

# Smaller brains in laying hens: New insights into the influence of pure breeding and housing conditions on brain size and brain composition

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**ABSTRACT** During domestication, many different chicken breeds have been developed that show many alterations compared with their wild ancestors and large variability in parameters such as body size, coloring, behavior, and even brain morphology. Among the breeds, one can differentiate between commercial and noncommercial strains, and commercial strains do not usually show variability as high as noncommercial breeds but exhibit a high production rate of eggs (or meat). The breeding of high-performing laying hens, including the housing conditions of hens, is often a focus of concern for animal welfare, and to date, little is known about the correlation between housing conditions and artificial selection on brain structure.

Based on an allometric approach, we compared the relative brain sizes of 2 inbred strains of laying hens (WLA and R11) with those of 7 other noncommercial chicken breeds. In addition, we examined the brain composition of laying hens and analyzed the relative sizes

of the telencephalon, hippocampus, tectum opticum, and cerebellum. Half of WLA and R11 lines were kept in floor-housing systems, and the other half were kept in a single cage-housing system.

Both strains of laying hens showed significantly smaller brains than the other chicken breeds. In addition, there was a significant difference between WLA and R11 hens, with R11 hens having larger brains. There was no difference in the relative brain sizes of floor-housed and cage-housed hens. WLA and R11 hens did not differ in their brain composition, but floor-housed hens showed a significantly larger cerebellum than cage-housed hens.

Apparently, pure breeding over a long time and strong artificial selection for a high production of eggs is accompanied by (unintentional) selection for smaller brains. Further studies may also reveal differences in brain composition and the influence of housing conditions on brain composition.

**Key words:** laying hen, brain size, brain composition, cerebellum, housing condition

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## INTRODUCTION

The domestication of animals is a recent event in human history and is defined as the condition wherein the breeding, care, and feeding of animals are more or less controlled by humans (Hale, 1969). Domestication is associated with several alterations and higher variability in many traits in domestic animals than their wild ancestors (Price, 1999). In every domesticated species, a variety of different breeds have developed. Breed-specific differences have been described since the beginning of domestication and the development of different breeds. In addition to breed-

specific alterations in, for example, body size, coloring, or behavior, alterations in brain size or brain composition have also been observed (Rehkämper et al., 2003; 2008). Most empirical data on brain sizes show smaller brains in domestic animals than in their wild ancestors, which led to the so-called “regression hypothesis” (Hemmer, 1990). This hypothesis claims domestication as “the decline of environmental appreciation.” In contrast to the “regression hypothesis” is the “adaptation hypothesis,” which states that domestication is a dynamic evolutionary process and that all alterations during domestication are of an adaptive character and are correlated with the conditions of a man-made environment (Hafez, 1968; Rehkämper et al., 1988, 2008). Thus, it would be more suitable to define domestication as an adaptation to a man-made environment via population genetic mechanisms in which natural selection is largely replaced by artificial selection (Sossinka, 1982). Clutton-Brock (1981) defines a breed as a group of animals from

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one species with typically heritable traits. Such a breed is a product of man's selection.

Among the population of domesticated animals, one can generally differentiate between economical strains and strains that are bred just for the pleasure of their breeder. The latter group, the so-called fancy or noncommercial breeds, do not generally produce high volumes of meat, milk, wool, or eggs but show numerous differences compared with their wild ancestors. This is the same in economical or commercial strains but with lower variability in phenotypes and genotypes due to more pure breeding and, for example, a high milk, meat, or egg production as a major breeding target (Tixier-Boichard et al., 2007).

As mentioned previously, a specific phenomenon of domestication is a reduction in brain size from wild to domestic animals. This decrease is not uniform but varies in different species and in different brain parts. As observed for domestic mammals (Kruska, 1980; Ebinger, 1984), brains of highly encephalized bird species (birds with large brains in relation to the body size) are more affected by domestication than those of species with normally smaller brains (e.g., birds of the duck or pigeon family show a low, but different, level of cephalization; Senglaub, 1960). In addition, the time of domestication and the intensity of breeding or housing play a role in brain alterations in domestic animals. Nevertheless, it has to be mentioned that a reduction in brain (parts) size does not necessarily reflect information about the cognitive abilities of these birds (Rogers, 1995).

