33	16,830,000	37,170			6,405,000	13,370		13,060,000	27,360	

(Continues)

TABLE 2 (Continued)

Nidopallium													
	Weight			All cells				Neurons			Nonneuronal cells		
Species	Structure (mg)	% telenc.	Body weight (g)	Total	Cells / mg	% telenc.	% neurons	Total	Neurons / mg	% telenc.	Total	Non Neuronal cells / mg	% telenc.
Pigeon Mean	171	41.94	469	33,220,000	195,400	41.93	65.02	20,790,000	122,700	44.22	12,430,000	72,670	44.22
Pigeon *SEM*	5		8	3,437,000	21,790			2,200,000	15,360		4,547,000	26,720	
Chicken Mean	213	45.74	1557	45,640,000	216,300	40.65	64.75	29,460,000	140,200	40.19	16,180,000	76,100	40,19
Chicken *SEM*	12		83	1,886,000	13,570			1,699,000	12,320		2,084,120	9990	0.61
Ostrich Mean	3166	38.63	100,000	207,600,000	64,670	36.40	39.67	82,760,000	25,600	35.22	124,800,000	39,070	36.71
Ostrich *SEM*	248		0	30,800,000	5,250			25,570,000	6670		30,930,000	7740	
Carrion Crow Mean	1175	46.81	463	270,900,000	230,500	45.61	66.97	182,700,000	155,400	44.66	88,190,000	75,060	44.66
Carrion Crow *SEM*	2		16	22,880,000	19,120			21,120,000	17,690		6,980,000	5950	
Hooded Crow Mean	1230	46.04	406	253,300,000	212,200	45.40	62.06	156,900,000	133,300	44.44	96,460,000	78,840	44.44
Hooded Crow *SEM*	86		26	15,720,000	30,430			20,620,000	28,100		19,330,000	14,530	
Rook Mean	1103	45.95	463	302,700,000	275,000	44.97	70.70	214,200,000	194,300	44.11	88,440,000	80,700	44.11
Rook *SEM*	13		33	29,560,000	28,040			25,470,000	22,770		14,520,000	14,410	
Arcopallium/Amygdala Complex													
	Weight			All cells				Neurons			Nonneuronal cells		
Species	Structure (mg)	% telenc.	Body weight (g)	Total	Cells / mg	% telenc.	% neurons	Total	Neurons / mg	% telenc.	Total	Non Neuronal cells / mg	% telenc.
Pigeon Mean	10	2.47	469	1,839,000	184,200	2.31	50.10	875,400	85,840	2.07	966,800	92,040	2.07
Pigeon *SEM*	1		8	231,200	20,100			25,750	6,570		303,900	26,960	
Chicken Mean	19	4.15	1,557	3,758,000	200,600	3.36	59.19	2,249,000	119,300	3.06	1,509,000	81,320	3.06
Chicken *SEM*	2		83	480,400	10,890			354,900	11,310		158,100	5,570	
Ostrich Mean	261	3.21	100,000	20,910,000	79,000	3.78	39.87	8,785,000	31,390	3.81	12,130,000	47,630	3.83
Ostrich *SEM*	37		0	3,592,000	4,070			2,579,000	6,460		2,166,000	7,230	
Carrion Crow Mean	73	2.92	463	10,820,000	153,200	1.89	63.94	6,848,000	98,620	1.74	3,973,000	54,590	1.74
Carrion Crow *SEM*	9		16	924,500	16,940			469,500	14,490		669,500	8,020	
Hooded Crow Mean	65	2.47	406	10,010,000	153,400	1.78	56.95	5,688,000	87,450	1.66	4,320,000	65,980	1.66
Hooded Crow *SEM*	1		26	1,740,000	25,410			1,030,000	15,680		1,017,000	14,680	
Rook Mean	62	2.56	463	11,950,000	204,500	1.75	46.30	5,573,000	96,580	1.12	6,375,000	107,900	1.12
Rook *SEM*	9		33	1,619,000	39,640			1,792,000	29,400		1,690,000	27,190	

(Continues)

TABLE 2 (Continued)

