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A comparative analysis of the dopaminergic innervation of the executive caudal nidopallium in pigeon, chicken, zebra finch, and carrion crow

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Abstract

Despite the long, separate evolutionary history of birds and mammals, both lineages developed a rich behavioral repertoire of remarkably similar executive control generated by distinctly different brains. The seat for executive functioning in birds is the nidopallium caudolaterale (NCL) and the mammalian equivalent is known as the prefrontal cortex (PFC). Both are densely innervated by dopaminergic fibers, and are an integration center of sensory input and motor output. Whereas the variation of the PFC has been well documented in different mammalian orders, we know very little about the NCL across the avian clade. In order to investigate whether this structure adheres to species-specific variations, this study aimed to describe the trajectory of the NCL in pigeon, chicken, carrion crow and zebra finch. We employed immunohistochemistry to map dopaminergic innervation, and executed a Gallyas stain to visualize the dorsal arcopallial tract that runs between the NCL and the arcopallium. Our analysis showed that whereas the trajectory of the NCL in the chicken is highly comparable to the pigeon, the two Passeriformes show a strikingly different pattern. In both carrion crow and zebra finch, we identified four different subareas of high dopaminergic innervation that span the entire caudal forebrain. Based on their sensory input, motor output, and involvement in dopamine-related cognitive control of the delineated areas here, we propose that at least three morphologically different subareas constitute the NCL in these songbirds. Thus, our study shows that comparable to the PFC in mammals, the NCL in birds varies considerably across species.

KEYWORDS

birds, convergent evolution, dopamine, executive functions, NCL, PFC

Abbreviations: AA, anterior arcopallium; AC, anterior commissure; AD, dorsal arcopallium; AI, intermediate arcopallium; Aid, dorsal intermediate arcopallium; AIv, ventral intermediate arcopallium; AM, medial arcopallium; AP, posterior arcopallium; AV, ventral arcopallium; CDL, dorsolateral corticoid area; DA, dorsal arcopallial tract; dMI, dorsal intermediate mesopallium; E, entopallium; Eb, entopallial belt; HA, apical part of the hyperpallium; HD, densocellular part of the hyperpallium; HP, hippocampus; IHA, interstitial part of HA; L, field L; LAD, dorsal arcopallial lamina; LSt, lateral striatum; M, mesopallium; NC, caudal nidopallium; NCC, caudocentral nidopallium; NCIF, island fields of the caudal nidopallium; NCL, nidopallium caudolaterale; NCLd, dorsal nidopallium caudolaterale; NCLdc, caudal aspect of the dorsal nidopallium caudolaterale; NCldr, rostral aspect of the dorsal nidopallium caudolaterale; NCLl, lateral nidopallium caudolaterale; NCLm, medial nidopallium caudolaterale; NCLv, ventral nidopallium caudolaterale; NCM, caudomedial nidopallium; NF, frontal nidopallium; Ov, nucleus ovoidalis; PFC, prefrontal cortex; RA, robust nucleus of the arcopallium; SN, substantia nigra; VTA, ventral tegmental area.

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1 | INTRODUCTION

The lineages of birds and mammals diverged in the Carboniferous about 312 million years ago (Benton & Donoghue, 2006). Since then both groups developed differently organized cerebra that are dominated by six layered cerebral cortices in mammals, and a nuclear structure in birds (Güntürkün, Stacho, & Ströckens, 2017). Despite these radically differently structured telencephalia, detailed studies revealed astonishing similarities in the neural organization processing perceptual features. Jack Pettigrew showed in a series of seminal studies that the visual wulst of barn owls and the primary visual cortex of monkeys have virtually identical coding mechanisms for orientation and binocular disparity (Pettigrew, 1980; Pettigrew & Konishi, 1976). Since the optic chiasm and the thalamopallial projection are differently organized in birds and mammals, it is likely that the similarities of the visual pallium result from convergent evolution that unfolded due to similar selection processes. But birds and mammals are also similar with respect to their rich, behavioral repertoire that is present across the phylogenetic tree of both lineages (Nicolakakis, Sol, & Lefebvre, 2003; Reader & Laland, 2002). This reflects various mental faculties such as object permanence (pigeon: Zentall & Raley, 2019; parrot: Pepperberg & Funk, 1990; magpie: Pollok, Prior, & Güntürkün, 2000; cat/dog: Triana & Pasnak, 1981; primate: de Blois, Novak, & Bond, 1998), perception of biological motion (chicken; Mascalzoni, Regolin, & Vallortigara, 2010; jackdaw; Greggor, McIvor, Clayton, & Thornton, 2018; cats: Blake, 1993; common marmoset: Brown, Kaplan, Rogers, & Vallortigara, 2010), numerical competence (pigeon: Scarf, Hayne, & Colombo, 2011; chicken: Rugani, Vallortigara, Priftis, & Regolin, 2015; Rhesus macaque: Brannon & Terrace, 1998; chimpanzee: Boysen & Berntson, 1989), tool use (New Caledonian crow: Hunt & Gray, 2004; chimpanzee: McGrew, 2004), mental time travel (pigeon: Zentall, Clement, Bhatt, & Allen, 2001; Western scrub jay: Clayton & Dickinson, 1998; rat: Babb & Crystal, 2006; chimpanzee: Martin-Ordas, Haun, Colmenares, & Call, 2010), and even theory of mind (ravens: Bugnyar, Reber, & Buckner, 2016; chimpanzee: Krupenye, Kano, Hirata, Call, & Tomasello, 2016).

In mammals, the seat for executive functioning is the prefrontal cortex (PFC), situated at the anterior pole of the frontal lobe of the neocortex (Fuster, 2015; Miller & Cohen, 2001). The avian functional equivalent to the PFC is known as the nidopallium caudolaterale (NCL) and was first described in pigeons (Mogensen & Divac, 1982). It is located at the posterior pole of the avian forebrain and shows striking parallels to the mammalian PFC in terms of connectivity, neurochemical modulation, and function (Güntürkün, 2005, 2012). Like the PFC (Fuster, 2015; Goldman-Rakic, 1987), the NCL is a higher order associative area that is reciprocally connected to secondary and tertiary sensory areas from all modalities, connects to limbic, visceral and memory-related structures, and sends descending projections to basal ganglia and premotor areas (Kröner & Güntürkün, 1999; Leutgeb, Husband, Riters, Shimizu, & Bingman, 1996; Shanahan, Bingman, Shimizu, Wild, & Güntürkün, 2013).

A crucial function of an executive structure is to gate, maintain, and manipulate incoming information and subsequently initiate the appropriate action. The key neurotransmitter involved in these processes is dopamine (Ott & Nieder, 2019), and we observe several parallels in the dopaminergic architecture of the PFC and NCL. Both structures are strongly innervated by dopaminergic fibers that arise from the mesencephalic ventral tegmental area (VTA) and substantia nigra (SN, rat; Fallon & Moore, 1978; Lindvall, Björklund, & Divac, 1978; monkey: Felten & Sladek, 1983; Gaspar, Stepniewska, & Kaas, 1992; pigeon: Kitt & Brauth, 1986; Waldmann & Güntürkün, 1993). The dopaminergic terminals follow a common theme visible as small symmetric synapses that occasionally form synaptic triads (Durstewitz, Kröner, & Güntürkün, 1999; Metzger, Jiang, & Braun, 2002; Schnabel et al., 1997). The fibers chiefly contact dendritic arbors and spines that are rich in D1 receptors, with relatively lower levels of D2 receptors (Durstewitz, Kröner, Hemmings, & Güntürkün, 1998; Herold et al., 2011). Moreover, the dopamine reuptake rate is low and as a consequence it activates extrasynaptic dopamine receptors located outside the synaptic cleft via diffusion-mediated volume transmission (rat: Zoli et al., 1998; pigeon: Bast, Diekamp, Thiel, Schwarting, & Güntürkün, 2002).

On a functional level, the PFC and NCL have been implicated in a range of behaviors that recruit self-control, working memory and cognitive flexibility—the core concepts of executive functioning (Diamond, 2013; Fuster, 2015; Güntürkün, 2005, 2012; Nieder, 2017). Lesion- and pharmacological blockade studies demonstrate that ablation of these executive structure interferes with performance on spatial and nonspatial working memory tasks (pigeon: Diekamp, Diekamp, Gagliardo, & Güntürkün, 2002; Gagliardo, Bonadonna, & Divac, 1996; Güntürkün, 1997; Lissek & Güntürkün, 2004; Mogensen & Divac, 1982; rat: Wikmark, Divac, & Weiss, 1973; cat: Divac, 1973; monkey: Rosvold & Szwarcbart, 1964; human: Müller & Knight, 2006), memory consolidation and learning (Hartmann & Güntürkün, 1998; Lengersdorf, Marks, Uengoer, Stüttgen, & Güntürkün, 2015; Lengersdorf, Stüttgen, Uengoer, & Güntürkün, 2014; Lissek, Diekamp, & Güntürkün, 2002; Lissek & Güntürkün, 2003, 2005), and choice behavior (Kalenscher, Diekamp, & Gunturkun, 2003). Even more striking is that the PFC and NCL show a high degree of similarity in the neural code on both single neuron as well as neuronal population level. Namely, a subset of neurons in both structures specifically increase their firing rate during the delay period in working memory tasks (pigeon: Diekamp, Kalt, & Güntürkün, 2002; Johnston, Anderson, & Colombo, 2017; Kalenscher et al., 2005; Rose & Colombo, 2005; crow: Veit, Hartmann, & Nieder, 2014; rat: Sakurai & Sugimoto, 1986; monkey: Fuster, 1973; Fuster & Alexander, 1971; Miller, Erickson, & Desimone, 1996; Procyk & Goldman-Rakic, 2006). Moreover, neurons in the PFC and NCL encode abstract rules (crow: Veit & Nieder, 2013; monkey: Wallis, Anderson, & Miller, 2001), are critically involved in multimodal learning processes (Moll & Nieder, 2015, 2017; Starosta, Stüttgen, & Güntürkün, 2014; Veit, Pidpruzhnykova, & Nieder, 2015), and represent reward or value (Dykes, Klarer, Porter, Rose, & Colombo, 2018; Johnston et al., 2017; Kalenscher et al., 2005; Koenen, Millar, & Colombo, 2013; Scarf et al., 2011; Starosta, Güntürkün, & Stüttgen, 2013). To conclude, despite differences in their gross morphological layout, the PFC and NCL are the key

structure involved in cognitive control and achieve this in a highly comparable manner.

Besides the similarities and differences in executive structure between these two vertebrate classes, there is considerable variation within the classes. Across mammals, the PFC is not a uniform structure. In line with the wide range of variation in cognitive capacities, the PFC does not only differ in absolute and relative size, but also varies in the extent of parcellation and thus number of subdivisions (Kaas, 2019). For example, the dorsolateral PFC is considered a specialization of the primate order and cannot be identified in rodent species (Carlén, 2017; Wise, 2008).

As already suggested by Iwan Divac, Mogensen, and Björklund (1985): "Given the variation of topography of the PFC in different mammalian species, one might expect some such variability also in different species of birds ". However, whereas the PFC has been described and studied in numerous mammalian species (Passingham & Wise, 2012), in birds the boundaries of the NCL have been delineated in pigeons only. Besides some data on the NCL in chicken (Braun, Bock, Metzger, Jiang, & Schnabel, 1999) and recent pioneering work in the carrion crow (Nieder, 2017), which only concerns in vivo recordings and lacks a neuroanatomical analysis, we know close to nothing about this structure in other bird species. This is problematic, because there are considerable differences both in behavior as well as in brain anatomy across the avian phylogenetic tree. For example, whereas members of the corvid family can readily learn and transfer abstract rules, pigeons either show no evidence of transfer at all (Wilson, Mackintosh, & Boakes, 1985), or need considerably more training (Wright et al., 2017). A similar pattern applies to self-control. Different corvid species demonstrate the capability to delay gratification for a higher quality reward for up to 320 s (Dufour, Wascher, Braun, Miller, & Bugnyar, 2012), whereas pigeons will opt for the smaller immediate reward even after a short delay of 4 s is introduced (Green, Fisher, Perlow, & Sherman, 1981).

These differences in behavior are mirrored by differences in brain structure, both at a macroscopic as well as a microscopic level. Namely, bird species that are behaviorally on par with nonhuman primates, such as some members of Psittacines (parrots) and Passeriformes (songbirds), have a brain with higher neuronal densities and expanded mesopallial and nidopallial territories. In contrast, more basal birds such as Columbiformes (pigeon) and Galliformes (chicken) have a brain with lower neuron numbers and a relatively larger brainstem and proportional cerebellar and telencephalic structures (Iwaniuk & Hurd, 2005; Olkowicz et al., 2016). Moreover, Psittacines and Passeriformes are both vocal learners, and thus their cerebral *bauplan* includes unique vocal and auditory nuclei, which are not present in nonvocal learners (Chakraborty & Jarvis, 2015; Reiner, Perkel, Mello, & Jarvis, 2004). Another striking difference, particularly in the caudal telencephalon, is the relative organization of the striatum and arcopallium. In pigeons (Karten, Karten, & Hodos, 1967) and chickens (Puelles, 2007) the arcopallium is situated lateral and caudal to the striatum, whereas in Passeriformes (Izawa & Watanabe, 2007; Nixdorf-Bergweiler & Bischof, 2007) it emerges medial and caudal to the striatum. Given the vast differences in both behavior and telencephalic morphology between pigeons and other bird species, it is likely the NCL shows a similar degree of variation as well.

In order to investigate if the NCL is a uniform structure across birds or whether we are confronted with a variety as observed in mammals, this study set out to identify and delineate the boundaries of the NCL in several bird species. We selected the scientifically relevant and well-studied species pigeon, chicken, zebra finch and carrion crow to represent diversity in body and brain size and cognitive capacities. We delineated the boundaries of the NCL based on an immunohistochemical stain against tyrosine hydroxylase (TH), which is the rate limiting enzyme in the production of dopamine. The NCL was then defined as the area of highest TH+ fiber density in the caudal nidopallium combined with the presence of characteristic "baskets," which are TH+ fibers that coil multiple times around an unstained perikaryon (Waldmann & Güntürkün, 1993; Wynne & Güntürkün, 1995). In addition, we visualized the strong reciprocal connection between the NCL and the intermediate arcopallium (AI) with a Gallyas stain against myelin. This projection is known as the dorsal arcopallial tract (DA) and was used as a second criterion to identify NCL (Kröner & Güntürkün, 1999; Zeier & Karten, 1971). With this approach, we tried to recognize and describe the trajectory of the NCL in pigeon, chicken, zebra finch and carrion crow and verify whether we need a species-specific approach to the NCL in birds.

2 | MATERIALS AND METHODS

2.1 | Specimen

For this study, we analyzed two homing pigeons (*Columba livia*), six chickens (*Gallus Gallus domesticus*, White Leghorn), five zebra finches (*Taeniopygia guttata*) and four carrion crows (*Corvus corone*). The adult pigeons, chickens and zebra finches were obtained from a registered breeder and the carrion crows were obtained in the wild. All animal care and procedures were in concordance with the German guidelines for care and use of animals in science, and approved by the national ethics committee of the State of North Rhine-Westphalia, Germany. This is all in agreement with the European Communities Council Directive 86/609/EEC regarding the care and use of animals for experimental purposes. Since pigeon have been studied very often to analyze diverse properties of the NCL, the two individuals functioned as a control for our method of analysis. The other species were selected to represent phylogenetic diversity, variation in cognitive performance and differences in brain morphology. In terms of phylogenetic position, chickens represent the most basal group. As part of the Galloanserae clade, they diverged from the other Neoaves species of our study approximately 72 mya. Although pigeons are Neoaves, their Columbaves branch separated from the remaining two species in our study 64 mya and thus represent intermediates between the basal chickens and the relatively modern zebra finches and carrion crows of the Passeriformes clade that emerged 56 mya (Prum et al., 2015). Regarding cognitive performance, pigeon and chicken demonstrate rather low levels of complex cognitive control (Güntürkün, Ströckens,

Scarf, & Colombo, 2017; Marino, 2017), while the carrion crow, as a member of the corvids, is considered on par with nonhuman primates (Emery & Clayton, 2004; Güntürkün & Bugnyar, 2016). Unfortunately, not much is known about the cognitive capacities of zebra finches since the main focus of research is on their song system. Likely as a result of phylogenetic position and cognitive performance (see above), brain morphology differs considerably between the selected species. Passeriformes (zebra finch and carrion crow) have a brain that consists of a relatively large forebrain, in comparison to pigeon and chicken (Iwaniuk & Hurd, 2005). In addition, the Passeriformes can be characterized by a positional shift of the arcopallium, now situated caudal and medial to the striatum, whereas in pigeon and chicken it is located lateral to the striatum (Mello, Kaser, Buckner, Wirthlin, & Lovell, 2019).

Pigeon, chicken and zebra finch were injected with 1,000 IU heparin (Ratiopharm, Ulm, Germany) about 15 min before anesthesia. Next, animals were deeply anesthetized with 0.45 ml/100 g body weight 1% pentobarbital—4.5% chloral hydrate solution (pigeon and zebra finch) or 0.03 ml/100 g body weight 10% pentobarbital solution (chicken) intramuscularly. Afterwards, animals were transcardially perfused with saline (0.9% NaCl, 40°C) followed by 4% paraformaldehyde (PFA) in 0.12 M sodium phosphate buffered saline (PBS, pH 7.4, 4°C). Brains were dissected and postfixated in 30% sucrose in PFA for 1 (chicken and pigeon) or 12 (zebra finch) hr and cryoprotected in 30% sucrose in PBS until sunken to the bottom (approx. 12–48 hr). The carrion crow brains were obtained from a licensed hunter from the Netherlands during regular pest control. After a crow was shot, it was instantly fetched by a trained hunting dog. Immediately after death, the brain was dissected on the spot and within 5–10 min postmortem immersed in 4% PFA. The total duration of immersion fixation was determined based on formaldehyde diffusion speed in brain tissue, which is 1 mm/hr, followed by a minimum of 24–48 hr to ascertain covalent bonding and crosslinking (Howat & Wilson, 2014). Each crow brain was fixated for a total of 120 hr with 3 days in 4% PFA, and 2 days postfixated in 30% sucrose in PFA. Next, it was transferred to 30% sucrose in PBS for cryoprotection for 24–48 hr. Brains were sectioned with a microtome (Leica Microsystems, Wetzlar, Germany) in the frontal plane into 10 parallel series of 40 μm sections, and stored in 0.1% sodium azide in PBS at 4°C.

2.2 | Tyrosine hydroxylase (TH) immunohistochemistry

In order to delineate the boundaries of the NCL, we conducted an immunohistochemical stain against tyrosine hydroxylase (TH), which is the rate-limiting enzyme in the production of dopamine and a well-established method to visualize the strong dopaminergic input to identify the NCL (Waldmann & Güntürkün, 1993; Wynne & Güntürkün, 1995). In pigeons, the NCL is the area of highest TH+ fiber density in the caudal nidopallium and contains characteristic dopaminergic "baskets" representing multiple TH+ fibers that coil around large unstained perikarya. The NCL is not the only structure that contains baskets, but it is the area where baskets are constituted by the highest density of dopaminergic fibers (Waldmann & Güntürkün, 1993; Wynne & Güntürkün, 1995). Thus, TH+ fiber densities and the occurrence of baskets are the most important indicators to identify the NCL histologically.