Domestic fowl, or chickens, are kept throughout the world. They are the most widely used of all poultry species. Similar to other domesticated species, there are commercial and noncommercial breeds, and commercial chicken breeds are divided into layer lines, meat lines, and dual-purpose lines. In commercial strains, (genetic) variability is low, whereas in noncommercial strains, there is higher genetic, morphological, and behavioral variability. Breeds can have alterations in feathering (curly feathers, feather crests, foot feathering, and the prolongation of tail feathers), in morphological features (dwarfism, gigantism, shortened legs, rumplessness, an absence of or increase in the comb), or in behavioral peculiarities (fighting/game breeds or breeds with special crowing behavior) (Wood-Gush, 1959; Mehlhorn and Rehkämper, 2013). In addition to all these alterations, domestic chicken breeds show large variability in brain morphology (Rehkämper et al., 2003).

As mentioned previously, commercial chicken strains show lower variability in several features. In layer lines, pure breeding, or even inbreeding, over a long period of time (e.g., with the intention of increasing egg production) has led to strains with laying performances of approximately 320 eggs per yr. Generally, these strains all originate from a small parental generation. It is well established that inbreeding deteriorates the physiological and reproductive performance of most organisms and reduces fitness in general (Charlesworth and Willis, 2009). Despite a long-standing research tradition, many crucial aspects of pure breeding or inbreeding have

been poorly investigated, for example, its effect on brain morphology.

In recent years, several studies have shown an influence of housing system on spatial learning ability behavior and levels of working memory in laying hens (Krause et al., 2006; Tahamtani et al., 2015; Campbell et al., 2018; Dudde et al., 2018). In addition, different housing environments can also be accompanied by variations in the brains of laying hens, such as in the hippocampus and the nidopallium caudolaterale (Patzke et al., 2009). However, to date, nothing is known about the effect of housing conditions on brain size or general brain morphology.

The aim of this study was to compare the brain sizes of 2 typical lines of white laying hens (WLA and R11) with the brain sizes of noncommercial breeds. WLA is a high-performing line that lays approximately 320 eggs per yr. R11 is a low-performing line with an average laying performance of 200 eggs per yr. Because environmental complexity affects the morphology of the central nervous system (Diamond, 2001; Mohammed et al., 2002; Cnotka et al., 2008; Mehlhorn et al., 2010), individuals of both strains were kept in 2 different housing systems (floor housing or cage housing).

As a preliminary study, we also examined the brain composition of a portion of our WLA and R11 laying hens and took a first look at the possible influence of strain and housing conditions on brain composition.

## MATERIALS AND METHODS

### Breeds

We examined 2 closely related pure-bred lines of white laying hens (*Gallus gallus* f.d.), the high-performing WLA (Lohmann Tierzucht GmbH, Cuxhaven, Germany) and the low-performing R11 (Friedrich-Loeffler-Institut, Institute of Farm Animal Genetics, Mariensee, Germany). Body weight measures and the brains of 36 hens (18 WLA, 18 R11) were collected and compared with the body and brain data of hens of 7 other domestic chicken breeds ( $n = 33$ ) from our existing brain collection. These "fancy" breeds are well defined according to the German Standard of Perfection (Deutscher Rassegeflügelstandard) and cover a large range of major groups of domestic chickens: Bantams (Japanese Bantams, Peking Bantams), Mediterranean chickens (Brown Leghorns), Crested chickens (Bredas), chickens originally used as game birds (Malay), and those with a peculiar phenotype, such as tailless Araucanas and Silky chickens with silky feathers.

Individuals of fancy breeds originated from private breeders and were extensively kept in aviaries or gardens and in small groups with free-ranging possibilities. All hens were at least 6 mo old. All WLA and R11 hens were hatched on the same day and were raised together in a floor housing system (compartments of 24 m<sup>2</sup> with 2 wooden ladders as perches and wood shavings and straw as litter) until 16 wks of

age. Food (wk 1–7: 12.97 MJ AMEN/kg DM, 189.61 g/kg crude protein, 31.38 g/kg crude fat, 9.14 g/kg Ca, 6.94 g/kg P; wk 8–16: 12.82 MJ AMEN/kg DM, 151.67 g/kg crude protein, 30.21 g/kg crude fat, 15.83 g/kg Ca, 8.11 g/kg P) and water were provided ad libitum. On the first 2 D of life, light was provided for 24 h before it was reduced to 15 h on D 3. From wk 1, light period was reduced to 9 h and in wk 7 by 1 h per wk and maintained until the end of rearing (wk 16 of age). At 16 wks of age, 9 individuals of WLA and 9 individuals of R11 were randomly chosen and moved into conventional single cages (50 cm × 46 cm × 43 cm). Each cage was equipped with a food trough, 2 drinking nipples, and a perch. The rest of the individuals were moved into floor housing compartments (2 m × 2 m) in groups of 15 hens per line. Compartments were littered with wood shavings and were equipped with perches and nests mounted on an elevated slatted floor 0.5 m above the littered area. The remaining 6 were used in another study. In both housing systems, animals had ad libitum access to food (11.68 MJ AMEN/kg DM, 168.11 g/kg crude protein, 29.43 g/kg crude fat, 50.05 g/kg Ca, 5.06 g/kg P) and water. From 16th to 23rd wk of age, the light period was increased in steps of 30 min from 9 h to 14 h and stayed constant for the rest of laying phase. Both housing conditions were in the same room, and the temperature was kept between 18°C and 25°C. At the age of 72 wks from each pen, 9 individuals were selected randomly and euthanized for the brain dissection.