Hippocampal formation													
	Weight			All cells				Neurons			Non neuronal cells		
Species	Structure (mg)	% telenc.	Body weight (g)	Total	Cells / mg	% telenc.	% neurons	Total	Neurons / mg	% telenc.	Total	Non Neuronal cells / mg	% telenc.
Pigeon Mean	46	11.36	469	6,632,000	140,800	8.03	54.31	3,624,000	78,570	7.40	3,007,000	62,240	7.40
Pigeon *SEM*	3		8	1,341,000	23,680			940,100	20,700		998,700	17,040	
Chicken Mean	30	6.31	1,557	7,848,000	305,600	7.04	67.02	5,351,000	201,700	7.20	2,496,000	104,000	7.20
Chicken *SEM*	6		83	868,900	66,960			951,100	39,610		414,000	33,000	
Ostrich Mean	524	6.39	100,000	36,700,000	73,070	6.57	40.51	14,500,000	31,600	6.78	22,190,000	41,450	6.71
Ostrich *SEM*	83		0	2,790,000	8,100			3,154,000	10,020		4,707,750	2,440	
Carrion Crow Mean	109	4.32	463	15,320,000	145,600	2.65	54.00	8,372,000	77,340	2.09	6,946,000	68,310	2.09
Carrion Crow *SEM*	14		16	1,368,000	14,660			1,229,000	6,130		579,200	11,670	
Hooded Crow Mean	97	3.67	406	13,690,000	141,600	2.44	55.93	7,645,000	78,960	2.25	6,047,000	62,650	2.25
Hooded Crow *SEM*	7		26	1,345,000	12,470			726,700	6,110		652,100	6,610	
Rook Mean	105	4.36	463	19,430,000	187,300	2.95	50.75	9,864,000	95,310	2.07	9,564,000	91,970	2.07
Rook *SEM*	4		33	1,830,000	21,730			969,900	12,230		942,500	10,110	
Subpallium													
	Weight			All cells				Neurons			Non neuronal cells		
Species	Structure (mg)	% telenc.	Body weight (g)	Total	Cells / mg	% telenc.	% neurons	Total	Neurons / mg	% telenc.	Total	Non Neuronal cells / mg	% telenc.
Pigeon Mean	62	15.19	469	13,880,000	222,800	17.10	53.56	7,164,000	116,000	14.69	6,715,000	106,800	14.69
Pigeon *SEM*	1		8	2,316,000	32,990			1,520,000	24,890		2,226,000	34,280	
Chicken Mean	57	12.10	1557	16,610,000	300,600	14.82	68.16	11,320,000	206,300	15.51	5,291,000	94,250	15.51
Chicken *SEM*	5		83	239,100	26,950			480,900	24,670		503,400	8480	
Ostrich Mean	671	8.58	100,000	48,170,000	71,830	9.24	47.49	23,660,000	35,330	9.60	24,520,000	36,500	9.12
Ostrich *SEM*	26		0	4,864,000	6930			6,853,000	10,200		2,592,224	3280	
Carrion Crow Mean	363	14.45	463	99,550,000	271,700	16.32	73.15	73,890,000	200,800	17.58	25,660,000	70,890	17.58
Carrion Crow *SEM*	15		16	15,330,000	36,600			13,380,000	32,750		3,467,000	9310	
Hooded Crow Mean	352	13.29	406	87,170,000	253,500	15.24	58.54	50,840,000	147,300	14.25	36,340,000	106,100	14.25
Hooded Crow *SEM*	17		26	13,640,000	49,120			8,831,000	31,000		6,354,000	21,540	
Rook Mean	326	13.59	463	102,900,000	312,100	15.01	70.50	74,290,000	223,900	14.99	28,640,000	88,240	14.99
Rook *SEM*	13		33	15,010,000	38,880			13,470,000	35,940		3,163,000	10,040	

Note: Mean values and standard error of the mean (*SEM*) for all data acquired in our study. Data are based on $n = 4$ for each species. Large numbers were rounded to the fourth significant digit. The body weight of ostriches could not be measured when brains were acquired. Thus, in line with the literature, an estimate of 100 kg is given (Literature values for the Common ostrich, *Struthio camelus*, range from 63 to 145 kg, with a mean body weight for females of 100 kg and for males of 115 kg; Davies, 2003).

