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Research report

Unusual brain composition in Crested Ducks (*Anas platyrhynchos* f.d.)—Including its effect on behavior and genetic transmission

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ABSTRACT

Crested Ducks (CR) occasionally show intracranial fat bodies. Additionally, behavioral abnormalities such as motor incoordination can be observed. Here, it is shown that a behavioral test helps to identify CR that have a problematical fat body. The ducks were put on their backs, and the time required for them to stand up was measured. Ten CR exhibited suboptimal motor coordination. The appropriateness of this test has been proved in a special breeding program. To investigate the influence of fat bodies on brain composition, an allometrical comparison of 26 CR brains with those of three uncrested duck breeds was done.

The fat bodies of CR varied from 0.3% to 41% of total brain volume, but two CR did not show a fat body. CR with motor incoordination show significantly larger fat bodies and require significantly more time in the test than "normal" CR. Total brain volume was significantly larger in CR, but brain volume minus fat body was significantly smaller compared to reference breeds. Cerebellum, apical hyperpallium, tegmentum and olfactory bulb are significantly reduced in CR. Obviously the behavioral deficits cannot be explained by the existence of a fat body, but they could be explained by functionally suboptimal cerebella and tegmenta. Fat body size seems to be a decisive factor. The relationship between fat body and reduced structures is discussed.

By breeding with test-selected ducks the hatching rate increased and the number of ducklings with malformations or motor incoordination decreased.

1. Introduction

During the course of domestication of ducks many breeds have been developed that show special features in size and/or behavior. These so-called fancy breeds are bred just for the pleasure of their breeder and mostly do not show high production of meat, eggs or feathers. Domestication can be seen as a selective process associated with a lot of alterations in the genome and greater variability in one or more characters in domestic animals in contrast to their wild ancestors [20]. Along with alterations in such characteristics as body size, coloring, habitat or behavior there may also be alterations in brain size and brain composition [e.g. 6,15] not only in comparison to wild animals but also in comparison to other breeds [23,21]. One example for this is the Crested Duck (*CR*, Fig. 1), a special breed of domestic ducks with a feather crest as its special characteristic.

The Crested Duck is a variety with high pre- and post-natal mortalities and malformations in skull and brain anatomy [1,3,10]. Additionally, intracranial fat accumulations have been found in a lot of Crested Ducks. Presumably, depending on its size and probably its position in relation to the brain, the fat body has a deleterious effect on behavior [5]. Occasionally motor incoordination can be observed (demonstrated by a tottering walk), and some animals are even unable to right themselves after having fallen on their backs. There is an assumption of a correlation between deficits in motor coordination and intracranial fat bodies but, until now, detailed examinations have been lacking.

To come to grips with these problems, a careful analysis of the observed problems, their possible origin and of the brains of Crested Ducks was necessary. Additionally, a behavioral test and a breeding strategy had to be developed that would allow a breeder to discover animals with large fat bodies even when the undesirable defect is not obvious and behavior in daily life looks quite normal. The goal is to eliminate detrimental traits from their breeding populations, assuming that the condition is heritable [16]. It has been demonstrated that the existence of a crest and its size is not a reliable predictor of the presence of a fat body or behavioral deficits [1,3,7,9,10,14] and thus, other pheno-

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Fig. 1. Habitus of a Crested Duck (CR).

typic measures must be identified that are associated with this trait.

It is the goal of the present paper to draw attention to a behavioral test that indicates motor coordination deficits. To prove the appropriateness of this test, individuals preselected by this test were used in a special breeding program. In parallel we have analyzed the brain size and brain composition of this breed in detail and analyzed whether individuals identified as malfunctional have a more or less prominent fat body inside their skulls. In contrast to a previous paper [4], we have enlarged the number of investigated specimens and have included the data from the breeding program to come to grips with the question about a genetic contribution to the observed peculiarities.

2. Material and methods

For the behavioral test and the brain analysis we investigated 26 adult CR (11 drakes, 15 ducks) from our own stock. The behavioral test was designed to check the motor abilities and challenge the system of motor coordination of CR. The idea for the 'righting test' test came from the fact that ducks with serious problems in motor coordination often fall on their backs and then are unable to right themselves and stand up. For this test, we placed all CR on their backs and measured the time they required to stand up on both feet. This procedure was repeated 13 times per animal over a period of 3 months. The average time was calculated for each individual.

