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5 Neuroanatomical and Behavioural Studies on Dominance Linearity in the Crow

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Summary

Mechanisms for dominance linearity as logic in social structure were investigated by (1) quantitative neuroanatomical comparison of pallial nucleic volumes between jungle crows and zebra finches, and by (2) examining the function of allopreening in group-housing crows. In experiment-1, proportions of the pallial nuclei to the entire telencephalon were compared between jungle crows and zebra finches. Ratios of those nuclei to basal ganglion volume were also compared between the two species. We found that proportions of pallial nuclei were relatively similar in the two species but major enlargement of crow brain in comparison to finch brain emerged in dorsal ‘association’ pallium, including mesopallium and nidopallium, based on the ratio index. In experiment-2, allopreening and agonistic interactions were recoded in a form of matrices and analyzed for examining the potential relationship between the two social interactions. We found no reciprocity between preeners and preenees but significant relationships between preeners and dominant individuals (i.e., preenees and subordinates). This result suggest the function of allopreening as dominance status signal which may contribute to maintain dominance linearity.

Introduction

Corvids are known to have sophisticated ‘logic’ which is suggested to be evolved based on the complex socio-ecology and the brain enlargement (de Waal & Tyack, 2003, Emery & Clayton, 2004). Their logical behaviours have been reported in both physical (Taylor et al., 2008) and social domain (Paz-y-Miño et al., 2004). Like other corvids, Japanese jungle crows (*Corvus macrorhynchos*) are also assumed to use social logics in their social lives. Indeed, they were found to form a strict and stable linearity in inter-individual dominance relationships (Izawa & Watanabe, 2008). However, little has been known what behavioural and brain mechanisms underlie the formation and/or maintenance of the linear dominance relationships. In the following studies, we tackled investigating its neuroanatomical basis by volumetric measurement of pallial nuclei and its behavioural basis, in terms of inter-individual social interactions, by an observational study on group-living crows.

Experiment-1: A volumetric analysis of crow ‘cortex’

Methods

Subject

Four wild-caught adult Japanese jungle crows (2 males and 2 females) and four adult zebra finches (*Taeniopygia guttata*; 2 males and 2 females) were used. Crows were provided from Tokyo Ueno Zoo (Permission No.698) and finches were obtained commercially from a local supplier.

Tissue preparation

Under deep anesthesia by an overdose injection of veterinary pentobarbital (Nembutal, 0.45-0.6 ml / crow, 0.3-0.4 ml / finch), birds were perfused with 0.9% saline with heparine (0.2 ml / 50 ml saline) followed by 4% paraformaldehyde in 0.1M PBS. After post-fixation over one night in the 4% paraformaldehyde, the brain was transferred into a 30% sucrose

solution for 4-5 days before sectioning. The brain then was sectioned in the transverse plane at 40- μ m thickness by using a cryostat. Every tenth or second section was mounted on gelatinized slides for crow or finch brain, respectively, and stained with cresyl violet for Nissle staining. Tissues were prepared only from left hemispheres.

Volume measurement of telencephalic nuclei

To evaluate the characteristics of the brain organization between jungle crows and zebra finches, we measured the entire volume of telencephalon and the following telencephalic nuclei; hippocampal formation (HP), hyperpallium apicale (HA), hyperpallium densocellulare (HD), mesopallium (M), nidopallium (N), entopallium (E), arcopallium (A), and basal ganglia (BG). Note that BG was comprised of medial stratum (MSt), lateral striatum (LSt) and globus pallidus (GP). Determination of these areas was followed to the brain atlas by Izawa and Watanabe (2007) for the crow and by Stokes et al. (1974) for the zebra finch.

Images of sections were digitized by using a scan scope (Aperio Technologies Inc., Vista), and the digitizer software Image-J (NIH, Bethesda). The area of a given brain region of every section was measured in square-millimeters scale based on the following formula:

$$t \cdot k \cdot \sum a,$$

where a = area (in square millimeters), t = thickness of sections (in millimeters), k = periodical distance between the sections.