Since preliminary experiments revealed that antibody binding in the two Passeriformes species was rather low in comparison to pigeons and chickens, we pretreated slices of zebra finches and carrion crows with a heat-induced epitope retrieval (HIER, Jiao et al., 1999). The low antibody binding is a possible consequence of the prolonged fixation times, in which a higher number of crosslinks mask the epitope of interest. Heating the tissue will cleave these bridges and unmask the antigen binding site (Yamashita, 2007). In order to make sure this procedure did not generate any false-positives, we executed a control in pigeon brain tissue. We tested the HIER-procedure in immersion-fixated pigeon tissue, which generated a highly comparable result pattern to perfused untreated tissue. For the HIER procedure, sections were rinsed in 0.12 M PBS and placed in preheated 10 mM sodium citrate buffer (pH 9.0) at 80°C for 20 min before the actual staining. Immunohistochemical staining for all species was executed in one series of free-floating sections and unless stated otherwise conducted at room temperature. All rinsing steps consisted of three rinses of 10 min in tris-buffered saline (TBS, pH 7.6) on a slow 5° rotator. After an initial rinsing step, endogenous peroxidases were deactivated by incubation in 0.6% H_2O_2 in distilled water. The slides were then rinsed and incubated in 10% Normal Goat Serum (NGS) in 0.3% Triton X-100 in TBS to block nonspecific binding. After another rinsing step, incubation in the primary antibody (polyclonal rabbit anti-TH, AB152, RRID:AB_390204, Merck Millipore, Darmstadt, Germany), 1:500 in 1% NGS in TBST took place overnight at 4°C on a slow 7° rotator. The following day, the slides were rinsed and incubated in the secondary antibody (polyclonal goat-anti-rabbit, Vectastain Elite ABC Kit, Rabbit IgG, Vectorlabs, Burlingame) for 2 hr. Next, the slides were rinsed in TBST and incubated in an avidin/biotin complex solution (Vectastain Elite ABC Kit, 1:100 in TBST) for 1 hr. This complex was visualized by a nickel enhanced 3,3′-diaminobenzidine (DAB) reaction, using a commercially available kit (DAB Peroxidase Substrate Kit, Vectastain, Vectorlabs, Burlingame). Afterwards, the sections were mounted on gelatin-covered slides, and after drying for 30 min, dehydrated in an alcohol series, rehydrated in xylene and cover slipped using DePex (Sigma–Aldrich, Darmstadt, Germany). We verified the specificity of our antibody with a western blot, see Appendix.

2.3 | Gallyas staining

The second criterion to delineate the boundaries of the NCL is the strong reciprocal connection to the AI. This projection shows a high degree of overlap with the area identified as the NCL based on connectivity and dopaminergic innervation in pigeons (Kröner & Güntürkün, 1999). This connection is also known as the dorsal arcopallial tract (DA) and since it is heavily myelinated it can be visualized with the silver impregnation Gallyas stain. This stain makes use of the inherent argyrophilic characteristic of myelin to bind silver particles

and consequently labels myelinated structures black (Gallyas, 1971; Merker, 1983).

One series of free-floating sections was mounted on gelatin coated slides and dried for 1 hr at 40°C. Slides were incubated for 1 hr in 70% ethanol and rinsed 3 × 5 min in distilled water. Next, sections were incubated for 1 hr in an infiltration solution (0.1% ammonium nitrate, 0.1% silver nitrate, 0.012% sodium hydroxide solution in distilled water), and consecutively rinsed 3 × 10 min in 0.5% acetic acid in the dark. Following, slides were incubated for 10 min in a developing solution (a 3:2:1 solution of (a) 5% sodium carbonate, (b) 0.2% ammonium nitrate, 0.2% silver nitrate, 1% silicotungstic acid, and (c) 0.2% ammonium nitrate, 0.2% silver nitrate, 0.4% 37% Formalin in distilled water) and rinsed 3 × 10 min in 0.5% acetic acid followed by one rinse of 5 min in distilled water. To enhance contrast, sections were incubated for 5 min in 1% gold chloride solution and washed 4 × 10 min in distilled water. Sections were then fixed in 1% sodium thiosulfate and rinsed for 5 min in distilled water. After drying, slides were dehydrated, rehydrated and cover slipped following the same procedure described above.

2.4 | Data acquisition

For each species and stain, pictures of the caudal part of the forebrain were taken with a 20× objective on a Zeiss Axio Imager M1 microscope (Carl Zeiss MicroImaging, Göttingen, Germany). The exact selection was based on previous description of the trajectory of the NCL in the pigeon (Waldmann & Güntürkün, 1993; Wynne & Güntürkün, 1995). As a conservative topological approach, this translated into analysis of the telencephalon situated caudal to the disappearance of the medial striatum. In the pigeon, the NCL was visible in the sections ranging from A7.00 to A3.75 (Karten et al., 1967), and in the zebra finch from A1.35 to P0.36 (Nixdorf-Bergweiler & Bischof, 2007). For adult chicken and carrion crow there are currently no brain atlases available, and we employed the chick brain atlas (Puelles, 2007) and the brain atlas of the Japanese jungle crow (Izawa & Watanabe, 2007) to guide identification of different areas. To ease referencing in the discussion of the results, we labeled the analyzed sections with ascending Roman numbers from anterior to posterior.

2.5 | Estimation of TH-fiber density

For the quantitative analysis of TH-fiber densities, we employed a custom written automatic counting program that recognizes fiber-like structures from high magnification images overlaid with a Hessian filter. The fiber quantification program is based on Sathyanesan, Ogura, and Lin (2012) and makes use of a Hessian based curvilinear feature extraction in ImageJ (version 1.48, U.S. National Institutes of Health, Bethesda, MD). The filtered images were analyzed with a custom written program in MATLAB (version R2016a, MathWorks, Natwick, MA). The first step includes a baseline adjustment to increase the signal to noise ratio. The baseline adjustment was executed with the "msbackadj" command of the MATLAB bioinformatics toolbox. This command employs an Expectation–Maximization algorithm to group the data points into "background "or "peak "(e.g., stained fibers). Both groups have a normal distribution. The final baseline corresponds to the mean value of the background group. Next, a peak detection function ("mspeaks" from the MATLAB bioinformatics toolbox) quantified the number of high-intensity pixels (e.g., the stained fibers) above a threshold on a projected gridline. The grid consisted of squares that measured 100 μm × 100 μm and overlaid the entire brain slice. The threshold was determined by parallel manual quantification of fibers by an experienced researcher in three brain slices per series. For this, the program generated four random squares of 100 μm × 100 μm, and the threshold was adjusted until a difference of less than 10% was obtained between manual and automatic fiber quantification. After determination of the threshold, the program automatically generated detailed fiber counts per 100 μm^2 over the whole brain slice, which was visualized in heat maps.

2.6 | Close-up analysis

The second criterion for the identification of the NCL is the presence of characteristic TH+ baskets. We used the generated heat map as a guideline to closely analyze the areas of highest fiber density in a qualitative manner. We first verified the presence of baskets, and next distinguished differences in basket morphology. This concerned the size of the innervated unstained perikarya, and the intensity of innervation (e.g., how many fibers). Moreover, a closer analysis of the areas of high fiber density allowed us to inspect morphology of the fibers, this regarded directionality of the fibers (linear or disperse), and identification of varicosities. As a third criterion, we analyzed the trajectory of the DA tract that runs between parts of the arcopallium and NCL in pigeon (Kröner & Güntürkün, 1999). We noted both the progression of fibers as well as the morphology of the myelinated tracts. These three parameters, basket morphology, fiber network, and course of the DA, aided in differentiating separate areas of the caudal telencephalon. It should be noted that the areas were primarily defined based on TH fiber density, and close up analysis was to verify the presence of baskets and further investigate the borders of possible subdivisions of the NCL.

3 | RESULTS

3.1 | TH+ fiber innervation in different species

The general staining pattern after TH immunohistochemistry was in all four species in line with previous reports from the literature. In the mesencephalon, we observed strongly labeled cells in both the VTA and SN in all four species (Figure 1), representing the well described dopaminergic projection of these areas (Smeets & González, 2000). In the caudal telencephalon both the LSt and AD were strongly innervated by high numbers of fibers and appeared as almost uniformly

darkfields. In pigeon and chicken the AD appeared lateral to the LSt, whereas in the zebra finch and carrion crow the AD was situated medial to the LSt, as has been described before (Mello et al., 2019). In contrast, the thalamo-recipient auditory region Field L, which is known to be void of dopaminergic input, showed no labeled fibers at all (Kröner & Güntürkün, 1999; Waldmann & Güntürkün, 1993; Wynne & Güntürkün, 1995). Within the caudal nidopallium, we could identify several areas of higher fiber density in each of the four species, which we further differentiated based on absolute fiber density, fiber organization and if applicable basket morphology.

3.1.1 | Pigeon

The area of highest TH+-fiber innervation in the caudal nidopallium appeared ventrolateral around A7.00 immediately adjacent to the lateral ventricle. Moving caudal, this area stretched in a semi-lunar shape along the ventrolateral and dorsomedial borders of the nidopallium. From A5.50 on, the ventromedial border of this area expanded further ventromedial into the surrounding nidopallium and the overall fiber density in the whole area intensified (Figure 2). The observed innervation pattern had a very high degree of overlap to previous descriptions of the NCL (Durstewitz et al., 1999; Kröner & Güntürkün, 1999; Waldmann & Güntürkün, 1993; Wynne & Güntürkün, 1995), and was therefore labeled as NCL. Within the NCL, we corroborated previous reports of a lateral NCL and a medial NCL subdivision based on differences in fiber density (Herold et al., 2011; Waldmann & Güntürkün, 1993). Medial NCL was visible between A7.00 and A4.50 as a smaller area curved by lateral NCL and showed a lower density than lateral NCL. The subdivision was separated by a thin band with low TH innervation.

The fiber network of both NCL subdivisions in pigeons (Figure 3b,c) consisted of curvilinear (as opposed to straight) fibers, and the fibers did not seem to have a clear plane of progression but were predominantly dispersed. At more anterior levels (A7.00–A 5.00) a subset of fibers demonstrated a clear directionality moving in parallel to the dorsal roof of the nidopallium. However, from A5.00 onwards, this fiber stream became less pronounced and turned into a disperse network. The entire NCL showed much higher fiber densities compared to the surrounding caudal nidopallium (NC, Figure 3d). Within NCL, we could identify numerous baskets consisting of multiple fibers coiling densely around an unstained perikaryon. These fibers are likely to form multiple synapses with the soma and possibly the initial dendrites. We observed chiefly singular baskets that were equally distributed, and sometimes would form conglomerations of several baskets in close proximity. Beside baskets, we identified multiple possible en-passant contacts. This type of contact is visible as a varicosity in a part of a fiber that is not coiled around a soma (Durstewitz et al., 1999). Corroborating previous reports (Waldmann & Güntürkün, 1993), the fiber density in medial NCL (Figure 3c) was of much lower density compared to lateral NCL (Figure 3b), and the fewer baskets we observed received a less dense innervation. Baskets were predominantly singularly distributed and occasionally formed conglomerations of 2–4 baskets. Clear boutons were numerous within baskets, as well as outside of baskets possibly forming en-passant contacts with other unstained structures.

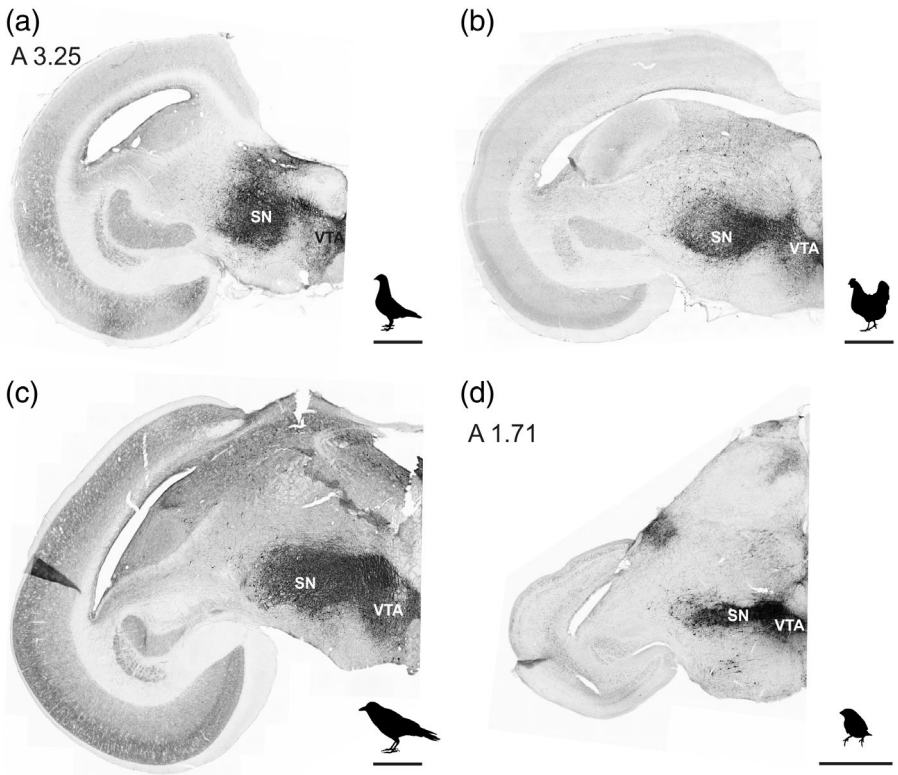


FIGURE 1 Frontal sections of TH labeling in the mesencephalon of pigeon (a), chicken (b), carrion crow (c), and zebra finch (d). In all species, we observed TH positive neuronal somata, axons and neuropil in the well-described dopaminergic cell groups VTA and SN. The labeling of these dopamine-rich structures is highly comparable across the four species. Shown here is one hemisphere of the mesencephalon without the forebrain. Stereotaxic coordinates are only given for pigeon (A3.25) and zebra finch (A1.71), since there is currently no atlas available for adult chicken and carrion crow. Scale bar in (a)–(d) depicts 1,000 μm. For abbreviations, see list

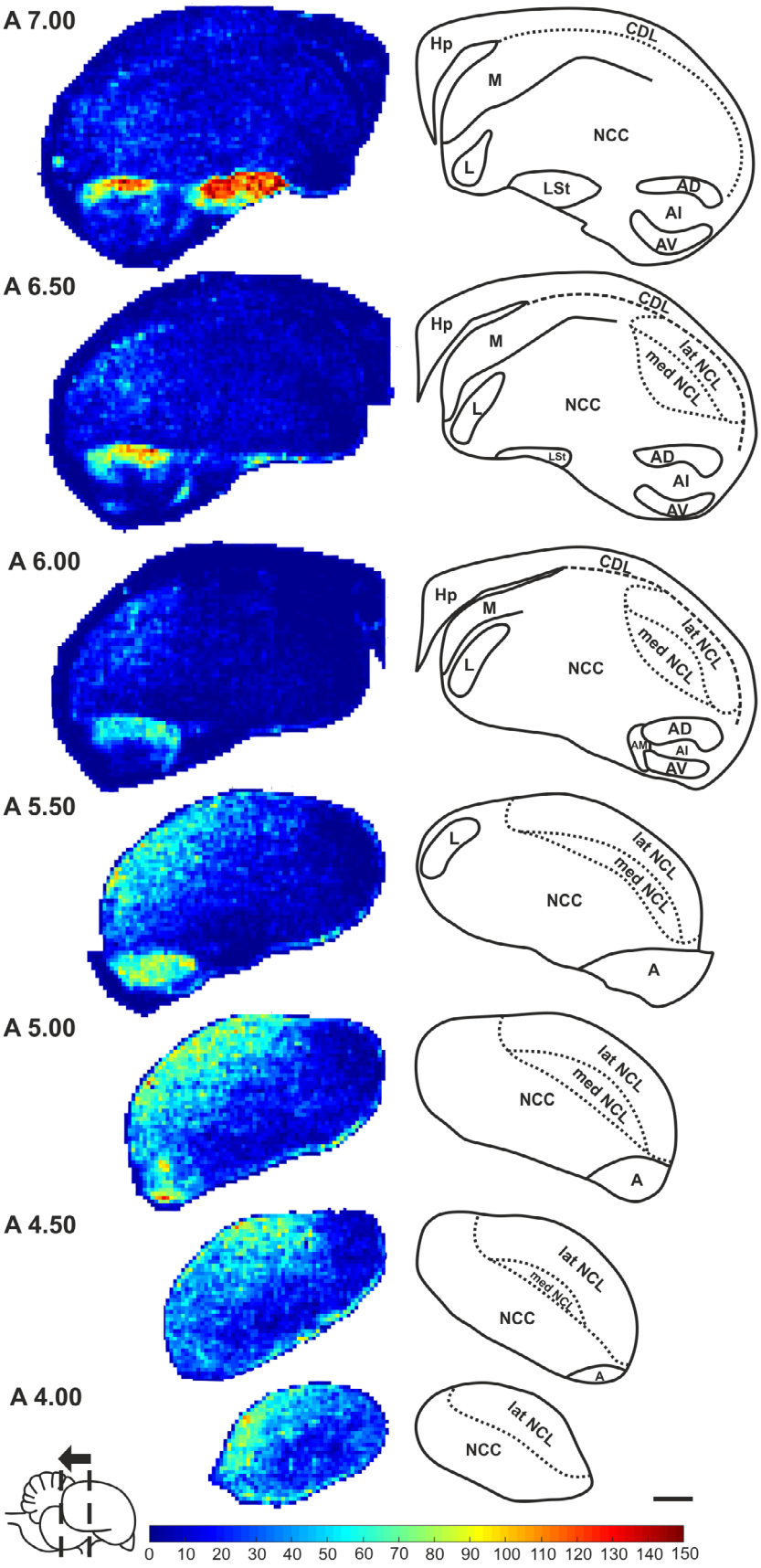


FIGURE 2 Exemplary heat maps representing densities of tyrosine hydroxylase (TH) positive fibers of one pigeon (left side) and schematic outlines of areas high in TH fiber density based on all analyzed pigeons (right side) in the caudal telencephalon (A7.00 – A4.00, Karten & Hodos, 1967). The maps are based on an immunohistochemical staining against TH visualized by nickel enhanced 3,3′-diaminobenzidine (DAB) reaction. Fiber densities were quantified in 150 μm^2 squares with a custom-made software making use of a Hessian based curvilinear feature extraction. Highest TH fiber densities could be observed in AD and the LSt, whereas field L was completely void of TH containing fibers. In the nidopallium, based on its relative higher TH fiber density and a qualitative assessment of fiber morphology and the occurrence/characteristics of fiber baskets (compare Figure 3), we were able to identify the previously defined pigeon NCL including two distinct subareas (lat NCL and med NCL) within the caudal nidopallium. Between A5.50 and A4.00 the dorsolateral corticoid area (CDL) and hippocampal complex (Hp) were lost during the staining process. Scale bar depicts 1,000 μm. For abbreviations, see list [Color figure can be viewed at wileyonlinelibrary.com]

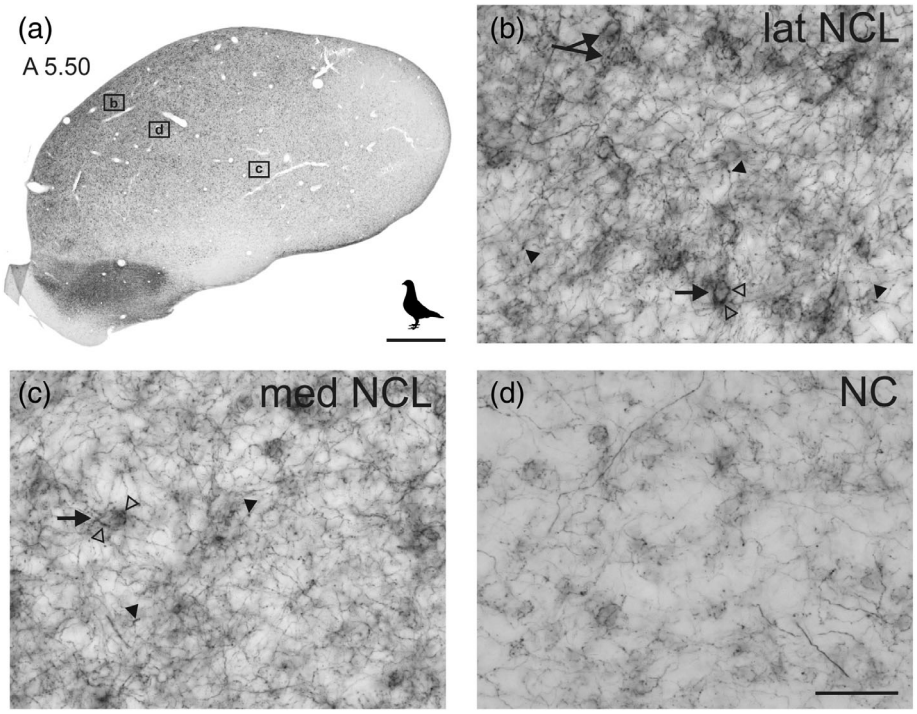


FIGURE 3 Morphology and characteristics of TH positive fibers and baskets in different areas of the caudal nidopallium in pigeons. (a) Overview of a pigeon brain slice at A5.50 (Karten et al., 1967) stained against TH. (b)–(d) Magnification of the rectangles shown in (a). As known from previous studies, the NCL of pigeons (b) + (c) showed a much higher TH fiber and basket density than the surrounding nidopallium (d). Within NCL, we could identify two subareas, which differed in TH fiber density and staining intensity of found baskets, with the lateral subarea (lat NCL, (b)) showing a denser TH innervation than the medial one (med NCL, (c)). In both subdivisions, numerous baskets (bold arrows) were visible, which were occasionally conglomerated (double arrow), and axons formed axonal boutons at these baskets (unfilled arrowheads). Furthermore, in both subareas we could observe fibers with multiple varicosities, possibly indicating en-passant contacts (filled arrow heads). (d) The surrounding nidopallium displayed low levels of fiber innervation, and only few barely innervated baskets. Scale bar in (a) depicts 1,000 μm, scale bar in (d) (representative for (b)–(d)) 50 μm

3.1.2 | Chicken

In chicken, we could delineate two separate areas of high TH+ fiber density (Figure 4). The first appeared at Section III at the border of nidopallium to AD, forming a small patch dorsal to the arcopallium. Moving posterior, this patch stretched dorsomedial below the lateral ventricle and increased in fiber density reaching its highest extent and density at Section VI. Here, the area is best described as a semilunar shaped band situated ventromedial to the lateral ventricle and extending approximately to the middle of the slide. Caudal to Section VI, the band decreased in size and fiber density and vanished before Section VIII. The trajectory of this area closely corresponded to the lateral subdivision of pigeon NCL. We therefore termed this area NCL. We could not identify any subdivisions based on TH+ innervation.

