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A Relationship between the Characteristics of the Oval Nucleus of the Mesopallium and Parrot Vocal Response to Playback

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Keywords
Neuron number · Oval nucleus of the mesopallium · Parrot · Stereology · Vocal learning

Abstract
Correlations between differences in animal behavior and brain structures have been used to infer function of those structures. Brain region size has especially been suggested to be important for an animal's behavioral capability, controlled by specific brain regions. The oval nucleus of the mesopallium (MO) is part of the anterior forebrain vocal learning pathway in the parrot brain. Here, we compare brain volume and total number of neurons in MO of three parrot species (the peach-fronted conure, *Eupsittula aurea*, the peach-faced lovebird, *Agapornis roseicollis*, and the budgerigar, *Melopsittacus undulatus*), relating the total neuron numbers with the vocal response to playbacks of each species. We find that individuals with the highest number of neurons in MO had the shortest vocal latency. The peach-fronted conures showed the shortest vocal latency and largest number of MO neurons, the peach-faced lovebird had intermediary levels of both, and the budgerigar had the longest latency and least number of neurons. These findings indicate the MO nucleus as one candidate region that may be part of what controls the vocal capacity of parrots.

Introduction

Differences in brain or brain region size and behavior have been linked in many studies [Healy and Krebs, 1992; Reader and Laland, 2002; Sol et al., 2007; Walløe et al., 2010; Eda-Fujiwara et al., 2016]. Songbirds, hummingbirds, and parrots provide examples of a specialized neural circuitry, i.e., the vocal control nuclei, dedicated to a specialized behavior, vocal production learning. They have evolved similar vocal pathway circuitry independent of a common ancestor [Jarvis, 2004; Jarvis et al., 2014]. This convergent circuit consists of two pathways, the posterior vocal pathway (PVP) and the anterior forebrain vocal pathway (AFP) [Nottebohm and Arnold, 1976; Paton et al., 1981; Smith, 1996; Jarvis and Mello, 2000; Jarvis et al., 2000]. In songbirds, the PVP has been shown to be primarily responsible for producing imitated songs,

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whereas the AFP is mainly responsible for the actual imitation, which is vocal learning. There is strong evidence that volume and number of neurons in the songbird vocal control circuitry correlate with the behavioral vocal communication capacity of these birds [Nottebohm et al., 1986; Kirn et al., 1989; Devoogd et al., 1993; Smith, 1996; Ward et al., 1998]. Studies on parrots have focused on anatomical descriptions, neural connectivity, molecular and sexual differences in vocal control nuclei, and in only one species, the budgerigar, *Melopsittacus undulatus* [Paton et al., 1981; Brauth et al., 1997; Jarvis and Mello, 2000; Brauth et al., 2001, 2005; Feenders et al., 2008], but have not examined relative size differences across species. Further, in songbirds, compared to the PVP, the AFP has received little attention on size differences. A recent study of ours has found a similar vocal control system in eight other species of parrots, spanning the entire parrot phylogeny, but with some apparent size differences in brain regions [Chakraborty et al., 2015].

The AFP in parrots consists of the oval nucleus of the mesopallium (MO), the oval nucleus of the anterior nidopallium, and the magnocellular nucleus of the medial striatum. The parrot MO song nucleus is visibly and clearly as large as other song nuclei relative to its very small counterpart in songbirds and hummingbirds [Jarvis and Mello, 2000], making it intriguing as a nucleus that may be related to parrots' greater ability for vocal learning. Further, it has recently been discovered that the parrot vocal control system has unique shell nuclei surrounding the core nuclei, the latter similar to songbirds and hummingbirds, where the shell system, particularly that of MO, is relatively larger than the core in species with greater vocal learning abilities [Chakraborty et al., 2015]. However, the behavioral differences among species were not determined quantitatively. Neurons in MO can respond to complex auditory stimuli, and lesions to MO in adult budgerigars cause a progressive loss of frequency modulation [Brauth et al., 1997]. This indicates that the parrot AFP may also play an important part in vocal learning, though the roles of each of the nuclei remain unclear. Further, the exceptional vocal learning abilities of parrots [Healy and Krebs, 1992; Vehrencamp et al., 2003; Pepperberg, 2006; Feenders et al., 2008; Balsby and Bradbury, 2009; Scarl and Bradbury, 2009; Balsby et al., 2012; Chakraborty et al., 2015] and the characteristics of MO make it interesting to investigate if various characteristics of MO are associated with vocal learning phenotypes.

Although parrots are renowned for their vocal learning abilities, there are anecdotal differences between species. African grey parrots (*Psittacus erithacus*) are known for the ability to imitate human speech [Pepperberg, 2006], whereas other species among aviculturist are known not to be talkers. Imitation among conspecifics has been shown in the orange-fronted conures (*Eupsittula canicularis*) and galahs (*Elophus roseicapillus*), where they have been shown to imitate conspecific contact calls within seconds [Vehrencamp et al., 2003; Balsby and Bradbury, 2009; Scarl and Bradbury, 2009; Balsby et al., 2012; Bradbury and Balsby, 2016]; whereas the budgerigar requires 1–2 weeks to do so [Farabaugh et al., 1994; Bartlett and Slater, 1999; Hile et al., 2000]. In a companion study [Walløe et al., 2015], we show that three species (budgerigar; peach-fronted conure, *Eupsittula aurea*; peach-faced lovebird, *Agapornis roseicollis*) differ in their short-term modifications of their contact calls in response to playback of conspecific contact calls, with the peach-fronted conure making the largest change to their contact call compared to the peach-faced lovebird and the budgerigar. In this study, we relate stereological estimated volumes of MO and total neuron numbers in MO with the behavioral data of the same individuals of these distantly related species [Walløe et al., 2015]. We find that individuals with the fastest vocal response to playbacks are also the individuals with the highest total number of neurons in MO.