Due to the shrinkage through the processes of tissue preparation, we corrected the calculated volume to estimate the actual volume of the fresh brain for each individual brain based on the percentage of shrinkage which was determined as the ratio of rostro-caudal telencephalic length between the fresh brain (i.e., immediately after the perfusion) and the value calculated from the summation of 40- μ m-thick sections for each brain sample.

Results and Discussion

We found that dorsal pallial nuclei such as HA, M, and N in the crow brain was significantly enlarged more than those in the finch brain. Volume of telencephalic nuclei and their proportion to the entire telencephalon were summarized in table 1. This table showed that proportion of telencephalic nuclei to the entire telencephalon was similar between crows and zebra finches. Overall trend that dorsal pallium such as HA, HD, M, and N occupied large proportion of telencephalon was commonly found in both crow and zebra finch brains. However, this ‘proportion’ comparison could be inappropriate to evaluate the degree of enlargement of a focal nucleus between different species because this comparison did not consider to offset the potential effect of larger occupation of one nucleus causing small occupation of another nucleus (Rehkämper et al., 1991). To offset this potential effect within pallial nuclei, we performed a standardization using the ‘ratio’ value of each pallial nucleus to the BG volume. This standardization comparison (fig. 1) showed that HA, M, and N of the crow were significantly larger than those of the zebra finch (two-tailed *t*-test, HA, $t_s = 8.28$, $p = 0.001$; M, $t_s = 7.10$, $p = 0.01$; N, $t_s = 7.39$, $p = 0.01$). HD and E of the crow were found to be smaller than those of the zebra finch (two-tailed *t*-test, HD, $t_s = -3.16$, $p = 0.02$; E, $t_s = -5.45$, $p = 0.03$). These results suggest that pallial association areas are enlarged in the crow brain in comparison to the zebra finch.

Our present study revealed the enlargement of dorsal pallial nuclei, such as M and N, in both crow and zebra finch brain in comparison to other pallial nuclei. This finding is the first quantitative evidence illustrating the enlargement of ‘association cortices’ in jungle crows. The present results are in good accordance with the previous brain-volumetric studies on other corvids such as jackdaws, rooks, carrion crows, (Volnov et al., 1994, Emery & Clayton, 2004) and New Caledonian crows (Cnotka et al., 2008). These studies including the present our own suggest that telencephalic enlargement is common traits in *Corvus* birds and may play a role to underlie their extraordinary cognitive abilities. Functional investigation on the crow brain at the next step is necessary to understand the common or different mechanisms for higher cognition between mammalian cortical structures and nucleic structures avian brain.

Table 1. Volume of telencephalic nuclei and their proportion to entire telencephalic volume of crows and finch brains

	<i>Tel</i>	<i>Hp</i>	<i>HA</i>	<i>HD</i>	<i>M</i>	<i>N</i>	<i>E</i>	<i>A</i>	<i>BG</i>
Mean volume (mm ³ , ±1SD)									
Jungle crow	4132.7	110.7	502.2	69.0	807.7	1989.4	23.0	127.0	413.6
(n=4)	563.8	13.5	54.7	13.1	100.5	287.2	5.7	17.8	53.8
Zebra finch	129.9	6.0	11.5	4.7	20.2	60.9	2.1	5.2	20.0
(n=3)	25.2	0.6	3.1	0.8	4.8	8.1	0.3	1.4	5.3
% to entire Tel (% , ±1SD)									
Jungle crow	100.0	2.7	12.2	1.7	19.6	48.1	0.6	3.1	10.0
(n=4)	0.0	0.5	0.7	0.3	1.1	1.1	0.1	0.4	0.8
Zebra finch	100.0	4.7	8.8	3.7	15.5	47.2	1.6	4.2	15.3
(n=3)	0.0	0.4	0.7	0.1	0.7	2.9	0.1	1.9	1.2