### Brain Size and Brain Composition

All WLA and R11 hens were anesthetized with Isoflurane (WDT, Garbsen, Germany). Because we needed the body weights for the allometric approach, hens were weighed after anesthetizing. After this, the vena jugularis was opened for bleeding out. The brains were removed immediately, weighed, and embedded in Bodian's fluid for fixation (Romeis, 1989). All birds of fancy poultry breeds were euthanized with an overdose of pentobarbital and weighed. After cardiac arrest was confirmed, they were perfused with physiological saline solution to wash out the blood, followed by Bodian's fluid to fix the brain. The brains were removed immediately after the perfusion process to ensure that they were not significantly different from fresh brain weights (Stephan et al., 1988). Thus, a correction for shrinkage due to fixation was not necessary.

In addition, brains of R11 and WLA hens were embedded in paraffin, and 17 of them (WLA n = 9, R11 n = 8) were serially sectioned (20 µm) on the coronal plane. Every fifth section was mounted and stained for perikarya using the silver technique (Gallyas, 1971).

### Analyses-Brain Size

Because brain weight scales allometrically with body weight (Snell, 1892; Dubois, 1897; Harvey 1988;

Striedter, 2005), we used allometric methods. The relationship between the brain and body weights is represented best by the following allometric formula:

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

where  $y$  represents the brain weight,  $b$  is the intercept of the allometric regression with the abscissa,  $x$  is the body weight, and  $a$  is the slope of the regression (Snell, 1892). To obtain reliable slopes, the data should originate from a sample that covers a reasonable body weight range and whose individual members are part of a biologically significant group, for example, a taxonomic unit. Both criteria are given with the inclusion of all 69 investigated individuals. There is a reasonable body weight range, and all individuals belong to the taxon *Gallus gallus*. This approach allowed us to test whether the brain is larger or smaller in laying hens than that in other domestic chicken breeds independently of the different body sizes.

We compared fresh brain weight of WLA and R11 to that of all other chickens by carrying out a regression analysis and calculating allometric encephalisation indices (E). To calculate these indices, we divided the actual brain weight of an individual by its predicted brain mass obtained from the regression (Stephan et al., 1986). All points on the regression line represent an E of 1.0, so an E of 2.0 would mean that a brain was twice as heavy as the predicted weight based on the data.

To determine differences in the whole data set, we first applied one-way ANOVA. To determine differences between the 2 lines R11 and WLA and the 2 housing conditions, we first applied a 2-way ANOVA. In the case of significance, we used Student  $t$  test to compare encephalisation indices and the Mann-Whitney U rank sum test in cases of nonnormal distribution. The level of significance was 5%. The software package SigmaPlot/SigmaStat version 12.0 (Systat Software Inc., San Jose, CA) was used for all statistical calculations.

### Analyses-Brain Composition

In a second step, the stained sections of WLA and R11 brains were scanned for digitalization using a microscope camera AxioCam 506 mono (Zeiss, Oberkochen, Germany). We determined the relative size of the telencephalon, hippocampus, tectum, and cerebellum by delineating them in mm<sup>2</sup> using the software ZEN2 (Zeiss, Oberkochen, Germany). We decided to focus on the total telencephalon because it is the center of complex behavior (including social behavior) and receives input from all sensory systems; the hippocampus because of its important role in spatial cognition and behavior; the optic tectum as, at least in birds, the major structure for processing visual information; and the cerebellum as the center for motor coordination (Ito, 1984; Güntürkün, 1991; Bingmann, 1993; Nieuwenhuys et al., 1998; Nadel and Hardt, 2004).