Material and Methods

Brain Tissue Collection

Brains were obtained from adults of three different parrot species (budgerigar: 2 females, 4 males; peach-fronted conure: 3 females, 3 males; peach-faced lovebird: 5 females, 3 males). All individuals used in this study were part of a behavioral comparative study testing vocal learning abilities through playback experiments [Walløe et al., 2015]. The birds came from various private breeders throughout Denmark.

Stereological Design

Two methods were combined to identify the anatomical boundaries and perform quantitative measures of the nuclei: stereological techniques for quantitative estimations of total number of neurons [Pakkenberg and Gundersen, 1997; Eriksen and Pakkenberg, 2007; Walløe et al., 2010, 2011; Mortensen et al., 2014] and in situ hybridization of genes specialized in the song nuclei [Chakraborty et al., 2015]. Because the two techniques require different section thicknesses, it was necessary to use both right and left hemispheres for each technique. One hemisphere was stained for *Parvalbumin* mRNA gene expression by in situ hybridization (Fig. 1, left) [Chakraborty et al., 2015] and used to help confirm the delineation of the boundaries of the region of interest, MO, especially in the peach-fronted conure and the peach-faced lovebird. The other hemisphere was stained for the neuronal marker NeuN (mouse anti NeuN antibody, catalogue number MAB377, Milli-

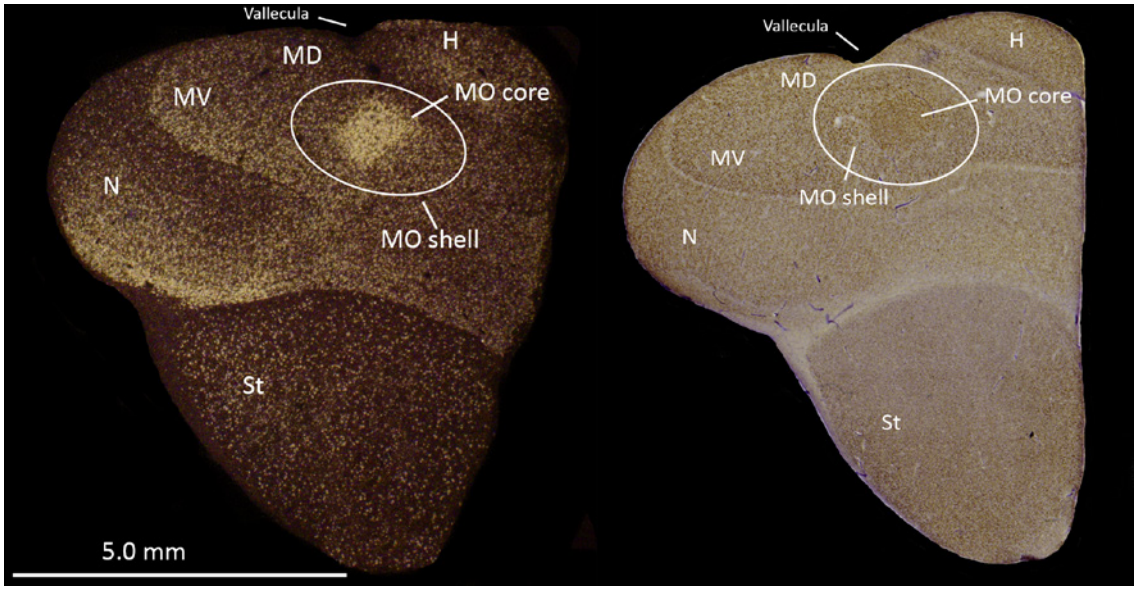

Fig. 1. The oval nucleus of MO. Left: Parvalbumin in situ hybridized tissue section with the bright field indicating the location of the MO core. Right: NeuN-stained tissue section showing the MO core and shell. In this study, the core and shell were counted as one. The NeuN-stained tissue sections were used for counting. The eclipse shows the outer boundaries of the MO shell. MO, mesopallium.

pore) and used for stereological analysis (Fig. 1, right). Left and right hemispheres from different animals were alternatively used for NeuN staining and in situ hybridization (Table 1). All hemispheres from all three species were cut into coronal sections and had a postprocessing (i.e., post NeuN staining) average thickness of 26 μm for the peach-faced lovebird and 27 μm for the budgerigar and peach-fronted conure. The sections designated for in situ hybridization were cut at 12 μm for all species. A stereotaxic atlas of the budgerigar brain (http://brauthlab.umd.edu/atlas.htm) and specialized gene expression profile of budgerigar song nuclei [Jarvis and Mello, 2000] were used to indicate where to start and finish the section sampling.

For cell counting, a combination of the Cavalieri principle and the optical disector [Gundersen and Jensen, 1987; Gundersen et al., 1988, 1999] was applied. The optical disector includes a microscope with a motorized *x*–*y* stage, a PCI-card (IK220, Heidenhain) used for measuring movements in the *z*-direction (applying a precision of 0.5 μm), and the New Cast, Visiopharm Integrator System (Visiopharm, Hørsholm, Denmark). The method is explained in detail in Gundersen [1986] and Gundersen et al. [1988]. In short, the volume, V_{ref}, was obtained using the Cavalieri principle, and the cell density, N_v, was calculated as the total number of cells counted divided by the volume of the disector times the number of counted disectors. The total number of cells was estimated by multiplying the volume, V_{ref}, by the cell density, N_v. The precision with which the volume and the total number of neurons were estimated was determined by the coefficient of error (CE) [Gundersen et al., 1999]. For more stereological details, see Table 2.

