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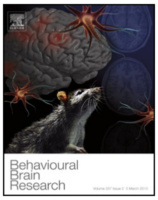

Research report

Selection for low mortality in laying hens affects catecholamine levels in the arcopallium, a brain area involved in fear and motor regulation

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HIGHLIGHTS

- Laying hens were selected for low mortality due to feather pecking and cannibalism.
- We used a novel genetic selection method, based on survival of group-housed siblings.
- We studied brain monoamines in this low mortality line (LML) and controls.
- LML hens had lower dopaminergic activity in the arcopallium than controls.
- This suggests a role for motor deficits in severe feather pecking and cannibalism.

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ABSTRACT

Feather pecking (FP) in laying hens may cause mortality due to cannibalism. Novel breeding methods using survival days of group-housed siblings allow for the genetic selection of laying hens with low mortality (LML: low mortality line) due to cannibalism. Previous studies have demonstrated less fear-related behavior and also less FP in LML hens compared to CL. Selection also caused changes in locomotor behavior in an open field. It is unknown, however, whether selection for low mortality affects central neurotransmitter levels. In this study, brain monoamine levels were measured in the dorsal thalamus, medial striatum, hippocampus and arcopallium of adult laying hens of both LML and CL using HPLC. Brain samples were collected after 5-min of manual restraint. The most prominent line differences were found in the arcopallium. Compared to CL, LML had lower levels of noradrenaline (NA) and 3,4-dihydroxyphenylacetic acid (DOPAC) and tended to have lower levels of dopamine (DA), homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA). Levels of serotonin (5-HT), 5-HT- and DA-turnover in this brain area were not affected by line. LML showed less fear-related behavior during the restraint than CL. These findings show that selection for low mortality in hens leads to changes of predominantly the dopaminergic system in the chicken's arcopallium, a forebrain somatomotor area also related to fear. This suggests a relationship between catecholamine functioning in this brain area and FP and cannibalistic behavior in chickens and underpins previously found relationships between FP, fear and high activity.

1. Introduction

Welfare concerns have led to a European ban on conventional battery cages for laying hens. Since 2012, only alternative housing systems ranging from "furnished" or "enriched" cages to non-caged aviaries or free-range systems are allowed [1]. Although these systems allow hens more freedom of movement, recent studies report increased mortality rates within alternative housing systems and many casualties are due to cannibalism [2]. Cannibalism in laying hens is the act of a bird pecking at the skin and devouring the flesh of other birds [3], which may ultimately lead to the death of the victims. Cannibalistic pecking is often preceded by severe

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feather pecking (FP) which is pecking at and removing of feathers of conspecifics causing denuded areas in the plumage which subsequently is very attractive for others to peck at [4]. To reduce problems related to severe feather pecking and cannibalism, many hens are exposed to beak-trimming, i.e. removal of the sharp tip of the upper beak. There is a growing societal resistance against animal mutilations, as it in itself beak-trimming induces stress, pain and fear in hens [5,6]. Thus, there is an urgent need for alternatives to reduce severe FP and cannibalism in laying hens.

Multiple factors, such as rearing and housing conditions [7,8], and diet [9,10] may contribute to the development of FP and cannibalism in laying hens. There are, however, also large individual differences in the vulnerability to develop severe FP and cannibalism and a genetic background for these differences has been found [11,12]. Traditionally, laying hens are selected for individual performance, such as egg production [13]. Selecting on individual performance can have potentially negative side-effects on group members [14,15]. By focusing on group performance and survival, Craig and Muir successfully decreased cannibalism-induced mortality in non-beak trimmed hens [16,17]. Recently, a novel selection method has been developed in which selection of individually housed candidates is partly based on the survival of their group-housed female siblings [18]. The advantage of this selection method is that candidates for breeding remain unaffected by group interactions, as they are housed individually, and vital information on individual performance is combined with the information on group performance. Already in the first generation a markedly decreased mortality rate was established in the low mortality line (LML) compared to the unselected control line (CL) [19,20]. In the third generation, LML showed less cannibalistic toe and comb pecking than CL [21]. Behavioral tests further show that LML hens displayed less fear-related and more active behavior compared to the unselected CL hens in several behavioral tests, both at young and at adult age [22–25]. Interestingly, LML and CL hens do not only differ in damaging and emotional behavior, but also in possible underlying physiological mechanisms. For example, differences in the peripheral serotoninergic system have been found, with LML hens having higher whole blood serotonin (5-hydroxytryptamine; 5-HT) levels [21,23] and a lower platelet 5-HT uptake [23] than CL. Also, lower plasma corticosterone levels were measured in LML after a manual restraint [21,22], possibly reflecting decreased fearfulness in the low mortality line. In animals and humans, anxiety (or fearfulness) has been related to brain 5-HT [26]. Several genetic and pharmacological studies further established the involvement of both central 5-HT and DA in FP [27–33] and cannibalism [12,21,22,27,34,35]. More recently, a lower concentration of tyrosine hydroxylase, the rate-limiting enzyme in DA production, was reported in the nidopallium, a "prefrontal" area [36–38] of LML hens compared with CL hens [39]. Unknown, however, is whether and how selection for low mortality in laying hens affects central neurotransmitter levels in the brain.