The second field of high fiber density also first appeared at Section III, but as a circular area in the dorsal half of the central caudal nidopallium. Following its trajectory caudal, it formed a sphere-like structure with increasing diameter and fiber density. It reached its maximum in both diameter and density at Section VI almost extending to NCL, where the dorsomedial-most tip of the NCL appeared to arch around the dorsal border of this central area. More posterior, the structure decreased in size remaining in the center of the tapering caudal nidopallium. This second structure corresponds in its trajectory and shape highly to an area labeled by Puelles (2007) island fields of the caudal nidopallium (NCIF). Thus, we decided to adopt the nomenclature.

NCL and NCIF differed strongly in fiber organization. The TH+ fiber innervation in putative chicken NCL (Figure 5b) was highly comparable to the NCL in pigeons. The fiber network in NCIF (Figure 5c) was of a lower density, more disperse, and with fewer but more strongly innervated baskets compared to NCL. Moreover, the baskets displayed a high degree of conglomeration forming spots of multiple strongly interconnected baskets. These spots corresponded to what Puelles (2007) termed "island fields." Both putative NCL and NCIF showed higher fiber densities compared to the surrounding NC (Figure 5d).

3.1.3 | Carrion crow

Overall, TH+ fiber innervation in the carrion crow differed greatly in extent and diversification compared to pigeon and chicken, but showed many similarities to the zebra finch (see below). Based on

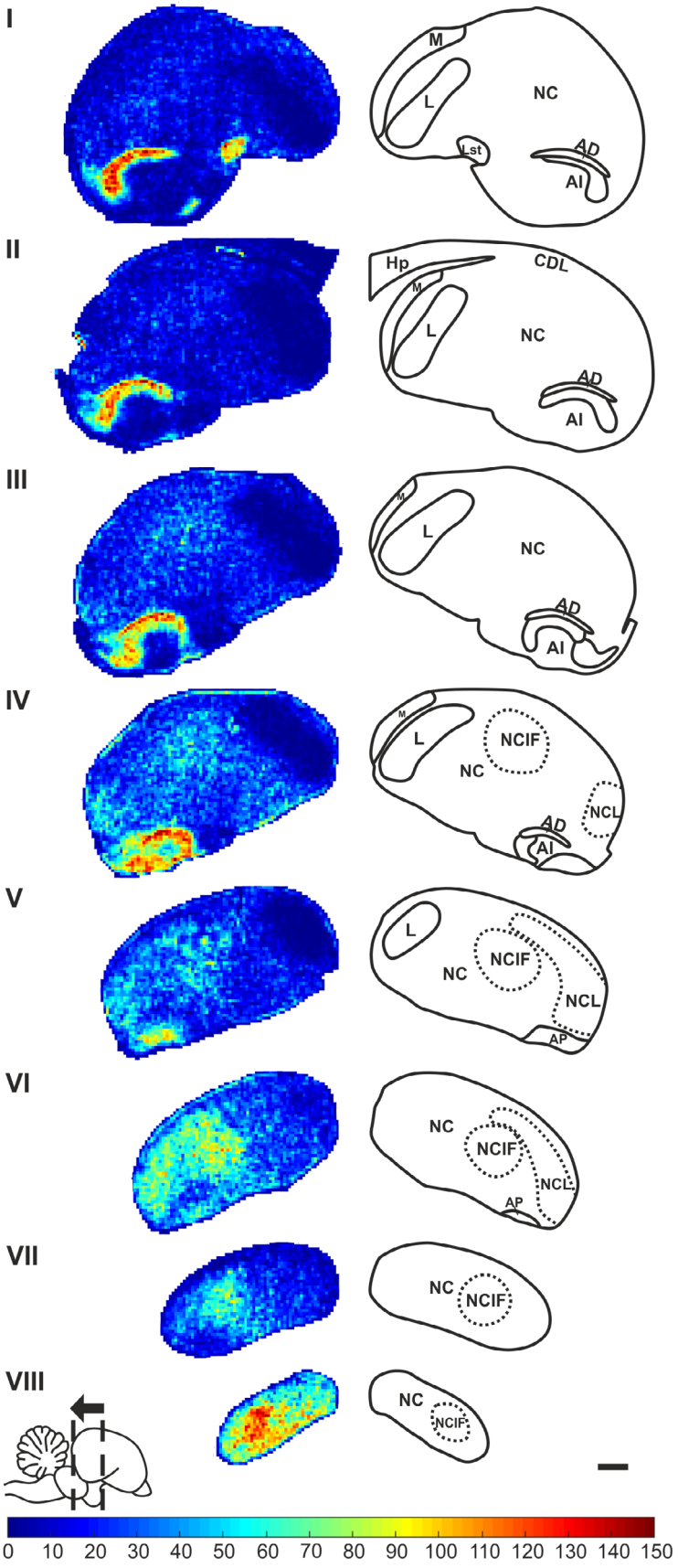


FIGURE 4 Exemplary heat maps representing densities of tyrosine hydroxylase (TH) positive fibers of one chicken (left side) and schematic outlines of areas high in TH fiber density based on all analyzed chickens (right side) in the caudal telencephalon (Puelles, 2007). We employed ascending Roman numbers from anterior to posterior since there is currently no brain atlas available for adult chicken. For information on data acquisition see main text and figure captions of Figure 2. Like in pigeons, AD and LSt showed by far the highest innervation with TH positive fibers while field L did not contain any. Within the caudal nidopallium, we observed two areas with an increased TH fiber density. The first field mirrored in trajectory, location and fiber/basket configuration the NCL in pigeons (compare Figures 2, 3) und was thus labeled NCL. The second field, located ventromedial to NCL resembled the in previous studies described NCIF and was thus labeled accordingly. Between Section III and Secion VIII CDL and Hp were lost in the staining process. Scale bar depicts 1,000 μm. For abbreviations, see list [Color figure can be viewed at wileyonlinelibrary.com]

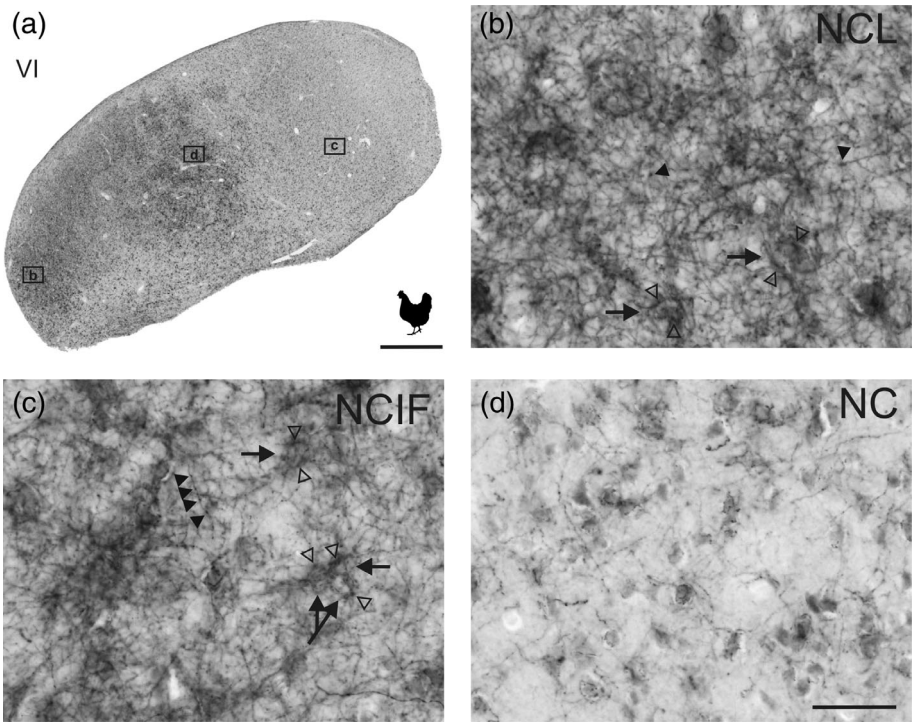
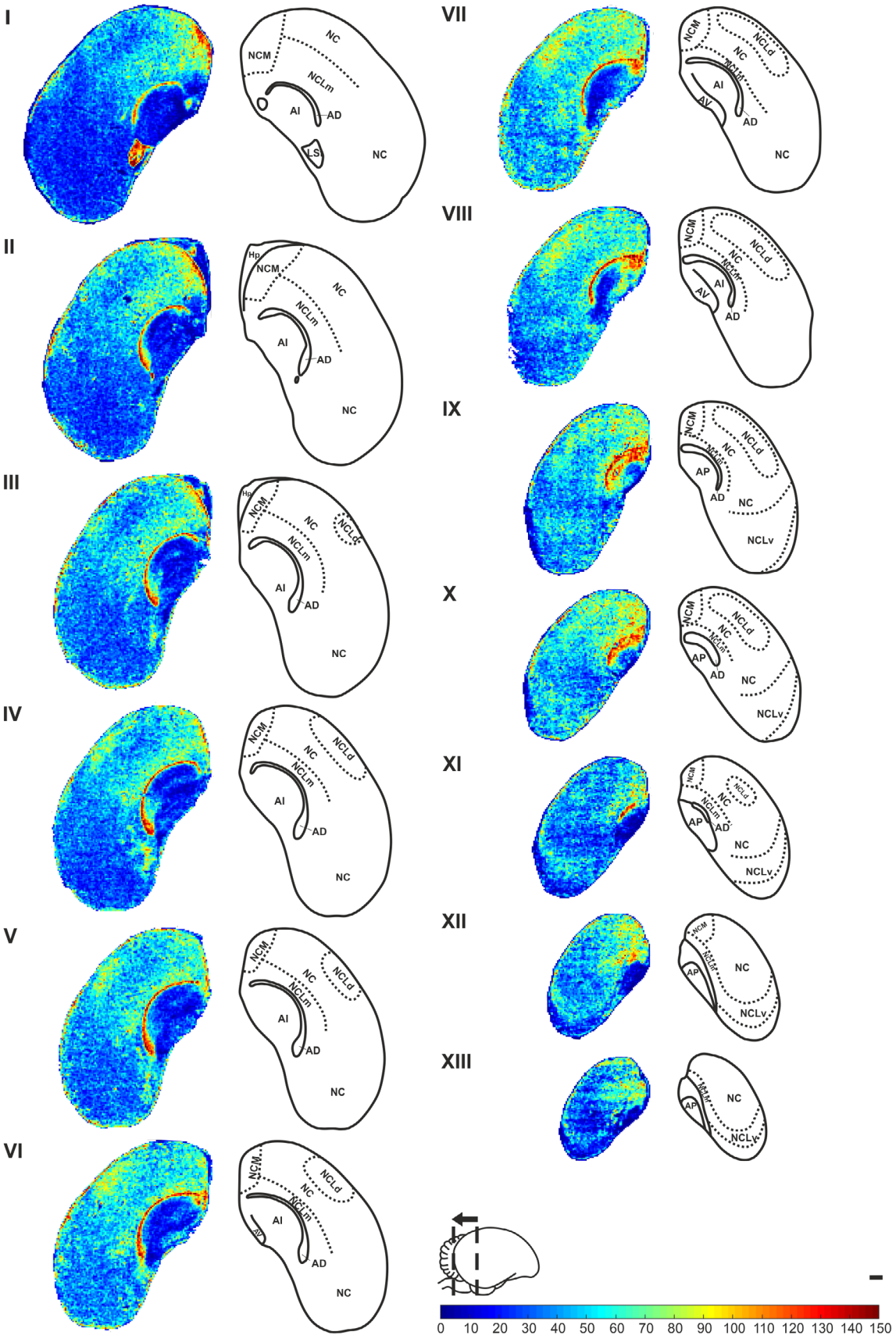


FIGURE 5 Morphology and characteristics of TH positive fibers and baskets in different areas of the caudal nidopallium in chickens. The Roman number corresponds to a section number of Figure 4. (a) Overview of a chicken brain slice at Section VI stained against TH. (b)–(d) Magnification of the rectangles shown in (a). TH fiber density in NCL (b) was higher in comparison to NCIF (c) and both were much higher in comparison to the adjacent NC (d). Fibers formed numerous baskets (bold arrows) that appeared equally distributed over the whole area, with occasional conglomerations of 2–3 baskets. Axonal boutons were visible at the baskets (unfilled arrowheads) as well as in progressing fibers possibly forming en-passant types of contact (filled arrowheads). In comparison to NCL, NCIF (c) fiber density was lower, fibers were more disperse, and contained fewer but more strongly innervated baskets (bold arrows). The baskets displayed a high degree of conglomeration forming spots of strongly interconnected baskets (double arrows). NCIF also contained both boutons at baskets (unfilled arrowheads) and in passing fibers (filled arrowheads). While the caudal nidopallium (d) in general contained TH positive fibers and baskets, fiber density was much lower and basket morphology clearly different from NCIF and NCL. Scale bar in (a) depicts 1,000 μm, scale bar in (d) (representative for (b)–(d)) 50 μm. For abbreviations, see list

fiber densities, basket and fiber morphology, we could identify four distinct fields of high TH+ innervation (Figure 6). The first was situated in the dorsomedial corner of the caudal nidopallium spanning almost the entire field between AD and the dorsal border of the nidopallium. It reached from Section I to the caudal-most border of the forebrain, stretching between 1 and 3 mm lateral. This area overlaps with what is known as the caudal medial nidopallium (NCM) and we thus adopted the term. A second territory of high fiber innervation was visible as a 1–2 mm wide band immediately adjacent to the dorsal border of AD. It first appeared at Section I and reached from NCM over the whole length of the arcopallium to the central nidopallium. Moving posterior, the area expanded ventrolateral, closely following the arch of AD. At Section IV–Section V it appeared to extend ventrally beyond the border of the arcopallium. Since this area has not been described before, we termed it medial part of the NCL (NCLm). The third field of higher fiber innervation became visible at Section III as a small circular shape at the dorsolateral roof of the nidopallium. Going posterior, it extended into a band aligned to the arch of the dorsal nidopallial border. The band reached its largest extent at Section VII stretching medial towards NCM, and up to the ventral most tip of AD. From there on, it decreased in size with the medial border retracting away from NCM, and the entire field disappeared before Section XII. We named this area the dorsal part of the NCL (NCLd). The fourth field that showed a high TH+ fiber density appeared at Section VIII and continued until the caudal end of the forebrain. Situated in the ventral nidopallium, it stretched as a 2–3 mm wide band from the medial to lateral border of the nidopallium. Moving more posterior, the band turned more narrow and curved, aligning to the ventrolateral nidopallial border, omitting a small stripe of low TH+ fiber density at the ventrolateral tip of the brain. From Section XII onwards, the area seemed to merge with NCLm. We labeled this area as the ventral part of the NCL (NCLv).

Each of the four subareas consisted of a dense fiber network with numerous baskets and contained axonal boutons on both baskets as well as in passing fibers. We observed differences in the morphological details (Figure 7). The fiber network of NCM (Figure 7b) was dispersed, and the baskets rarely formed conglomerations. In contrast, a subset of the fibers in NCLm (Figure 7c) showed directionality and transgressed in parallel to the lamina



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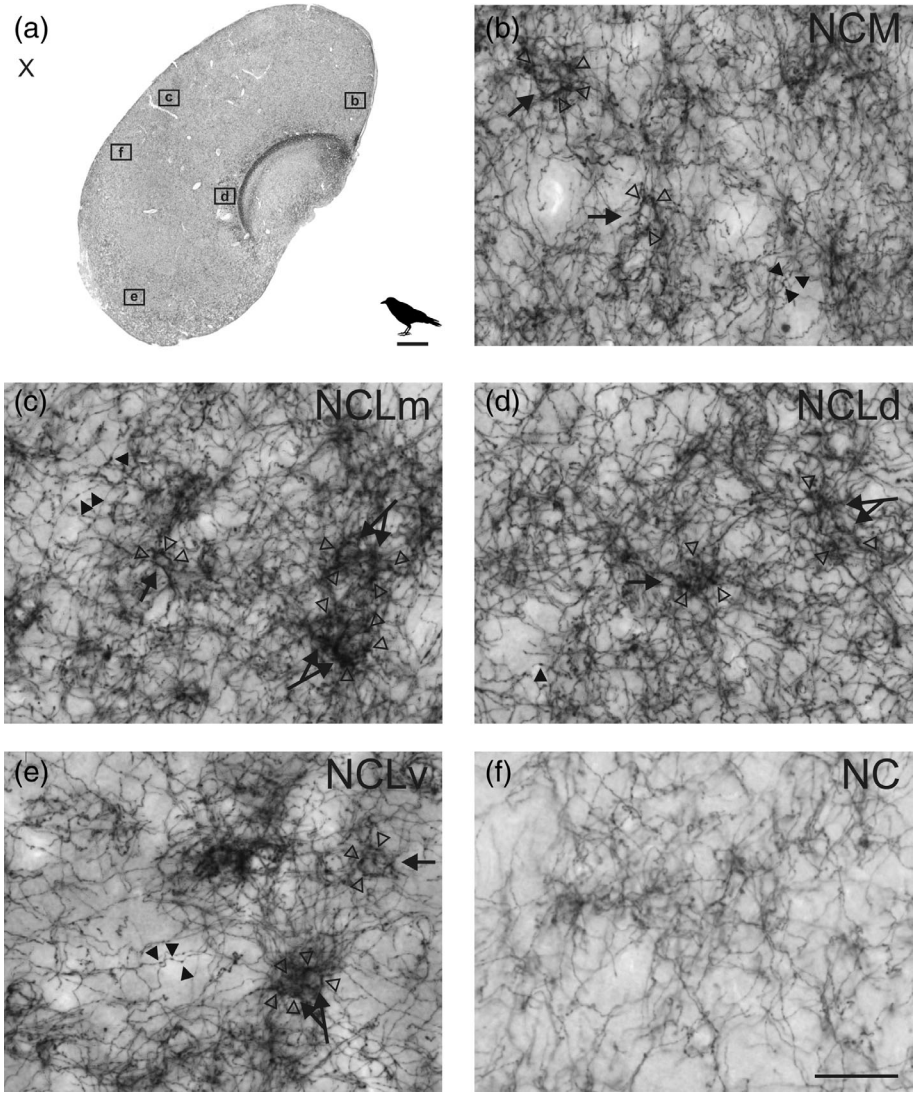


FIGURE 7 Morphology and characteristics of TH positive fibers and baskets in different areas of the caudal nidopallium in carrion crow. The Roman number corresponds to a section number of Figure 6. (a) Overview of a carrion crow brain slice at Section X stained against TH. (b)–(f) Magnification of the rectangles shown in (a). TH-fiber density in the four identified subareas NCM (b), NCLm (c), NCLd (d), and NCLv (e) was much higher compared to surrounding nidopallium (f). All subareas contain baskets (bold arrows) and axonal boutons at baskets (unfilled arrowheads) as well as in progressing fibers (filled arrowheads) were visible in all subareas. Baskets in NCM (b) were equally distributed and rarely formed conglomerations with other baskets. In contrast to NCM, baskets in NCLm (c) occasionally formed conglomerations (double arrow) and baskets were much denser innervated. NCLd baskets (d) also formed conglomerations, but innervation of baskets was lower in comparison to NCLm. NCLv (e) differed from the other three areas in showing a lower average TH fiber density, whereas dense and conglomerated baskets could still be identified. Scale bar in (a) depicts 1,000 μm, scale bar in (d) (representative for (b)–(d)) 50 μm. For abbreviations, see list

arcopallialis dorsalis (LAD), and we identified occasional interconnected conglomerations. These baskets were so densely innervated they appeared as tiny black dots that could readily be discerned from the slide with the naked eye. Another specific feature of this region was that almost all fibers displayed numerous varicosities along their whole length and thus possibly many en-passant type contacts. Characteristically, TH+ fibers in NCLd (Figure 7d) were largely dispersed and showed a higher degree of curvilinearity (i.e. the fibers made more curves as opposed to transgressing straight) in comparison to NCM, NCLd and NCLm. Furthermore, baskets appeared less dense compared to the other three areas. The NCLv (Figure 7e) had a relatively low TH+ fiber density compared to the other described areas with a rather dispersed fiber network. The baskets appeared mostly in interconnected conglomerations of 3–5 baskets that were very densely innervated. Across all fields, even though the fiber and basket typology was consistent, the entire staining pattern intensified moving from anterior to posterior, and was always higher compared to surrounding NC (Figure 7f).