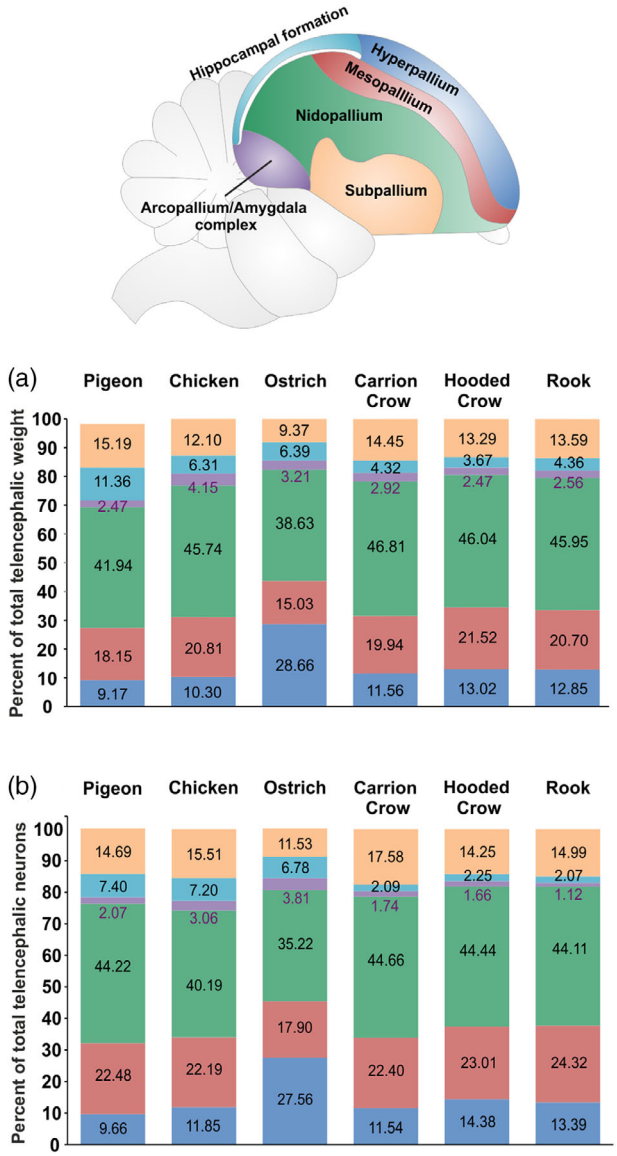


FIGURE 4 Mass (a) and neuron (b) distribution in the telencephalon of six avian species. Ostriches accumulate a significant higher percentage of their telencephalic mass and neurons in their hyperpallium (blue) than all other species while their meso (red) and nidopallium (green) contains a significant lower percentage of mass in comparison to almost all other birds (all $p < .05$ except pigeon—ostrich comparison in the nidopallium). In addition, the nidopallium of ostriches contains a significantly lower percentage of neurons in comparison to carrion crows and hooded crows, while the comparison to pigeons ($p < .053$) and rooks ($p < .058$) barely missed significance. See Table 1 for further significance values

$p < .05$, Table 2). In contrast, the three corvid species (81–116 million) had at least twice the number of mesopallial neurons than the ostrich (41 million) and even more than pigeons and chicken (10–16 million, $p < .05$ for all corvids compared to all non-corvids, Figure 5, Table 2). Similarly, while the ostrich nidopallium weighs 3.2 g, and thus twice as much as that of corvids (1.1–1.2 g), all three corvid species had at least twice as many nidopallial neurons (157–214 million) than the ostrich (83 million) and 5–10 times more than pigeon and chicken (21–29 million, $p < .05$ for all corvids in comparison to all noncorvids, except for the hooded crow vs. ostrich comparison (n.s.), Figure 5, Table 2).

This pattern stands in contrast to other pallial areas. Ostriches have a large hyperpallium (2 g per hemisphere) while that of corvids weighs less (0.3 g) and pigeon and chicken reach just about 0.05 g (all $p < .05$, Tables 1 and 2). In terms of neuron numbers, ostriches (61 million) and corvids (49–63 million) are about equal (all $p > .05$, Tables 1 and 2) and surpass pigeons and chicken ($p < .05$ for all corvid/ostrich vs. pigeon/chicken comparisons, Figure 5, Tables 1 and 2). Arcopallium/amygdala complex neuron numbers vary between 9 million (ostrich) and 1 million (pigeon) with only the ostrich versus pigeon comparison being significant ($p < .05$; Table 2) while all other comparisons (including corvids) failed to reach significance. A comparable pattern was also observed for the hippocampal formation where neuron numbers ranged between 14.5 million (ostrich) and 4–5 million (pigeons/chicken) with only ostrich vs. pigeon/chicken comparisons being significant (all $p < .01$, Tables 1 and 2).