To exclude the body weight differences between WLA and R11, we took the ratio of the values of our regions of

interest (ROIs) to whole brain measures. Because there were many lost and damaged sections, we selected 5 different atlas levels for each ROI to represent it from its anterior to its posterior extension (Karten and Hodos, 1967). For the telencephalon, we analyzed atlas levels ( $\pm 500 \mu\text{m}$ ) A12.75, A10.25, A8.00, A5.75, and A4.00; for the hippocampus, we analyzed A10.00, A8.75, A7.25, A6.00, and A4.00; for the optic tectum, we analyzed A6.00, A4.25, A3.00, A1.50, and A0.75; and for the cerebellum, we analyzed A3.75, A2.00, AP0.00, P2.00, and P3.50. Thus, we acquired value percentages that represented the relationship between the ROI and the whole brain for each specific section at the atlas level. Finally, we compared WLA and R11 and cage-housed individuals vs. floor-housed individuals. Because of the small number of animals and the limited number of measurable sections, we pooled the data of floor-housed WLA and R11 and cage-housed WLA and R11 for comparisons. The values of ROIs were averaged and compared using Student *t* test (level of significance was 5%). The brain regions and atlas levels for all sections were identified and named in accordance with “The stereotaxic atlas of the brain of the pigeon” (Karten and Hodos, 1967) and “The chick brain in stereotaxic coordinates” (Puelles et al., 2007). We used the nomenclature recommended by the “Avian Brain Nomenclature Forum” (Reiner et al., 2004). The original research reported herein was performed under the guidelines of German law to prevent cruelty to animals and was approved by the Lower Saxony State Office for Consumer Protection and Food Safety (no.: 33.9–42,502-05–10A079).

## RESULTS

Average body weights, brain masses, and allometric size indices in the laying lines WLA and R11 and 7 other domestic chicken breeds are given in Table 1.

One-way ANOVA of encephalization indices showed a significant difference between chicken breeds ( $F = 16.84$ ,  $df = 8$ ,  $P < 0.001$ ). Single comparisons revealed that both WLA and R11 had significantly smaller encephalization indices for brain mass than the pooled data for the

other chicken breeds (Figure 1; WLA:  $t = -6.565$ ,  $df = 49$ ,  $P < 0.001$ ; R11:  $t = -4.855$ ,  $df = 48$ ,  $P < 0.001$ ).

Two-way ANOVA of encephalization indices of R11 and WLA including the housing condition as the second main effect showed a significant difference in the mean values among the 2 different lines after allowing for effects of differences in the housing condition ( $F = 7.524$ ,  $df = 10$ ,  $P = 0.010$ ). The comparison for the factor “housing condition” was not statistically significant ( $F = 0.0683$ ,  $df = 10$ ,  $P = 0.796$ ). The single comparisons showed that R11 have significantly larger encephalization indices than WLA (Figure 2,  $t = 2.694$ ,  $df = 39$ ,  $P = 0.011$ ).

There was no significant difference between cage-housed and floor-housed hens, either within the lines (WLA:  $t = -0.153$ ,  $df = 16$ ,  $P = 0.880$ ; R11:  $t = -0.269$ ,  $df = 16$ ,  $P = 0.791$ ) or between pooled WLA and R11 cage-housed hens and pooled WLA and R11 floor-housed hens ( $t = -0.528$ ,  $df = 34$ ,  $P = 0.601$ ).

Comparisons between the averaged relative brain structure size of different atlas levels (Table 2) revealed no significant differences between telencephalon, hippocampus, optic tectum, and cerebellum sizes in WLA and R11 (Figure 3; telencephalon:  $t = -1.119$ ,  $df = 12$ ,  $P = 0.285$ ; optic tectum:  $t = -0.532$ ,  $df = 15$ ,  $P = 0.603$ ; hippocampus:  $t = 0.225$ ,  $df = 10$ ,  $P = 0.827$ ; cerebellum:  $t = -0.177$ ,  $df = 13$ ,  $P = 0.863$ ).