FIGURE 6 Exemplary heat maps representing densities of tyrosine hydroxylase (TH) positive fibers of one carrion crow (left side) and schematic outlines of areas high in TH fiber density based on all analyzed crows (right side) in the caudal telencephalon (Izawa & Watanabe, 2007). We employed ascending Roman numbers from anterior to posterior since there is currently no brain atlas available for carrion crow. For information on data acquisition see main text and figure captions of Figure 2. TH density patterns in carrion crows differed considerably from pigeons and chickens. While again AD and LSt showed the highest density and Field L was again void of fibers (not shown), TH innervation in the remaining caudal nidopallium was much more diverse. In total, we could identify four different areas with a high TH fiber density, labeled, according to their established nomenclature and position: NCM, NCLd, NCLm, and NCLv. All four areas differed strongly in morphology of their baskets and fiber network (compare Figure 7). Between Section IV and Section XIII the CDL and Hp were lost in the staining process. Scale bar depicts 1,000 μm. For abbreviations, see list [Color figure can be viewed at wileyonlinelibrary.com]

3.1.4 | Zebra finch

In the zebra finch, TH+ fiber distribution within the caudal nidopallium demonstrated a high degree of similarity to what was observed in the carrion crow. We identified the four subareas NCM, NCLm, NCLd, and NCLv (Figure 8). Different from the carrion crow, we could identify a rostral and caudal subdivision of NCLd based on TH innervation and DA projection.

NCM first appeared at A1.08, caudoventral to Field L in between the arcopallium and the dorsal roof of the nidopallium. It stretched all the way to the caudal back of the forebrain and never extended beyond 1 mm lateral from the medial border. Next, we could identify NCLm as a narrow band of higher fiber density arching in parallel to the entire dorsal arcopallium, visible from A1.08 to the caudal end of the forebrain. At P0.09 it extended further ventrally and seemed to merge with NCLv (see below). NCLd was situated in the dorsolateral roof of the nidopallium and had a clear rostral and caudal subdivision separated by a field of lower TH+ fiber density. Rostral NCLd (NCLdr) was visible from A1.35 to A0.45 as a wide band that stretched from adjacent to Field L along and slightly away from the dorsal roof of the nidopallium. It was separated from the lateral border of the nidopallium by a field that was low in TH+ fibers. The caudal subdivision of NCLd (NCLdc) was a smaller field that first appeared at A0.18 and was visible until the caudal end of the forebrain. It was situated midway the section in the medial-lateral plane, and moving posterior, it expanded slightly towards the medial border. NCLv of the zebra finch appeared at A0.45, first emerging as a diffuse patch of TH+ fibers at the ventral tip of the arcopallium. From A0.18 onwards, the patch turned into a thin band following the ventral nidopallial border.

The innervation profile of each subarea consisted of a dense network with baskets, and varicosities were visible both on the baskets as well as on passing fibers. We observed slight differences with regards to fiber and basket morphology (Figure 9). The fiber and basket profile of NCM (Figure 9c) in the zebra finch was highly similar to the NCM in the carrion crow. The network was predominantly dispersed and baskets occurred singular. Compared to the other subareas in the zebra finch, it showed the highest TH+ fiber density of all nidopallial subareas, especially in its caudomedial extent. The fiber network of NCLm (Figure 9d) was also alike what was observed in the carrion crow, with a subset of fibers that traversed in parallel to the arcopallial border. The baskets were densely innervated and especially at caudal sections occurred frequently in conglomerations of 2–4 baskets. NCLdr (Figure 9e) is best described as a dense fiber network of a dispersed character with baskets that appeared less pronounced compared to the other subareas in zebra finches. The baskets were equally distributed and we identified only few conglomerations. NCLdc (Figure 9f) displayed an innervation profile that was comparable to NCLd in the carrion crow, where the distinct feature was the higher degree of curvilinearity of the dispersed fiber network. In addition, the fibers appeared to be more loosely wrapped around the unstained perikarya, giving the baskets a less dense appearance, and we observed only few conglomerations. The fibers in NCLv (Figure 9g) were predominantly disperse in the dorsal half, and curved in parallel to the ventral tip of the caudal. The baskets were characteristically pronounced and predominantly singular. All subareas displayed higher densities of TH+ fibers compared to surrounding NC (Figure 9h).

3.2 | Myelin trajectory between AI and NCL

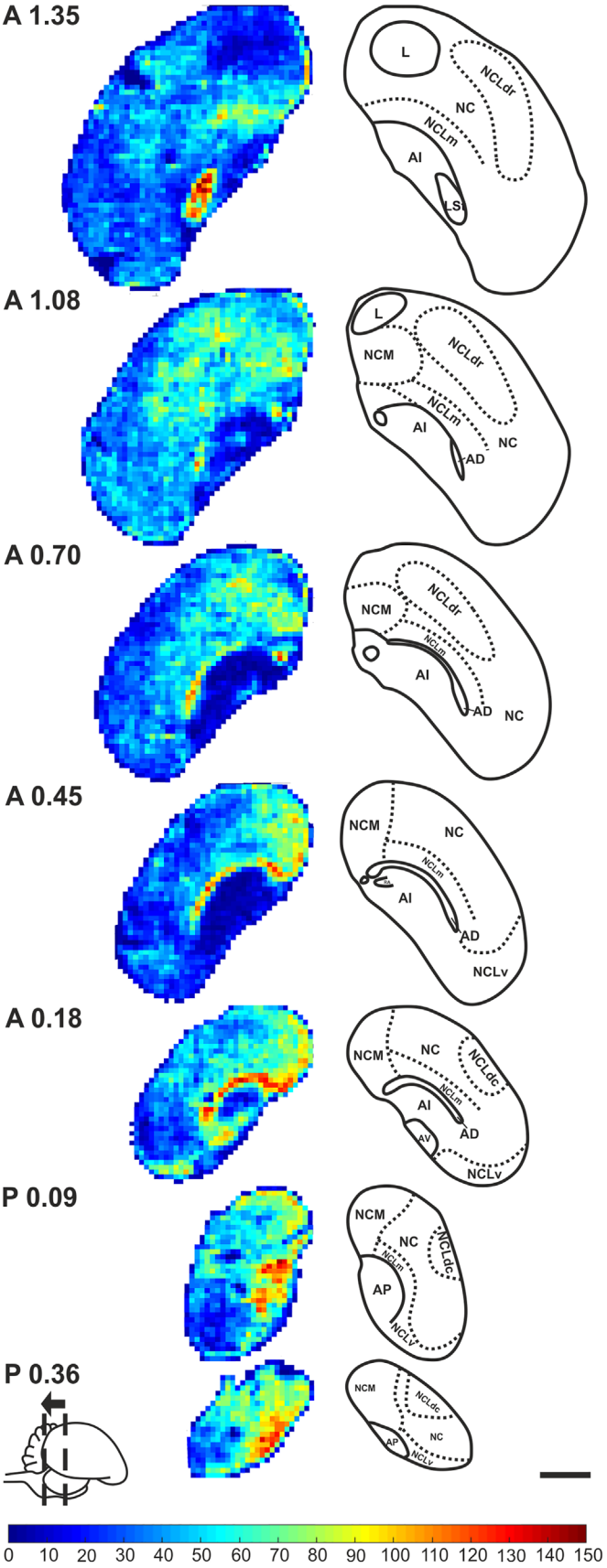
In all species, the DA was clearly visible in the caudal telencephalon extending between the arcopallium and the surrounding nidopallium (Figure 10). In pigeons (Figure 10a), it spread between AI and NCL, and arched along the lateral ventricle to the dorsal roof of the nidopallium. It showed a high degree of overlap with the NCL. In the chicken (Figure 10b), it appeared broader and extended slightly more medial, encompassing a larger area than what we designated the putative NCL. Similar to pigeons, it arched along the lateral ventricle dorsal, but did not reach beyond halfway the slice in the lateral-medial axis. It did not seem to target NCIF directly, but instead arched around it.

The DA in carrion crows and zebra finches showed a strikingly different pattern to pigeons and chickens, but was comparable in both Passeriformes species (Figure 10c–f). Namely, the arcopallium was situated midway instead of at the lateral-most border. It spanned almost the entire arcopallium in the frontal plane, and targeted the NCLd of the carrion crow (Figure 10c) and NCLdr in zebra finches (Figure 10e). At more caudal levels, DA extended towards the ventral tip of the arcopallium (Figures 10e,f). In the zebra finch, it projected to NCLdc and progressed past NCLv. This latter progression could not be observed in the carrion crow, and DA appeared to only target NCLd.

Myelinated fibers of DA varied between species (Figure 11). In the pigeon (Figure 11a), DA consisted almost exclusively of thin, likely singular fibers. The same was observed in chicken (Figure 11b), with also some thick fiber bundles being present lateral. Carrion crows (Figure 11c) showed a mix of both types at more anterior sections, while singular fibers dominated when moving caudal. This was also apparent in zebra finches, with NCLdr and NCLdc being reached by thick and thin fiber bundles, respectively (Figure 11d,e).

4 | DISCUSSION

The present study investigated the location and trajectory of the nidopallium caudolaterale (NCL) in pigeon, chicken, carrion crow and zebra finch based on TH+ fiber density and innervation pattern. Though this comparative analysis is far from exhaustive and many orders of the avian clade are not represented in this study, it is a sound first step to investigate the possible diversity of the NCL. Indeed, our analysis showed that the location of the NCL requires a species-specific approach, especially for the Passeriformes. In short, based on TH+ fiber innervation, the putative NCL in chicken is highly similar to pigeon, with the addition of one field known as island fields of the caudal nidopallium (NCIF, Puelles, 2007). The two Passeriformes species on the other hand show a strikingly different pattern. In both carrion crow and zebra finch, we could identify four



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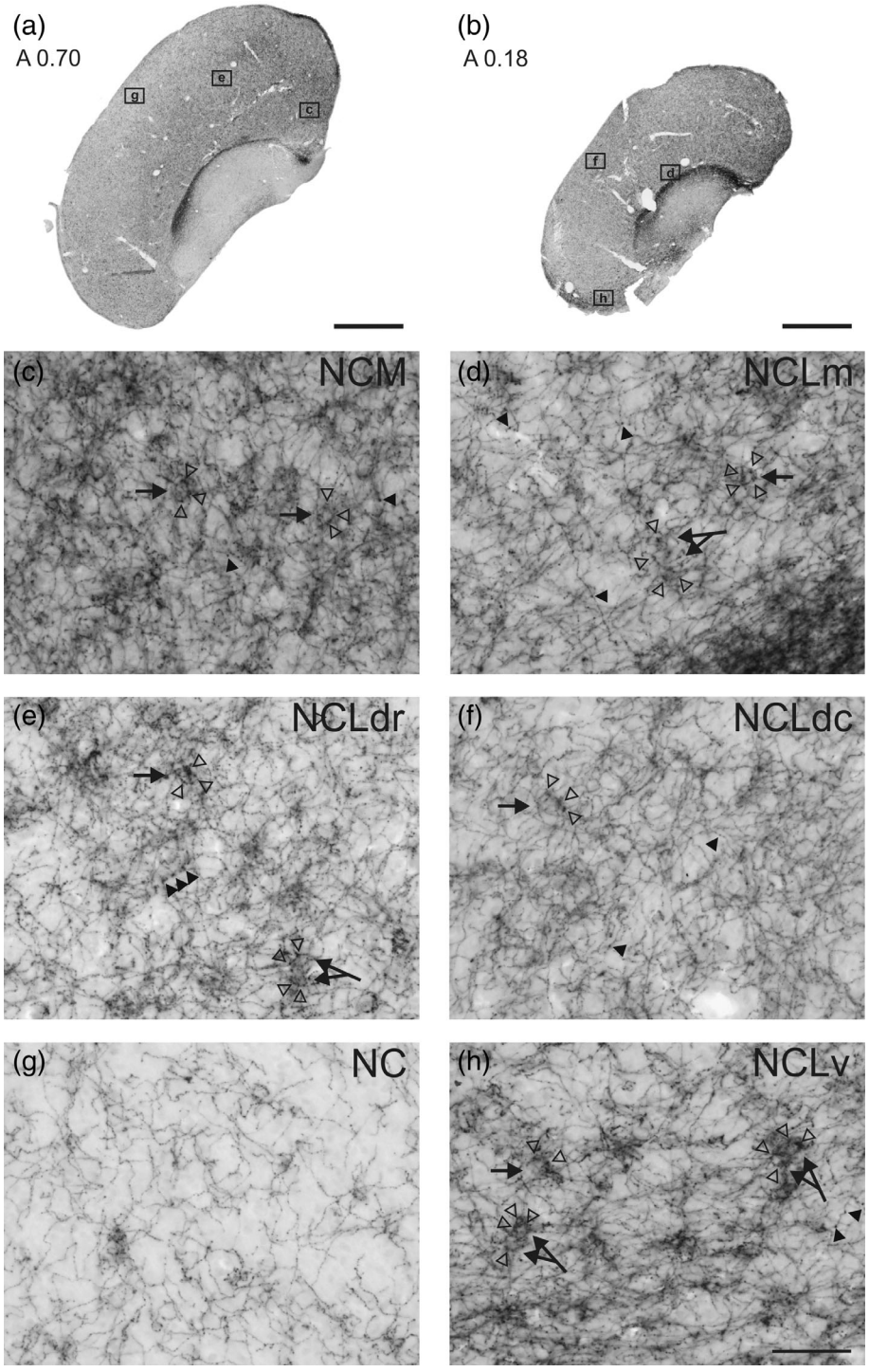

FIGURE 9 Morphology and characteristics of TH positive fibers and baskets in different areas of the caudal nidopallium in zebra finches. (a) Overview of a zebra finch brain slice in the rostral caudal nidopallium at A0.70 (Nixdorf-Bergweiler & Bischof, 2007) stained against TH. (c, e, g) Magnification of the rectangles shown in (a). (b) Overview of a more caudal section of the caudal nidopallium at A0.18 stained against TH. (d, e, h) Magnification of the rectangles shown in (b). All four identified subareas showed a higher TH fiber density than the surrounding NC (h), contained numerous baskets (bold arrows), as well as clearly visible boutons at baskets (filled arrowheads) as well as on fibers (unfilled arrowheads). Fiber density in NCM (c) was higher than in the other subarea and showed mostly singular, evenly distributed baskets. Like in carrion crow, baskets in zebra finch NCLm (d) formed conglomerations (double arrow) and were much denser innervated in comparison to the other subfields. Note that the high fiber density in the bottom right corner stems from the adjacent AD. NCLdr (e) and NCLdc (f) were very similar in appearance with lower basket counts in comparison to the other areas and baskets being less dense innervated in comparison to NCM and especially NCLm. Baskets appeared predominantly singular. In contrast, NCLv (g) showed pronounced baskets occasionally arranged in conglomerations. Note that the shown pictures stem from a female zebra finch in which song related nuclei (e.g., RA) are less pronounced than in males. Scale bar in (a) and (b) depict 1,000 μm, scale bar in (h) (representative for (c)–(h)) 50 μm. For abbreviations, see list

FIGURE 8 Exemplary heat maps representing densities of tyrosine hydroxylase (TH) positive fibers of one zebra finch (left side) and schematic outlines of areas high in TH fiber density based on all analyzed zebra finches (right side) in the caudal telencephalon (A1.35–P0.36, Nixdorf-Bergweiler & Bischof, 2007). For information on data acquisition see main text and figure captions of Figure 2. Distribution of TH+ fibers in zebra finches was different in comparison to pigeons and chickens but highly similar to carrion crow (compare Figure 6), likely due to their close phylogenetic relationship. As in the other species, AD and LSt showed the highest TH fiber density while Field L did not contain any TH positive fibers. Like in the carrion crow, we could identify the areas NCM, NCLm, NCLd, and NCLv, which showed a higher TH density than the surrounding nidopallium and differed basket and fiber network morphology. However, in contrast to crows, NCLd in zebra finches was separated by an area of low TH fiber density into a rostral and caudal aspect, labeled NCLdr and NCLdc, respectively. Between A1.35 and P0.36 the CDL and Hp were lost in the staining process. Scale bar depicts 1,000 μm. For abbreviations, see list [Color figure can be viewed at wileyonlinelibrary.com]

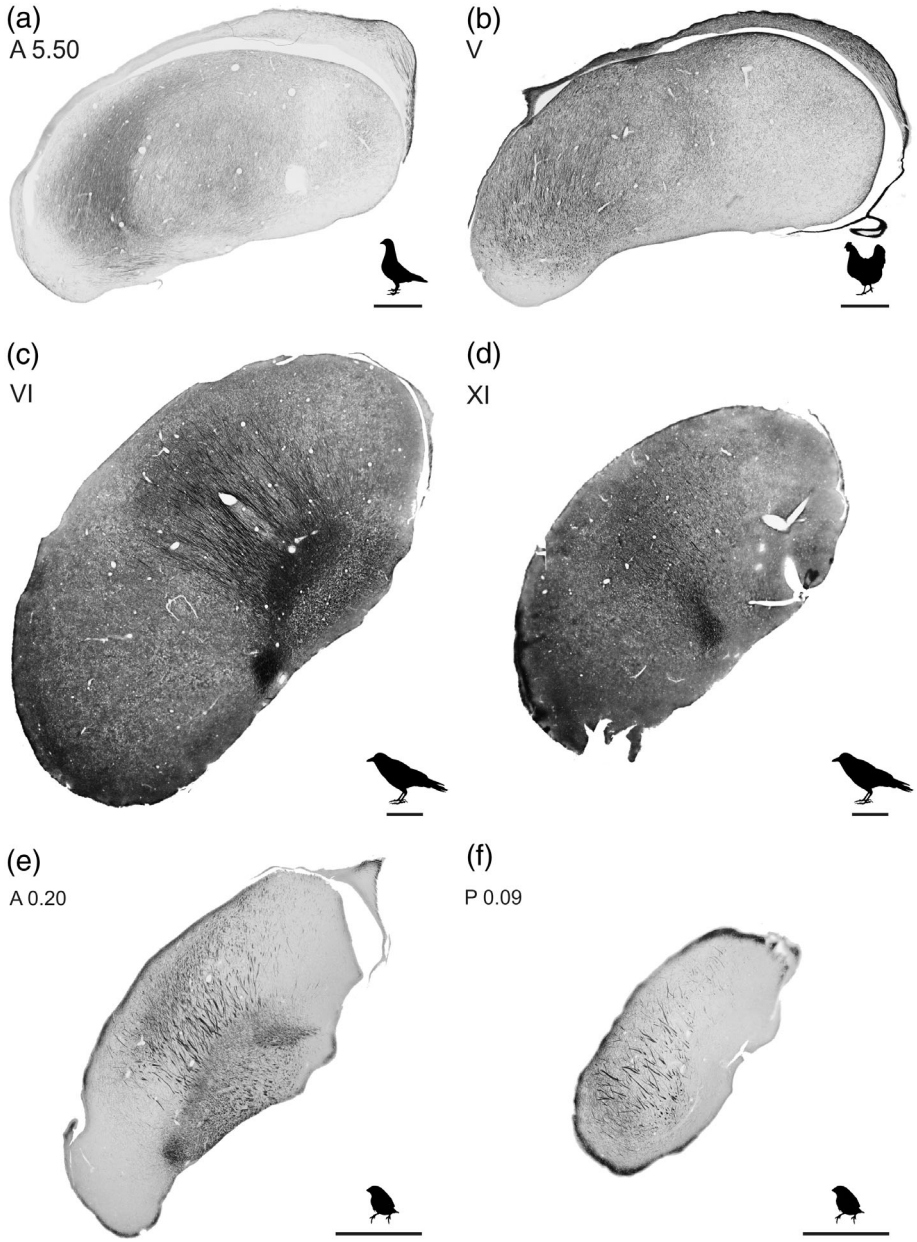

FIGURE 10 Representative overview slide of Gallyas myelin stain in different species. In both pigeon (a) and chicken (b), the progression of DA along the dorsolateral nidopallial border is clearly visible connecting the arcopallium with the area labeled as NCL. Note that the area defined as NCIF in chickens is mostly omitted by myelinated fibers. In concordance to the shift of the arcopallium in Passeriformes (see main text), the course of DA in carrion crow ((c), (e)) and zebra finch ((d), (f)) also shifted medial, connecting the acropallium with the area defined by us as NCLd (both NCLdr and NCLdc in zebra finches). Scale bar for all species depicts 1,000 μm. For abbreviations, see list

separate areas of a distinct high TH+ fiber density that span from medial to lateral across the entire caudal nidopallium. Besides the caudomedial nidopallium (NCM), none of these areas have been described before and we termed them based on their topography as dorsal (NCLd), medial (NCLm), and ventral NCL (NCLv).