The aim of the present study was to compare brain monoamine levels and DA and 5-HT turnover levels between the fourth generation of laying hens selected for low mortality (LML) and the control line (CL). In total, four target regions related to the modulation of fear and motor control were selected [40,41]: a combination of the dorsal (AD) and central (AI) region of the intermediate arcopallium (referred to as arcopallium), the medial striatum, the hippocampus, and the dorsal thalamus. The arcopallium receives input from various associative and sensory forebrain areas and is the source of a major down-sweeping pathway to brainstem motor structures; it thus is a somatomotor forebrain area [42]. The medial striatum is the limbic component of the avian striatal complex [42]. Given the behavioral and physiological differences between LML and CL, we expect lower levels for both DA and 5-HT in CL hens compared to LML hens, as the former are considered more fearful and display more FP and cannibalistic behavior leading with higher mortality rates.

2. Methods

2.1. Ethical statement

The experiment was approved by the Animal Care and Use Committee of Wageningen University, and in accordance with Dutch legislation on the treatment of experimental animals the ETS123 (Council of Europe 1985) and the 86/609/EEC Directive.

2.2. Birds and housing

In total, 40 adult female White Leghorns (*Gallus gallus domesticus*) of 33 weeks of age were selected for brain analyses. Half of these hens originated from CL ($n = 20$) and the other half from the fourth generation of LML ($n = 20$) aimed at breeding with selection candidates of which siblings showed low group mortality [18,25]. All non-beak trimmed hens came from the same population of 160 hens as described previously [25] and were obtained from ISA, the layer breeder division of Hendrix Genetics, the Netherlands. Hens were housed per line in groups of 10 birds per pen, 8 pens per line thus 16 pens in total. From each pen, two to three hens were randomly selected for brain analyses such that twenty birds were selected per line. Water and a commercial mash diet were provided ad libitum. Pen floors (1.9 by 1.2 m) were covered with sand (1/3) and wood shavings (2/3). For more details on housing conditions, see [25].

2.3. Manual restraint

At 33 weeks, each hen was subjected to a manual restraint test, using a method previously described [23]. Briefly, each hen was removed from her home pen and put in a cardboard box to be tested in an adjacent room. The experimenter used the right hand to place a hen on her right side on a table covered with cardboard and then covered the trunk and the left hand gently stretched the hen's legs. For 5 min, the frequency of consecutive struggles and the number of vocalizations was recorded as well as the latency to struggle and vocalize. After each struggle, hens were placed back into the original test position until the time of the test passed. The manual restraint took place on two consecutive days by one researcher and the order of testing was balanced for line. After the manual restraint, the hens selected for brain analyses were sacrificed by cervical dislocation.