Ten of the 26 investigated individuals showed motor incoordination such as staggering during moving or tumbling at least one time in their lives ("affected" CR hereafter). These ducks exhibited the symptoms in different ways but appeared otherwise quite normal in their daily lives. The deficits became apparent during stressful situations like when investigators were collecting or handling of the ducks. After such treatment, many of them recovered quickly and any deficits were inconspicuous again in daily life. The other 16 ducks had no detectable deficits in motor coordination ("normal" CR hereafter). On the basis of these general observations of their behavior, the following 'righting test' was employed on two distinguishable groups of ducks, "affected" CR ($n = 10$) and "normal" CR ($n = 16$).

For the brain and fat body analysis we compared the brains of these 26 CR with those of three other domestic duck breeds, called Pommeranian Ducks (*PO*, $n = 10$, 5 drakes/5 ducks), Abacot Ranger Ducks (*AR*, $n = 6$, 3 drakes/3 ducks) and "Hochbrutflugenten" (*HB*, $n = 10$, 5 drakes/5 ducks). All these breeds are uncrested and do not show, as far as is known, any peculiarities like those observed in CR. All specimens of were obtained from organized breeders and were at least one year old.

The animals were euthanased with an overdose of barbiturate. After cardiac arrest was confirmed they were weighed and then perfused with physiological saline solution to wash out the blood, followed by Bodian's fluid to fix the brain. The brains were carefully dissected, weighed and after embedding in paraffin they were cut into 20 μm thick coronal sections. A defined number of the sections were stained for perikarya using a silver technique [11]. Total brain volume, volume of the fat body and the volume of 14 distinct areas of the brain (hyperpallium apicale, hyperpallium densocellulare, mesopallium, nidopallium, globus pallidus/lateral striatum, septum, hippocampus, area prepiriformis, bulbus olfactorius, tegmentum, cerebellum, tectum, tractus opticus, diencephalon) were determined. For volume measurements the contours of brain structures were drawn using a digital pen and a camera lucida. To arrive at the fresh volume, the resulting area values were multiplied by the section thickness and the distance between the sections. Because the brain shrinks considerably during histological processing, there are differences between the measured volume in serial sections and the fresh brain volume. The extent of shrinkage is different in each brain. To obtain comparable values, each (structural) volume was multiplied by the conversion factor for shrinkage (C), where C = volume fresh brain/sum of serial section volumes.

To compare volumes of brain structures in different breeds with different body sizes, allometric methods were used. The relationship between volume of brain, fat body or brain structures and body weight is represented best by the formula:

$$\log y = \log b + a \times \log x$$

where y represent the brain or brain structure size, b the intercept of the allometric line with the abscissa, x the body weight and a the slope of the line [25]. A regression line was calculated for all data points of the CR and the reference breeds. For statistical analysis of the differences in brain structure volumes between the CR and the pooled reference breeds allometric size indices (SI) were calculated expressing the distance of individual data points from the regression line:

$$\mathrm{SI} = \left(\frac{\text{actual brain(structure)size}}{\text{expected brain(structure)size}}\right) \times 100$$

The expected brain (or brain structure) size is the value on the regression line that corresponds to a given individual body weight.

A detailed description of these methods for preparation, measurement and calculation is given in the literature [4,8,17,18,25].

Altogether 3 years of breeding have been completed. In the first year, three breeding couples were chosen from our own stock without any special selection. In the following 2 years the breeding couples (five in the second year and three in the third year) were preselected by the 'righting test'. Only the individuals with the shortest average righting times were chosen for breeding. All breeding couples were housed in a separate aviary with their own swimming facility. The eggs were collected every day and hatched by a common incubator. The ducklings were reared all together in a separate aviary with good observation conditions. Eggs that did not hatch were opened 3 days after the end of hatching time and the habitus and skull of extant embryos were investigated. Thus, we investigated fertilization rate, hatching rate, number of ducklings with skull malformations (perforations of the skull) and number of ducklings with behavioral deficits of these breeding couples.