Identification of the Oval Nucleus of the MO

Details of the location and histology of MO have previously been determined in the budgerigar brain [Brauth et al., 1994; Striedter, 1994; Jarvis and Mello, 2000; Brauth et al., 2001] and updated and determined in other parrot species in one of our companion studies [Chakraborty et al., 2015]. MO is identified as a semicircular nucleus recognized by larger cell bodies relative to surrounding cells and with loosely organized cell densities (Fig. 2a) [Durand et al., 1997; Jarvis and Mello, 2000]. The neurons tend to be arranged in clusters (Fig. 2b) [Brauth et al., 2001]. The location of MO is reliably found underneath a prominent sulcus in the surface of the brain called the vallecula, where a large blood vessel is located [Jarvis and Mello, 2000; Brauth et al., 2001] (Fig. 1). The MO core and a shell regions are easily identified with Nissl staining and with parvalbumin mRNA expression, which is higher in the core relative to the shell region [Chakraborty et al., 2015]. When the current study was conducted, the experiments to identify the core and shell regions using gene expression and Nissl staining, combined with neural tracing experiments, were still being performed [see methodological details in Chakraborty et al., 2015]. Consequently, due to lack of availability of

Table 1. Brain details

	Left hemisphere	Right hemisphere
Budgerigar (n = 6)	2	4
Peach-faced lovebird (n = 8)	3	5
Peach-fronted conure (n = 6)	3	3
Total	8	12

The uneven distribution of left and right hemispheres is due to differences in the quality of the sectioning.

Table 2. Stereological details of MO

	k	*A*, μm^2	*z*, μm	*x*–*y* step length, μm	$\overline{CE}$
Budgerigar (*n* = 6)	4	580	15	250	0.13
Peach-faced lovebird (*n* = 8)	4	581	15	200	0.12
Peach-fronted conure (*n* = 6)	4	576	15	300	0.12

k, sampling frequency; *A*, frame area, μm^2; *z*, depth of counting frame, μm; CE as in Gundersen et al. [1999].

the preliminary results from the companion study [Chakraborty et al., 2015], it was decided to count the core and shell as one region. To affirm that we were able to distinguish the neurons from other cell types we used a stain specific for neurons, NeuN (mouse anti NeuN antibody, catalogue number MAB377, Millipore). Further criteria for identification of neurons were presence of cytoplasm, a clearly defined nucleus, if visible a dark centrally located nucleolus, and, in the case of large neurons, size (Fig. 2a). Using these approaches, we were able to identify and quantify features of MO in serial coronal sections (Fig. 2c).

We weighed the brains of peach-fronted conures and peach-faced lovebirds, whereas the brain weight for the budgerigar is an average of five brains from other individuals.

Behavioral Data

The behavioral data with which we compared the stereological estimates comes from Walløe et al. [2015]. In brief, each test bird was subjected to a semi-interactive playback experiment, receiving playback stimuli of calls from both familiar and unfamiliar male and female conspecifics (a total of four playback trials for each test bird). The response calls of the test birds were recorded and compared to the playback stimuli calls, to show the vocal modification changes. Through spectrographic cross-correlation (SPCC) [Cortopassi and Bradbury, 2000, 2006], we measured the following five vocal variables: (1) the *maximum SPCC* similarity between each of the playback stimuli calls and the respective response calls; (2) the *mean value* of this same variable; (3) *relative change in similarity*, a measure of how much the response contact calls vary during a trial and a way to assess if this variation shows a tendency towards convergence with or divergence from the playback stimuli calls [Walløe et al., 2015]; (4) the *call rate*, which is the number of response calls per minute given during the playback trial; and (5) the *latency of first call response*, which is the time in seconds from the end of the playback call to the beginning of the first response call. For each of the four trials, there was one measure for each of the five vocal variables, leaving the test birds with four measures of each vocal variable. For the purpose of this study, we used the shortest latency, the highest call rate, and the highest maximum and mean SPCC similarity achieved across the four playback trials, and the relative similarity difference for every bird (Table 3). The strongest response was used as the birds sometimes only responded to part of the playback trials. In addition, we were interested in how well the birds responded at their best and not only how they responded in an average performance.

Statistical Analysis

Statistical analyses were performed in SAS version 9.4 (SAS Institute, Cary, NC, USA). The three species are not closely related within the parrot order. We, therefore, consider them as independent units in the analyses. To test for differences in MO volume and total number of neurons between the three species, we used an ANOVA and, if significant, it was followed by a post hoc least square means pairwise comparison of means. To test for sex differences in MO volume and total number of neurons in MO across species, a mixed model analysis was performed, which included sex as the dependent variable and species as a random factor. Having species as a random factor allowed us to combine data from all three species and extend the conclusion beyond the three species tested. Besides the number of neurons, we extracted the residual number of neurons, which was the residuals from the regression between number of neurons and brain weight, to create a measure adjusted for size differences.

For the analysis of the relation between the behavioral data and the stereological measures, we used only the total number of neurons, as the total number of neurons and volume showed a significant positive relation and the total number of neurons is not affected by possible shrinkage factors. We tested this relation using a random coefficient model, which converged with a common slope and intercept.