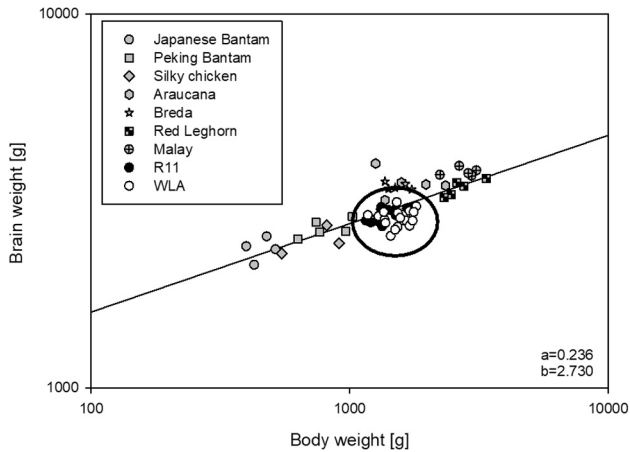
Comparisons between averaged relative brain structure sizes in floor-housed hens vs. cage-housed hens showed a significant difference in the relative cerebellum size (Figure 4;  $df = 10$ ,  $t = 2.412$ ,  $P = 0.037$ ). The other investigated structures did not show significant differences between floor-housed and cage-housed hens (Table 2; telencephalon:  $t = -0.975$ ,  $df = 12$ ,  $P = 0.349$ ; optic tectum:  $t = 0.773$ ,  $df = 11$ ,  $P = 0.456$ ; hippocampus:  $t = 0.368$ ,  $df = 8$ ,  $P = 0.722$ ).

## DISCUSSION

By analyzing the relative brain size of 2 lines of laying hens and comparing it to that of other chicken breeds, we observed that laying hens have significantly relative smaller brains. This relative decrease in brain size

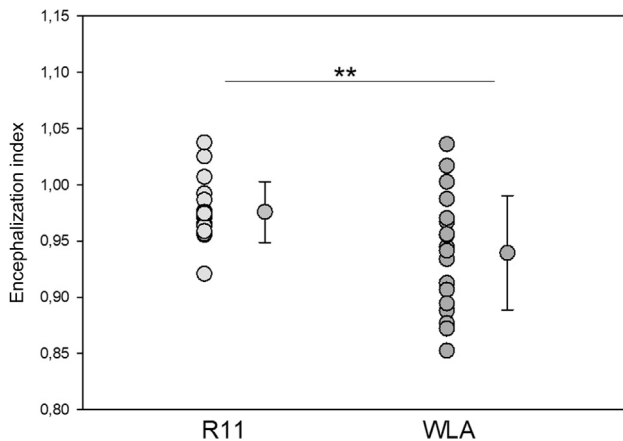
**Table 1.** Breed-typical means  $\pm$  SD of body weight (g), fresh brain weight (g), and calculated encephalisation indices.

Breed	Body weight (g)	Brain weight (g)	Encephalization index
Japanese Bantam (n = 4)	454.43 ( $\pm$ 52.54)	2.44 ( $\pm$ 1.74)	1.035 ( $\pm$ 0.07)
Peking Bantam (n = 5)	825.00 ( $\pm$ 164.66)	2.77 ( $\pm$ 1.52)	1.024 ( $\pm$ 0.04)
Silky chicken (n = 3)	757.33 ( $\pm$ 188.96)	2.57 ( $\pm$ 2.31)	1.024 ( $\pm$ 0.04)
Araucana (n = 5)	1,708.00 ( $\pm$ 450.98)	3.54 ( $\pm$ 1.50)	1.074 ( $\pm$ 0.05)
Breda (n = 5)	1,533.20 ( $\pm$ 155.36)	3.58 ( $\pm$ 0.76)	1.142 ( $\pm$ 0.04)
Red Leghorn (n = 5)	2,707.00 ( $\pm$ 410.91)	3.55 ( $\pm$ 1.74)	0.991 ( $\pm$ 0.03)
Malay (n = 5)	2,763.00 ( $\pm$ 334.94)	3.92 ( $\pm$ 0.98)	1.086 ( $\pm$ 0.04)
R11 all hens (n = 18)	1,390.39 ( $\pm$ 171.07)	2.99 ( $\pm$ 0.12)	0.976 ( $\pm$ 0.03)
R11 floor-housed (n = 8)	1,414.88 ( $\pm$ 220.36)	3.00 ( $\pm$ 0.14)	0.974 ( $\pm$ 0.03)
R11 cage-housed (n = 10)	1,370.80 ( $\pm$ 128.64)	2.99 ( $\pm$ 0.11)	0.977 ( $\pm$ 0.02)
WLA all hens (n = 18)	1,540.69 ( $\pm$ 174.20)	2.95 ( $\pm$ 0.15)	0.939 ( $\pm$ 0.05)
WLA floor-housed (n = 9)	1,594.80 ( $\pm$ 194.70)	2.96 ( $\pm$ 0.19)	0.935 ( $\pm$ 0.06)
WLA cage-housed (n = 9)	1,480.58 ( $\pm$ 133.82)	2.94 ( $\pm$ 0.09)	0.939 ( $\pm$ 0.04)