All research was performed in accordance with the official German regulations for research on animals.

3. Results

The 'righting test' was employed on two distinguishable groups of ducks (see above): the 10 individuals with several observed deficits in motor coordination and the 16 individuals without any behavioral deficits.

In contrast to the "normal" ducks, the "affected" CR performed poorly. On average they required 13.4 s ± 6.93 (mean ± standard deviation) with a range from 1.0 to 62.6 s for the 'righting test'. The "normal" CR required 1.4 s ± 0.20 s and ranged from 0.5 to 2.9 s. The average times of the two groups differ significantly (two-tailed t-test, $t = 2.21$; d.f. = 24; $p = 0.037$).

Affected CR show an average body weight of 2301 g ± 58.4 (ranged from 1725 to 2960 g) and an average brain size of 6855 mm^3 ± 209.1 (ranged from 5111 to 9547 mm^3). Normal CR ducks show an average body weight of 2300 g ± 81.7 (ranged from 1825 to 2960 g) and an average brain size of 6659 mm^3 ± 224.85 (ranged from 5111 to 8060 mm^3). Two (normal) CR ducks from the examined duck population did not show a fat body, the volumes of the fat bodies of the other 24 CR ducks varied from 19 to 3891 mm^3 or from 0.3% to 41% of total brain volume. The fat body size of the two groups (consisting of 10 "affected" and 16 "normal" ducks determined by general observations) differed significantly. "Affected" ducks show a significantly larger fat body (1512.2 mm^3 ± 371.29) than "normal" ducks (637.3 mm^3 ± 137.46; two-tailed t-test, $t = 2.583$, d.f. = 24, $p = 0.016$). Most frequently there are middle-sized fat bodies positioned in the tentorium cerebelli (Fig. 2). Additionally, the fat bodies often extend rostrally between the two hemispheres (Fig. 3). There was no significant correlation between the fat body size and the latency to right (Pearson product moment correlation, $r = 0.278$, $p = 0.169$)

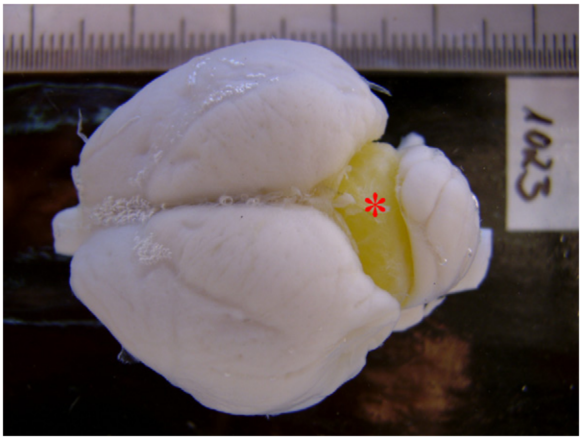

Fig. 2. Dorsal view of a Crested Duck brain with incorporated middle-sized fat body (*).

The allometric comparison of the brain and brain structure volume in relationship to the body weight of all ducks lead to the following results (Table 1): CR individuals (n = 26) exhibit a significantly larger total brain volume in comparison to the pooled reference ducks (n = 26, two-tailed t-test, t = 2.651, d.f. = 50, p = 0.011). The total brain volume included, if present, the volume of the fat body. However, net brain volume (total brain volume minus fat body volume and ventricular volume) of CR is significantly smaller in an allometric comparison to the reference breeds (t = −2.325, d.f. = 50, p = 0.024). Among the 14 brain structures delineated, 10 were not reduced in CR compared to reference breeds. Relatively reduced in comparison to the reference group were cerebellum, tegmentum, apical hyperpallium and olfactory bulb (cerebellum: t = −2.882, d.f. = 50, p = 0.006; tegmentum: t = −2.567, d.f. = 50, p = 0.013; apical hyperpallium: t = −2.056, d.f. = 50, p = 0.045; olfactory bulb: t = −4.392, d.f. = 48, p < 0.001). An expected significant positive correlation between the fat body volume and the total brain volume of CR was observed (Fig. 4, Pearson product moment correlation, r = 0.908, n = 26, p < 0.001). There were no significant correlations between the fat body size and the net brain (total brain volume minus fat body volume and ventricular volume, r = −0.271, p = 0.18) or the reduced structures cerebellum (r = −0.142, p = 0.49), tegmentum (r = −0.0147, p = 0.949), bulbus olfactorius (r = −0.0759, p = 0.725) or hyperpallium apicale (r = −0.174, p = 0.394).