A test of a general relation between the total number of neurons in MO and the behavioral data across species would have been challenged if we had used a simple regression, as larger birds would have more neurons and the analysis would assume that all data points were independent. We, therefore, used a random coefficient mixed model [Littell et al., 2006], which estimated the general relationship between neuron number and behavioral data while accounting for species differences. In the random coefficient model, species were coded as subjects to account for species differences, such as brain weight. The model included total number of neurons in MO and sex as independent fixed variables, and maximum SPCC similarity, mean SPCC similarity, relative change in similarity, call rate and latency as dependent variables. Including sex as a fixed variable accounted for variations between species in the ratio between males and females used in this study. The random coefficient model converged with a common intercept, but not with a common slope. Call rate and latency had to be log-transformed for residuals to follow normal distribution. We conducted the same analysis for residual number of neurons.

Fig. 2. MO consist of both large and small neurons and neurons tend to be in clusters. **a** Large neurons in budgerigar MO. **b** The thin arrow points to a typical neuron cluster in MO, and the thick arrow points to a glia cell. Scale bar = 20 μm. **c** Serial coronal sections showing MO (shown is a male budgerigar left hemisphere). MO, mesopallium.

(For figure see next page.)

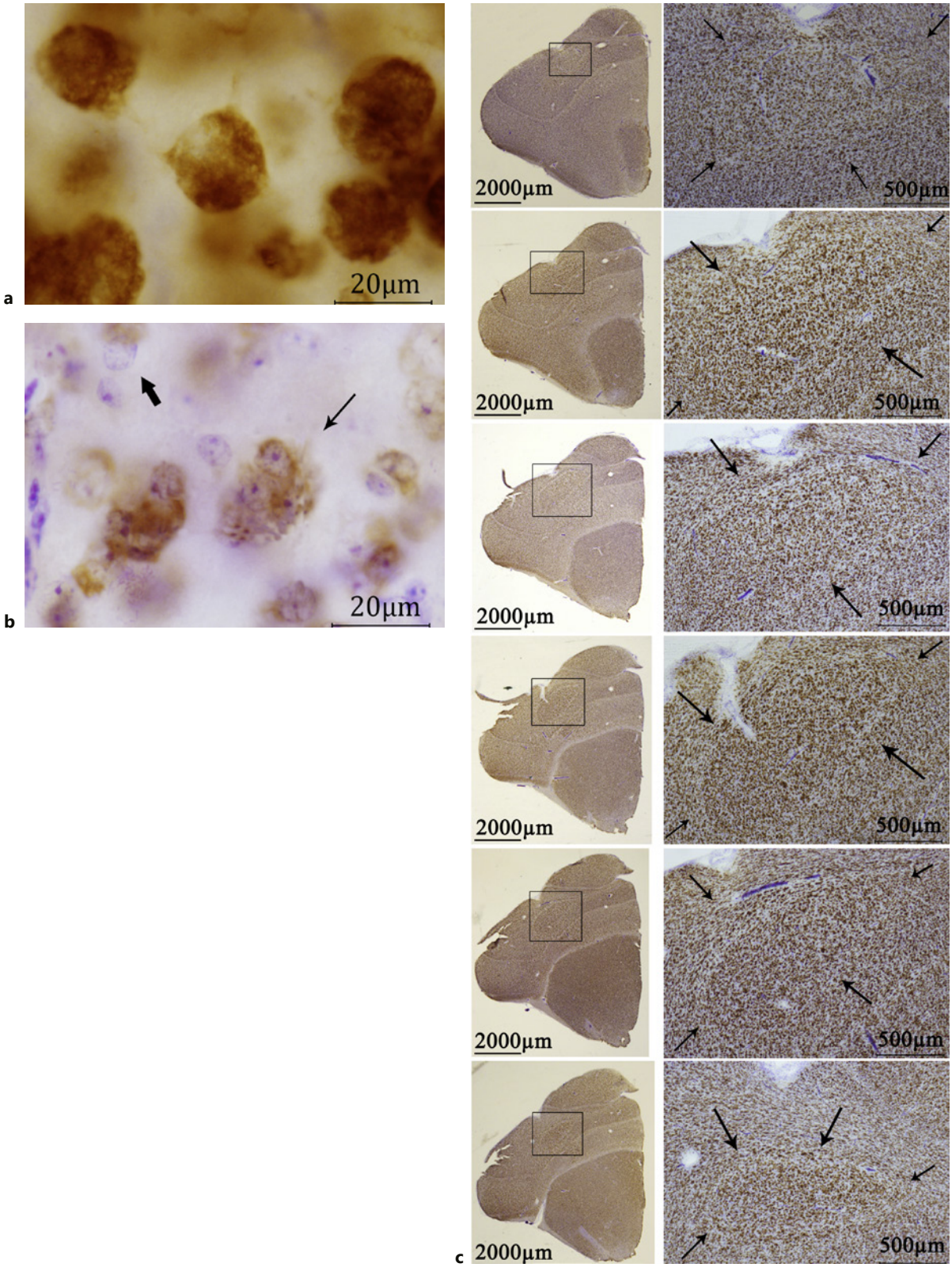

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Table 3. Means of highest call rate, shortest latency, and spectrographic measures for the birds that replied in response to the playback calls

	Call rate, calls/min	Latency, s	Mean SPCC	Max. SPCC	Relative difference
Budgerigar (n = 5)	0.396 (0.080–0.670)	7.72 (0.26–9.19)	0.676 (0.505–0.811)	0.703 (0.517–0.844)	0.057 (0.031–0.079)
Peach-faced lovebird (n = 4)	0.360 (0.040–1.140)	0.395 (0.108–0.578)	0.658 (0.635–0.703)	0.731 (0.720–0.741)	0.224 (0.064–0.390)
Peach-fronted conure (n = 6)	15.5 (10.1–21.8)	0.273 (0.060–0.600)	0.704 (0.611–0.747)	0.779 (0.689–0.818)	0.181 (0.150–0.295)

For details on measurements, see Walløe et al., 2015. The range is given in parentheses.