Based on a large body of research, the NCL is considered the avian analogue to the mammalian prefrontal cortex. Anatomically, this is supported by several lines of evidence: (a) a dense dopaminergic projection from mesencephalic VTA/SN, (b) innervation from the secondary sensory areas of each modality, and (c) downstream efferents to premotor and motor structures. In concordance, this dopamine-modulated integration area of sensory input and motor output is involved complex mental faculties such as working memory, rule learning and reward coding (Güntürkün & Bugnyar, 2016). In order to elucidate what constitutes the NCL in different bird species, we will discuss whether each subarea delineated by high dopaminergic innervation receives multisensory innervation, sends projections to (pre) motor areas, and is involved in complex behavioral capacities.

4.1 | Pigeon

In accordance with previous suggested definitions (Herold et al., 2011; Kröner & Güntürkün, 1999; Waldmann & Güntürkün, 1993; Wynne & Güntürkün, 1995), the NCL in pigeons is best described as a semi-lunar structure situated in the dorsolateral roof of the caudal nidopallium. Our analysis uncovered a medial and lateral subdivision of the NCL, separated by a narrow streak of lower fiber and basket density. This segregation reiterates a previous differentiation based on receptor density differences (Herold et al., 2011). Our data is thus

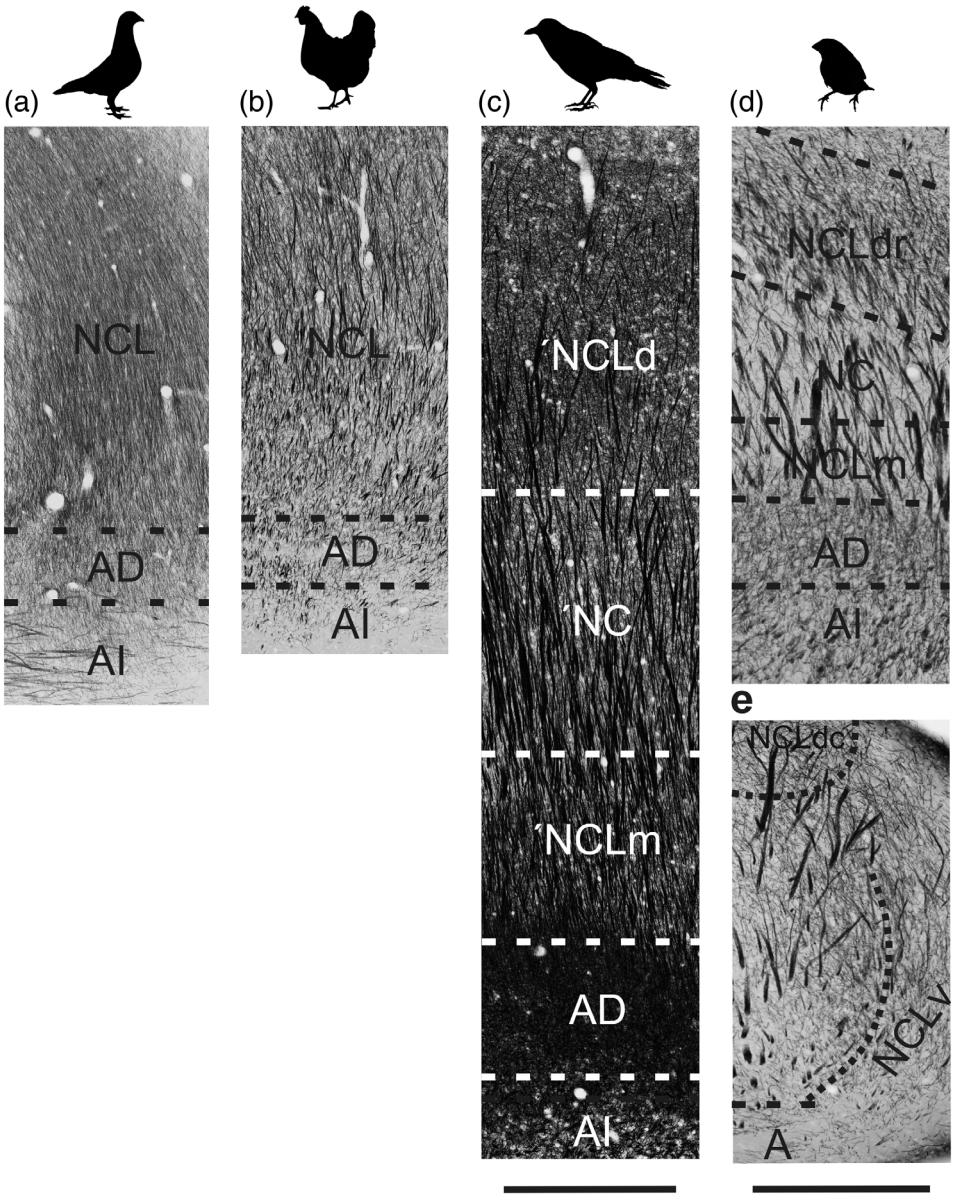

FIGURE 11 Close up pictures of the dorsal arcopallial tract (DA) visualized by a Gallyas myelin stain in the different species. In pigeon (a) and chicken (b), the DA connects the arcopallium with NCL situated directly dorsal adjacent to AD. While the DA in pigeon consists of rather thin, mostly singular fibers, thin fibers in the chicken DA are intermixed with thicker processes that likely represent small fiber bundles. In the carrion crow (c) and zebra finch (d, e), DA connects NCLd with the arcopallium, passing through NCLm and NC. Comparable to the chicken, the fiber plexus in the two Passeriformes is a mix of thin singular fibers and small bundles of fibers. In the zebra finch, the plexus targeting NCLdr appeared dominated more by bundles (d), whereas the projection to NCLdc consisted of more singular fibers (e). Scale bar in (c) (representative for (a)–(c)) depicts 1,000 μm. Scale bar in (e) (representative for (d), (e)) depicts 500 μm. For abbreviations, see list

in complete concordance with earlier descriptions of the NCL, proving the effectiveness and reliability of our method of analysis.

In pigeons, it has been well established that NCL, and especially the lateral subdivision, receives sensory input from all modalities, organized in a dorsomedial to ventrolateral manner of auditory, visual (thalamofugal and tectofugal), somatosensory, and trigeminal input. Each sensory modality reaches the NCL via a common flow where a sensory specific thalamic nucleus projects onto the primary pallial recipient which relays information to a neighboring secondary area that then projects onto the NCL (Kröner & Güntürkün, 1999; Leutgeb et al., 1996). In short, the primary pallial recipient of auditory information is field L2, which projects to the flanking fields L1 and L3 that innervate the dorsomedial most part of the NCL (Wild, Karten, & Frost, 1993). Interestingly, this part of the NCL is the only subarea targeted by an additional thalamic input (Kröner & Güntürkün, 1999). This projection stems from the shell region of the nucleus ovoidalis (Ov) and represents a parallel auditory projection. The central NCL receives an overlapping innervation from the two visual streams and somatosensory pathway (Kröner & Güntürkün, 1999; Leutgeb et al., 1996). In the tectofugal visual pathway, the entopallium is the primary pallial recipient (Benowitz & Karten, 1976) and sends projections to the entopallial belt (Watanabe, Ito, & Ikushima, 1985), transferring information to central NCL. The thalamofugal visual pathway first targets the caudolateral part of the densocellular (HD) and interstitial part of the hyperpallium apicale (IHA, Hodos, Karten, & Bonbright, 1973), which then project onto the caudal hyperpallium apicale (HA, Shimizu, Cox, & Karten, 1995) that targets central NCL. Somatosensory information first reaches rostral aspects of HD and IHA (Funke, 1989), is then send to rostral parts of HA (Wild, 1987) from where it also projects to central NCL. The primary telencephalic field of the trigeminal pathway is the nucleus basalis (Schall, Gunturkun, & Delius, 1986), which sends efferents to the frontal nidopallium (NF, Wild,

Arends, & Zeigler, 1985), which then projects to the ventrolateral part of NCL (Kröner & Güntürkün, 1999; Leutgeb et al., 1996).

Next to being a main recipient of multisensory information, the NCL is involved in motor output functions. Originating largely from the medial subdivision, the NCL sends efferents to the sensorimotor division of the medial and lateral striatum (Kröner & Güntürkün, 1999; Veenman, Wild, & Reiner, 1995) while the lateral NCL subdivision is predominantly reciprocally connected to anterior, dorsal, and intermediate arcopallium (AA, AD, AI, Kröner & Güntürkün, 1999), which are considered to be somatomotor (Herold, Paulitschek, Palomero-Gallagher, Güntürkün, & Zilles, 2018; Zeier & Karten, 1971). In addition, the projection between AI and NCL partly conveys input from the contralateral hemisphere via the anterior commissure (AC, Letzner, Simon, & Güntürkün, 2016). AI is the main terminal field of the DA (Zeier & Karten, 1971), and, as shown here, NCL and DA display a high degree of overlap. Taken together, the pigeons' NCL is highly innervated by dopaminergic fibers that form characteristic baskets. It is in addition an executive area that constitutes the apex of multisensory input and descending output (Kröner & Güntürkün, 1999; Leutgeb et al., 1996; Shanahan et al., 2013).

4.2 | Chicken

Our study shows that the putative NCL in chicken, situated in the dorsolateral roof of the caudal nidopallium, is highly comparable to the NCL in pigeon. We identified a second area of high TH+ fiber density situated ventromedial to the putative NCL known as island fields of the caudal nidopallium (NCIF, Puelles, 2007). In accordance with our findings, high densities of TH positive fibers in the dorsolateral caudal nidopallium in chicken have been described before (Schnabel et al., 1997), but others reported a rather homogenous distribution of TH+ fibers and baskets in the entire caudal nidopallium, with the exception of an empty field L (Metzger, Jiang, Wang, & Braun, 1996; Moons, van Gils, Ghijsels, & Vandesande, 1994). These differences could be the result of variations in the used staining protocols, or it is possible that our computerized fiber quantification program was better able to identify even subtle innervation differences.

Comparable to the pigeon, the putative chicken NCL is a multimodal integration center that is organized in a similar dorsolateral to ventromedial fashion of sensory modalities (Martin Metzger, Jiang, & Braun, 1998; Metzger et al., 1996). The dorsal medial NCL is the main recipient of auditory information as targeted by afferents from field L1/L3 and the shell region of Ov (Wang, Zorio, & Karten, 2017). The center region of the putative NCL receives visual information from Ep and caudal division of HA, and somatosensory input is relayed via the rostral part of HA. Lastly, the ventrolateral area of the putative NCL is the main target of the trigeminal stream from NF (Metzger et al., 1996; Metzger et al., 1998). In contrast to pigeons, the overlap of sensory input seems to be less apparent. It is possible that the sensory modalities are more segregated in the chicken NCL, or alternatively the sensory overlap is only fully revealed when placing anterograde tracers in the secondary sensory areas as has been conducted in pigeons (Kröner & Güntürkün, 1999).

The efferent projections of the dorsolateral caudal nidopallium in chickens are highly comparable to pigeons. The putative NCL sends downstream projections to the basal ganglia, and is homotopically and reciprocally connected to AD, AId, and AIv (Metzger et al., 1996; Metzger et al., 1998). Injections into NCL also revealed interhemispheric connectivity to the contralateral arcopallium (Metzger et al., 1996; Metzger et al., 1998). As in pigeons, AI is the main terminal field of DA (Davies, Csillag, Székely, & Kabai, 1997), and we observe a high degree of overlap between DA and NCL. The tract does not seem to target NCIF, but instead arches around it to reach the medial subdivision of NCL. One difference between the DA of pigeons and chickens concerns the morphology of its myelinated processes. In pigeon, the DA only consists of thin fibers, whereas in chicken the thin plexus is intermixed with thick processes, most probably representing bundles of fibers.

For NCIF, very little is known about the connectivity pattern. The term island fields was first introduced by Redies and Puelles (2001) to designate groups of cells with a specific cadherin expression profile surrounded by a nidopallial matrix expressing different cadherin subtypes (Redies et al., 2002). In addition, these fields have a comparable neural birth date (Heyers, Kovjanic, & Redies, 2003; Striedter & Keefer, 2000) and are strongly innervated by TH+ fibers (Puelles, 2007).

Compared to pigeons, NCIF corresponds topographically to the caudocentral nidopallium (NCC), which is characterized by an elaborate intrinsic circuitry and receives a predominant projection from the dorsal intermediate mesopallium (dMI), and sends efferents to both AI and AM. Because of this circuitry, it has been suggested to be limbic in nature and to be an important player in neuroendocrine and autonomic functions (Atoji & Wild, 2009). The NCC in pigeons is dopaminergically innervated (Waldmann & Güntürkün, 1993; Wynne & Güntürkün, 1995), but to a much lesser extent as we observe in NCIF in the chicken.

In summary, based on the highly comparable innervation pattern of sensory input and motor output, we propose the area of high dopaminergic innervation in the dorsolateral roof of the caudal nidopallium is the NCL in chicken. However, there is currently not enough data to include or exclude NCIF as part of NCL.

4.3 | Carrion crow and zebra finch

As mentioned above, in comparison to pigeon and chicken, the carrion crow and zebra finch show a strikingly different pattern of dopaminergic innervation of the caudal nidopallium. Both birds are members of the oscine branch of the Passeriformes order, and their last common ancestor existed approximately 28 mya (Prum et al., 2015). Their brains demonstrate a high degree of similarity in organization and architecture (Izawa & Watanabe, 2007; Nixdorf-Bergweiler & Bischof, 2007). This comparability is confirmed by our analysis of TH innervation. Consequently, we will discuss these species together. In both

Passeriformes, we could identify four distinct subareas. NCM is a well-defined auditory area (Maney & Pinaud, 2011) that sits at the caudomedial border of the nidopallium. Lateral adjacent, NCLd stretches along the dorsal roof and mirrors the arch and extent of the arcopallium. In the zebra finch, we could observe a rostral (NCLdr) and a caudal (NCLdc) subdivision of NCLd that were not apparent in the carrion crow. In both species, NCLm is located immediately adjacent to the arcopallium, separated only by the LAD. The last defined subarea NCLv is visible in the ventrolateral aspect of the caudal nidopallium.

In the carrion crow, our findings corroborate previous descriptions of dopaminergic innervation of the caudal nidopallium of a different crow species; the house crow (*Corvus splendens*; Sen, Parishar, Pundir, Reiner, & Iyengar, 2019), though we diverge in our interpretation of subdivisions and nomenclature. What we consider NCM, Sen et al. (2019) labeled as DNC. The current designated NCLd corresponds to their mNCL, and what we termed NCLv relates to their lNCL. Sen et al. (2019) do not explicitly describe NCLm, but the area can be distinguished from their stained slices (compare Figure 7 in Sen et al., 2019). We propose this change in nomenclature for two reasons. First, we think it is important to facilitate consistency and therefore adhere to existing songbird literature, as applies to NCM (Maney & Pinaud, 2011). Second, because of the addition of one further NCL-like territory (NCLm), their proposed topological denominations do not hold anymore and thus require a revision. It is possible that our analysis and interpretation differ as a result of general anatomical differences between the two crow species. The house crow is significantly smaller in body (295 g.) and brain size (5.7 g.) compared to the carrion crow (470 g., 8.5 g.; Jønsson, Fabre, & Irestedt, 2012), which could be reflected in differences in the neuroanatomy. Previous reports on the dopaminergic innervation of the caudal nidopallium of zebra finches do not mention any of the subareas identified by us, except for a thin band along the medial edge of the caudal nidopallium (Bottjer, 1993). This area would correspond to NCM, for which the dense dopaminergic innervation has been well-documented in other Passeriformes (White-throated sparrow (*Zonotrichia albicollis*): LeBlanc, Goode, MacDougall-Shackleton, & Maney, 2007; Matragrano, Sanford, Salvante, Sockman, & Maney, 2011; canary (*Serinus canaria*): Appeltants, Ball, & Balthazart, 2001).

In the zebra finch, the caudal nidopallium receives sensory input from all modalities that largely overlaps with our identified NCL-subareas, but also targets the surrounding caudal nidopallium. Interestingly, comparable to pigeon and chicken, the sensory modalities are partly overlapping and are organized in the same dorsomedial to ventrolateral manner of auditory, tectofugal and thalamofugal visual, somatosensory, and trigeminal input, correspondingly. Namely, the dorsomedial NCM is part of the auditory pathway, and based on its connectivity pattern, it can be subdivided in a rostral and a caudal part. Exclusively the rostral part of NCM receives a projection from field L2 and a thalamic input from the shell region Ov, whereas the caudal NCM is predominantly innervated by field L1/3 (Mello, Vates, Okuhata, & Nottebohm, 1998; Vates, Broome, Mello, & Nottebohm, 1996). Parts of NCLd are targeted by the tectofugal visual stream from Ep and the thalamofugal visual stream from the caudal part of HA (Sadananda, Korte, & Bischof, 2007), while the rostral part of HA relays a somatosensory projection to NCLd (Wild & Williams, 1999). Lastly, NCLv is the main recipient of trigeminal input from NF (Wild & Farabaugh, 1996). There is currently no data available on connectivity of NCLm.

The projection onto the arcopallium stem from different parts of the caudal nidopallium. NCM sends efferents to the mediodorsal and medioventral part of AI (Mandelblat-Cerf, Las, Denisenko, & Fee, 2014), which partly overlaps with the RA-cup that sends downstream projections to subtelencephalic auditory nuclei (Mello et al., 1998). The caudal nidopallium that includes NCLd projects homotopically onto AD and AIv (Mandelblat-Cerf et al., 2014; Paterson & Bottjer, 2017). In the ventrolateral part, NCLv sends a strong projection onto the lateral subdivision of AD and AIv. In addition, AIv relays the interhemispheric projection via the AC to contralateral AV that in turn projects homotopically to the contralateral caudal nidopallium (Paterson & Bottjer, 2017). Comparable to pigeon and chicken, AD and AI in songbirds are most probably involved in general motor generation (Bottjer, Brady, & Cribbs, 2000; Feenders et al., 2008; Mandelblat-Cerf & Fee, 2014; Dugas-Ford, Rowell, & Ragsdale, 2012; Stetner & Fee, 2017).

As mentioned above, the arcopallium of songbirds appears to have shifted medial within the caudal telencephalon in comparison to pigeons and chickens. Our results show that DA shifted in concordance with the arcopallium, and predominantly connects to NCLd in both zebra finch and carrion crow. Comparable to chicken, DA of the zebra finch appears to have two types of innervation. The projection to NCLdr consists predominantly of bundles of fibers that arise from the entire medial-lateral extent of the arcopallium. Moving posterior, the tract expands to the ventral tip of the arcopallium and we observed an increase in singular processes especially in the ventral most section. The DA in the carrion crow also consists of in intermixed plexus of thin and thick myelinated processes.

To conclude, the densely dopaminergically innervated subareas in carrion crows and zebra finches receive a multimodal sensory input and send efferent projections to sensorimotor related areas. We therefore propose that NCM, NCLd and NCLv constitute the NCL of the Passeriformes analyzed in this study. We currently do not have enough data for NCLm to disclose whether this is a possible fourth subdivision or not.