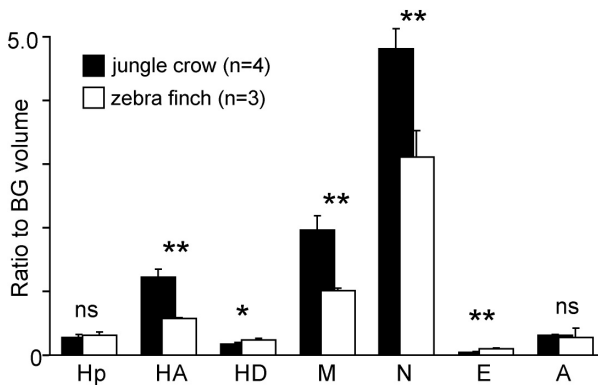


Figure 1. Volumetric comparison of pallial nuclei in the ratio to BG volume between jungle crows (black bar) and zebra finches (white bar). Dorsal 'association' pallial nuclei, including HA, M, and N, of crows were larger than those of zebra finches. Asterisks indicate significant difference at the level of 0.05 (single asterisk) and 0.01 (double asterisks). See text for abbreviations.

Experiment-2: Linearity of dominance relationships and the maintaining mechanism in a flock of crows

Methods

Animals and Housing Conditions

Eight jungle crows were used in this study (4 males, *W*, *Or*, *RY*, *WW*, and 4 females, *S*, *RB*, *G*, *Y*). Female *S* was captured at Mita campus of Keio University in May 2003 when she fledged, and hand-reared thereafter. The other seven birds, including four males and three females, were captured in the wild at Tokyo Ueno Zoo during January – March 2008. At the time of capturing, these seven crows were determined as the first year-round young adults according to their tongue coloration (Kitagawa, 1980). The birds were identified with colored rings on their legs, and their sex was determined by genetic analysis of blood DNA samples (Fridolfsson & Ellegren, 1999). In May 2008, these eight birds were housed together in an outside aviary (5 x 5 m and 3 m high). They received dog foods containing meat, fish, and vegetables, with supplemental foods such as dry fruits and cheese daily at 0900-1200. They always had access to fresh water.

Observations

Observational data analyzed for this study was obtained during mid July - end of September 2008. We conducted ad libitum sampling (Altmann, 1974) for 30 min daily at 1000-1200. Ad libitum observations were carried out in a total of 720 min. Observations were used notably to analyse instances of allopreening and agonistic interactions. Allopreening was defined as one bird passing its beak through the feathers of another bird. Behaviours categorized as agonistic interactions were summarized in table 2. We recorded the initiator and the recipient for each event of allopreening and agonistic interaction. Obtained data of allopreening and agonistic interactions was represented in a form of an initiator-recipient matrix and an aggression-submission matrix for the analysis.

Data analysis

We examined the function of allopreening by testing the three hypotheses, reciprocity and status signaling. To test these hypotheses, matrix correlation analyses were carried out with the Mantel Z test with 10 000 permutations and Pearson r correlation (Hemelrijk, 1990a, b). The reciprocity hypothesis considered reciprocal occurrence of allopreening between preeners (i.e., initiators) and preenees (i.e., recipients), and, thus, a correlation was expected between the preener-preenee matrix and the preenee-preener matrix. The second hypothesis, status signaling, considered the equivalent expression of social status (e.g. dominance) in the different types of social interactions like between allopreening and agonistic interactions, and, thus, the correlation was expected between the preener-preenee matrix and the aggression-submission matrix.

The dominance hierarchy was also determined using the MatMan software package version 1.0 (Noldus, Wageningen) based on all aggressive and submissive behaviours according to the behavioural category shown in table 2. If the significant linearity was found in dominance structure, I&SI method was performed to determine the dominance rank (see the detailed methods for de Vries, 1998).

Table 2. Behavioural category involved in agonistic interactions.