Corvids thus surpassed the other species selectively in associative neuron numbers, which tightly mirrored numbers in the subpallium (see above and Table 2). Indeed, using linear regressions with a Bonferroni corrected significance level at $p < .001$ we found that subpallial neuron numbers scale tightly across all six species together with mesopallial ($r^2 = .850$) and nidopallial ($r^2 = .879$) neuron numbers. This relation was considerably weaker for hyperpallium ($r^2 = .487$) and not significant for arcopallium/amygdala complex and hippocampal formation (Figure 6).

4 | DISCUSSION

We departed from the hypothesis that a part of the remarkable cognitive prowess of corvids results from their high absolute numbers of associative pallial neurons. To test this assumption, we estimated the neuron numbers of all major telencephalic components in three corvid species as well as in noncorvid chicken, pigeon, and ostrich using the isotropic fractionator method. Especially the comparison with the ostrich was a tall task, given that it is the animal with the largest bird brain. Indeed, we found that the mass of the associative meso- and nidopallium of ostrich brains is more than twice of those of corvids, but nevertheless only holds less than half the number of neurons as corvids have. In contrast, such a difference was not visible in the other nonassociative pallial areas. Finally, especially meso- and nidopallium scale closely with subpallial neuron numbers. These findings constitute a strong support for our hypothesis that the numbers of associative pallial neurons drive cognitive prowess and thus constitute a key fundament of the outstanding cognitive abilities of corvids (Abeyesinghe et al., 2005; Bagotskaia et al., 2010; Balakhonov & Rose, 2017; Bird & Emery, 2009a, 2009b; Dufour et al., 2012; Güntürkün, Ströckens et al., 2017; Scarf et al., 2016; Wright et al., 2017).

The avian associative meso- and nidopallium are not functionally homogenous areas. This is especially true for the nidopallium, which contains three primary sensory subregions (auditory, visual, trigeminal) that make up 7% of the nidopallial volume (calculated from