2.4. Brain tissue preparation

Brains were removed and immediately deep frozen in n-heptane, put on dry ice and stored at −80 °C (protocol by [43,44]). Slicing of brains was executed in a cryostat (Frigocut Jung Mod_700) under cold conditions (−10 °C). Slice thickness was 400 μm. The four regions of interest were located using the brain atlas for 2-week-old chickens [45] and with considering literature on the avian brain [46,47,48], thereby also taking into account the increased brain size in our hens at 33-weeks of age. Fig. 1 is a schematic drawing depicting the location of, respectively, the MSt (A) and the dorsal thalamus, hippocampus and the arcopallium (B). The gray dotted shapes illustrate the cutting lines per brain area (carefully cut with a scalpel). Tissue samples were taken from multiple slices, with corresponding figure numbers in the atlas: medial striatum (MSt; 7.56–5.68 mm anterior to the interaural line) including the accumbens (Acb; 8.08–7.56 mm anterior to the interaural line), hippocampus (HIPP; Hi1, Hi2, PHiM, PHil, PHil1, PHil 2, and PHiA;

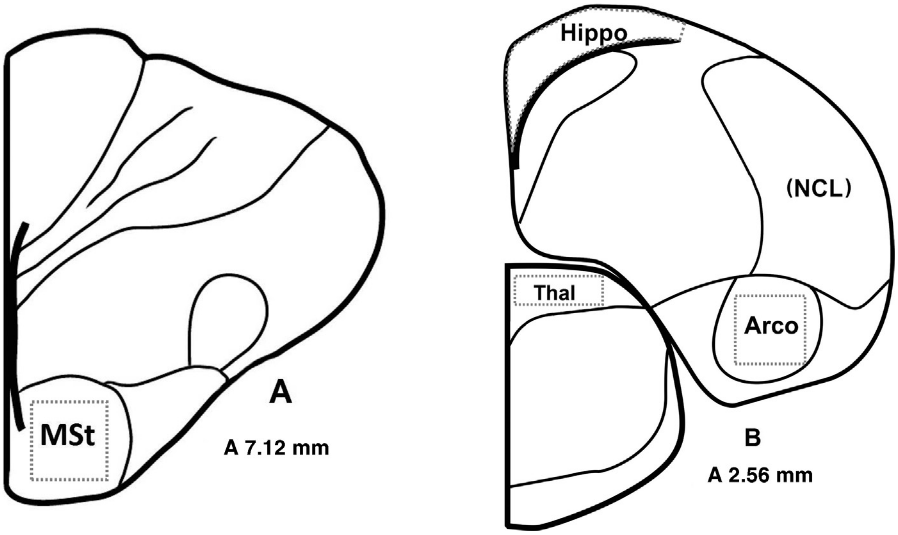

Fig. 1. Coronal schematic views of the chicken brain illustrating the medial striatum, thalamus, hippocampus, and arcopallium. The schematic views of the left hemisphere of a chicken's brain are drawn after [46,47,48] with brain coordinates based on the chicken brain atlas [45]. Depicted are the medial striatum (MSt) at 7.12 mm anterior to the interaural line (A), and the thalamus, arcopallium and hippocampus at 2.56 mm anterior to the interaural line (B). The location of the NCL is indicated between brackets (B), because this area was analyzed by [39], and here compared with our results and discussed. At 33 weeks of age, chicken brains were sampled from both the left (shown here) and right hemisphere (not shown); the gray dotted shapes illustrate the cutting lines per brain area. *Abbreviations*: A = anterior to the interaural line; MSt = medial striatum; Thal = Thalamus; Arco = Arcopallium; Hippo = hippocampus; NCL = nidopallium caudolaterale.

6.16–0.40 mm anterior to the interaural line), and the dorsal thalamus (DPe, DMA, DIA, DLA; 3.04–1.36 mm anterior to the interaural line). For the arcopallium, the area referred to as amygdala core by [45] was sampled (4.24–2.08 mm anterior to the interaural line). Brain samples of the left and right hemisphere were taken together and analyzed as one.

