ASSOCIATIVE BRAIN REGIONS ARE RELATIVELY ENLARGED IN ROOK (*Corvus frugilegus*)

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Bird species vary in terms of cognitive skills. The skills of corvids have been suggested to be at level with the cognitively advanced great apes. We wanted to identify whether corvid brains have noticeably different neural features from other birds. Specifically, we studied whether associative brain structures (mesopallium and nidopallium) are enlarged in rooks *(Corvus frugilegus)* compared to three other bird species: Japanese jungle crow (*Corvus macrorhynchos*), budgerigar (*Melopsittacus undulatus*) and chicken *(Gallus gallus)*. Our results indicate that relative proportions of associative brain regions are larger in rook and Japanese jungle crow compared to chicken and budgerigar. In mammals, analogous associative areas are proportionally enlarged in cognitively sophisticated species, hence our findings for a similar enlargement in corvid species suggest similar neurobiological principles for advanced cognition in both birds and mammals.

INTRODUCTION

Among passerine birds, corvid species are known to exhibit complex cognitive abilities and behavioral flexibility (Sol et al. 2005; Iwaniuk & Hurd, 2005), and they are regarded to exhibit cognitive skills similar to those of apes (Emery & Clayton, 2004). What are the neural components that make corvids cognitively special compared to other avian species? The traditional approach is to make gross morphological comparisons such as measuring overall brain volume relative to body size, and the resulting encephalization quotient in general correlates with behavioral flexibility (Reader & Laland, 2002). Corvid species do indeed have larger brains compared to body size (Iwaniuk & Hurd, 2005). Although encephalization quotient is an overall robust measure, these kind of gross comparisons might miss the differential growth of specific brain regions which might have stronger influence on behavioral flexibility and complex cognition (Rehkämper et al. 2008). In this context it is interesting to note that the size of two associative avian pallial regions - mesopallium and nidopallium – have been suggested to correlate with innovative behavior (Timmermans et al. 2000) tool use, (Lefebvre, et al. 2002) and behavioural flexibility in general in certain avian species (Mehlhorn et al. 2010). Our aim in this study is to investigate if associative pallial regions in the brain of a corvid species, rook (*Corvus frugilegus)*, differs from three other avian species in terms of relative proportions. Brain morphology in this species has not been previously studied. Rooks are colonial species that live in large groups all year long, and their cognitive skills in the social domain has been documented showing that they are able to cooperate for problem solving (Seed et al. 2008). Somewhat more surprisingly, they also exhibit high cognitive skills in the physical domain (Seed et al. 2006, Bird & Emery, 2010), including tool use such as bending a

wire into a hook to get a reward in a laboratory setting, although they have not been reported using tools in the wild (Bird & Emery, 2009). We want to understand if these cognitive abilities are associated with a differential enlargement of associative brain areas in their brain, in particular mesopallium and nidopallium. We compared regions of interest of rook brains with the corresponding brain regions of three other avian species: Japanese jungle crow (*Corvus macrorhynchos*), budgerigar (*Melopsit-tacus undulatus*) and chicken *(Gallus gallus)).* The selection for these species of comparison was based both on availability of online brain atlases for these species as well as on phylogenetic considerations. Whereas Japanese jungle crow is a corvid species that was separated from rook around 11 million years ago (Jønsson et al. 2012), budgerigar and chicken are distantly related species to rook, with the common ancestor of rook and budgerigar dating back around 91 million years while the common ancestor of rook and chicken lived around 106 million years ago. Thus, our study might help establishing broad phylogenetic patterns in terms of the relative proportions of associative brain regions; but it can also help spotting more recent changes those brain regions underwent after the separation of rook and Japanese jungle crow.

MATERIALS AND METHODS

Subjects

We collected brains of 2 rooks (*Corvus frugilegus)* and 2 chickens (*Gallus gallus*). Rooks consisted of an adult female (4-years old) and a juvenile male (yearling). Chickens were kindly provided by a local chicken farmer.