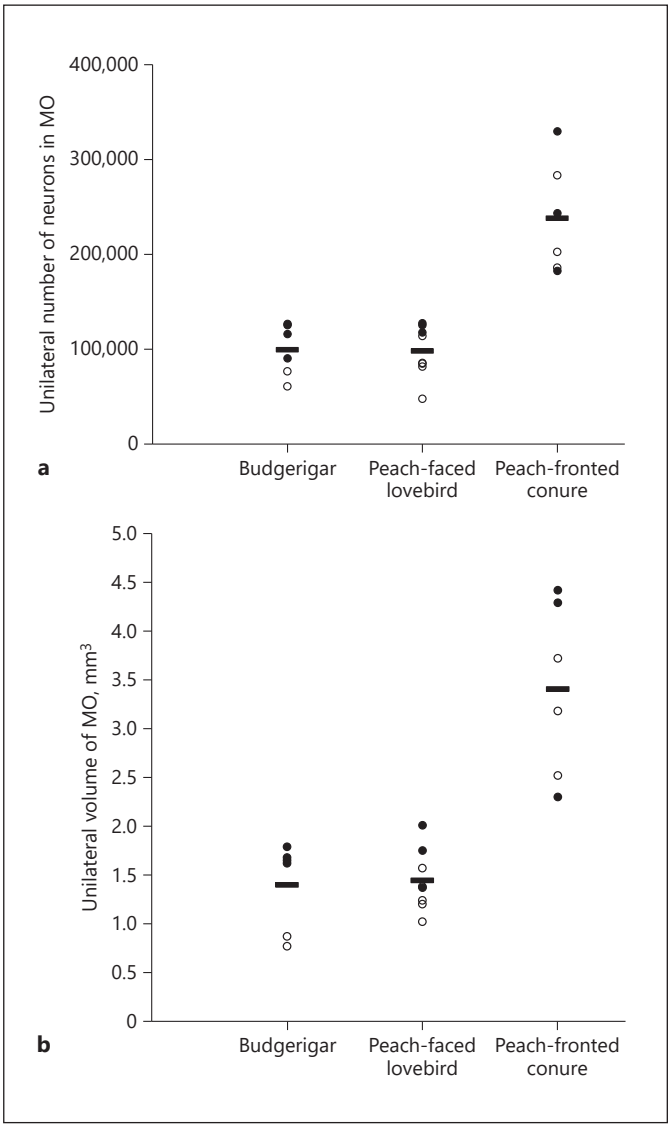

Fig. 3. MO neuron number and volume across species. **a** The total number of neurons in MO (unilateral). The dots represent single individuals, and the line shows the mean of each species. **b** The volume of MO (unilateral). The dots represent single individuals, and the line shows the mean of each species. MO, mesopallium.

To illustrate the relation between total number of neurons and behavioral capacity, we estimated the residual variation in a random coefficient model, which only included sex. These residuals account for differences between sexes and thus illustrate the relation between total number of neurons and behavioral data, which was tested in the statistical model.

Results

Stereological Results

The peach-fronted conure had the highest average number of neurons in MO (females: 230,000; males: 252,000) compared to the peach-faced lovebird (females: 83,100; males: males: 130,000) and the budgerigar (females: 68,000; males: 115,000; Fig. 3a; Table 4). The differences in total number of neurons in MO between the peach-fronted conure and the other two species were significant (one-way ANOVA: $F_{2,17} = 25.97$, $p < 0.001$; least square means pairwise comparison: peach-faced lovebird vs. budgerigar, $p = 0.950$; peach-fronted conure vs. budgerigar, $p < 0.001$; Peach-fronted conure vs. peach-faced lovebird, $p < 0.001$). There was a similar significant difference in the volume of MO (one-way ANOVA: $F_{2,17} = 13.1$, $p < 0.001$; least square means pairwise comparison: peach-faced lovebird vs. budgerigar, $p = 0.691$; peach-fronted conure vs. budgerigar, $p < 0.001$; peach-fronted conure vs. peach-faced lovebird, $p < 0.001$; Fig. 3b; Table 5). Consistent with these results, we found a positive relation between the number of neurons and the volume of MO across species (Fig. 4; random coefficient model, $F_{1,2} = 47.86$, $p = 0.02$). The number of neurons and volume showed a negative, but nonsignificant relation with brain size (mixed model, neuron: $F_{1,11} = 0.11$, $p = 0.742$, slope = –10,571; volume: $F_{1,11} = 0,87$, $p = 0.371$, slope = –0.164). The number of neurons and the volume of MO differed significantly between males and females across species (mixed model, number of neurons: $F_{1,16} = 5.73$, $p = 0.03$; volume: $F_{1,16} = 6.23$, $p = 0.02$, respectively), with

Table 4. Test of sex differences in number of neurons and volume in MO using general linear models

	Number of neurons			Volume		
	df	*F*	*p*	df	*F*	*p*
Budgerigar	1.4	11.5	0.028	1.4	296.2	<0.001
Peach-faced lovebird	1.6	8.3	0.028	1.6	3.6	0.108
Peach-fronted conure	1.4	0.29	0.621	1.4	0.2	0.721