2.5. Central monoamine analysis with HPLC

Brain samples were analyzed using a high performance liquid chromatography (HPLC) method. For that, the tissue samples were homogenized in an ice-cold solution containing 5 μM clorgyline, 5 μg/ml glutathione and 1.2 μM N-methylserotonin (NMET, internal standard) using sonication. To 80 μl homogenate, 20 μl 2 M HClO4 was added and mixed. After 15 min in ice water, the homogenates were centrifuged during 15 min at 15,000 × *g* (4 °C). The supernatants were diluted 10 times with water before HPLC analysis. The concentration of serotonin [5-HT] and its metabolite 5-hydroxyindoleacetic acid [5-HIAA], and dopamine [DA] with corresponding metabolites 3-methoxytyramine [3-MT], 3,4-dihydroxyphenylacetic acid [DOPAC], and homovanillic acid [HVA], and also noradrenaline [NA] in the tissue extracts were measured by HPLC with electro chemical detection (ECD). The HPLC system consisted of a pump model P100, a model AS300 autosampler (both from Thermo Separation Products, Waltham, MA, USA), a ERC-3113 degasser (Erma CR. Inc., Tokyo, Japan), an ESA Coulochem II detector with 5011 analytical cell set at potential +550 mV (ESA Inc., Bedford, MA, USA), a data acquisition program (Atlas 2003, Thermo Separation Products) and a column (150 mm × 4.6 mm i.d.) packed with Hypersil BDS C18, 5 μm particle size (Alltech Associates, USA). The mobile phase solution consisted of 50 mM citric acid, 50 mM phosphoric acid, 0.1 mM EDTA, 45 μl/l dibutylamine, 77 mg/l 1-octanesulfonic acid sodium salt, 10% methanol; the pH of the buffer was adjusted to 3.4 with NaOH. Separation was performed at 45 °C using a flow rate of 0.8 ml/min. The concentration of each compound was calculated by comparison with both the internal and the external standards. The protein content of each homogenate sample was determined using the DC protein Assay (Bio-Rad). Monoamine concentrations are expressed as nmol/g protein. Turnover levels of serotonin (5-HIAA/5-HT) and dopamine ((DOPAC + HVA + 3-MT)/DA) were calculated as an index for the activity of the serotonergic and dopaminergic system [44]; high levels indicate a quicker metabolic pathway due to higher biosynthetic enzyme activity.

2.6. Statistical analysis

SAS version 9.2 was used for statistical calculations [49]. Monoamine and metabolite levels per brain area were tested with a mixed model that included the fixed effects of line (LML vs. CL) and day (test day 1 and 2). Pen nested within line was added as a random effect to the model, thus, effectively, pen ($n = 16$) was used as experimental unit for testing line effects. Post hoc least square means were used to detect pair-wise differences. A log transformation for DA turnover in the arcopallium and HVA in the dorsal thalamus was executed to obtain normality of residuals. Data are presented as mean ± SEM.

Effects of line on the behavioral responses of hens during the manual restraint test were analyzed using Kruskal–Wallis tests as data were not normally distributed. If significantly different, values are presented as median (M) with the interquartile range, i.e. lower ($Q1$) and upper ($Q3$) quartiles. Many birds did not struggle or vocalize at all during manual restraint. Therefore, struggling and vocalizing during the test was also analyzed as a binary (yes/no) variable using a generalized mixed model with logit link function. Line and day were fixed effects in this model, and pen nested within line was added as random effect. Data are presented as mean ± SEM.

3. Results

3.1. Effects on DA

In Fig. 2, the levels of DA, its three metabolites DOPAC, HVA, and 3-MT, NA and the calculated DA turnover in the arcopallium are shown. DA levels ($F_{(1,14)} = 4.1$, $P = 0.06$) and levels of its metabolite HVA ($F_{(1,14)} = 4.1$, $P = 0.06$) tended to be lower for LML birds than for CL birds. LML birds also showed significant lower NA levels ($F_{(1,14)} = 5.6$, $P = 0.03$) and DOPAC levels ($F_{(1,14)} = 7.1$, $P = 0.02$) in