Brain collection

The chickens were decapitated and the skulls containing the brains were immersed in fixative, 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer, pH 7.4, at 4°C for 2 days. Brains were then carefully dissected from the skull and postfixed in the same media for 1 day. After this, brains were cryoprotected in 20% sucrose solution and frozen. The frozen brains were cryo-sectioned coronally at 30 µm thickness, and mounted onto SuperFrost® plus slides (Mänzel-Gläser, Germany). Mounted sections were Nissl stained with cresyl violet and coverslipped for histological examination.
Rook specimens were collected at the event of their death and skulls including brains were stored in PFA at 4°C. The procedures for tissue handling were performed as for the chicken brains, apart from that rook brains were stored, dissected and postfixed for an extended time period. This prolonged fixation immersion was necessitated to be able to procure the chicken brains, and establish the treatment protocols using these tissues.

Staining protocol

Chicken brain: Mounted sections were air dried at room temperature for 30 minutes after which they were immersed in ethanol/chloroform (1:1), overnight at room temperature. The following day they were moved through decreasing concentrations of ethanol (100%, 95%; 5 minutes for each step) to achieve rehydration. This initial alcohol treatment also helps removing lipids and fixation chemicals from the tissue. Then the slides were rinsed in distilled water (2 minutes), stained with cresyl violet (Life Science products & services company; 0,1% in acetic acid, 5 minutes), and rinsed in distilled water (1 minute). Tissues were then dehydrated in ethanol baths (95%, 100% X 2; 5 minutes for each step). Finally, slides were cleared in xylene (100%; 2 times, 5 minutes each), and coverslipped with DPX mounting media (Fluka, Germany).

Rook brain: The prolonged immersion fixation increased tissue detachment of rook brains from the microscope slides when applying the same procedure as for chicken brains. To circumvent this problem the procedure was slightly modified so that the rinsing step in distilled water immediately before cresyl violet staining was omitted. This alteration reduced the tissue detachment to some extent.

Since we focused on the relative proportions of the associative brain regions of mesopallium and nidopallium in this study, we aimed to collect brain sections covering the full

anteroposterior range of these brain regions. We sectioned and stained a large number of sections that lie within this range. However technical problems related to the sectioning procedure hindered us from picking up sections from the entire region. In particular, in this material we lack sections from some parts of the Nidopallium caudo laterate (NCL). Overall, our collected sections from the rook brain spanned 59 % of the pallium (7.3 mm. – 16.7 mm anterior-posterior direction). Thus, to allow for comparisons with the other avian species, the corresponding part of the pallium was identified and measured using brain atlases for each of these species (japanese jungle crow (*Corvus macrorhynchos*), budgerigar (*Melopsittacus undulatus*) and chicken *(Gallus gallus))*.

Volumetric calculations

The sections with the highest morphological quality for each region of interest were selected, and digital photos were taken under 16x magnification using a Leica stereo microscope and Leica application suite 2.8.1 software .The collected images were then analyzed off-line using the software ImageJ (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2014.). Since different structures in the subpallial region were not our main interest in this study, and also because the current staining methods did not allow for a fine discrimination of different structures in subpallium, we decided to group all subpallial structures together, henceforth referred to as the ”subpallial region”. Borders of mesopallium, nidopallium and subpallial region were identified and the respective areas were calculated for every section. The volume element of a specific brain region in between each pair of sections selected for area measurements was estimated through linear interpolation. That is, the mean value of the areas in the two measured sections was multiplied with the distance between the two sections: (Area1 + Area2)/2 x distance. The volumes between all measured sections were added up in the end to calculate the total volume of a particular region throughout the anteroposterior range of our sample. The total volume of the brain for our anteroposterior range of interest was calculated by the same method, this time measuring the whole brain area in the same sections. The relative volumes of regions of interest were calculated as follows: Volume of a region/total volume of the brain. The same method for volumetric calculations were used both for sections of rook brain and for the brains examined using the atlases available for Japanese jungle crow, chicken and budgerigar.