4.4 | Dopamine-modulated functions of the NCL

The original delineation of the NCL in pigeons and our study is based on the criterion of a strong dopaminergic innervation arising from the mesencephalic VTA and SN (Divac et al., 1985; Waldmann & Güntürkün, 1993; Wynne & Güntürkün, 1995). This criterion originated from mammalian research, where the dense dopaminergic innervation was considered a defining feature of the PFC (Güntürkün, 2005). The dopaminergic system is highly conserved across vertebrates (Smeets & González, 2000), and as mentioned in the introduction, we

can find a comparable dopaminergic architecture in both NCL and PFC (Puig, Rose, Schmidt, & Freund, 2014). A large body of research supports the notion that dopamine is of critical importance to facilitate executive functioning, and goal-directed behavior in particular (Ott & Nieder, 2019). Several lines of evidence demonstrate the executive involvement of the NCL as delineated by our current analysis in pigeon, chicken, zebra finch and carrion crow.

In pigeons, the best-studied phenomenon is working memory, conceptualized as the capacity to maintain and manipulate relevant information that is no longer perceptually present (Baddeley & Hitch, 1974; Diamond, 2013; Miller & Cohen, 2001). Indeed, the first behavioral evidence supporting the analogy of the PFC and NCL came from the group of Ivan Divac who showed that ablation of the NCL impaired alternation between two choice-keys with an introduced delay, but not visual discrimination in pigeons (Mogensen & Divac, 1982). That the NCL is crucial for working memory was furthermore corroborated by single unit recordings that found increased firing rates linked to the delay phase of a working memory task (Diekamp, Kalt, & Güntürkün, 2002; Johnston et al., 2017; Kalenscher, Windmann, et al., 2005; Rose & Colombo, 2005). Moreover, this function is highly dependent on dopamine as dopamine levels in the NCL rise especially during the delay phase (Karakuyu, Diekamp, & Güntürkün, 2003).

In chicken, the NCL has been implicated to play a role in imprinting. This is an important and robust learning mechanism facilitating social attachment and occurs during a sensitive period in the early life of a chick (Bateson, 1966). Whereas some brain areas are preferentially activated by either visual or auditory stimuli, the center part of the NCL demonstrates heightened metabolic activity following the presentation of both (Bock, Schnabel, & Braun, 1997). These results confirm the associative character of this part of the NCL in chickens, and refer to a possible involvement of emotional processes and memory recall (Braun et al., 1999).

The data on cognitive capacities in zebra finch is unfortunately scarce, since the main focus in this model species is on the song system. From this body of literature, we know that NCM is a well-defined higher order auditory area of songbirds that selectively responds to behaviorally relevant songs, as opposed to simple tones (Pinaud & Terleph, 2008). Importantly, dopamine plays a crucial role in modulating the incentive salience of a song; infusion of dopamine agonists or antagonist into the NCM of female zebra finches could increase or decrease their preference for that particular song, respectively (Barr, Wall, & Woolley, 2019). Research on other parts of the caudal nidopallium demonstrated that the NCLdc shows an increase in 2DG-uptake following the first courtship display (Bischof & Herrmann, 1988) and being chased around the cage (Sadananda et al., 2007). The authors interpret this finding as an involvement of this area in regulation of arousal.

Most of what is known in the carrion crow comes from single unit recordings that target an area that most closely corresponds to the rostrolateral parts of NCLv (Veit & Nieder, 2013). Based on a range of experiments, NCLv appears to be chiefly involved in prospective encoding of different visual and multimodal stimuli over a delay period. Among other capacities, they found that neurons in this part of the NCLv encode abstract rules (Veit & Nieder, 2013), cross modal associations (Moll & Nieder, 2015, 2017), and visual or spatial working memory (Rinnert, Kirschhock, & Nieder, 2019; Veit et al., 2014). It is interesting to note that whereas this area does contain neurons specifically encoding different visual stimuli and cross modal associations, auditory selective neurons have not been identified (Moll & Nieder, 2015). Thus, as has been overwhelmingly supported for the NCL in pigeons (Güntürkün, 2005, 2012; Güntürkün & Bugnyar, 2016), there is a small body of evidence that pinpoint complex associative mechanisms that facilitate goal-directed behavior to the subareas identified as NCL in our current analysis.

4.5 | The evolutionary changes of the caudal nidopallium

Our and previous data (Mello et al., 2019) make it obvious that the caudal telencephalon of songbirds is strikingly differently organized in comparison to pigeons and chickens. It has been suggested that this reorganization occurred with the rise of Passeriformes 56 mya and is thus unique to songbirds (Mello et al., 2019; Prum et al., 2015). There are currently two hypotheses that try to explain how the caudal forebrain shifted. The first is known as the rotational axis hypothesis (Mello et al., 2019), which postulates that the caudal forebrain rotated such that the medial-lateral axis shifted into an anterior–posterior axis. This could explain why the arcopallium in pigeon and chicken is situated mostly lateral to the striatum while in the Passeriformes it is arranged caudal and medial to the striatum (Mello et al., 2019). The second hypothesis suggests that the arcopallium in Passeriformes appears more medial due to the presence of additional nidopallial territory lateral to the arcopallium (Wang et al., 2015). Indeed, a characteristic feature of the Passeriformes in comparison to other birds (with the exception of the Psittaciformes) is an expanded meso- and nidopallium (Iwaniuk & Hurd, 2005; Mehlhorn, Hunt, Gray, Rehkämper, & Güntürkün, 2010), which could possibly have played a role in the shift of the arcopallium. In line with the rotational axis hypothesis, it is possible that in Passeriformes the entire NCL-like area as present in the ancestral condition rotated such that it now spans the entire back of the forebrain. This does not exclude the nidopallial expansion hypothesis, since the rotation alone does not explain the expanded protrusion of NCLv. Thus, it is possible that a combination of both have been at play. In any case, this reorganization has had a considerable impact on the topography of the caudal nidopallium and we observe an expected effect on the position and trajectory of the NCL in the Passeriformes analyzed in this study (Figure 12).

5 | CONCLUSION

As referred to in the introduction, across mammals the PFC is not a uniform structure. Compared to rat and mouse, the primate branch is

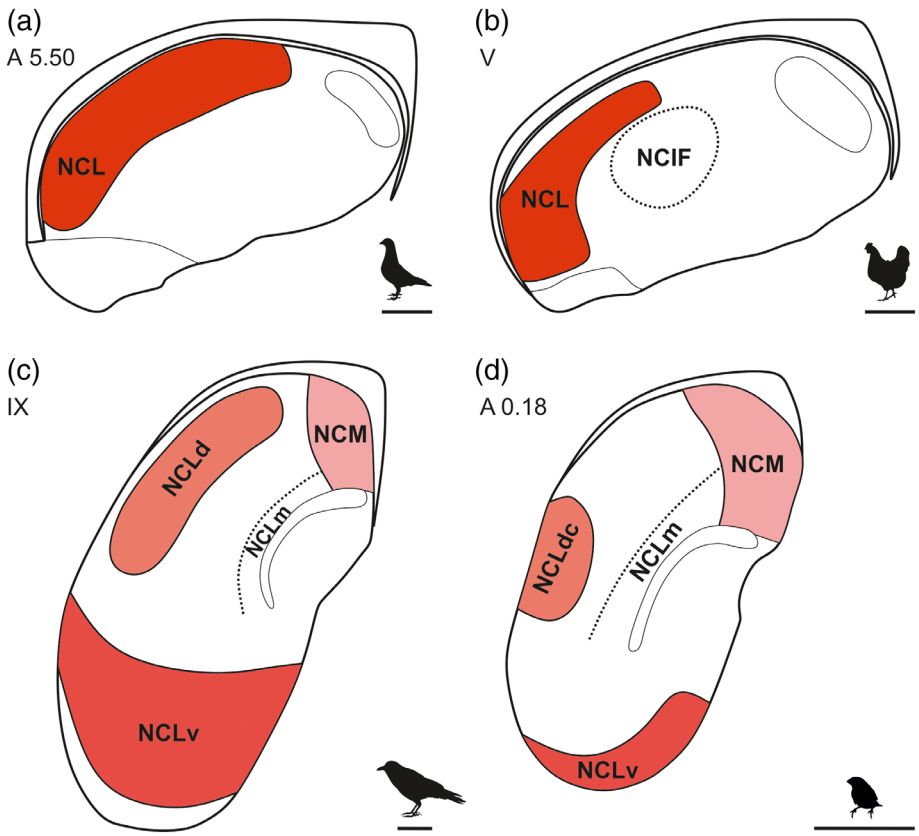

FIGURE 12 Schematic representation of the NCL in pigeon (a), chicken (b), carrion crow (c), and zebra finch (d). The NCL in pigeon and chicken is best described as a semilunar structure in the dorsolateral part of the caudal nidopallium. The NCL in carrion crow and zebra finch consists of at least three subareas (NCM, NCLd, and NCLv) that span across the entire back of the caudal forebrain. NCLd in zebra finch consists of a rostral and caudal subdivision, of which only the latter is depicted here. There is currently not enough data on NCIF in chicken, and NCLm in carrion crow and zebra finch to include or exclude them as part of the NCL. Scale bar in (a)–(d) depicts 1,000 μm. For abbreviations, see list [Color figure can be viewed at wileyonlinelibrary.com]

characterized by an expanded frontal lobe that is more gyrified and parcellated (Passingham & Wise, 2012). Moreover, rodents lack the granular PFC areas that are characteristic of the PFC in monkeys and apes (Brodmann, 1909). There is an unresolved debate whether primates have evolved unique prefrontal territories, or whether it expanded from existing areas in early mammals. The latter would imply that all PFC subdivisions are shared between rodents and primates, but in a condensed or more expanded form (Preuss, 1995; Uylings, Groenewegen, & Kolb, 2003). Interestingly, in parallel to the expansion of prefrontal territories, the dopaminergic innervation of the PFC extended and diversified (Berger, Gaspar, & Verney, 1991). Our findings show impressive parallels to these observations in mammalian research. Compared to pigeon and chicken, the Passeriformes are characterized by an expanded meso- and nidopallium (Iwaniuk & Hurd, 2005; Mehlhorn et al., 2010), that, at least in carrion crow and zebra finch, is more densely and diversely innervated by dopaminergic fibers. Concomitantly, the NCL of both the carrion crow and the zebra finch consists of at least three different subareas that span across the entire caudal nidopallium. It is also interesting to note the parallels in mental faculties, since crows are considered behaviorally on par with chimpanzees (Emery & Clayton, 2004; Güntürkün & Bugnyar, 2016). Thus, this study discloses another instance of the remarkable convergent evolution of the executive structure in mammals and birds. Nearly 40 years ago, Jack Pettigrew (1979) remarked in a paper on the convergently evolved properties of binocular vision in owls and macaques "…that there may exist for nervous systems only a very small number of possible solutions, perhaps a unique one, to the problem of stereopsis " (p. 435). It seems, something quite similar can be said for executive functions.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

Appeltants, D., Ball, G. F., & Balthazart, J. (2001). The distribution of tyrosine hydroxylase in the canary brain: Demonstration of a specific and sexually dimorphic catecholaminergic innervation of the telencephalic song control nuclei. *Cell and Tissue Research*, *304*(2), 237–259. https://doi.org/10.1007/s004410100360

Atoji, Y., & Wild, J. M. (2009). Afferent and efferent projections of the central caudal nidopallium in the pigeon (*Columba livia*). *Journal of Comparative Neurology*, *517*(3), 350–370. https://doi.org/10.1002/cne.22146

Babb, S. J., & Crystal, J. D. (2006). Discrimination of what, when, and where is not based on time of day. *Learning & Behavior*, *34*(2), 124–130. https://doi.org/10.3758/BF03193188

Baddeley, A. D., & Hitch, G. (1974). Working memory. *Psychology of Learning and Motivation*, *8*, 47–89. https://doi.org/10.1016/S0079-7421(08)60452-1

Barr, H. J., Wall, E. M., & Woolley, S. C. (2019). Dopamine in the songbird auditory cortex shapes auditory preference. *BioRxiv*, *761783*. https://doi.org/10.1101/761783

Bast, T., Diekamp, B., Thiel, C., Schwarting, R. K. W., & Güntürkün, O. (2002). Functional aspects of dopamine metabolism in the putative prefrontal cortex analogue and striatum of pigeons (*Columba livia*). *Journal of Comparative Neurology*, *446*(1), 58–67. https://doi.org/10.1002/cne.10187

Bateson, P. P. G. (1966). The characteristics and context of imprinting. *Biological Reviews*, *41*(2), 177–217. https://doi.org/10.1111/j.1469-185X.1966.tb01489.x

Benowitz, L. I., & Karten, H. J. (1976). Organization of the tectofugal visual pathway in the pigeon: A retrograde transport study. *The Journal of Comparative Neurology*, *167*(4), 503–520. https://doi.org/10.1002/cne.901670407

Benton, M. J., & Donoghue, P. C. J. (2006). Paleontological evidence to date the tree of life. *Molecular Biology and Evolution*, *24*(1), 26–53. https://doi.org/10.1093/molbev/msl150

Berger, B., Gaspar, P., & Verney, C. (1991). Dopaminergic innervation of the cerebral cortex: Unexpected differences between rodents and primates. *Trends in Neurosciences*, *14*(1), 21–27. https://doi.org/10.1016/0166-2236(91)90179-X

Bischof, H.-J., & Herrmann, K. (1988). Isolation-dependent enhancement of 2-[14C]deoxyglucose uptake in the forebrain of zebra finch males. *Behavioral and Neural Biology*, *49*(3), 386–397. https://doi.org/10.1016/S0163-1047(88)90402-5

Blake, R. (1993). Cats perceive biological motion. *Psychological Science*, *4*(1), 54–57. https://doi.org/10.1111/j.1467-9280.1993.tb00557.x

Bock, J., Schnabel, R., & Braun, K. (1997). Role of the dorso-caudal neostriatum in filial imprinting of the domestic chick: A pharmacological and autoradiographical approach focused on the involvement of NMDA-receptors. *The European Journal of Neuroscience*, *9*, 1262–1272. https://doi.org/10.1111/j.1460-9568.1997.tb01481.x

Bottjer, S. W. (1993). The distribution of tyrosine hydroxylase immunoreactivity in the brains of male and female zebra finches. *Journal of Neurobiology*, *24*(1), 51–69. https://doi.org/10.1002/neu.480240105

Bottjer, S. W., Brady, J. D., & Cribbs, B. (2000). Connections of a motor cortical region in zebra finches: Relation to pathways for vocal learning. *Journal of Comparative Neurology*, *420*(2), 244–260. https://doi.org/10.1002/(SICI)1096-9861(20000501)420:2<244::AID-CNE7>3.0.CO;2-M

Boysen, S. T., & Berntson, G. G. (1989). Numerical competence in a chimpanzee (*Pan troglodytes*). *Journal of Comparative Psychology*, *103*(1), 23–31. https://doi.org/10.1037/0735-7036.103.1.23

Brannon, E. M., & Terrace, H. S. (1998). Ordering of the numerosities 1 to 9 by monkeys. *Science*, *282*(5389), 746–749. https://doi.org/10.1126/science.282.5389.746

Braun, K., Bock, J., Metzger, M., Jiang, S., & Schnabel, R. (1999). The dorsocaudal neostriatum of the domestic chick: A structure serving higher associative functions. *Behavioural Brain Research*, *98*(2), 211–218. https://doi.org/10.1016/S0166-4328(98)00086-2

Brodmann, K. (1909). *Vergleichende Lokalisationslehre der Grosshirnrinde in ihren Prinzipien dargestellt auf Grunddes Zellenbaues*. Leipzig: Berth.

Brown, J., Kaplan, G., Rogers, L. J., & Vallortigara, G. (2010). Perception of biological motion in common marmosets (*Callithrix jacchus*): By females only. *Animal Cognition*, *13*(3), 555–564. https://doi.org/10.1007/s10071-009-0306-0

Bugnyar, T., Reber, S. A., & Buckner, C. (2016). Ravens attribute visual access to unseen competitors. *Nature Communications*, *7*, 1–6. https://doi.org/10.1038/ncomms10506

Carlén, M. (2017). What constitutes the prefrontal cortex? *Science*, *358*(6362), 478–482. https://doi.org/10.1126/science.aan8868

Chakraborty, M., & Jarvis, E. D. (2015). Brain evolution by brain pathway duplication. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *370*(1684), 20150056. https://doi.org/10.1098/rstb.2015.0056

Clayton, N., & Dickinson, A. (1998). Episodic-like memory during cache recovery by scrub jays. *Nature*, *395*. Retrieved from https://www.nature.com/articles/26216, 272–274.

Davies, D. C., Csillag, A., Székely, A. D., & Kabai, P. (1997). Efferent connections of the domestic chick archistriatum: A phaseolus lectin anterograde tracing study. *The Journal of Comparative Neurology*, *389*(4), 679–693. https://doi.org/10.1002/(SICI)1096-9861(19971229)389:4<679::AID-CNE10>3.0.CO;2-7

de Blois, S., Novak, M., & Bond, M. (1998). Object permanence in orangutans (*Pongo pygmaeus*) and squirrel monkeys (*Saimiri sciureus*). *Journal of Comparative Psychology*, *112*(2), 137–152. https://doi.org/10.1037/0735-7036.112.2.137

Diamond, A. (2013). Executive functions. *Annual Review of Psychology*, *64*(1), 135–168. https://doi.org/10.1146/annurev-psych-113011-143750

Diekamp, B., Kalt, T., & Güntürkün, O. (2002). Working memory neurons in pigeons. *The Journal of Neuroscience*, *22*(4), RC210. https://doi.org/10.1523/JNEUROSCI.22-04-j0002.2002

Diekamp, B., Gagliardo, A., & Güntürkün, O. (2002). Nonspatial and subdivision-specific working memory deficits after selective lesions of the avian prefrontal cortex. *The Journal of Neuroscience*, *22*(21), 9573–9580. https://doi.org/10.1523/JNEUROSCI.22-21-09573.2002

Divac, I. (1973). Delayed response in cats after frontal lesions extending beyond the gyrus proreus. *Physiology & Behavior*, *10*(4), 717–720. https://doi.org/10.1016/0031-9384(73)90151-0

Divac, I., Mogensen, J., & Björklund, A. (1985). The prefrontal "cortex" in the pigeon. Biochemical evidence. *Brain Research*, *332*(2), 365–368. https://doi.org/10.1016/0006-8993(85)90606-7

Dufour, V., Wascher, C. A. F., Braun, A., Miller, R., & Bugnyar, T. (2012). Corvids can decide if a future exchange is worth waiting for. *Biology Letters*, *8*(2), 201–204. https://doi.org/10.1098/rsbl.2011.0726

Dugas-Ford, J., Rowell, J. J., & Ragsdale, C. W. (2012). Cell-type homologies and the origins of the neocortex. *Proceedings of the National Academy of Sciences*, *109*(42), 16974–16979. https://doi.org/10.1073/pnas.1204773109

Durstewitz, D., Kröner, S., & Güntürkün, O. (1999). The dopaminergic innervation of the avian telencephalon. *Progress in Neurobiology*, *59*(2), 161–195. https://doi.org/10.1016/S0301-0082(98)00100-2

Durstewitz, D., Kröner, S., Hemmings, H. C., & Güntürkün, O. (1998). The dopaminergic innervation of the pigeon telencephalon: Distribution of DARPP-32 and co-occurrence with glutamate decarboxylase and tyrosine hydroxylase. *Neuroscience*, *83*(3), 763–779. https://doi.org/10.1016/S0306-4522(97)00450-8

Dykes, M., Klarer, A., Porter, B., Rose, J., & Colombo, M. (2018). Neurons in the pigeon nidopallium caudolaterale display value-related activity. *Scientific Reports*, *8*(1), 5377. https://doi.org/10.1038/s41598-018-23694-8

Emery, N. J., & Clayton, N. S. (2004). The mentality of crows: Convergent evolution of intelligence in corvids and apes. *Science*, *306*(5703), 1903–1907. https://doi.org/10.1126/science.1098410

Fallon, J. H., & Moore, R. Y. (1978). Catecholamine innervation of the basal forebrain IV. Topography of the dopamine projection to the basal forebrain and neostriatum. *The Journal of Comparative Neurology*, *180*(3), 545–579. https://doi.org/10.1002/cne.901800310

Feenders, G., Liedvogel, M., Rivas, M., Zapka, M., Horita, H., Hara, E., … Jarvis, E. D. (2008). Molecular mapping of movement-associated areas in the avian brain: A motor theory for vocal learning origin. *PLoS One*, *3*(3), e1768. https://doi.org/10.1371/journal.pone.0001768

Felten, D. L., & Sladek, J. R. (1983). Monoamine distribution in primate brain V. monoaminergic nuclei: Anatomy, pathways and local organization. *Brain Research Bulletin*, *10*(2), 171–284. https://doi.org/10.1016/0361-9230(83)90045-X

Funke, K. (1989). Somatosensory areas in the telencephalon of the pigeon. II. Spinal pathways and afferent connections. *Experimental Brain Research*, *76*(3), 620–638. https://doi.org/10.1007/bf00248918

Fuster, J. M. (1973). Unit activity in prefrontal cortex during delayed-response performance: Neuronal correlates of transient memory. *Journal of Neurophysiology*, *36*(1), 61–78. https://doi.org/10.1152/jn.1973.36.1.61

Fuster, J. M. (2015). *The prefrontal cortex*. Cambridge, CA: Academic Press. https://doi.org/10.1016/C2012-0-06164-9

Fuster, J. M., & Alexander, G. E. (1971). Excitation and inhibition of neuronal firing in visual cortex by reticular stimulation. *Science*, *133*(3469), 2011–2012. https://doi.org/10.1126/science.133.3469.2011

Gagliardo, A., Bonadonna, F., & Divac, I. (1996). Behavioural effects of ablations of the presumed 'prefrontal cortex' or the corticoid in pigeons. *Behavioural Brain Research*, *78*(2), 155–162. https://doi.org/10.1016/0166-4328(95)00243-X

Gallyas, F. (1971). Silver staining of Alzheimer's neurofibrillary changes by means of physical development. *Acta Morphologica Academiae Scientiarum Hungaricae*, *19*(1), 1–8. https://doi.org/10.1007/BF00296118

Gaspar, P., Stepniewska, I., & Kaas, J. H. (1992). Topography and collateralization of the dopaminergic projections to motor and lateral prefrontal cortex in owl monkeys. *The Journal of Comparative Neurology*, *325*(1), 1–21. https://doi.org/10.1002/cne.903250102

Goldman-Rakic, P. (1987). Circuit basis of a cognitive function in non-human primates. In S. Stahl, S. Iversen, & E. Goodman (Eds.), *Cognitive neurochemistry* (Vol. 4, pp. 90–110). Oxford, UK: Oxford University Press. https://doi.org/10.1002/acp.2350040108

Green, L., Fisher, E., Perlow, S., & Sherman, L. (1981). Preference reversal and self control: Choice as a function of reward amount and delay. *Behavioural Analysis Letters*, *1*, 43–51.