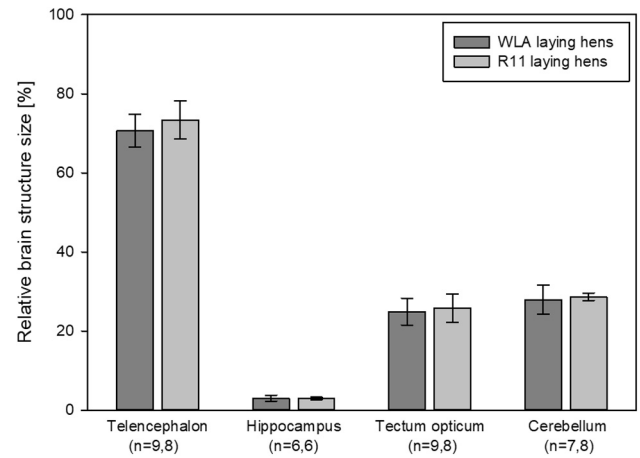


**Figure 1.** Fresh brain weight (g) of 2 lines of laying hens (WLA and R11) and 7 domestic chicken breeds in relation to body weight (g). Allometric size indices are given in Table 1.

applies for both WLA and R11 laying hens. Apparently, pure breeding over a long period of time and strong selection for one physiological feature, a high laying rate in this case, can be accompanied by a reduction in relative brain size. Generally, most domesticated animals show a decreased relative brain size compared with that of their wild ancestors (see Introduction), and of course it cannot be excluded completely that this decrease is caused by an increase in body size. But it seems to be that the brain sizes of these 2 laying lines are at the low end of the brain size scale among the investigated chicken breeds and thus represent the result of a very intensive form of artificial selection. Both lines have been selected for their laying performance: WLA are so-called “high performer” that lay approximately 320 eggs per yr, and the R11 line is a low-performing line with an average laying performance of 200 eggs per yr (Lieboldt et al., 2015). The R11 line has not been bred further for high laying performance since the 1970s. Larger brains in R11 hens than in WLA hens may indicate that stronger selection for laying performance leads to a smaller brain. Selection for egg production seems to be associated with a decrease



**Figure 2.** Encephalization indices of the fresh brain weight of R11 and WLA laying hens (means  $\pm$  SD).  $**P = 0.011$ .



**Figure 3.** Relative brain structure size of WLA and R11 laying hens summarized and averaged of 5 different atlas levels (means  $\pm$  SD).

in the size of other organs, such as the brain, which is not primarily essential for high egg production.

Since the breeding of high-performing strains of laying hens began, the most common housing condition was either the single-cage system or the battery cage system. In addition, alternative housing systems such as floor housing or free-range housing arose approximately 25 yr ago. In particular, conventional caging systems have focused on animal welfare, and much research has focused on how chickens behave and perform in different housing systems. For example, conventional caging systems can restrict behavioral expression and increase the risk of skeletal degradation (Whitehead, 2004; Eusemann et al., 2018), whereas newer noncage (aviaries, barns [“floors”] or free-range systems) or furnished cage systems may increase behavioral anomalies, incidences of skeletal injuries, and mortality (Lay et al., 2011; Weeks et al., 2016). It appears that each system has unique challenges and that no housing system is ideal from a laying hen welfare perspective. Although environmental complexity increases behavioral opportunities, it also introduces difficulties in terms of disease and pest control. In addition, environmental complexity can evoke behavioral patterns that may be detrimental to hen welfare. Meanwhile, there have been changes in the thinking about animal welfare, and since the European ban on conventional battery cages for laying hens in 2012, housing systems such as the floor housing system, free-range system or, at least, cage-housing with enriched cages and more space have replaced the old battery cage system.

However, the aforementioned problems still exist; one reason could be that species-specific behaviors and basic behavioral needs, such as dust bathing or perching, were neglected during the selection and development of high-performing lines. These lines were bred for high egg production in a small cage without enrichment and without the requirement of well-developed motoric, sensory, or cognitive abilities, for example. The intense selection of chickens for production traits such as egg laying is thought to have caused undesirable side effects and changes in behavior. Trade-offs resulting from energy