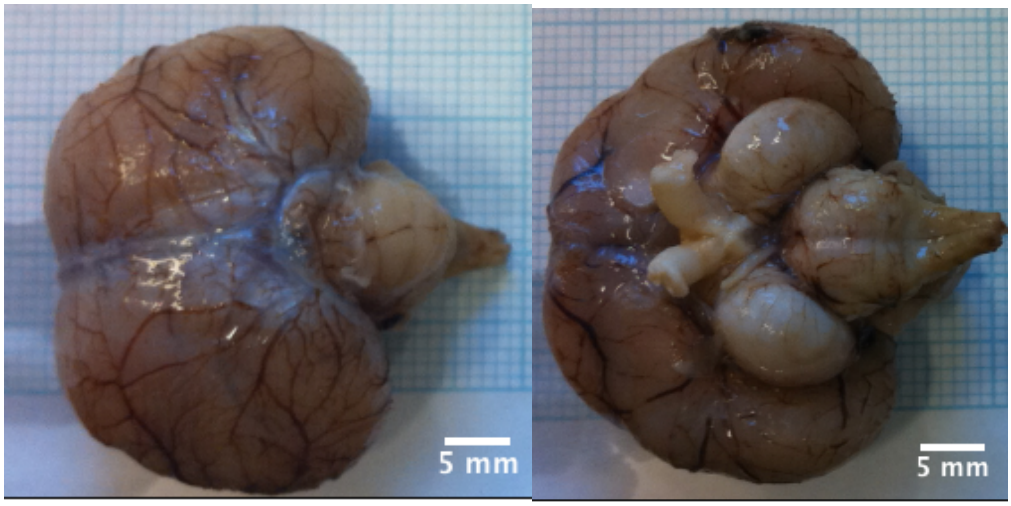

FIGURE 1: Dorsal and ventral view of a rook brain.

RESULTS

Method Validation

In order to validate the methodological approach used in this study we decided to first compare the staining method to previously published material. For this purpose we included chickens in the study given that a relatively detailed full brain atlas has been published for this species. In particular, we needed to make certain the different pallidal regions were clearly distinguishable using the chosen methodological approach.

In Figure 2 two sections are shown exemplifying the methods used for area measurements throughout the study. Note that, the lamina that separates mesopallium and nidopallium is clearly visible, and that delineations of the three different regions analyzed in the stained section is consistent with the previously published chicken atlas (www.avianbrain.org).