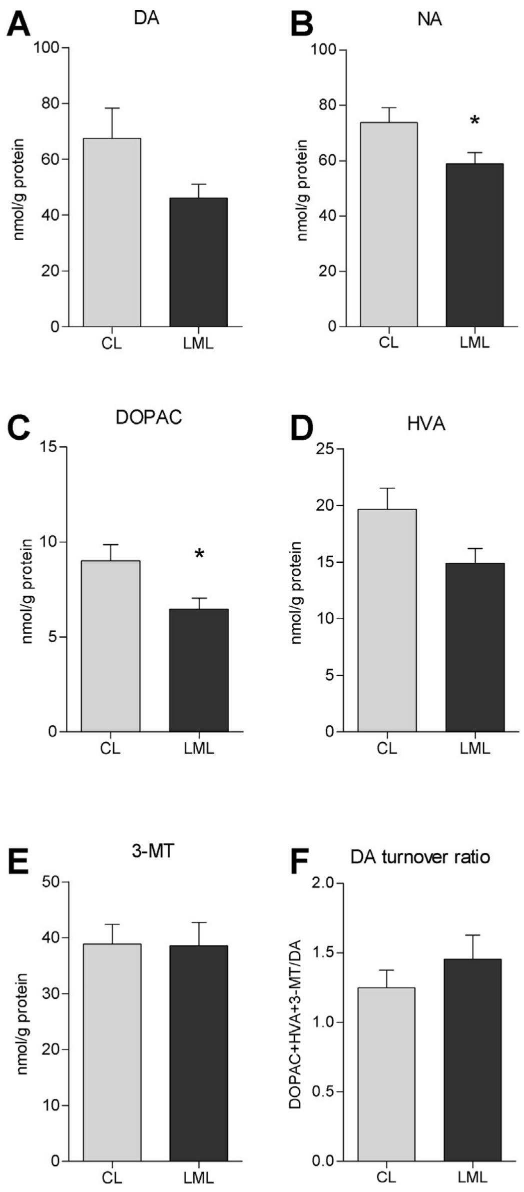

Fig. 2. Catecholamine, metabolite, and turnover levels in the arcopallium of adult laying hens. Mean (±SEM) values for catecholamines dopamine (A) and noradrenaline (B), the DA-metabolites DOPAC (C), HVA (D), and 3-MT (E), and the DA turnover ratio ((DOPAC + HVA + 3-MT)/DA) (F) in the arcopallium of hens from the control line (CL) and low morality line (LML). $n = 20$/group; $^{*}P < 0.05$.

this brain area compared to CL birds. Levels of 3-MT ($F_{(1,14)} = 0.0$, $P = 0.95$) and the DA turnover ($F_{(1,14)} = 1.0$, $P = 0.24$) in the arcopallium were unaffected by line. Table 1 shows the levels of DA, DOPAC, HVA, 3-MT, DA turnover, and NA in the dorsal thalamus, medial striatum, and hippocampus per line. DOPAC levels in the thalamus were lower for LML birds compared to CL hens ($F_{(1,14)} = 6.1$, $P = 0.03$). LML hens showed a higher DA turnover in the hippocampus ($F_{(1,14)} = 5.2$, $P = 0.04$) than CL hens. No other line effects were found for DA and its metabolites in the dorsal thalamus, medial striatum, and hippocampus.

3.2. *Effects on 5-HT*

Fig. 3 shows levels of 5-HT, 5-HIAA, and 5-HT turnover in the arcopallium. There, 5-HIAA levels tended to be lower for LML hens compared to CL hens ($F_{(1,14)} = 3.6$, $P = 0.08$). Levels of 5-HT ($F_{(1,14)} = 2.3$, $P = 0.15$) and the 5-HT turnover ($F_{(1,14)} = 0.6$, $P = 0.45$) in this area were unaffected by line. In the three other brain areas, i.e. the dorsal thalamus, medial striatum, and hippocampus, no effects of line on 5-HT, 5-HIAA, or 5-HT turnover were found (Table 2).

Table 1
Catecholamine, metabolites, and DA turnover levels in the dorsal thalamus, medial striatum, and hippocampus of hens from the control line (CL) and low morality line (LML).