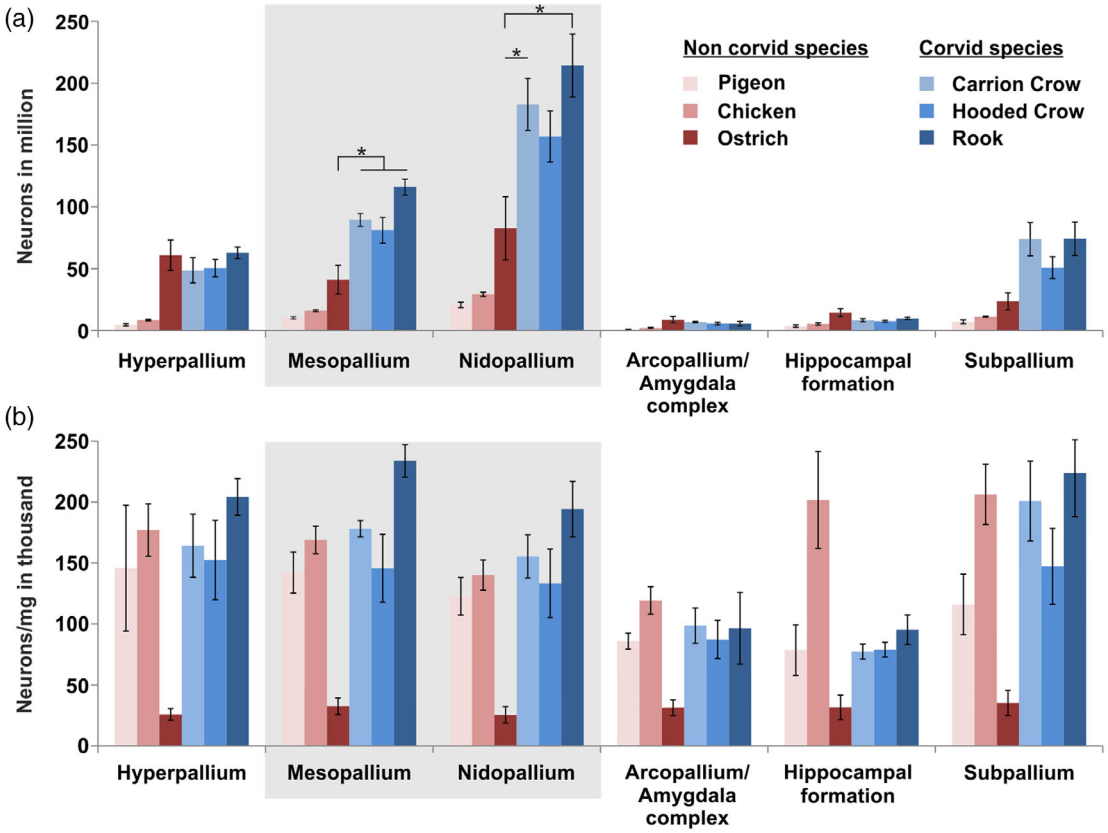


FIGURE 5 (a) Total number of neurons within six telencephalic areas of one hemisphere in three corvid and three noncorvid species. Noncorvid species are labelled with red colors while corvid species are labeled with blue tones. Areas, which are known to play a role in higher cognitive functions (meso- and nidopallium), are highlighted in grey. Within these associative areas, corvids possessed in all cases significantly more neurons than pigeons and chickens and, with one exception (the nidopallium of hooded crows), also higher neuron numbers than ostriches. While there were only few, species-specific differences in the nonassociative arcopallium/amygdala complex and hippocampal formation, the neuron numbers in the mostly sensory hyperpallium were on par in ostriches and corvids. Differences in the subpallium followed the pattern of the associative areas. (b) Neuron densities of the areas shown in (a). For both associative areas, we found ostriches to have significantly lower neuron densities than all other species. All other comparisons between species in these areas (except for higher mesopallial neuron densities in rooks in comparison to hooded crows and pigeons) failed to reach significance. Significantly lower neuron densities in ostriches in comparison to all other species were also present when analyzing the whole telencephon and pallium. However, this difference reached not in all cases significance, when analyzing the nonassociative areas separately. Furthermore, hippocampal neuron densities were significantly higher in chicken in comparison to the other species. For the sake of clarity, only the most important significance values were plotted. All other values can be found in Table 1. Error bars indicate standard error of the mean

Güntürkün et al., 2013; Mehlhorn, Haastert et al., 2010), but also the large nidopallium caudolaterale (NCL), which is thought to be the functional equivalent to the mammalian prefrontal cortex (Eugen et al., 2020; Güntürkün & Bugnyar, 2016; Güntürkün et al., 2021). The NCL is highly relevant for cognitive functions and is involved in processes like working memory, decision making, reward prediction, and is suggested to play a role in episodic-like memory (Allen & Fortin, 2013; Ditz & Nieder, 2016; Güntürkün & Bugnyar, 2016; Lengersdorf et al., 2014; Moll & Nieder, 2015; Nieder et al., 2020). Unfortunately, the borders of the NCL are, like the borders of the PFC in mammals, quite difficult to identify without detailed neurochemical analyses (Eugen et al., 2020; Wynne & Güntürkün, 1995), especially in species that have been less anatomically scrutinized up to now. We therefore refrained from delineating the NCL in our study to avoid any unwarranted conclusions. Besides NCL, nidopallium also hosts further associative areas like nidopallium caudocentrale (NCC), a higher-order limbic forebrain area, and the nidopallium caudomediale (NCM), an auditory associative structure (Atoji & Wild, 2009; van Ruijssevelt et al., 2018).

The mesopallium, in contrast, does not contain any primary sensory areas and does not project out of the telencephalon (Atoji & Wild, 2012), despite the fact that, like the nidopallium, it can also be subdivided into several subregions. Thus, the mesopallium can be considered a purely associative area that receives only indirect input from all sensory modalities and the limbic system (Atoji & Wild, 2012). Studies have shown that the mesopallium is highly involved in learning and memory tasks like imprinting (Atoji & Wild, 2012; Chaves & Hodos, 1997; Horn, 1998) or associative learning (Marzluff et al., 2012), and some of its subareas play a major role in song processing and vocal learning (for review see Hahnloser & Kotowicz, 2010). Thus, the massively larger absolute numbers of mesopallial neurons in corvids compared to