**Table 2.** Relative brain structure size of 4 brain structures.

Breed	Telencephalon	Hippocampus	Tectum opticum	Cerebellum
R11	73.41 ( $\pm 4.86$ )	2.99 ( $\pm 0.33$ )	25.83 ( $\pm 3.62$ )	28.66 ( $\pm 0.93$ )
WLA	70.71 ( $\pm 4.17$ )	3.06 ( $\pm 0.76$ )	24.93 ( $\pm 3.40$ )	27.94 ( $\pm 3.69$ )
Floor-housed hens	70.28 ( $\pm 2.48$ )	3.24 ( $\pm 0.63$ )	25.83 ( $\pm 3.11$ )	30.08 ( $\pm 1.19$ )
Cage-housed hens	72.75 ( $\pm 3.26$ )	3.13 ( $\pm 0.24$ )	24.42 ( $\pm 3.45$ )	27.03 ( $\pm 2.62$ )

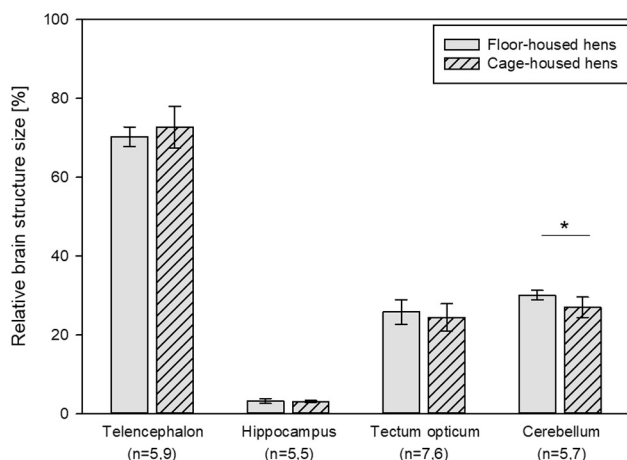
Values are means  $\pm$  SD of 5 different structure-specific atlas levels.

expenditure for productivity may influence other traits: To sustain energy costs for high egg production, energy expenditure may be redirected away from specific behavioral traits. For example, such energy trade-offs may change the hens' cognitive abilities (Dudde et al., 2018), which could manifest in smaller brain volumes. Here, we have shown that these lines have small brains and have suggested that, perhaps, this is one reason for the difficulty of adapting these hens to new housing systems in an appropriate manner, and it is possible that this brain size reduction has functional consequences. It is known that there is a positive correlation between brain (component) size and brain (component) function (Bennett and Harvey, 1985; Rehkämper et al., 1988; Iwaniuk and Hurd, 2005), and in particular, higher cognitive abilities correlate with larger relative brain size (Rehkämper et al., 1991; Lefebvre et al., 2002; Iwaniuk and Hurd, 2005). Selective breeding for specific housing systems or desired traits, such as improved bone strength and decreased feather pecking and cannibalism, may help to improve welfare (Kops et al., 2017; Lutz et al., 2017; Raymond et al., 2018; Riddle et al., 2018).

All hens were reared in floor-housed compartments until the age of 16 wks. At this point, the brain was already well developed, and thus, an influence of housing on total brain size was improbable. However, it is possible that the difference between brain sizes of noncommercial chicken breeds and WLA and R11 could be the result of different rearing and housing conditions. All investigated individuals of fancy breeds were reared

and housed in small groups and in extensive systems (aviaries, gardens, and so on). In addition, it is known that the avian brain shows a high level of plasticity during its whole life and is sensitive to different experiences (Clayton and Krebs, 1994; Cnotka et al., 2008; Mehlhorn et al., 2010; Herold et al., 2019). Thus, the influence of housing conditions on brain composition seems to be possible, unless the shared experiences in the first 16 wks exerted irreversible effects on the chicken brain, reducing adult plasticity. In fact, it has been shown that rearing conditions have major effects on behavioral development and social behavior (Rodenburg et al., 2008), but to date, little is known about the influence of rearing conditions on brain plasticity in adult hens. It is known that, for example, rearing with a foster hen can lead to morphological changes such as differences in cell soma size in the hippocampus of the adult hens' brain (Nordquist et al., 2013). Besides, rearing with a foster hen or dark breeder rearing have an influence on the hypothalamic dopaminergic and vasotinergetic system and the corticosterone level in feathers (Hewlett et al., 2014; Nordquist et al., 2020). But these findings describe rather an altered brain development and cannot be transferred to our hens because they were reared together 16 wks after hatching. Generally, these observations strengthen the notion that brain measures may be useful as potential readouts for animal welfare (Nordquist et al., 2013).