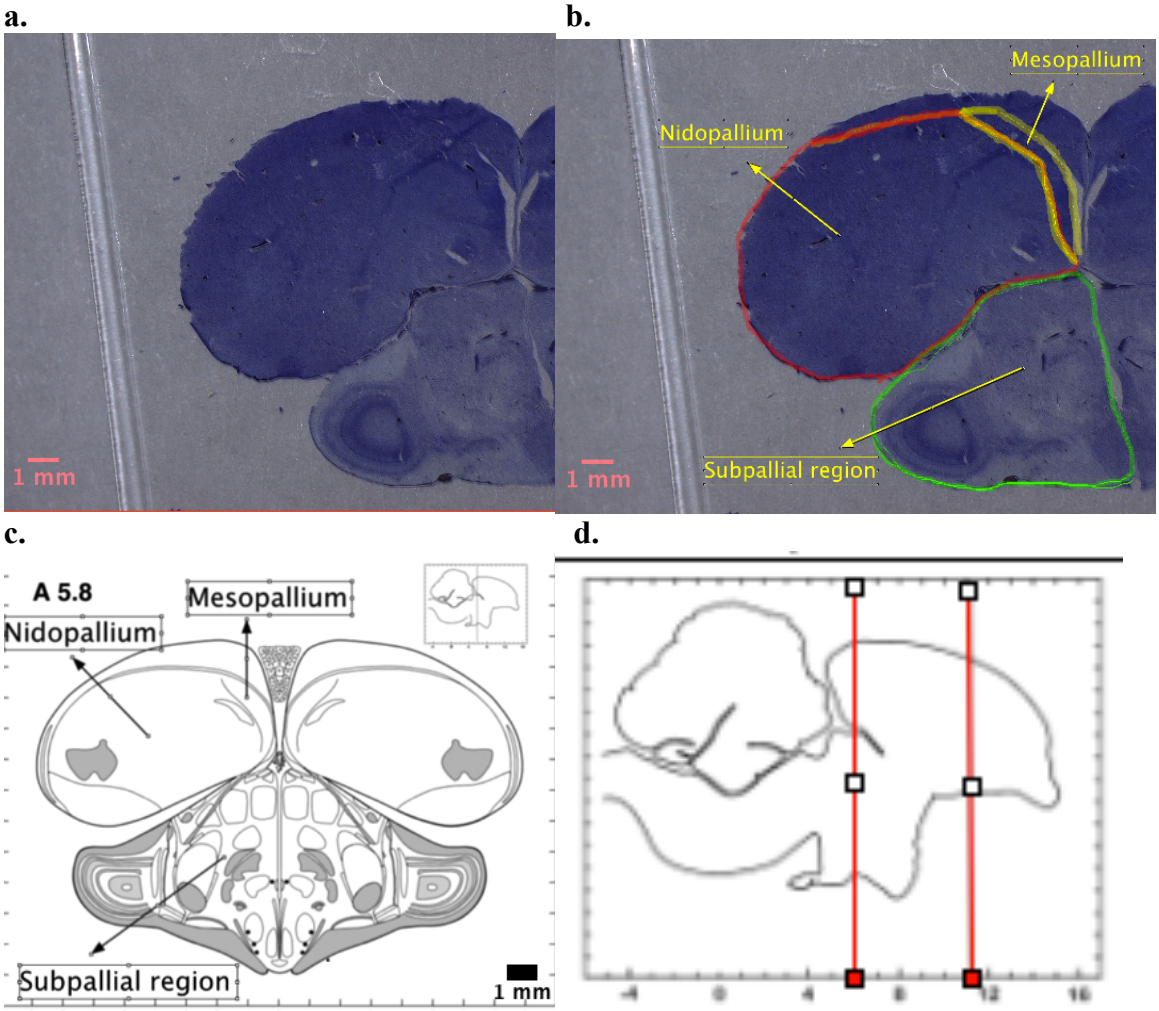

FIGURE 2: The histological methods used were validated by comparing sectioned chicken brains with a previously published chicken atlas (www.avianbrain.org). a) Unlabeled section of chicken brain. b) For the same section, laminae that separates the regions of interest were identified and demarcated by different colours. c) Corresponding section from the chicken atlas: the corresponding regions of interest are indicated by arrows. d) Overall sagittal view of the chicken brain from the atlas. Volumetric calculations were conducted for the pallial region that lies in between two red vertical lines.

In the analyses of the different species, one thing immediately caught our attention. For each section, the area of the mesopallium was relatively enlarged in rook and Japanese jungle crow compared to chicken and budgerigar (Figure 3).