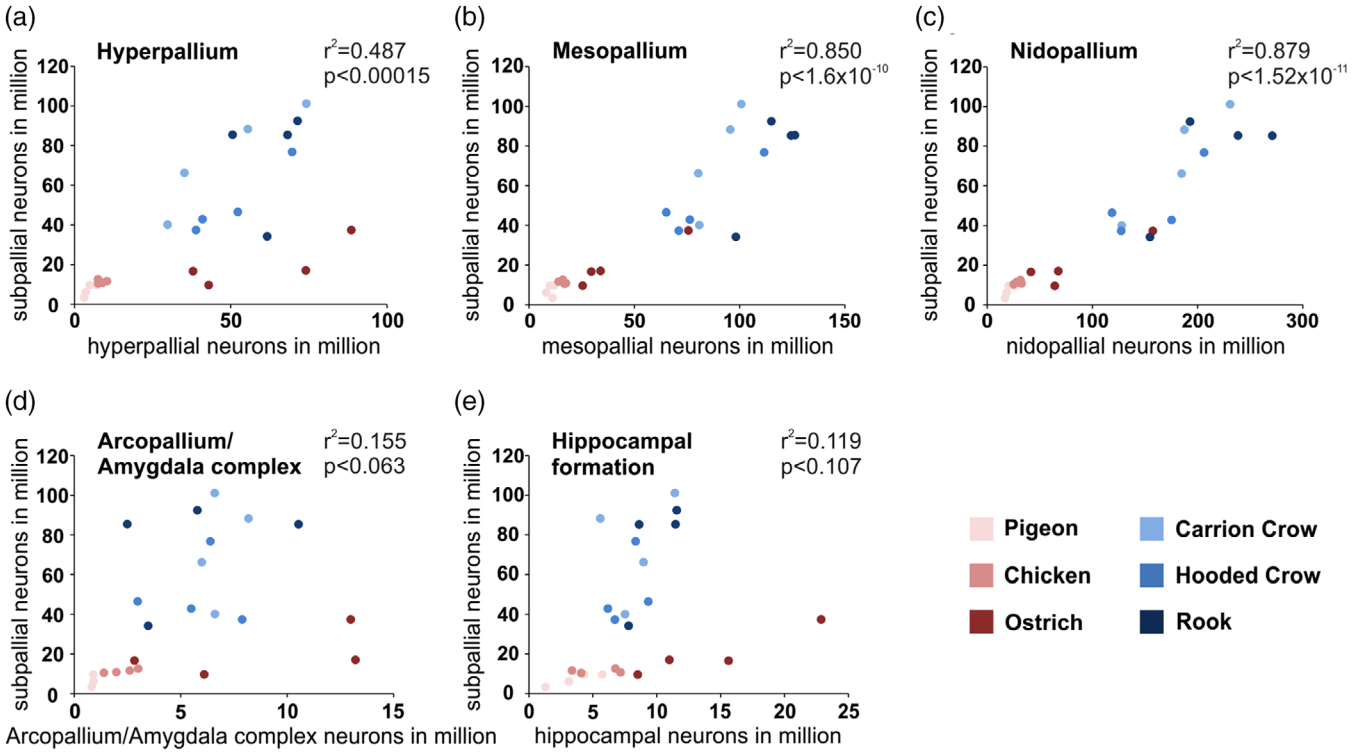


FIGURE 6 Relationship of neuron numbers in the subpallium with neuron numbers in the pallial areas hyperpallium (a), mesopallium (b), nidopallium (c), arcopallium/amygdala complex (d), and hippocampal formation (e) over all species. Regression analysis revealed a significant (Bonferroni corrected at $p < .01$) and high correlation of subpallial neurons with neurons in the associative mesopallium (b) and nidopallium (c). However, subpallial neuron numbers correlated much more weakly or not at all with neurons in the nonassociative hyperpallium, arcopallium/amygdala complex, and hippocampal formation

noncorvids falls in line with studies showing that the relative size of the mesopallium is increased in species with more flexible and innovative behavior (Lefebvre et al., 2002, 2004; Mehlhorn, Hunt et al., 2010; Timmermans et al., 2000). Still, our finding that the ostrich mesopallium is about three times as large in mass as the mesopallium of corvids, but it is the corvid mesopallium that has at least twice as many neurons as the ostrich mesopallium, underscores the risk of relying on absolute brain structure size as a proxy for comparisons of neuron-based functional properties across species.