The cerebellum performs important tasks in the control of motor skills. It is responsible for the coordination, fine-tuning, unconscious planning, and learning of movement sequences. We found a smaller relative cerebellum size in cage-housed hens than that in floor-housed hens. Although we need more data, a clear tendency toward smaller cerebellum size in cage-housed hens cannot be denied. This finding could be explained by the limited amount of space in the cages and, thus, an unchallenged cerebellum. The cerebellum can be regarded as a center of motor coordination (Ito, 1984), and as mentioned previously, comparative neuromorphometry has elucidated that there is a correlation between the size of a brain part and how well it works. Hence, suboptimal functioning in smaller structures is quite feasible. The differences in the cerebellums of hens living in different housing systems support the idea that there are external parameters that influence brain composition and that brain morphology is correlated with individual life history and not exclusively based on heritable traits. This idea is supported by, for example, homing pigeons whose brain composition depends on navigational experience (Cnotka et al., 2008; Mehlhorn et al., 2010).



**Figure 4.** Relative brain structure size of floor-housed and cage-housed laying hens summarized and averaged over 5 different atlas levels (means  $\pm$  SD, \* $P = 0.037$ ).

Our data did not show any other differences in the brain composition of floor-housed and cage-housed hens, and there was no difference in the brain composition of WLA and R11. However, to date, we only analyzed a few brain structures from a limited number of animals. It cannot be excluded that further investigations with more animals would reveal differences in more structures or that there could still be volumetric differences in other brain regions. WLA hens have smaller brains than R11 hens, and thus, we have to investigate whether this reduction is due to a uniform decrease in all brain regions or whether there are just specific regions that are affected.

Interestingly, there are no differences in the hippocampus size between WLA and R11 as well as between animals in the 2 housing systems. The hippocampus is known to be especially sensitive to environmental enrichment and shows adaptive alterations in different domesticated species (Cnotka et al., 2008; Rehkämper et al., 2008). In addition, the hippocampus shows morphological and functional changes and suppressed neurogenesis in response to chronic stress (McEwen, 1999; Mirescu and Gould, 2006; Robertson et al., 2017; Smulders, 2017).

Patzke et al. (2009) even described affected hippocampi (and nidopallia caudolateralis) in battery-caged and free-ranged hens and explained this effect by the higher spatial complexity of the free-range system, and Gualtieri et al. (2019) showed that unpredictable chronic mild stress leads to fewer proliferating cells at the caudal pole of the hippocampal formation. The number of newly generated neurons surviving, potentially to the point of functional integration, in this region is reduced in line with negative welfare experience. Perhaps there is an effect in the hippocampus, but it is not volumetrically measurable with our approach. It is also possible that this alteration takes place on a neurochemical level or involves connectivity, cell size, or cell number. Another explanation is that both housing systems may have caused chronic stress, and thus, no differences can be detected. In addition, former studies indicate that other parameters, such as dendritic branching or spine density, may also be affected by different housing conditions (Rosenzweig and Bennett, 1996). It would be interesting to focus on the hippocampus in further studies. Measuring neurogenesis in the caudal hippocampal formation postmortem may provide a sensitive measure of cumulative welfare state in poultry, which could allow comparison of stress engendered by different commercial housing systems (Gualtieri et al., 2019). That there is no difference in telencephalon size could be explained by the fact that the telencephalon consists of many different brain structures with different functions. Thus, a more detailed analysis of single-telencephalon components would likely be more promising. All hens received similar inputs of optic cues and were kept under the same light conditions; thus, it is not surprising that we found no differences in the optic tectum.

In fancy poultry breeds, there are obvious differences in the brain composition, and in some breeds, such as

the White Crested Polish chicken, these differences are so large that it is believed these breeds are on the way to becoming a new species (Rehkämper et al., 2003). In the case of the White Crested Polish chicken, breeding for a large feather crest led to a cranial protuberance accompanied by a differently composed brain. In the case of laying hens, breeding for a high production of eggs led to, at least, smaller brains. The functional consequences of this reduction and a possible influence on animal welfare must be examined.

The small brains of WLA and R11 compared with those of other domestic chicken breeds and the fact that WLA have even smaller brains than R11 could be discussed as a further consequence of extreme artificial selection. Laying hens are an example of extreme intensive breeding and represent the highest level of artificial selection.

In further investigations, all brain regions will be analyzed in more detail. Besides, further analysis will include not only volumetric analyses but also cell number and cell density analyses. Apparently, there is also an influence of housing condition on brain morphology (and maybe brain size), but further investigations are necessary to obtain more insight and to exclude possible constraints on animal welfare. The avian brain shows a high level of plasticity in response to external parameters such as experience. In addition, the neuronal consequences of reduced social and cognitive behavior, the effects in cage-housed systems, the level of plasticity in adult brains, and the influence of (social) stress are further interesting topics for our research.

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