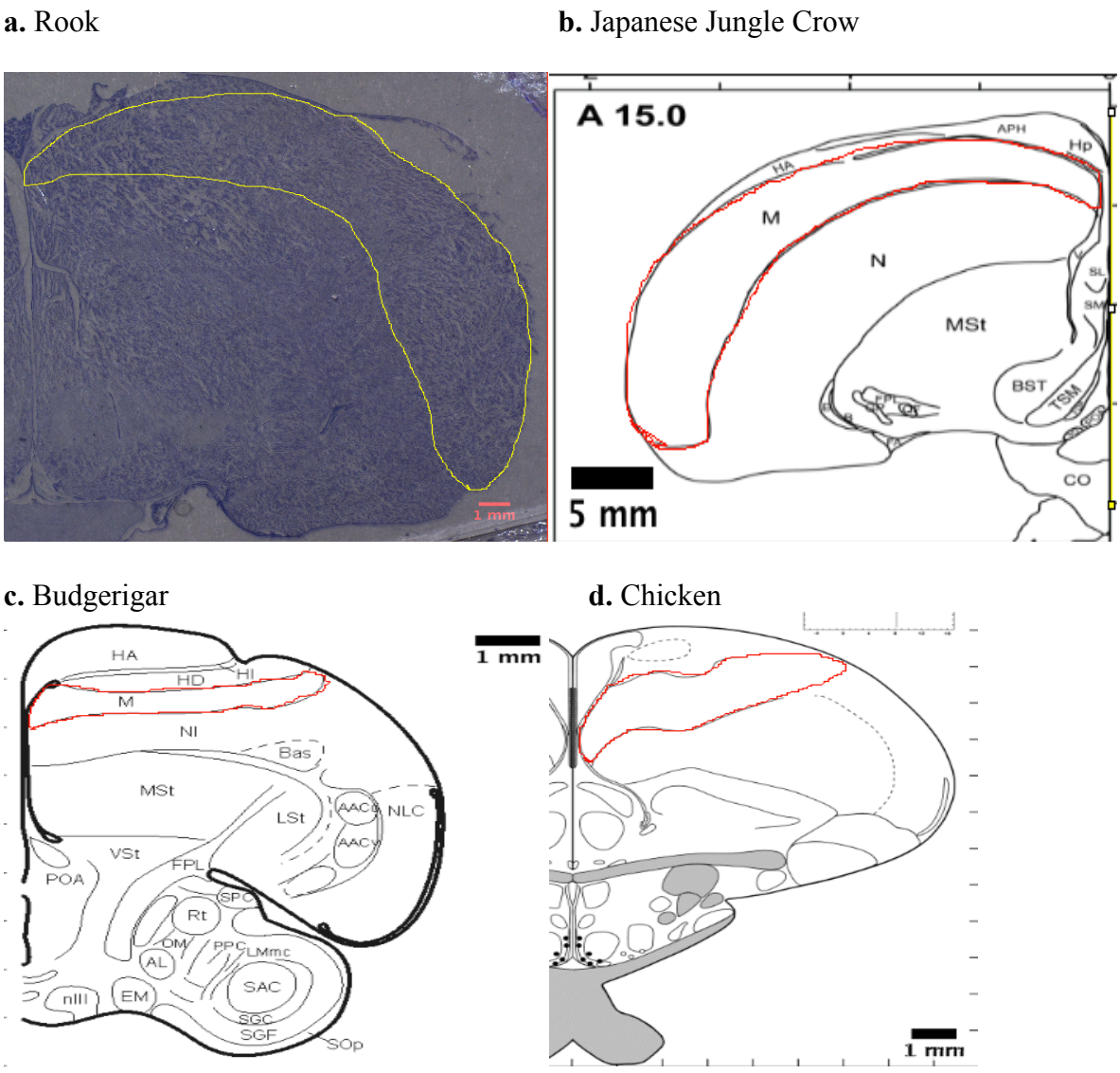

FIGURE 3: Comparison of the relative size of the mesopallium (enclosed structure in different colors) in four different avian species. Note that, mesopallium constitutes a substantially larger proportion of the brain in two corvid species (rook and Japanese jungle crow) compared to budgerigar and chicken.

Volumetric analyses based on the corresponding anteroposterior regions in the different species supported this preliminary analysis: rook and Japanese jungle crow have significantly higher relative mesopallium volume compared to budgerigar and chicken. (Mesopallium volume / Total brain volume: rook: 24.5%, Japanese jungle crow: 21.3%, chicken: 10.4%, budgerigar: 6,8%., for the region analyzed; Figure 4b). We also found that corvid brains have a relatively larger nidopallium than budgerigar and chicken brains, however this difference is not as large as in the case of mesopallium (Nidopallium volume/Total brain volume: rook: 41.3%, Japanese jungle crow: 36.8%, chicken: 31.6%, budgerigar: 25%; Figure 4c).