	Control (CL)		Low mortality (LML)		P value
	Mean	SEM	Mean	SEM	
Dorsal thalamus					
DA	12.33	1.64	8.72	0.98	0.105
NA	94.90	6.71	81.77	4.23	0.145
DOPAC	0.94	0.13	0.55	0.07	0.027*
HVA	3.91	0.65	2.89	0.25	0.200
3-MT	6.79	0.65	6.73	0.56	0.944
DA turnover	1.05	0.09	1.18	0.08	0.219
Medial striatum					
DA	748.75	34.11	701.90	59.08	0.536
NA	38.25	4.28	31.20	3.17	0.211
DOPAC	33.70	2.20	30.34	2.89	0.421
HVA	45.60	3.03	43.00	4.01	0.726
3-MT	86.10	5.93	95.55	8.28	0.369
DA-turnover	0.23	0.01	0.24	0.01	0.291
Hippocampus					
DA	4.40	1.09	2.95	0.67	0.320
NA	66.95	3.70	65.20	3.01	0.723
DOPAC	0.95	0.26	1.15	0.26	0.600
HVA	1.35	0.40	2.45	0.59	0.145
3-MT	18.05	1.91	19.25	2.45	0.774
DA turnover	0.65	0.15	1.52	0.39	0.043*

* $P < 0.05$; Mean (±SEM) in nmol/g protein; $n = 20$/group.

3.3. *Manual restraint*

During the 5-min manual restraint, birds from the two lines differed in their latency to vocalize ($\chi^2 = 5.9$, df = 1, $P = 0.02$) and frequency ($\chi^2 = 6.1$, df = 1, $P = 0.01$), with LML birds vocalizing sooner ($M_{LML} = 296$ s, $Q1 = 161$, $Q3 = 300$) and more ($M_{LML} = 0.5$, $Q1 = 0.0$, $Q3 = 16.3$) compared to CL birds ($M_{CL} = 300$ s, $Q1 = 300$, $Q3 = 300$, and $M_{CL} = 0.0$, $Q1 = 0.0$, $Q3 = 0.0$, respectively). When vocalizing was expressed as a binary variable, 50% of all LML hens vocalized, whereas only 15% of CL hens did ($F_{(1,14)} = 3.60$, $P = 0.08$). Lines did not differ significantly in their latency to struggle ($\chi^2 = 1.9$, df = 1, $P = 0.167$; $M_{LML} = 300$, $Q1 = 241$, $Q3 = 300$ vs. $M_{CL} = 300$, $Q1 = 300$, $Q3 = 300$) or in frequency of struggling ($\chi^2 = 2.6$; df = 1; $P = 0.11$; $M_{LML} = 0$, $Q1 = 0$, $Q3 = 1$ vs. $M_{CL} = 0$, $Q1 = 0$, $Q3 = 0$). Also, the percentage of birds that struggled during the test did not differ between LML (45%) and CL birds (20%) ($F_{(1,14)} = 2.31$, $P = 0.15$).

Table 2
Serotonin, metabolite, and 5-HT turnover levels HT and 5-HIAA, and 5-HT turnover (5-HIAA/5-HT) in the dorsal thalamus, medial striatum, and hippocampus of hens from the control line (CL) and low morality line (LML).

	Control (CL)		Low mortality (LML)		P value
	Mean	SEM	Mean	SEM	
Dorsal thalamus					
5-HT	92.84	5.56	98.96	5.33	0.533
5-HIAA	10.16	0.65	10.01	0.51	0.919
5-HT turnover	0.11	0.01	0.10	0.00	0.246
Medial striatum					
5-HT	159.60	10.23	162.30	10.88	0.850
5-HIAA	15.45	0.97	14.75	1.26	0.733
5-HT turnover	0.1	0.00	0.09	0.00	0.161
Hippocampus					
5-HT	177.25	11.00	174.00	10.13	0.853
5-HIAA	23.65	1.15	23.85	1.29	0.904
5-HT turnover	0.14	0.01	0.14	0.00	0.982