The fertilization rate of all pooled breeding couples in all 3 years amounted to 96%. There are no significant differences between the years. The average hatching rate of the (unselected) breeding cou-

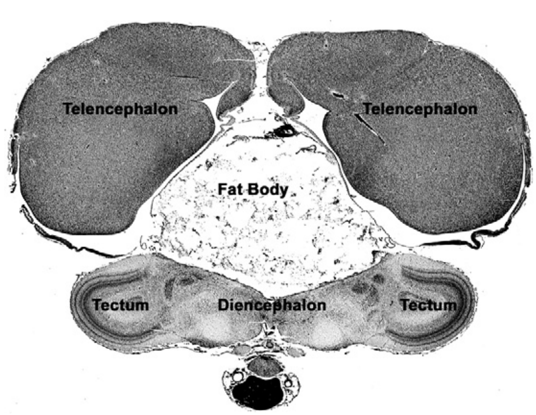

Fig. 3. Coronal section through the brain of a Crested Duck with an intracranial fat body.

Table 1
Allometric size indices of the brain and different brain structures in four breeds of domestic ducks (HB = "Hochbrutflugenten", PO = Pommeranian Ducks, AR = Abacot Ranger Ducks

Structure	HB + PO + AR (n = 26)	Crested Ducks (n = 26)
Total brain volume		
a = 0.35, b = 2.63	97.98 ± 1.32	107.26 ± 3.22*
Net brain volume		
a = 0.17, b = 3.18	102.72 ± 1.53	97.49 ± 1.64*
Hyperpallium apicale		
a = 0.21, b = 2.02	104.98 ± 2.91	96.87 ± 2.66*
Hyperpallium densocellulare		
a = 0.19, b = 1.57	101.63 ± 2.45	100.63 ± 2.34
Mesopallium		
a = 0.15, b = 2.23	101.42 ± 2.22	97.87 ± 1.92
Nidopallium		
a = 0.10, b = 2.87	103.11 ± 2.09	98.47 ± 2.3
Globus pallidus/lateral striatum		
a = 0.14, b = 2.22	101.44 ± 1.69	99.86 ± 3.03
Hippocampus		
a = 1.87, b = 6.58	101.05 ± 1.94	100.97 ± 4.27
Septum		
a = 0.21, b = 0.84	102.24 ± 1.69	99.21 ± 3.38
Area prepiriformis		
a = 0.28, b = 0.26	99.66 ± 2.68	103.28 ± 3.62
Bulbus olfactorius		
a = 0.12, b = 1.05	109.44 ± 3.14	92.49 ± 2.12***
Diencephalon		
a = 0.25, b = 1.62	102.33 ± 1.74	98.77 ± 1.98
Tractus opticus		
a = 0.15, b = 1.36	104.39 ± 2.33	97.98 ± 3.04
Tectum		
a = 0.11, b = 1.99	101.51 ± 2.07	99.23 ± 1.86
Tegmentum		
a = 0.13, b = 2.41	103.40 ± 1.85	97.38 ± 1.45*
Cerebellum		
a = 0.29, b = 1.82	104.89 ± 2.24	96.34 ± 1.94**

Values are means ± S.E.M.
* $p \leq 0.05$.
** $p \leq 0.01$.
*** $p \leq 0.001$.