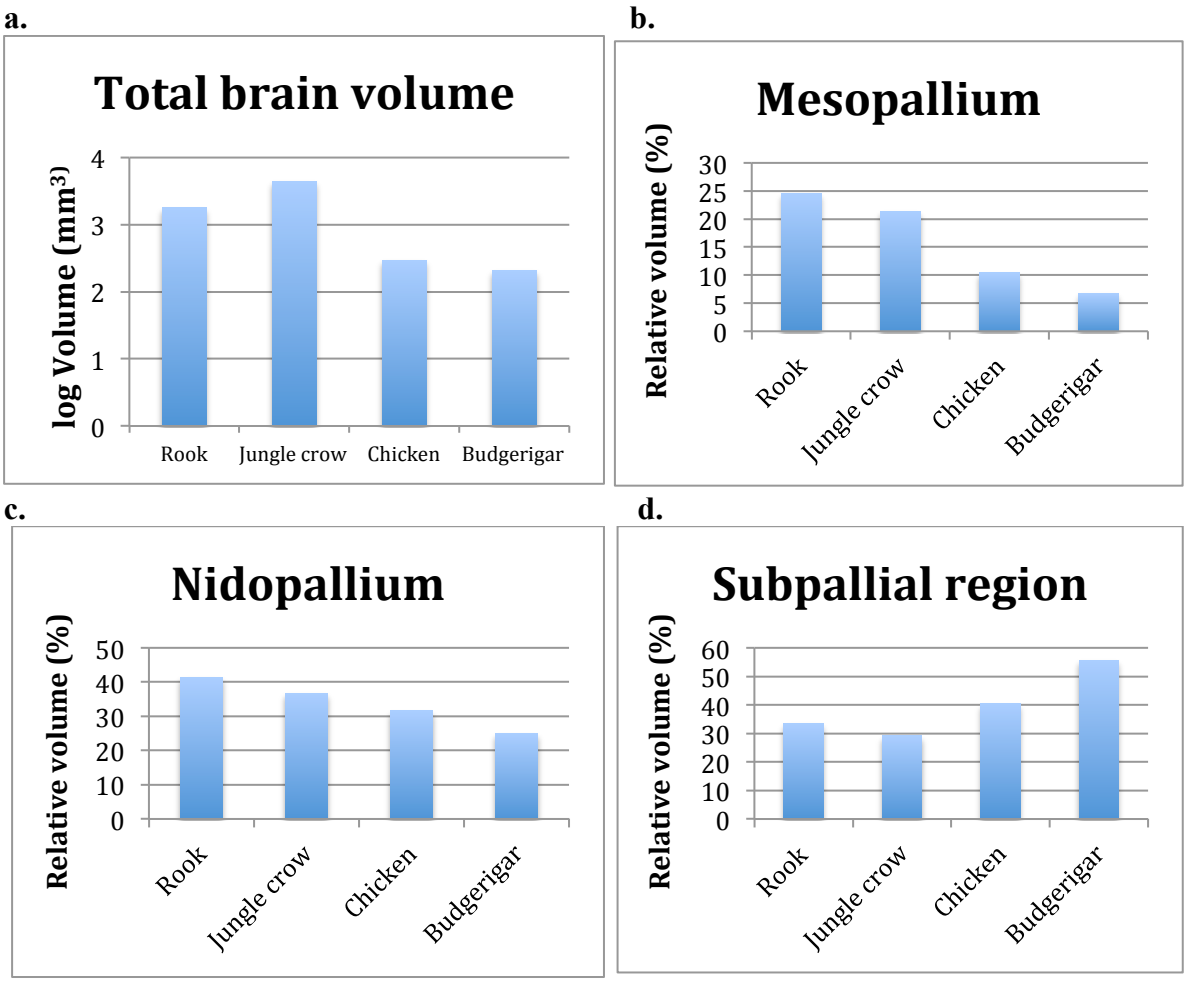

FIGURE 4: Comparison of the total brain volume and relative volume of different brain regions across different avian species for our dataset. a) Total brain volume for the sampled brain region. The relative proportions of different brain areas in the brain region sampled for 4 different species: b) mesopallium, c) nidopallium, d) the subpallial region.