Table 5. Measures of brain weight, volume, and number of neurons

	Brain weight, g	Volume (CV), mm^3	Neurons (CV)
Budgerigar: MO			
Female (n = 2)	1.32	0.82 (0.09)	68,000 (0.16)
Male (n = 4)	1.32	1.68 (0.04)	115,000 (0.15)
Peach-faced lovebird: MO			
Female (n = 5)	1.88	1.28 (0.16)	83,100 (0.28)
Male (n = 3)	1.93	1.71 (0.19)	130,000 (0.16)
Peach-fronted conure: MO			
Female (n = 3)	2.91	3.14 (0.19)	230,000 (0.23)
Male (n = 3)	3.52	3.67 (0.32)	252,000 (0.29)

All values are unilateral. CV, coefficient of variation = SD/mean. The volume estimates given here are calculated from the original section thickness (peach-faced lovebird and peach-fronted conure = 80 μm, budgerigar = 60 μm). For total number of neurons estimation, the post-processed volume (not shown) was used.

males having higher values than females. When sex differences were tested within species, only budgerigars showed significant differences for both number of neurons and volume (Tables 4, 5). Peach-faced lovebirds showed significant differences in number of neurons, whereas sex differences in peach-fronted conures could not be detected (Table 5). In terms of the magnitude of these differences, a qualitative assessment suggested that the biggest differences between sexes occurred in budgerigars (Table 5).

Relation between Behavioral Data and Total Number of Neurons

For the three species, we found a significant negative relation between total neuron number and the log-transformed latency to vocally respond to call playbacks (Fig. 5a; random coefficient mixed model, $F_{1,10}$ = 8.57, p = 0.015); i.e., the higher the number of neurons, the shorter the latency to generate a response call. The residual number of neurons showed the same trend but was

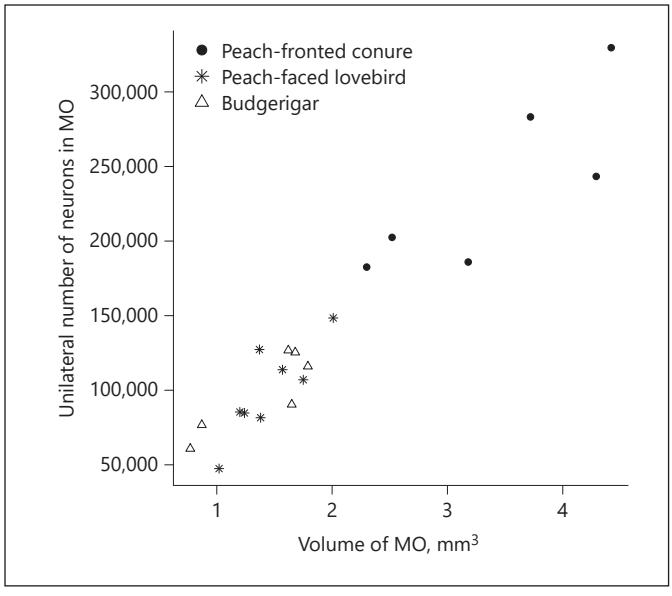

Fig. 4. Correlation between total number of neurons in MO and the volume of MO. Both values are unilateral. MO, mesopallium.