Greggor, A. L., McIvor, G. E., Clayton, N. S., & Thornton, A. (2018). Wild jackdaws are wary of objects that violate expectations of animacy. *Royal Society Open Science*, *5*(10), 181070. https://doi.org/10.1098/rsos.181070

Güntürkün, O. (1997). Cognitive impairments after lesions of the neostriatum caudolaterale and its thalamic afferent in pigeons: Functional similarities to the mammalian prefrontal system? *Journal Fur Hirnforschung*, *38*(1), 133–143.

Güntürkün, O. (2005). The avian "prefrontal cortex" and cognition. *Current Opinion in Neurobiology*, *15*(6), 686–693. https://doi.org/10.1016/j.conb.2005.10.003

Güntürkün, O. (2012). The convergent evolution of neural substrates for cognition. *Psychological Research*, *76*(2), 212–219. https://doi.org/10.1007/s00426-011-0377-9

Güntürkün, O., & Bugnyar, T. (2016). Cognition without cortex. *Trends in Cognitive Sciences*, *20*(4), 291–303. https://doi.org/10.1016/j.tics.2016.02.001

Güntürkün, O., Stacho, M., & Ströckens, F. (2017). The brains of reptiles and birds. In J. Kaas (Ed.), *Evolution of nervous systems* (2nd ed., pp. 171–221). Amsterdam: Elsevier. https://doi.org/10.1016/B978-0-12-804042-3.00007-5

Güntürkün, O., Ströckens, F., Scarf, D., & Colombo, M. (2017). Apes, feathered apes, and pigeons: Differences and similarities. *Current Opinion in Behavioral Sciences*, *16*, 35–40. https://doi.org/10.1016/j.cobeha.2017.03.003

Hartmann, B., & Güntürkün, O. (1998). Selective deficits in reversal learning after neostriatum caudolaterale lesions in pigeons: Possible behavioral equivalencies to the mammalian prefrontal system. *Behavioural Brain Research*, *96*(1–2), 125–133. https://doi.org/10.1016/S0166-4328(98)00006-0

Herold, C., Palomero-Gallagher, N., Hellmann, B., Kröner, S., Theiss, C., Güntürkün, O., … Zilles, K. (2011). The receptor architecture of the pigeons' nidopallium caudolaterale: An avian analogue to the mammalian prefrontal cortex. *Brain Structure and Function*, *216*(3), 239–254. https://doi.org/10.1007/s00429-011-0301-5

Herold, C., Paulitschek, C., Palomero-Gallagher, N., Güntürkün, O., & Zilles, K. (2018). Transmitter receptors reveal segregation of the arcopallium/amygdala complex in pigeons (*Columba livia*). *Journal of Comparative Neurology*, *526*(3), 439–466. https://doi.org/10.1002/cne.24344

Heyers, D., Kovjanic, D., & Redies, C. (2003). Cadherin expression coincides with birth dating patterns in patchy compartments of the developing chicken telencephalon. *The Journal of Comparative Neurology*, *460*(2), 155–166. https://doi.org/10.1002/cne.10631

Hodos, W., Karten, H. J., & Bonbright, J. C. (1973). Visual intensity and pattern discrimination after lesions of the thalamofugal visual pathway in pigeons. *The Journal of Comparative Neurology*, *148*(4), 447–467. https://doi.org/10.1002/cne.901480404

Howat, W. J., & Wilson, B. A. (2014). Tissue fixation and the effect of molecular fixatives on downstream staining procedures. *Methods*, *70*(1), 12–19. https://doi.org/10.1016/j.ymeth.2014.01.022

Hunt, G. R., & Gray, R. D. (2004). The crafting of hook tools by wild new Caledonian crows. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *271*(Suppl. 3), S88–S90. https://doi.org/10.1098/rsbl.2003.0085

Iwaniuk, A. N., & Hurd, P. L. (2005). The evolution of cerebrotypes in birds. *Brain, Behavior and Evolution*, *65*(4), 215–230. https://doi.org/10.1159/000084313

Izawa, E.-I., & Watanabe, S. (2007). A stereotaxic atlas of the brain of the jungle crow (*Corvus macrorhynchos*). In S. Watanabe & M. A. Hofman (Eds.), *Integration of comparative neuroanatomy and cognition* (pp. 215–273). Tokyo, Japan: Keio University Press.

Jiao, Y., Sun, Z., Lee, T., Fusco, F. R., Kimble, T. D., Meade, C. A., … Reiner, A. (1999). A simple and sensitive antigen retrieval method for free-floating and slide-mounted tissue sections. *Journal of Neuroscience Methods*, *93*(2), 149–162. https://doi.org/10.1016/S0165-0270(99)00142-9

Johnston, M., Anderson, C., & Colombo, M. (2017). Neural correlates of sample-coding and reward-coding in the delay activity of neurons in the entopallium and nidopallium caudolaterale of pigeons (*Columba livia*). *Behavioural Brain Research*, *317*, 382–392. https://doi.org/10.1016/j.bbr.2016.10.003

Jønsson, K. A., Fabre, P. H., & Irestedt, M. (2012). Brains, tools, innovation and biogeography in crows and ravens. *BMC Evolutionary Biology*, *12*(1), 72. https://doi.org/10.1186/1471-2148-12-72

Kaas, J. H. (2019). The origin and evolution of neocortex: From early mammals to modern humans. *Progress in Brain Research*, *250*, 61–81. https://doi.org/10.1016/bs.pbr.2019.03.017

Kalenscher, T., Diekamp, B., & Gunturkun, O. (2003). Neural architecture of choice behaviour in a concurrent interval schedule. *European Journal of Neuroscience*, *18*(9), 2627–2637. https://doi.org/10.1046/j.1460-9568.2003.03006.x

Kalenscher, T., Güntürkün, O., Calabrese, P., Gehlen, W., Kalt, T., & Diekamp, B. (2005). Neural correlates of a default response in a delayed GO/NO-GO task. *Journal of the Experimental Analysis of Behavior*, *84*(3), 521–535. https://doi.org/10.1901/jeab.2005.86-04

Kalenscher, T., Windmann, S., Diekamp, B., Rose, J., Güntürkün, O., Colombo, M., … Colombo, M. (2005). Single units in the pigeon brain integrate reward amount and time-to-reward in an impulsive choice task. *Current Biology*, *15*(7), 594–602. https://doi.org/10.1016/j.cub.2005.02.052

Karakuyu, D., Diekamp, B., & Güntürkün, O. (2003). Comparative neurochemistry of the avian forebrain and striatum: A microdialysis study.

Neuroscience Research Communications, *33*(2), 139–146. https://doi.org/10.1002/nrc.10090

Karten, H. J., Karten, H. J., & Hodos, W. (1967). *Stereotaxic atlas of the brain of the pigeon (Columba livia)*. Baltimore, MD: John Hopkins Press.

Kitt, C. A., & Brauth, S. E. (1986). Telencephalic projections from midbrain and isthmal cell groups in the pigeon. II. The nigral complex. *The Journal of Comparative Neurology*, *247*(1), 92–110. https://doi.org/10.1002/cne.902470106

Koenen, C., Millar, J., & Colombo, M. (2013). How bad do you want it? Reward modulation in the avian nidopallium caudolaterale. *Behavioral Neuroscience*, *127*(4), 544–554. https://doi.org/10.1037/a0033551

Kröner, S., & Güntürkün, O. (1999). Afferent and efferent connections of the caudolateral neostriatum in the pigeon (*Columba livia*): A retro- and anterograde pathway tracing study. *Journal of Comparative Neurology*, *407*(2), 228–260. https://doi.org/10.1002/(SICI)1096-9861(19990503)407:2<228::AID-CNE6>3.0.CO;2-2

Krupenye, C., Kano, F., Hirata, S., Call, J., & Tomasello, M. (2016). Great apes anticipate that other individuals will act according to false beliefs. *Science*, *354*(6308), 110–114. https://doi.org/10.1126/science.aaf8110

LeBlanc, M. M., Goode, C. T., MacDougall-Shackleton, E. A., & Maney, D. L. (2007). Estradiol modulates brainstem catecholaminergic cell groups and projections to the auditory forebrain in a female songbird. *Brain Research*, *1171*, 93–103. https://doi.org/10.1016/J.BRAINRES.2007.06.086

Lengersdorf, D., Marks, D., Uengoer, M., Stüttgen, M. C., & Güntürkün, O. (2015). Blocking NMDA-receptors in the pigeon's "prefrontal" caudal nidopallium impairs appetitive extinction learning in a sign-tracking paradigm. *Frontiers in Behavioral Neuroscience*, *9*, 85. https://doi.org/10.3389/fnbeh.2015.00085

Lengersdorf, D., Stüttgen, M. C., Uengoer, M., & Güntürkün, O. (2014). Transient inactivation of the pigeon hippocampus or the nidopallium caudolaterale during extinction learning impairs extinction retrieval in an appetitive conditioning paradigm. *Behavioural Brain Research*, *265*, 93–100. https://doi.org/10.1016/J.BBR.2014.02.025

Letzner, S., Simon, A., & Güntürkün, O. (2016). Connectivity and neurochemistry of the commissura anterior of the pigeon (*Columba livia*). *Journal of Comparative Neurology*, *524*(2), 343–361. https://doi.org/10.1002/cne.23858

Leutgeb, S., Husband, S., Riters, L. V., Shimizu, T., & Bingman, V. P. (1996). Telencephalic afferents to the caudolateral neostriatum of the pigeon. *Brain Research*, *730*(1–2), 173–181. https://doi.org/10.1016/S0006-8993(96)00444-1

Lindvall, O., Björklund, A., & Divac, I. (1978). Organization of catecholamine neurons projecting to the frontal cortex in the rat. *Brain Research*, *142*(1), 1–24. https://doi.org/10.1016/0006-8993(78)90173-7

Lissek, S., Diekamp, B., & Güntürkün, O. (2002). Impaired learning of a color reversal task after NMDA receptor blockade in the pigeon (*Columba livia*) associative forebrain (neostriatum caudolaterale). *Behavioral Neuroscience*, *116*(4), 523–529. https://doi.org/10.1037//0735-7044.116.4.523

Lissek, S., & Güntürkün, O. (2003). Dissociation of extinction and behavioral disinhibition: The role of NMDA receptors in the pigeon associative forebrain during extinction. *The Journal of Neuroscience*, *23*(22), 8119–8124. https://doi.org/10.1523/JNEUROSCI.23-22-08119.2003

Lissek, S., & Güntürkün, O. (2004). Maintenance in working memory or response selection? Functions of NMDA receptors in the pigeon "prefrontal cortex". *Behavioural Brain Research*, *153*(2), 497–506. https://doi.org/10.1016/J.BBR.2004.01.007

Lissek, S., & Güntürkün, O. (2005). Out of context: NMDA receptor antagonism in the avian "prefrontal cortex" impairs context processing in a conditional discrimination task. *Behavioral Neuroscience*, *119*(3), 797–805. https://doi.org/10.1037/0735-7044.119.3.797

Mandelblat-Cerf, Y., & Fee, M. S. (2014). An automated procedure for evaluating song imitation. *PLoS One*, *9*(5), e96484. https://doi.org/10.1371/journal.pone.0096484

Mandelblat-Cerf, Y., Las, L., Denisenko, N., & Fee, M. S. (2014). A role for descending auditory cortical projections in songbird vocal learning. *eLife*, *3*, e02152. https://doi.org/10.7554/eLife.02152

Maney, D., & Pinaud, R. (2011). Estradiol-dependent modulation of auditory processing and selectivity in songbirds. *Frontiers in Neuroendocrinology*, *32*(3), 287–302. https://doi.org/10.1016/j.yfrne.2010.12.002

Marino, L. (2017). Thinking chickens: A review of cognition, emotion, and behavior in the domestic chicken. *Animal Cognition*, *20*(2), 127–147. https://doi.org/10.1007/s10071-016-1064-4

Martin-Ordas, G., Haun, D., Colmenares, F., & Call, J. (2010). Keeping track of time: Evidence for episodic-like memory in great apes. *Animal Cognition*, *13*(2), 331–340. https://doi.org/10.1007/s10071-009-0282-4

Mascalzoni, E., Regolin, L., & Vallortigara, G. (2010). Innate sensitivity for self-propelled causal agency in newly hatched chicks. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(9), 4483–4485. https://doi.org/10.1073/pnas.0908792107

Matragrano, L. L., Sanford, S. E., Salvante, K. G., Sockman, K. W., & Maney, D. L. (2011). Estradiol-dependent catecholaminergic innervation of auditory areas in a seasonally breeding songbird. *European Journal of Neuroscience*, *34*(3), 416–425. https://doi.org/10.1111/j.1460-9568.2011.07751.x

McGrew, W. C. (2004). Primatology: Advanced ape technology. *Current Biology*, *14*(24), 1046–1047. https://doi.org/10.1016/j.cub.2004.11.042

Mehlhorn, J., Hunt, G. R., Gray, R. D., Rehkämper, G., & Güntürkün, O. (2010). Tool-making new caledonian crows have large associative brain areas. *Brain, Behavior and Evolution*, *75*(1), 63–70. https://doi.org/10.1159/000295151

Mello, C. V., Kaser, T., Buckner, A. A., Wirthlin, M., & Lovell, P. V. (2019). Molecular architecture of the zebra finch arcopallium. *Journal of Comparative Neurology*, *527*(15), 2512–2556. https://doi.org/10.1002/cne.24688

Mello, C. V., Vates, E., Okuhata, S., & Nottebohm, F. (1998). Descending auditory pathways in the adult male zebra finch (*Taeniopygia guttata*). *The Journal of Comparative Neurology*, *395*(2), 137–160. https://doi.org/10.1002/(SICI)1096-9861(19980601)395:2<137::AID-CNE1>3.0.CO;2-3

Merker, B. (1983). Silver staining of cell bodies by means of physical development. *Journal of Neuroscience Methods*, *9*(3), 235–241. https://doi.org/10.1016/0165-0270(83)90086-9

Metzger, M., Jiang, S., & Braun, K. (2002). A quantitative immuno-electron microscopic study of dopamine terminals in forebrain regions of the domestic chick involved in filial imprinting. *Neuroscience*, *111*(3), 611–623. https://doi.org/10.1016/S0306-4522(01)00611-X

Metzger, M., Jiang, S., & Braun, K. (1998). Organization of the dorsocaudal neostriatal complex: A retrograde and anterograde tracing study in the domestic chick with special emphasis on pathways relevant to imprinting. *Journal of Comparative Neurology*, *395*(3), 380–404. https://doi.org/10.1002/(SICI)1096-9861(19980808)395:3<380::AID-CNE8>3.0.CO;2-Z

Metzger, M., Jiang, S., Wang, J., & Braun, K. (1996). Organization of the dopaminergic innervation of forebrain areas relevant to learning: A combined immunohistochemical/retrograde tracing study in the domestic chick. *The Journal of Comparative Neurology*, *376*(1), 1–27. https://doi.org/10.1002/(SICI)1096-9861(19961202)376:1<1::AID-CNE1>3.0.CO;2-7

Miller, E. K., & Cohen, J. D. (2001). An integrative theory of prefrontal cortex function. *Annual Review of Neuroscience*, *24*, 167–170. https://doi.org/10.1146/annurev.neuro.24.1.167

Miller, E. K., Erickson, C. A., & Desimone, R. (1996). Neural mechanisms of visual working memory in prefrontal cortex of the macaque. *The Journal of Neuroscience*, *16*(16), 5154–5167. https://doi.org/10.1523/JNEUROSCI.16-16-05154.1996

Mogensen, J., & Divac, I. (1982). The prefrontal "cortex" in the pigeon. *Brain, Behavior and Evolution*, *21*(3), 60–66. https://doi.org/10.1159/000121617

Moll, F. W., & Nieder, A. (2015). Cross-modal associative mnemonic signals in crow endbrain neurons. *Current Biology*, *25*(16), 2196–2201. https://doi.org/10.1016/j.cub.2015.07.013

Moll, F. W., & Nieder, A. (2017). Modality-invariant audio-visual association coding in crow endbrain neurons. *Neurobiology of Learning and Memory*, *137*, 65–76. https://doi.org/10.1016/j.nlm.2016.11.011

Moons, L., van Gils, J., Ghijsels, E., & Vandesande, F. (1994). Immunocytochemical localization of L-DOPA and dopamine in the brain of the chicken (*Gallus domesticus*). *The Journal of Comparative Neurology*, *346*(1), 97–118. https://doi.org/10.1002/cne.903460107

Müller, N. G., & Knight, R. T. (2006). The functional neuroanatomy of working memory: Contributions of human brain lesion studies. *Neuroscience*, *139*(1), 51–58. https://doi.org/10.1016/J.NEUROSCIENCE.2005.09.018

Nicolakakis, N., Sol, D., & Lefebvre, L. (2003). Behavioural flexibility predicts species richness in birds, but not extinction risk. *Animal Behaviour*, *65*(3), 445–452. https://doi.org/10.1006/anbe.2003.2085

Nieder, A. (2017). Inside the corvid brain—Probing the physiology of cognition in crows. *Current Opinion in Behavioral Sciences*, *16*, 8–14. https://doi.org/10.1016/j.cobeha.2017.02.005

Nixdorf-Bergweiler, B. E., & Bischof, H.-J. (2007). *A stereotaxic atlas of the brain of the zebra finch, Taeniopygia guttata, with special emphasis on telencephalic visual and song system nuclei in transverse and sagittal sections*. Kiel, Germany: University of Kiel.

Olkowicz, S., Kocourek, M., Luèan, R. K., Porteš, M., Fitch, W. T., Herculano-Houzel, S., … Němec, P. (2016). Birds have primate-like numbers of neurons in the forebrain. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(26), 7255–7260. https://doi.org/10.1073/pnas.1517131113

Ott, T., & Nieder, A. (2019). Dopamine and cognitive control in prefrontal cortex. *Trends in Cognitive Sciences*, *23*(3), 213–234. https://doi.org/10.1016/J.TICS.2018.12.006

Passingham, R., & Wise, S. (2012). *The neurobiology of the prefrontal cortex: Anatomy, evolution, and the origin of insight*. Oxford, UK: Oxford University Press.