DISCUSSION

Our results confirm the general finding that associative brain areas constitute a higher proportion of the brain in species displaying behavioural flexibility (Timmermans et al. 2000). In particular, we here show that the rook shows a relative enlargement of the mesopallidal region. A relative enlargement of this brain region has previously been reported in another corvid species – the New Caledonian crow. In fact, the biggest difference in terms of the relative proportions of different brain areas resides in mesopallium. For technical reasons, section quality deteriorated somewhat towards both the posterior and anterior end in our specimens which necessitated a restriction of the volumetric measures to an anteroposterior interval excluding a small proportion of the pallium (As shown in Fig 2). Importantly however, the mesopallium was completely included in our dataset for the rook brain. Hence, we believe that our conclusion indicating a relative enlargement of the mesopallium is robust. Mesopallium is thought to be a true associative brain region with no direct sensory input. Its function has been experimentally investigated and it is associated with innovation and flexible behavior in New Caledonian crow (Mehlhorn et al. 2010), human face recognition in the context of reward and punishment in American crow (Marzluff et al. 2012) imprinting and avoidance learning in chicken (Rose, 2000, Horn 2004), song learning in budgerigar and zebra finch (Jarvis & Melo 2000, Bauer et al. 2008). The relative enlargement of the mesopallium in rooks is thus consistent with aforementioned cognitive abilities of rooks in the domains of innovative problem solving and tool use (Seed et al. 2006), (Bird & Emery,

2009), where rapid learning of the contingencies of a faced problem leads to a successful solution. Besides, because corvids are opportunistic generalist feeders, rapid learning of changes in the environment may carry high fitness benefits for these species. Given the assumed important role of the mesopallium in various domains of learning, a relative enlargement of mesopallium might consequently have enabled corvids to build their behavioural flexibility and learning capacities.

The relative size of nidopallium was also larger in the two corvid species analyzed compared to chicken and budgerigar, but the difference was smaller compared to mesopallium. It should be noted, however, that while our dataset covered a large part of the range of nidopallium, the most caudal part of the nidopallium (NCL), was not completely included due to technical limitations. The NCL has been suggested to play a role in executive function and working memory (Güntürkün, 2012) and is by some researchers regarded as the avian equivalent of the mammalian prefrontal cortex (Güntürkün, 2005).

It is interesting to note that the relative enlargement of the mesopallium in corvid species may be regarded as evidence for an independent similar evolution of mammalian and avian brains. Associative brain area functionally related to the mesopallium in the cerebral cortex are also enlarged in relative proportions in primates compared to other mammals (Rehkämper et al. 1991, Sol et al. 2008). This indicates that corvid and primate brains have independently converged to similar solutions to support complex cognitive skills.

In conclusion, our study provides additional support to previous studies documenting an enlargement of mesopallium in innovative species (Timmermans et al. 2000). More specifically, our study shows relative enlargement of mesopallium is present in the in the rook. In order to achieve a more fine-tuned discrimination of the roles of different brain regions, further studies should test additional corvid species and compare them with phylogenetically close out-groups in terms of both relative size and cell density, which may enable us to disentangle adaptive cognitive specializations from historical and phylogenetic constraints. It is also of interest to compare other brain areas like cerebellum and basal ganglia, as they are also known to be involved in higher cognitive abilities like decision making, planning, working memory etc. (Barton 2012, Anderson et al. 2005). Also, from the embodied cognition perspective, cognition is inseparable from sensorimotor processes, thus it can be speculated that these sensorimotor related brain regions may be especially important for cognition with their tight connections with pallial areas and the motor pathways.

REFERENCES

Anderson JR, Albert MV, Fincham JM. (2005).Tracing problem solving in real time: fMRI analysis of the subject-paced Tower of Hanoi. Journal of Cognitive Neuroscience 17:1261–1274.