It should be noted that the delineated areas were based on definitions of the Avian Brain Nomenclature Consortium (Reiner et al., 2004) in combination with clear anatomical landmarks to ease dissection in unstained slices (see Figure 1 for outlines). However, over the last decade several studies that were based on transcriptomic data suggested different pallial subdivisions and borders (Chen et al., 2013; Gedman et al., 2021; Jarvis et al., 2013). Application of these borders would have resulted in a different pattern of pallial partitions. Figure 1 depicts the delineation that we used. According to the series of studies from the Jarvis lab, the green labelled region constitutes a combination of nidopallium and intercalated pallium, the red area is ventral mesopallium, while the blue labelled region is a combination of hyperpallium, intercalated hyperpallium, and dorsal mesopallium. We refrained from using these borders because the pallial subdivisions identified by genetic expression studies are in partial conflict with some of the existing connectivity data. For example, a recent study could show a strong, columnar-like organized interconnectivity within the hyperpallium (hyperpallium apicale [HA]; interstitial nucleus of HA [IHA]; hyperpallium intercalatum [HI]; hyperpallium dorsale [HD] as well as a second and different one within the dorsal ventricular ridge, encompassing nidopallium as well as ventral and dorsal mesopallium; Stacho et al., 2020). Delineating the avian pallium according to transcriptomic data (Chen et al., 2013; Gedman et al., 2021; Jarvis et al., 2013) would have wrongly rearranged the whole columnar connectivity profile of the avian sensory pallium as discovered by Stacho et al. (2020). In addition, the thalamic sensory input to the hyperpallial layers IHA, HI, and HD (Karten et al., 1973) are crucial components that guide the differentiation between associative and nonassociative areas. Since our differentiation of "associative" and "nonassociative" areas are based on connectivity, we decided to apply the borders suggested by the Avian Brain Nomenclature Consortium, because they reflect in our view the connectional results better than borders that depend on genetic expression data. We hope that future evidence will help to support one hypothesis over another.

The Avian Brain Nomenclature Consortium also defines the sensory input zone of trigeminal, visual, and auditory pathways as part of the nidopallium (Reiner et al., 2004). As outlined above we were not able to reliably separate the partly lamina-like thalamopallial input areas from

the nonsensory nidopallial areas. However, the primary sensory component of the nidopallium only makes up 7% of the nidopallial volume. Despite this small number, it is important to alert the reader that our number of associative nidopallium neurons is certainly overestimated for all species studied.

4.1 | Do subpallial neurons numbers contribute to associative cognitive functions?

Besides the expected difference in the number of associative pallial neurons between corvids and noncorvids, our study also revealed a strong association between increased numbers of nido/mesopallial and subpallial neurons across species. This finding is in line with the massive projections from nido- and mesopallium onto striatal territories that serve as motor output structures (Atoji & Wild, 2009; Kröner & Güntürkün, 1999; Shanahan et al., 2013; Veenman et al., 1995). Thus, it is possible that the high numbers of subpallial neurons in corvids result from coordinated scaling with associative areas due to functional and anatomical coupling between these areas. Importantly, the scaling of numbers of subpallial neurons in coordination with numbers of neurons in the associative mesopallium and nidopallium supports the suggestion that the striatum is more than a simple sensorimotor coordination center and is tightly involved in cognitive processes. This overlaps with findings in mammals that demonstrate the relevance of the striatum for diverse cognitive processes (Antzoulatos & Miller, 2011; Boot et al., 2017), along with an a essential role in selecting between cognitively guided response options that compete with each other (Provost et al., 2015). In contrast, we found that the number of hippocampal as well as neurons of the arcopallium/amygdala complex did not scale with subpallial neurons. In case of the hippocampal formation, this is likely due to the lack of direct connections between the subpallium and the hippocampal formation (Atoji & Wild, 2004), removing one of the main drivers of concerted neuronal scaling. While a fraction of arcopallium/amygdala complex neurons in specific subdivisions project to subpallial targets, other subdivisions are completely void of subpallial projections (Hanics et al., 2017; Letzner et al., 2016), possibly overshadowing a significant correlation of projection neurons.

We found that corvids possess more neurons in the hyperpallium in comparison to pigeons and chicken, but not in comparison to ostriches. The hyperpallium is mostly a sensory and especially visual area (Güntürkün, Stacho et al., 2017; Iwaniuk & Wylie, 2020). The large size and high neuron numbers of the ostrich hyperpallium could reflect a clade-specific feature of paleognath birds, since all other neognath avian species of our analysis were similar to each other, but different from the ostrich. The existence of such a clade specific effect is supported by data from skull endocast studies, reporting a pronounced wulst in several paleognath species (Ashwell & Scofield, 2008; Corfield et al., 2008).

Large relative and absolute numbers of neurons in the hyperpallium could, however, also be a visual adaptation to the specific foraging style of ostriches. Ostriches have the largest eyes of any land vertebrate (Boire et al., 2001; Martin et al., 2001) and fixate an item on the ground binocularly at ca. 10 cm distance, to then produce a rapid, precise, and ballistic peck (Martin & Katzir, 1995). Whatever its origin, the large hyperpallial neuron numbers in ostriches underline the importance of area-specific analyses, since analysis restricted to total numbers of pallial neurons fail to capture local peaks in numbers of neurons that might be the neural substrate of species differences in cognitive capability.