Paterson, A. K., & Bottjer, S. W. (2017). Cortical inter-hemispheric circuits for multimodal vocal learning in songbirds. *Journal of Comparative Neurology*, *525*(15), 3312–3340. https://doi.org/10.1002/cne.24280

Pepperberg, I. M., & Funk, M. S. (1990). Object permanence in four species of psittacine birds: An African Grey parrot (*Psittacus erithacus*), an Illiger mini macaw (*Ara maracana*), a parakeet (*Melopsittacus undulatus*), and a cockatiel (*Nymphicus hollandicus*). *Animal Learning & Behavior*, *18*(1), 97–108. https://doi.org/10.3758/BF03205244

Pettigrew, J. D. (1979). Binocular visual processing in the owl's telencephalon. *Proceedings of the Royal Society of London. Series B. Biological Sciences*, *204*(1157), 435–454. https://doi.org/10.1098/rspb.1979.0038

Pettigrew, J. D. (1980). Comparative physiology of binocular vision. *Clinical and Experimental Optometry*, *63*(5), 204–210. https://doi.org/10.1111/j.1444-0938.1980.tb02933.x

Pettigrew, J. D., & Konishi, M. (1976). Neurons selective for orientation and binocular disparity in the visual Wulst of the barn owl (*Tyto alba*). *Science*, *193*(4254), 675–678. https://doi.org/10.1126/science.948741

Pinaud, R., & Terleph, T. A. (2008). A songbird forebrain area potentially involved in auditory discrimination and memory formation. *Journal of Biosciences*, *33*(1), 145–155. https://doi.org/10.1007/s12038-008-0030-y

Pollok, B., Prior, H., & Güntürkün, O. (2000). Development of object permanence in food-storing magpies (*Pica pica*). *Journal of Comparative Psychology*, *114*(2), 148–157. https://doi.org/10.1037/0735-7036.114.2.148

Preuss, T. M. (1995). Do rats have prefrontal cortex? The Rose-Woolsey-Akert program reconsidered. *Journal of Cognitive Neuroscience*, *7*(1), 1–24. https://doi.org/10.1162/jocn.1995.7.1.1

Procyk, E., & Goldman-Rakic, P. S. (2006). Modulation of dorsolateral prefrontal delay activity during self-organized behavior. *The Journal of Neuroscience*, *26*(44), 11313–11323. https://doi.org/10.1523/JNEUROSCI.2157-06.2006

Prum, R. O., Berv, J. S., Dornburg, A., Field, D. J., Townsend, J. P., Lemmon, E. M., & Lemmon, A. R. (2015). A comprehensive phylogeny of birds (Aves) using targeted next-generation DNA sequencing. *Nature*, *526*(7574), 569–573. https://doi.org/10.1038/nature15697

Puelles, L. (2007). *The chick brain in stereotaxic coordinates: An atlas featuring neuromeric subdivisions and mammalian homologies*. Cambridge, CA: Academic Press.

Puig, M. V., Rose, J., Schmidt, R., & Freund, N. (2014). Dopamine modulation of learning and memory in the prefrontal cortex: Insights from studies in primates, rodents, and birds. *Frontiers in Neural Circuits*, *8*, 93. https://doi.org/10.3389/fncir.2014.00093

Reader, S. M., & Laland, K. N. (2002). Social intelligence, innovation, and enhanced brain size in primates. *Proceedings of the National Academy of Sciences*, *99*(7), 4436–4441. https://doi.org/10.1073/pnas.062041299

Redies, C., Kovjanic, D., Heyers, D., Medina, L., Hirano, S., Suzuki, S. T., & Puelles, L. (2002). Patch/matrix patterns of gray matter differentiation in the telencephalon of chicken and mouse. *Brain Research Bulletin*, *57*(3–4), 489–493. https://doi.org/10.1016/S0361-9230(01)00680-3

Redies, C., & Puelles, L. (2001). Modularity in vertebrate brain development and evolution. *BioEssays*, *23*(12), 1100–1111. https://doi.org/10.1002/bies.10014

Reiner, A., Perkel, D. J., Mello, C. V., & Jarvis, E. D. (2004). Songbirds and the revised avian brain nomenclature. *Annals of the new York Academy of Sciences*, *1016*, 77–108. https://doi.org/10.1196/annals.1298.013

Rinnert, P., Kirschhock, M. E., & Nieder, A. (2019). Neuronal correlates of spatial working memory in the endbrain of crows. *Current Biology*, *29*(16), 2616–2624.e4. https://doi.org/10.1016/J.CUB.2019.06.060

Rose, J., & Colombo, M. (2005). Neural correlates of executive control in the avian brain. *PLoS Biology*, *3*(6), 1139–1146. https://doi.org/10.1371/journal.pbio.0030190

Rosvold, H., & Szwarcbart, M. (1964). Neural structures involved in delayed-response performance. In J. M. Warren and K. Akert (Eds.), *The frontal granular cortex and behavior* (pp. 1–15). New York: McGraw-Hill.

Rugani, R., Vallortigara, G., Priftis, K., & Regolin, L. (2015). Number-space mapping in the newborn chick resembles humans' mental number line. *Science*, *347*(6221), 534–536. https://doi.org/10.1126/SCIENCE.AAA1379

Sadananda, M., Korte, S., & Bischof, H.-J. (2007). Afferentation of a caudal forebrain area activated during courtship behavior: A tracing study in the zebra finch (*Taeniopygia guttata*). *Brain Research*, *1184*, 108–120. https://doi.org/10.1016/J.BRAINRES.2007.09.040

Sakurai, Y., & Sugimoto, S. (1986). Multiple unit activity of prefrontal cortex and dorsomedial thalamus during delayed go/no-go alternation in the rat. *Behavioural Brain Research*, *20*(3), 295–301. https://doi.org/10.1016/0166-4328(86)90229-9

Sathyanesan, A., Ogura, T., & Lin, W. (2012). Automated measurement of nerve fiber density using line intensity scan analysis. *Journal of Neuroscience Methods*, *206*(2), 165–175.

Scarf, D., Hayne, H., & Colombo, M. (2011). Pigeons on par with primates in numerical competence. *Science*, *334*(6063), 1664. https://doi.org/10.1126/science.1213357

Scarf, D., Miles, K., Sloan, A., Goulter, N., Hegan, M., Seid-Fatemi, A., … Colombo, M. (2011). Brain cells in the avian "prefrontal cortex" code

for features of slot-machine-like gambling. *PLoS One*, *6*(1), e14589. https://doi.org/10.1371/journal.pone.0014589

Schall, U., Gunturkun, O., & Delius, J. D. (1986). Sensory projections to the nucleus basalis prosencephali of the pigeon. *Cell and Tissue Research*, *245*(3), 539–546. https://doi.org/10.1007/BF00218555

Schnabel, R., Metzger, M., Jiang, S., Hemmings, H. C., Greengard, P., & Braun, K. (1997). Localization of dopamine D1 receptors and dopaminoceptive neurons in the chick forebrain. *Journal of Comparative Neurology*, *388*(1), 146–168. https://doi.org/10.1002/(SICI)1096-9861(19971110)388:1<146::AID-CNE10>3.0.CO;2-T

Sen, S., Parishar, P., Pundir, A. S., Reiner, A., & Iyengar, S. (2019). The expression of tyrosine hydroxylase and DARPP-32 in the house crow (*Corvus splendens*) brain. *Journal of Comparative Neurology*, *527*(11), 1801–1836. https://doi.org/10.1002/cne.24649

Shanahan, M., Bingman, V. P., Shimizu, T., Wild, M., & Güntürkün, O. (2013). Large-scale network organisation in the avian forebrain: A connectivity matrix and theoretical analysis. *Frontiers in Computational Neuroscience*, *7*, 1–17. https://doi.org/10.3389/fncom.2013.00089

Shimizu, T., Cox, K., & Karten, H. J. (1995). Intratelencephalic projections of the visual wulst in pigeons (*Columba livia*). *The Journal of Comparative Neurology*, *359*(4), 551–572. https://doi.org/10.1002/cne.903590404

Smeets, W. J., & González, A. (2000). Catecholamine systems in the brain of vertebrates: New perspectives through a comparative approach. *Brain Research Reviews*, *33*(2–3), 308–379. https://doi.org/10.1016/S0165-0173(00)00034-5

Starosta, S., Güntürkün, O., & Stüttgen, M. C. (2013). Stimulus-response-outcome coding in the pigeon Nidopallium Caudolaterale. *PLoS One*, *8*(2), e57407. https://doi.org/10.1371/journal.pone.0057407

Starosta, S., Stüttgen, M. C., & Güntürkün, O. (2014). Recording single neurons' action potentials from freely moving pigeons across three stages of learning. *Journal of Visualized Experiments*, *88*, e51283. https://doi.org/10.3791/51283

Stetner, M., & Fee, M. (2017). Quantification of single unit activity related to head and body movements in the intermediate arcopallium of the zebra finch. *Society for Neuroscience*. Retrieved from https://www.abstractsonline.com/pp8/index.html#!/4376/presentation/17555

Striedter, G. F., & Keefer, B. P. (2000). Cell migration and aggregation in the developing telencephalon: Pulse-labeling chick embryos with bromodeoxyuridine. *The Journal of Neuroscience*, *20*(21), 8021–8030. https://doi.org/10.1523/JNEUROSCI.20-21-08021.2000

Triana, E., & Pasnak, R. (1981). Object permanence in cats and dogs. *Animal Learning & Behavior*, *9*(1), 135–139. https://doi.org/10.3758/BF03212035

Uylings, H. B. M., Groenewegen, H. J., & Kolb, B. (2003). Do rats have a prefrontal cortex? *Behavioural Brain Research*, *146*(1–2), 3–17. https://doi.org/10.1016/J.BBR.2003.09.028

Vates, G. E., Broome, B. M., Mello, C. V., & Nottebohm, F. (1996). Auditory pathways of caudal telencephalon and their relation to the song system of adult male zebra finches (*Taenopygia guttata*). *The Journal of Comparative Neurology*, *366*(4), 613–642. https://doi.org/10.1002/(SICI)1096-9861(19960318)366:4<613::AID-CNE5>3.0.CO;2-7

Veenman, C. L., Wild, J. M., & Reiner, A. (1995). Organization of the avian 'corticostriatal' projection system: A retrograde and anterograde pathway tracing study in pigeons. *The Journal of Comparative Neurology*, *354*(1), 87–126. https://doi.org/10.1002/cne.903540108

Veit, L., Hartmann, K., & Nieder, A. (2014). Neuronal correlates of visual working memory in the corvid endbrain. *Journal of Neuroscience*, *34*(23), 7778–7786. https://doi.org/10.1523/JNEUROSCI.0612-14.2014

Veit, L., & Nieder, A. (2013). Abstract rule neurons in the endbrain support intelligent behaviour in corvid songbirds. *Nature Communications*, *4*(1), 2878. https://doi.org/10.1038/ncomms3878

Veit, L., Pidpruzhnykova, G., & Nieder, A. (2015). Associative learning rapidly establishes neuronal representations of upcoming behavioral choices in crows. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(49), 15208–15213. https://doi.org/10.1073/pnas.1509760112

Waldmann, C., & Güntürkün, O. (1993). The dopaminergic innervation of the pigeon caudolateral forebrain: Immunocytochemical evidence for a "prefrontal cortex" in birds? *Brain Research*, *600*(2), 225–234. https://doi.org/10.1016/0006-8993(93)91377-5

Wallis, J. D., Anderson, K. C., & Miller, E. K. (2001). Single neurons in prefrontal cortex encode abstract rules. *Nature*, *411*(6840), 953–956. https://doi.org/10.1038/35082081

Wang, R., Chen, C.-C., Hara, E., Rivas, M. V., Roulhac, P. L., Howard, J. T., … Jarvis, E. D. (2015). Convergent differential regulation of SLIT-ROBO axon guidance genes in the brains of vocal learners. *Journal of Comparative Neurology*, *523*(6), 892–906. https://doi.org/10.1002/cne.23719

Wang, Y., Zorio, D. A. R., & Karten, H. J. (2017). Heterogeneous organization and connectivity of the chicken auditory thalamus (*Gallus gallus*). *Journal of Comparative Neurology*, *525*(14), 3044–3071. https://doi.org/10.1002/cne.24262

Watanabe, M., Ito, H., & Ikushima, M. (1985). Cytoarchitecture and ultrastructure of the avian ectostriatum: Afferent terminals from the dorsal telencephalon and some nuclei in the thalamus. *The Journal of Comparative Neurology*, *236*(2), 241–257. https://doi.org/10.1002/cne.902360208

Wikmark, R. G. E., Divac, I., & Weiss, R. (1973). Retention of spatial delayed alternation in rats with lesions in the frontal lobes. *Brain, Behavior and Evolution*, *8*(5), 329–339. https://doi.org/10.1159/000124362

Wild, J. M., Arends, J. J. A., & Zeigler, H. P. (1985). Telencephalic connections of the trigeminal system in the pigeon (*Columba livia*): A trigeminal sensorimotor circuit. *The Journal of Comparative Neurology*, *234*(4), 441–464. https://doi.org/10.1002/cne.902340404

Wild, J. M., & Farabaugh, S. M. (1996). Organization of afferent and efferent projections of the nucleus basalis prosencephali in a passerine,*Taeniopygia guttata*. *The Journal of Comparative Neurology*, *365*(2), 306–328. https://doi.org/10.1002/(SICI)1096-9861(19960205)365:2<306::AID-CNE8>3.0.CO;2-9

Wild, J. M., & Williams, M. N. (1999). Rostral wulst of passerine birds: II. Intratelencephalic projections to nuclei associated with the auditory and song systems. *The Journal of Comparative Neurology*, *413*(4), 520–534. https://doi.org/10.1002/(SICI)1096-9861(19991101)413:4<520::AID-CNE3>3.0.CO;2-B

Wild, M. J. (1987). The avian somatosensory system: Connections of regions of body representation in the forebrain of the pigeon. *Brain Research*, *412*(2), 205–223. https://doi.org/10.1016/0006-8993(87)91127-9

Wild, M. J., Karten, H. J., & Frost, B. J. (1993). Connections of the auditory forebrain in the pigeon (*Columba livia*). *The Journal of Comparative Neurology*, *337*(1), 32–62. https://doi.org/10.1002/cne.903370103

Wilson, B., Mackintosh, N. J., & Boakes, R. A. (1985). Transfer of relational rules in matching and oddity learning by pigeons and corvids. *The Quarterly Journal of Experimental Psychology Section B*, *37*(4b), 313–332. https://doi.org/10.1080/14640748508401173

Wise, S. P. (2008). Forward frontal fields: Phylogeny and fundamental function. *Trends in Neurosciences*, *31*(12), 599–608. https://doi.org/10.1016/j.tins.2008.08.008

Wright, A. A., Magnotti, J. F., Katz, J. S., Leonard, K., Vernouillet, A., & Kelly, D. M. (2017). Corvids outperform pigeons and Primates in learning a basic concept. *Psychological Science*, *28*(4), 437–444. https://doi.org/10.1177/0956797616685871

Wynne, B., & Güntürkün, O. (1995). Dopaminergic innervation of the telencephalon of the pigeon (Columba-Livia): A study with antibodies against tyrosine hydroxylase and dopamine. *Journal of Comparative Neurology*, *357*(3), 446–464. https://doi.org/10.1002/cne.903570309

Yamamoto, K., Ruuskanen, J. O., Wullimann, M. F., & Vernier, P. (2011). Differential expression of dopaminergic cell markers in the adult zebrafish forebrain. *The Journal of Comparative Neurology*, *519*(3), 576–598. https://doi.org/10.1002/cne.22535

Yamashita, S. (2007). Heat-induced antigen retrieval: Mechanisms and application to histochemistry. *Progress in Histochemistry and Cytochemistry*, *41*(3), 141–200. https://doi.org/10.1016/j.proghi.2006.09.001

Zeier, H., & Karten, H. J. (1971). The archistriatum of the pigeon: Organization of afferent and efferent connections. *Brain Research*, *31*(2), 313–326. https://doi.org/10.1016/0006-8993(71)90185-5

Zentall, T. R., Clement, T. S., Bhatt, R. S., & Allen, J. (2001). Episodic-like memory in pigeons. *Psychonomic Bulletin & Review*, *8*(4), 685–690. https://doi.org/10.3758/BF03196204

Zentall, T. R., & Raley, O. L. (2019). Object permanence in the pigeon (*Columba livia*): Insertion of a delay prior to choice facilitates visible- and invisible-displacement accuracy. *Journal of Comparative Psychology*, *133*(1), 132–139. https://doi.org/10.1037/com0000134

Zoli, M., Torri, C., Ferrari, R., Jansson, A., Zini, I., Fuxe, K., & Agnati, L. F. (1998). The emergence of the volume transmission concept. *Brain Research Reviews*, *26*(2–3), 136–147. https://doi.org/10.1016/S0165-0173(97)00048-9

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APPENDIX

Western blot

Material and methods

To verify specificity of our antibody, we ran a western blot analysis with fresh pigeon tissue. After decapitation, one pigeon brain was quickly dissected, flash frozen in isopentane, and stored at −80°C until further processing. We further dissected the brain into brainstem, cerebellum, and left and right posterior and anterior forebrain. The tissue was homogenized in 200 µL 1× cell lysis buffer (Cell Signaling Technology, Frankfurt, Germany) containing 1:10 protease inhibitor (phenylmethane sulfonyl fluoride [PMSF]). The homogenized sample was centrifuged for 10 min at 13,000 rpm at 4°C, the supernatant was transferred to a new tube and centrifuged for 15 min at 13,000 rpm at 4°C. The protein concentration was determined using the Pierce™ BCA Protein Assay Kit (Thermo Scientific, Germany). Next, proteins were transferred onto a nitrocellulose membrane (Bio-Rad, Germany) electrophoresis was performed for 7 min at 25 V (Mixed MW [Turbo] protocol; Trans-Blot Turbo, Bio-Rad, Germany). Ponceau S staining (Santa Cruz Biotechnology, Germany) confirmed the complete transfer. After a rinse with TBST (1% Tween 20 in 1× TBS), the membrane was blocked for 30 min in Roti®-Block 1:10 in 1× TBS (Roth, Germany). Next, the membrane was incubated overnight at 4°C in primary antibody (polyclonal rabbit anti-TH [AB152, MerckMillipore, Darmstadt, Germany], 1:500 in TBST). The next day, the membrane was washed 5 × 5 min in TBST and incubated for 1 hr in the secondary horseradish-peroxidase-conjugated antibody (Vector Laboratories; anti-rabbit 1:5000 in Rotiblock-TBS). After 3 × 5 min wash in TBST, followed by 2 × 5 min wash in TBS, TH was visualized with the Western Blotting Luminol Reagent Kit (Santa Cruz Biotechnologies, Germany) and detected with Chemidoc XRS + Imaging System (Bio-Rad, Germany).

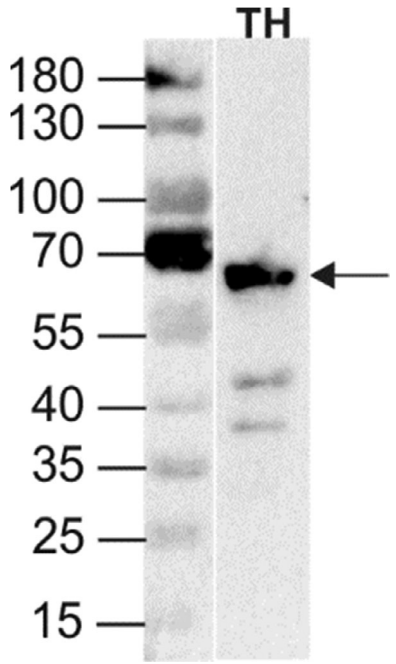

FIGURE A1 Results of the western blot analysis to confirm antibody specificity of the polyclonal rabbit anti-tyrosine hydroxylase antibody (AB152, MerckMillipore, Darmstadt, Germany) used in our study. Specificity was tested in pigeon brain tissue with an antibody concentration of 1:500. Analyses revealed one strong band at approximately 62 kDA (see arrow), which corresponds to the molecular weight of tyrosine hydroxylase. Two additional faint bands at around 42 and 37 kDA probably correspond to protein degradation products. These have been reported in a previous study (Yamamoto, Ruuskanen, Wullimann, & Vernier, 2011) as well as in the data sheet of the antibody vendor and were considered negligible

Results

In order to test the specificity of our antibody we performed a western blot on fresh pigeon brain tissue. The polyclonal antibody recognized TH proteins in the pigeon brain (Figure A1). Comparable to the data sheet provided by the antibody vendor (Millipore), the strongest band was visible at approximately 62 kDA (see arrow), which corresponds to the molecular weight of tyrosine hydroxylase. We could see two additional bands at lower molecular weights, which were also visible in the data sheet. These probably correspond to protein degradation products.