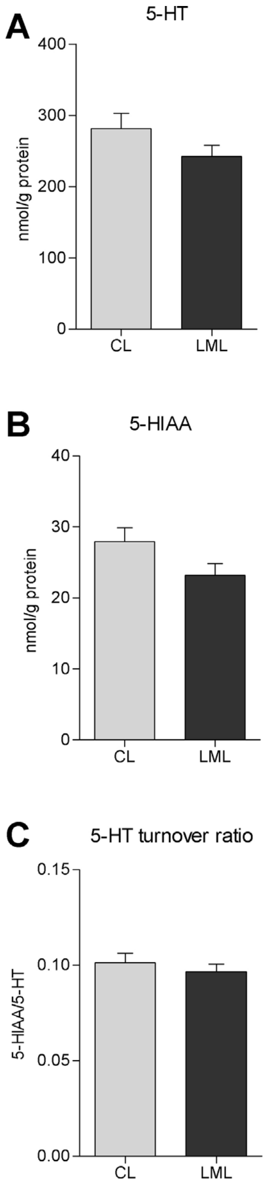

Fig. 3. Serotonin, metabolite, and turnover levels in the arcopallium of adult laying hens. Mean (±SEM) values for serotonin (5-HT) (A), its metabolite 5-HIAA (B), and the 5-HT turnover ratio (5-HIAA/5-HT) (C) in the arcopallium of hens from the control line (CL) and low morality line (LML). $n = 20$/group.

4. Discussion

The current study compared brain monoamine levels in four different brain regions of laying hens selected for low mortality using group selection of siblings (LML) with a control line (CL). Selection for low mortality resulted in changes in dopaminergic measures, most prominently present in the arcopallium, but did not significantly affect serotoninergic measures.

4.1. *Effects of selection for low mortality on dopaminergic measures*

Selection for low mortality resulted in lower levels of NA and DOPAC and a tendency for lower levels of DA and HVA, with no line differences for 3-MT levels and DA turnover levels in the arcopallium. In agreement with this observed difference in dopaminergic action, a recent immunohistochemistry study in the same selection lines showed lowered tyrosine hydroxylase concentrations in the nidopallium caudolaterale (NCL) in hens of the LML (second generation) as compared to the CL [39]. Tyrosine hydroxylase catalyzes the production of DA from tyrosine [50] and fewer enzymes might lead to a reduced synthesis of DA and diminished production of metabolites, as shown here. It is speculated that other mechanisms may be involved too, such as an altered activity of dopamine β hydroxylase and monoamine oxidase (MAO) that might affect monoaminergic neurotransmission [51]. While Nordquist et al. [39] focused on the NCL; we focused on the arcopallium, medial striatum, hippocampus, and the thalamus. The telencephalic areas arcopallium and medial striatum contain a higher distribution of dopaminergic fibers and D1 receptors than the NCL [40,52]. A microdialysis study in pigeons showed a high release of DA and high production of DA-metabolites in the striatal area; with a relatively low HVA/DOPAC ratio reflecting a fast reuptake by the dopamine transporter (DAT) [53]. In the present study, lines did not differ in their dopaminergic levels in the MSt. As the hippocampus has very little expression of the DAT [54], reuptake of released DA into the presynaptic terminal is hampered, resulting in low levels of DOPAC and HVA. This effect might be stronger in CL hens, which had a lower DA turnover ratio in this area than LML hens. Similar to the arcopallium, DOPAC levels in the thalamus were lower for CL hens than for LML hens.

As LML are selected for low mortality due to FP and cannibalism, these results confirm previously found relationships between FP and the dopaminergic system [28,55]. It is shown that LML hens had a lowered DA neurotransmission compared to CL hens, most prominently seen in the arcopallium. It remains unknown, however, how much DA is released by the presynaptic cell thereby contributing to the levels of DOPAC (via reuptake of DA), 3-MT (via released DA) and HVA (via forming of DOPAC and 3-MT) [51]. A microdialysis study could provide more details on the functional aspects of monoamines as this technique enables measuring presynaptic release of DA (and 5-HT) and its metabolites [53].

4.2. *Effects of selection for low mortality on serotonergic measures*

Selection for low mortality tended to lower 5-HIAA levels in the arcopallium of LML hens, but 5-HT levels, albeit numerically lower in LML hens, were unaffected by line. No significant serotonergic effects were found in any of the three other brain areas either. Previously, it has been shown that LML hens had higher peripheral whole blood 5-HT levels [21,23] and a lower platelet 5-HT uptake [21,23] than CL hens. In addition, it was shown that hens displaying severe FP had increased 5-HT turnover in the dorsal thalamus and higher levels of the 5-HT metabolite in the medial striatum compared to non-peckers and victims of FP [56]. Similar results were found when comparing 5-HT turnover between a flighty, FP-prone line and a more docile, low FP line [55]. This suggests that FP may be influenced by brain 5-HT, whereas selection for low mortality is probably affected by more or different traits, obscuring the link with central 5-HT.