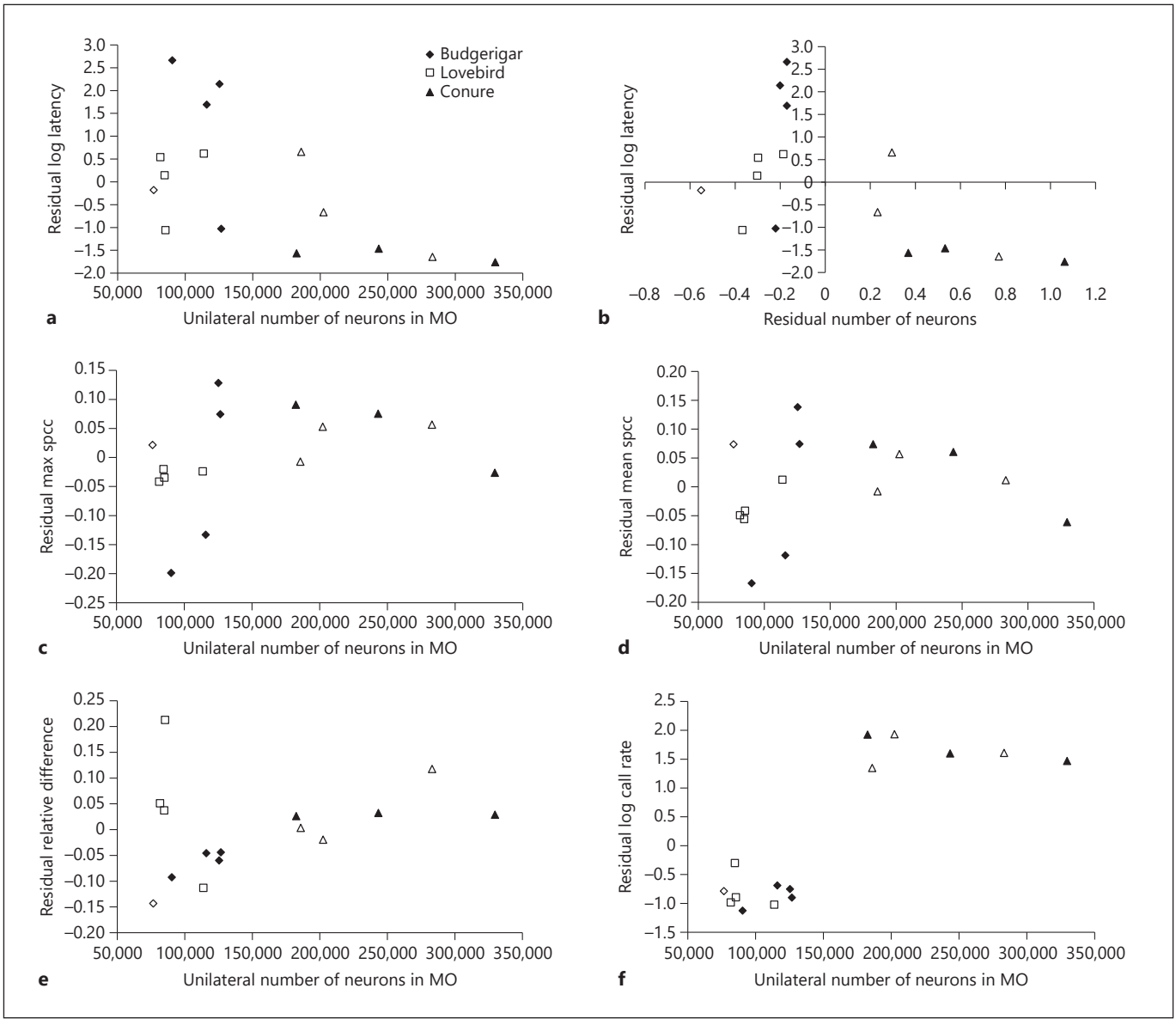

Fig. 5. Total number of neurons in relation to behavioral variables quantified as residuals. **a** Residual log latency. **b** Residual log latency versus residual number of neurons. **c** Residual maximum SPCC. **d** Residual mean SPCC. **e** Residual relative similarity difference. **f** Residual log call rate. For residuals, the effect of sex difference has been controlled for. The residuals for the behavioral variables originate from a mixed model that only included sex as a fixed variable. The statistical model also accounted for species differences, which are also marked in the graphs. The sex of the individual birds is marked as males = closed symbols; females = open symbols. MO, mesopallium.

not quite significant (Fig. 5b; random coefficient mixed model, $F_{1,10} = 4.08$, $p = 0.071$).

There was no relation between the number of neurons and the maximum SPCC similarity (Fig. 5c; random coefficient mixed model, neurons: $F_{1,10} = 1.60$, $p = 0.234$; residual number of neurons: $F_{1,10} = 1.69$, $p = 0.223$), the mean SPCC (Fig. 5d; random coefficient mixed model, neurons: $F_{1,10} = 0.29$, $p = 0.599$; residual number of neurons: $F_{1,10} = 0.33$, $p = 0.581$), the relative similarity difference (Fig. 5e; random coefficient mixed model, neurons: $F_{1,10} = 0.52$, $p = 0.486$; residual number of neurons: $F_{1,10} = 0.49$, $p = 0.502$), or the log-transformed call rate (Fig. 5f; random coefficient mixed model, neurons: $F_{1,10} = 0.04$, $p = 0.545$; residual number of neurons: $F_{1,10} = 0.70$, $p = 0.423$).

However, there were clear species differences, where the conures had a significantly higher call response rate relative to the lovebirds and budgerigars (ANOVA $F_{2,12}$ = 168.7, $p > 0.001$; all least square mean comparisons, $p < 0.001$).

A difference was found between sex and the log-transformed latency (random coefficient mixed model: neurons: $F_{1,10} = 12.01$, $p = 0.006$; residual number of neurons: $F_{1,10} = 6.79$, $p = 0.026$) with males having a shorter latency than females. There was, however, no relation between any of the four remaining behavioral measures and sex (random coefficient mixed model: maximum SPCC similarity: neurons: $F_{1,10} = 1.53$, $p = 0.24$; residual number of neurons: $F_{1,10} = 1.52$, $p = 0.246$; mean SPCC similarity: neurons: $F_{1,10} = 0.26$, $p = 0.62$; residual number of neurons: $F_{1,10} = 0.26$, $p = 0.619$; relative similarity difference: neurons: $F_{1,10} = 1.55$, $p = 0.24$; residual number of neurons: $F_{1,10} = 1.51$, $p = 0.247$; log-transformed call rate: neurons: $F_{1,10} = 0.95$, $p = 0.352$; residual number of neurons: $F_{1,10} = 0.26$, $p = 0.622$).

Discussion

This study is the first to present stereologically estimated values for the total number of neurons and volume of MO across species of parrots. The estimates show that of the three species studied, the peach-fronted conure has the largest volume and highest number of neurons in MO, along with the shortest latency in response to call playbacks and the highest call rates. The relation between residual number of neurons and latency, although not quite significant, showed a similar pattern, which suggested that the results were robust and did not arise because of size differences between the three species.

Although we found no correlation between vocal modification ability (maximum SPCC and mean SPCC) and the number of neurons or residual number of neurons, the pattern of the values was similar to those of latency with the peach-fronted conure having consistently higher modification values within species compared to the other species. The maximum SPCC may not represent the full imitative ability of the individuals. In this experiment, maximum SPCC varied considerably between trials within an individual. This is because maximum SPCC may depend on how well our playback stimulates the individual birds, and some individuals may not show their full imitative ability. Such variation within individuals has also been observed in orange-fronted conures [Balsby and Bradbury, 2009].