4.2 | Associative neuron numbers in relation to cognitive abilities in birds

A large number of behavioral experiments conducted in all three corvid species of our study show that their cognitive skills are on par with those found in primates (Bagotskaia et al., 2010; Balakhonov & Rose, 2017; Bird & Emery, 2009b; Dufour et al., 2012; Emery et al., 2007; Güntürkün & Bugnyar, 2016; Smirnova et al., 2002). Although pigeons and chicken have occasionally baffled the scientific community with unexpected cognitive abilities (Marino, 2017; Scarf et al., 2011S, 2016), a direct comparison to corvids reveals that, for instance, pigeons need much longer training to reach the cognitive level of corvids and are far less able to transfer their skills to related but different tasks or stimulus patterns (Güntürkün, Ströckens et al., 2017). As expected, we find that pigeons and chickens rank lower than corvids both in terms of varieties of cognitive behaviors and in numbers of pallial and associative pallial neurons. While no experimental data on cognitive skills have been collected so far in ostriches, a large analysis covering 2608 reports on 1018 bird species found that no field observation reported the presence of any sort of behavioral innovation in ostriches (Overington et al., 2009). In contrast, corvids consistently rank among the most innovative species (Overington et al., 2009). The absence of ostriches in this collection is noteworthy since these birds are abundant in animal parks and meat factories of industrialized countries, which generated the majority of contributions to the behavioral innovation data bank. Thus, we presume that ostriches rank cognitively lower in comparison to corvids until some future study shows otherwise.

Importantly, here we find similar or even higher numbers of neurons in associative pallial areas in corvids as in several primate species. According to our study, corvid mesopallium and nidopallium combined have between 200 and 300 million neurons per hemisphere (Figure 6, Table 2), which is more than the 68 million neurons estimated to compose one hemisphere of the prefrontal cortical region (anterior to the corpus callosum) of the rhesus monkey (*Macaca mulatta*) brain, and comparable to the 115 million neurons estimated for one hemisphere of the baboon (*Papio cynocephalus*) prefrontal cortical region (Gabi et al., 2010; Gabi et al., 2016). At 8% of all cortical neurons, we predict that the entire prefrontal region of the chimpanzee (*Pan troglodytes*) cortex contains fewer than 300 million neurons per hemisphere (Collins et al., 2016), which would be roughly on par with our corvid numbers. Considering that corvids and great apes perform at ceiling levels in the cognitive tests reported (Güntürkün, Ströckens et al., 2017), our results are compatible with our hypothesis that absolute numbers of cortical neurons, and associative neurons in particular, are a main determining factor of the cognitive capabilities of vertebrate species.

5 | CONCLUSION

Our inquiry into the question of what makes corvids smart shows that corvids have particularly large numbers of neurons in the associative nido- and mesopallium compared to pigeons, chickens, and even ostriches, which have the largest avian brain. In contrast, corvid neuron numbers in nonassociative pallial areas are not higher than those of ostriches. The large number of associative neurons in corvids is closely mirrored by similar quantities in subpallial structures, making it likely that associative pallio-striatal loops are key components of cognition in corvids as also described in primates. We propose that while comparisons of total numbers of pallial neurons are informative, cognitive capabilities of different species are more directly related to absolute numbers of neurons in associative structures that can support flexible and complex cognition. Future comparative studies of cognitive capabilities and their neurological underpinnings should therefore focus on associative areas separately from purely sensorimotor structures. Thus, although complex cognitive functions obviously also depend on many other variables like cellular morphology, connectivity patterns, neurochemical properties, and cognition-related regulatory genetic sequences (Audet et al., 2018; Dicke & Roth, 2016; Genç et al., 2019; Genç et al., 2018; Goriounova & Mansvelder, 2019; Güntürkün, Stacho et al., 2017; Sayol et al., 2016; Wirthlin et al., 2018), we propose that sheer large quantities of associative neurons constitute a key component of the outstanding cognitive capabilities of both corvids and primates.

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CONFLICT OF INTEREST

All authors declare that there was no conflict of interest.

DATA AVAILABILITY STATEMENT

All relevant data can be found in the manuscript.

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