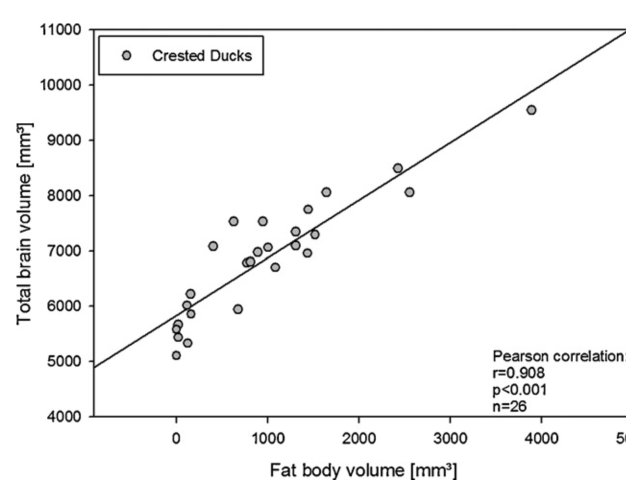

Fig. 4. Correlation analysis of total brain volume (mm^3) and fat body volume (mm^3) of 26 Crested Ducks.

ples of the first year amounted to 64% and was significantly different from that of the next 2 years. The hatching rate of the second year amounted to 86% and of the third year to 86% as well (first year vs. second year: $\chi^2 = 8.97$, $z = 2.995$, $p = 0.003$; first year vs. third year: $\chi^2 = 6.248$, $z = 2.5$, $p = 0.012$). The number of ducklings with malformations of the skull decreased also significantly in the course of the 3 years from 42% in the first year to 23% in the second year and to 10% in the third year (first year vs. second year: $\chi^2 = 9.498$, $z = 3.082$, $p = 0.002$; first year vs. third year: $\chi^2 = 15.713$, $z = 3.964$, $p < 0.001$). The number of ducklings with behavioral deficits decreased as well, but there were no significant differences (first year: 25%; second year: 19%; third year: 17%).

4. Discussion

In our study we could determine "affected" ducks by behavioral observations. But there is the assumption that there are a lot of CR with large fat bodies that seem to have no effect in motor coordination in daily life. Nevertheless, these individuals bear the risk of passing a negative genetic disposition to the next generation. Now, these animals can also be selected by applying the test presented here. None of these animals should be used for breeding.

It is reasonable to suggest a high correlation between fat body size and latency to right. However, our data set does not reveal a significant correlation. But the other results show, that the proposed 'righting test' is able to filter those individuals out of a population that had only slight difficulties in behavior but exhibit large fat bodies and presumably a tendency for motor incoordination. The breeding results demonstrate the appropriateness of the test and indicate that genetic transmission may play a key role. This is in agreement with studies where a genetic background of these peculiarities has been assumed [16,24]. Thus, the test might help to reduce the proportion of individuals with suboptimal brains and deficits in motor coordination.

By analyzing the brain of CR in detail, we observed that an intracranial fat body seems to be a typical neuromorphological peculiarity in Crested Ducks because 92% of our Crested Ducks show such a fat body. The intracranial fat body increases total brain volume and decreases net brain volume. The increased total brain volume is associated with the fat body. Not the existence but the size of these fat bodies seems to be a decisive factor for brain composition and behavior. The significantly smaller brains in CR are due to a reduction in size of several brain parts including the apicale hyperpallium and olfactory bulb. These results are in accordance with a previous paper [4] and confirm the previous results using a larger number of animals. The olfactory bulb is strictly associated with olfaction, and the apical hyperpallium is a multimodal integration center that serves cognitive functions and is part of the visual system [19]. The tegmentum and cerebellum are reduced as well and these findings would be in line with the observed behavioral deficits. The cerebellum can be regarded as a center of motor coordination [12], and also the tegmentum bears many structures that serve motor control [19]. Comparative neuromorphometry has elucidated that there is a correlation between the size of a brain part and how well it functions [2,13,22] and hence suboptimal functioning of reduced structures is quite feasible.

Apparently, the presence of the fat body reduces the volume of structures near its common position as opposed to those more distant. The fat body could reduce the size of structures just because it occupies space, giving them no place to develop, or it is possible that some substance of fat origin could influence neural development directly.

An oversized fat body and degenerated brain structures appear to be the origin of dysfunctions and should be eliminated. Our breeding experiments suggest a relatively high degree of heritability because only few generations are needed to increase hatching rates and decrease the percentage of malformations that are found in CR.

Doubtlessly the fat body is principally a negative peculiarity. However, there are open questions since there are individuals having a fat body without showing motor incoordination. The reason for that could be investigated in the future. It might be of heuristic value for human brain diseases since there is a similarity between these fat bodies and lipomas found in human [1].

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