4.3. *Effect of dopaminergic changes in the arcopallium on behavior and motor control*

The catecholamines, such as DA and NA, are known to play a role in motivational and reward-related motor and higher cognitive functions like impulsivity [57]. Pharmacological studies in both birds and mammals support the involvement of the central dopaminergic system in dysfunctional behaviors. For instance, increasing DA activity by administering a DA_2 receptor agonist induced stereotypies in pigeons [58], increased aggressive pecking in normally low aggressive chickens [59], and increased impulsivity in rats [60]. Administering a DA_2 receptor antagonist, thus decreasing DA activity, reduced FP ratios in laying hens [28], while a DA_1 receptor antagonist decreased the behavior of already high aggressive chickens [59] and high impulsive rats [60], but not in low aggressive chickens or low impulsive rats. Thus, high dopaminergic levels in CL might (at least partly) lie at the basis of FP or impulsivity. Also, a large number of studies have demonstrated that fearfulness is related to FP behavior [23,61–67]. It is assumed that more fearful animals are more prone to display defensive aggression and are more likely to perform severe FP [67,68] and cannibalism [69–71]. In addition, within a group both fearfulness and FP may be transmitted among pen-mates [25,66,72], and also victims of FP show increased fearfulness [61,62] with possible activation of the ascending DA system, as seen in rodents suffering from repeated aggressive attacks [73]. Here, the more active vocal behavior of LML hens during the manual restraint indicates lower fearfulness compared to CL. That is, a more active behavior during fear tests represents lower fear levels or a higher social reinstatement motivation in comparison to non-vocalizing and non-struggling behavior [74] as seen in CL hens. As described before LML showed reduced cannibalistic toe pecking [21] and are characterized as less fearful in numerous behavioral tests compared to the CL [21,23,25,75], including the one in this study. The differences in the dopaminergic activity between LML (low DA activity) and CL (high DA activity) might thus underlie the intergroup differences in damaging behaviors.

Importantly, the strongest dopaminergic effects were found in the arcopallium. Before the avian neuroanatomical nomenclature was changed, the area in the most ventrolateral and posterior part of the bird telencephalon was called archistriatum. Based on a large amount of neurobiological evidence, the archistriatum is now subdivided into the somatomotor arcopallium and a cluster of subnuclei that constitutes the amygdala [42,76,77]. Lesions in the amygdala of Japanese quail increase fear behavior in an open field test, while arcopallium lesions decrease anxiety [78], which fits with our results. It is also possible, though, that the quails' fearful state was mediated by motor output deficits as fearful quails remained longer immobile in the open field [78]. In addition, humans suffering from either obsessive-compulsive disorder or trichotillomania, a hair-pulling disease with similarities to FP [79], both have impaired inhibition of motor response [80]. L-DOPA (L-3,4-dihydroxyphenylalanine) is known to improve motor learning and motor memory by increasing DA levels [81,82]. Increased DA levels can, however, also induce stereotypies [83] and deficits in the control of posture and motor activity [84]. Dopaminergic terminals are abundant in the arcopallium [85] and activate primarily D1-receptors [86], thereby possibly increasing spike densities of pre-activated neurons [40,87]. Consequently, the higher DA activity might lead to a more active motor output system in CL hens, with impulsivity or even hyperactivity [88] as underlying problems associated with FP and cannibalistic behavior. Vice versa, it is hypothesized that lower dopaminergic neurotransmission in LML hens decreases the probability that motor-processes related to FP and cannibalism are activated and are executed.

5. Conclusion

The present study shows that selection for low mortality using a sibling group selection scheme affects the dopaminergic neurotransmission of laying hens with possible implications for the motor output of FP and cannibalistic behavior, as suggested by the strong results found in the arcopallium, a somatomotor area. The stronger effect of line in the arcopallium compared to the absence of significant effects for dopamine or serotonin in the limbic MSt suggests that deficits in motor functioning might be at the base of these behaviors, although fear and even impulsivity might also affect FP and cannibalism. Future studies are needed (e.g. in vivo microdialysis) on the role of dopamine in FP and cannibalism to further investigate the underlying neural mechanisms.

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