Bauer EE, Coleman MJ, Roberts TF, Roy A, Prather JF, Mooney R. (2008). A synaptic basis for auditory-vocal integration in the songbird. J Neurosci 28: 1509–1522.

Barton, R. A. (2012). Embodied cognitive evolution and the cerebellum. Philosophical Transactions of the Royal Society B. 367: 2097-2107.

Bird CD, Emery NJ. (2009). Insightful problem solving and creative tool modification by captive nontool-using rooks. Proc. Natl Acad. Sci. USA 106:10 370–375.

Bird CD, Emery NJ (2010) Rooks perceive support relations similar to six-month-old babies. Proc R Soc Lond Ser B-Biol Sci 277:147–151.

Emery NJ, Clayton N (2004). The mentality of crows: convergent evolution of intelligence in corvids and apes. Science 306:1903–1907.

Güntürkün O (2005). The avian ‘prefrontal cortex' and cognition. Curr Opin Neurobiol 15: 686–693.

Güntürkün O (2012). The convergent evolution of neural substrates for cognition. Psychol Res 76(2):212–219.

Horn G (2004). Pathways of the past: the imprint of memory. Nat Rev Neurosci 5:108–120.

Iwaniuk AN, Hurd PL (2005). The evolution of cerebrotypes in birds. Brain Behav Evol 65: 215–230.

Jarvis ED, Mello CV (2000). Molecular mapping of brain areas involved in parrot vocal communication. J Comp Neurol 419: 1–31.

Jønsson KA, Fabre P-H, Irestedt M. 2012. Brains, tools, innovation and biogeography in crows and ravens. BMC Evolutionary Biology 12: 72-83.

Lefebvre L, Nicolakakis N, Boire D (2002). Tools and brains in birds. Behavior 139:939–973.

Marzluff, J. M., Miyaoka, R., Minoshima, S., & Cross, D. J. (2012). Brain imaging reveals neuronal circuitry underlying the crow's perception of human faces. Proceedings of the National Academy of Sciences, 109, 15912-15917.

Mehlhorn, J., Rehkämper, G., Hunt, G. R., Gray, R., and Güntürkün, O. (2010). Tool making new caledonian crows have larger associative brain areas. Brain Behav. Evol. 75: 63–70.

Reader SM, Laland KN (2002). Social intelligence, innovation, and enhanced brain size in primates. Proc Nat Acad Sci 99: 4436–4441.

Rehkämper G, Frahm HD, Cnotka J (2008). Mosaic evolution and adaptive brain component alteration under domestication seen on the background of evolutionary theory. Brain Behav Evol 71:115–126.

Rehkämper G, Frahm HD, Zilles K (1991): Quantitative development of brain and brain structures in birds (Galliformes und Passeriformes) compared to that in mammals (insectivores and primates). Brain Behav Evol 37:125–143.

Rose SPR (2000). God's organism? The chick as a model system for memory studies. Learn Mem 7: 1–17.

Seed AM, Tebbich S, Emery NJ, Clayton NS (2006). Investigating physical cognition in rooks, *Corvus frugilegus*. Curr Biol 16:697–701.

Seed, A. M., Clayton, N. S. & Emery, N. J. (2008). Cooperative problem solving in rooks (*Corvus frugilegus*). Proc. R. Soc. B 275: 1421-1429.

Sol D, Duncan RP, Blackburn TM, Cassey P, Lefebvre L (2005). Big brains, enhanced cognition, and response of birds to novel environments. Proc Natl Acad Sci 102: 5460–5465.

Sol D, Bacher S, Reader SM, Lefebvre L (2008). Brain size predicts the success of mammal species introduced into novel environments. Am Nat 172: 63–71.

Timmermanns S, Lefebvre L, Boire D, Basu P (2000). Relative size of the hyperstriatum ventrale is the best predictor of feeding innovation rate in birds. Brain Behav Evol 56:196–203.