Aggression	
jab	pecking motion toward the recipient
peck	pecking at the recipient
approach	getting close to the recipient
Submission	
head droop	drooping head
retreat	stepping or shifting away from the initiator
begging call	begging call (see Izawa & Watanabe 2008)
fuzzy head	fluffing head feathers
avoid	flying away from the initiator

Results and Discussion

A total of 1029 agonistic interactions were observed (table 3). All these interactions involved only dyads but did not triads or more individuals at one time. We found a highly significant linearity of dominance structure in this group of crows ($h' = 0.833$, $\chi^2 = 43$, $df = 21$, $p = 0.003$). Dominance rank was also determined as $W > Or > RY > S > WW > RB > G > Y$, where W was the most dominant and Y was the most subordinate. Dominance relationships were more strict in males than in females. This was seen as more aggressive interactions in male-male dyads than in male-female or female-female dyads (one-way ANOVA, $F_{2,25} = 3.78$, $p < 0.05$, data from table 3), suggesting strict dominance relationship within males than within females in a flock of crows.

Table 3. Aggression and submission in agonistic interactions.

		Submission							
		<i>W</i>	<i>Or</i>	<i>RY</i>	<i>S</i>	<i>WW</i>	<i>RB</i>	<i>G</i>	<i>Y</i>
Aggression	<i>W</i>	-	153	85	18	64	192	59	50
	<i>Or</i>	3	-	42	7	15	87	44	16
	<i>RY</i>	8	4	-	4	3	7	14	31
	<i>S</i>	0	4	1	-	3	5	2	5
	<i>WW</i>	4	3	3	1	-	7	10	5
	<i>RB</i>	4	2	1	3	0	-	5	32
	<i>G</i>	0	2	0	2	1	3	-	1
	<i>Y</i>	2	3	2	0	1	5	1	-

A total of 1205 allopreening were observed. As shown in table 4, allopreening occurred in an asymmetrical fashion between birds. Over 83% of allopreening events were found in male-male dyads (Fisher's exact test, initiator/recipient \times male/female, $\chi^2 = 2213.3$, $df = 3$, $p = 0.0001$). Correlation analysis between preener-preene matrix and preene-preener matrix for testing the reciprocity hypothesis did not yielded significant correlation between matrices ($Z = 4562$, Pearson's $r = -0.03$, ns), suggesting no reciprocity of allopreening. Correlation analysis between preener-preene matrix and aggression-submission matrix for testing the

status-signaling hypothesis showed nearly significant correlation between matrices ($Z = 10723$, Pearson's $r = 0.28$, $p = 0.07$), suggesting that the direction of allopreening tends to be similar to that of dominance relationships. These results could support the status-signaling hypothesis.

Table 4. Matrix of allopreening in a flock of Jungle crows.

		Submission							
		<i>W</i>	<i>Or</i>	<i>RY</i>	<i>S</i>	<i>WW</i>	<i>RB</i>	<i>G</i>	<i>Y</i>
Initiator	<i>W</i>	-	212	52	0	406	20	2	4
	<i>Or</i>	0	-	167	0	122	1	28	0
	<i>RY</i>	0	6	-	0	7	0	1	0
	<i>S</i>	0	0	0	-	33	0	13	0
	<i>WW</i>	0	1	33	1	-	2	4	0
	<i>RB</i>	0	0	5	0	1	-	1	2
	<i>G</i>	0	1	3	64	7	0	-	2
	<i>Y</i>	0	0	2	0	2	0	0	-

Results in the experiment-2 clearly showed the status signaling function of allopreening behaviour in social corvids. In mammals, especially in old world primates such as baboons and macaques, allogrooming is generally known to occur typically from subordinate to dominant individuals as affiliative interaction interchangeable to the rescue in fighting by dominant groomees (Aureli & de Waal, 2000). However, this is not the case in jungle crows. The finding of asymmetrical occurrence of allopreening from dominants to subordinates in jungle crows indicated that allopreening could function to signal dominant status of preeners to preenees or even to the bystanders. Thus, allopreening in crows is not functionally equivalent to allogrooming but rather may correspond to mounting in old world primates. Verifying interchangeability of allopreening to other social interactions by analyzing more varieties of behavioural contexts could be important to understand what logics underlie the complexities of the crow society.

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