Little is known about peach-fronted conure behavioral ecology. However, we presume that its social structure is similar to its closest relative, the orange-fronted conure, which lives in a highly dynamic fission-fusion society [Hardy, 1965]. Throughout the day, they have several temporary fission-fusion encounters, e.g., overlapping visits of multiple flocks at the same foraging sites, which often results in fusion and fissions of small groups and flock switches by individual pairs [Hardy, 1965; Bradbury et al., 2001]. The complexity of their highly dynamic social system indicates a requirement for fast information processing as well as fast responses during a dynamic vocal interaction (e.g., at times convergent and at other times divergent) with other individuals and possible strangers. It is likely that having more neurons in a small particular area of the brain allows the information to be processed faster, thereby allowing the bird to respond faster and more accurately modify its contact call during a vocal interaction. This is supported by Olkowicz et al. [2016], who suggest that the higher density of neurons found in parrots relative to many other bird species likely results in faster information processing abilities. Further, the negative relationship between the total neuron number and latency time to respond to a playback suggests that individuals with a high number of neurons are able to respond faster and are perhaps more willing to respond.

Previous studies have shown a large sexual dimorphism in the vocal control nuclei of songbirds as well as in only one parrot species, the budgerigar [Nottebohm and Arnold, 1976; Ball et al., 1994; Tramontin et al., 1998; Deviche and Gulledge, 2000; Heaton and Brauth, 2000; Brauth et al., 2005]. Our findings extend this across parrot species for total number of neurons and volume of MO, with males having a significantly higher number of neurons and larger volume of MO. So, although male and female budgerigars both have the ability to acquire new calls throughout life [Farabaugh et al., 1994; Brittan-Powell et al., 1997; Hile et al., 2000; Hile and Striedter, 2000], a sexual dimorphism exists in this species. Assuming that the number of neurons is the determining factor of an individual's behavioral capability [Jerison, 1985; Olkowicz et al., 2016], this could explain why male budgerigars apparently learn calls more quickly than females, warble more and longer than females, and during breeding season converge their calls to that of the females [Brockway, 1964; Wyndham, 1980; Farabaugh et al., 1994; Hile et al., 2000; Hile and Striedter, 2000]. The differences between males and females in the peach-faced lovebird are similar but not as profound. We know very little about the vocal behavior of the peach-faced lovebird, but it has been

shown to perform pair duets, with both sexes taking equal part [Mebes, 1978]. For the peach-fronted conure, we found no difference between males and females in MO volume or number of neurons. Little is known about vocalizations in this species, but if we assume that it acts in a similar manner as its close relative, the orange-fronted conure, females would be expected to have similar vocal modification abilities and take part in warble interactions [Bradbury, 2003; Balsby and Scarl, 2008]. Similar differences have been found among songbird species. In songbird species with large sexual dimorphic vocalization patterns (e.g., the zebra finch, *Taeniopygia guttata*), there are large sex differences between male and female vocal control nuclei [Nottebohm and Arnold, 1976; Tramontin et al., 1998], whereas in species that vocalize in a comparable manner between sexes, there are less pronounced differences in the brain between the sexes (e.g., duetting bay wren, *Thryothorus nigricapillus*, and the buff-breasted wren, *Thryothorus leucotis* [Arnold et al., 1986], reviewed in Jarvis [2004]).

A study by Balsby and Scarl [2008] shows that male orange-fronted conures respond more to convergent than to divergent stimuli, whereas females respond equally to both divergent and convergent stimuli. As vocal interactions in the orange-fronted conure are complex and the participants may take turns, being the follower and the leader [Balsby and Scarl, 2008], it was suggested that this indicates that males are less willing to accept the role of follower in an interaction. In support of this, our results of faster response rates in male peach-fronted conures could indicate that males may respond fast under specific conditions, wanting to take on the leader role in the interaction. It is possible that having a large MO with more neurons can provide the opportunity for a quick response with a good imitation of the contact call. This could function as a way of courting a female or demonstrating superiority to the other individual and potential eavesdroppers in order to gain leadership after fusion of a flock. In a study by Walløe et al. [2015], there was no effect of sex on latency, but as we, in this study, have corrected for number of neurons, these results indicate the potential role of MO in behavioral capability. We acknowledge that not being able to distinguish the core and shell regions within MO, and using total number of neurons to describe the MO nucleus as one region, limits our interpretation of the current study. We speculate that the MO core and shell regions may play distinctive roles in behavioral learning capabilities in parrots, including the three species investigated in this study [Chakraborty et al., 2015]. However, our current study provides several advances in our knowledge required to guide future investigations of the role of the MO core and shell regions in behavioral learning in parrots. Hence, additional experiments on a wide variety of parrot species that differ in their communication abilities and relative sizes in core and shell regions will allow us to identify the specific functions of the MO region.

In summary, as in many other studies, we have used a correlative approach to infer a relationship between avian brain space and behavioral capability. However, although correlations can be informative about causation, they are not deterministic of the causal mechanism. Many factors may affect the evolutionary relationship between brain and behavior, but despite the small sample size and number of species (less than 1% of the known parrot species) in this study, we believe our findings provide a possible measure of an underlying mechanism involved in vocal speed and complexity control in parrots.

Statement of Ethics

The study of the parrots was performed following the guidelines provided by The Animal Experiments Inspectorate under the Ministry of Environment and Food of Denmark. At the time of the study, ethical approval was not required for the procedures according to national guidelines. Further, our caretaker team were all accredited by the Federation of European Laboratory Animal Science Associations (FELASA). Overall, this study was made in accordance with the rules for animal research at the Department of Biology and University of Copenhagen and consequently approved, no decision number was granted.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

S.W., T.D., B.P., and T.J.S.B. conceived the study. S.W., B.P., M.C., and E.D.J. analyzed the brain samples. S.W. analyzed the playback responses. T.J.S.B. and S.W. conducted the statistical analysis. S.W. wrote the manuscript assisted by T.J.S.B., E.D.J., M.C., and T.D., the other authors commented on the manuscript.

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