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Research report

Serotonin release in the caudal nidopallium of adult laying hens genetically selected for high and low feather pecking behavior: An *in vivo* microdialysis study

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HIGHLIGHTS

- Selection on high and low feather pecking (FP) affects serotonergic brain levels.
- High FP (HFP) had higher serotonin release in the caudal nidopallium than low FP (LFP).
- Serotonin release levels after D-fenfluramine were similar between lines.

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ABSTRACT

Severe feather pecking (FP) is a detrimental behavior causing welfare problems in laying hens. Divergent genetic selection for FP in White Leghorns resulted in strong differences in FP incidences between lines. More recently, it was shown that the high FP (HFP) birds have increased locomotor activity as compared to hens of the low FP (LFP) line, but whether these lines differ in central serotonin (5-hydroxytryptamine, 5-HT) release is unknown. We compared baseline release levels of central 5-HT, and the metabolite 5-HIAA in the limbic and prefrontal subcomponents of the caudal nidopallium by *in vivo* microdialysis in adult HFP and LFP laying hens from the ninth generation of selection. A single subcutaneous D-fenfluramine injection (0.5 mg/kg) was given to release neuronal serotonin in order to investigate presynaptic storage capacity. The present study shows that HFP hens had higher baseline levels of 5-HT in the caudal nidopallium as compared to LFP laying hens. Remarkably, no differences in plasma tryptophan levels (precursor of 5-HT) between the lines were observed. D-fenfluramine increased 5-HT levels in both lines similarly indirectly suggesting that presynaptic storage capacity was the same. The present study shows that HFP hens release more 5-HT under baseline conditions in the caudal nidopallium as compared to the LFP birds. This suggests that HFP hens are characterized by a higher tonic 5-HT release.

1. Introduction

Severe feather pecking (FP) is the pecking at and pulling out of feathers of conspecifics. This detrimental behavior causes welfare problems in laying hens and has multifactorial causes [1–3]. Genetic studies have shown a moderate heritability of FP [4,5] with genetic variations in several genes of the monoaminergic systems that seem to be related to FP behavior [6–8]. From neurobiological and pharmacological studies there is indeed a growing body of evidence on the involvement of brain monoamines such as serotonin (5-hydroxytryptamine; 5-HT) and dopamine (DA) in the propensity to develop FP [7,9–14]. Comparing brain monoamine levels in young chickens from commercial lines selected on production traits (*e.g.* egg size and egg quality), unintentionally also differed in levels of FP, revealed that the young chickens of the line with higher FP levels had lower 5-HT and DA turnover ratios

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than the line with lower FP [13]. Treatment of these young chickens with a tryptophan-rich diet [9] or pharmaceutical D2 receptor antagonist such as haloperidol [15] was very effective at reducing gentle FP ratios by increasing brain 5-HT and DA levels. In contrast, more gentle FP incidences were recorded after decreasing 5-HT levels by inhibiting 5-HT release *via* a 5-HT1A autoreceptor agonist [16]. Thus gentle FP may be related to low turnover of central 5-HT and DA as shown in the rostral forebrain of young chickens. It should be noted, though, that severe FP is mostly prevalent at an adult age [1,17]. Differences in brain monoamine levels were inconsistent in adult chickens of commercial lines selected for traits other than FP, that coincidentally also affected FP [18–20]. In 1997, Kjaer and Sørensen [5] started genetically selecting chickens on their individual display of severe FP behavior. This resulted in experimental lines called the high (HFP) and low (LFP) feather pecking selection lines. Next to strong divergences in FP ratios in the third and following generations [21,22], the HFP compared to LFP were more motivated to eat feathers [23] – a behavior related to FP [2,24] – and differed in gut flora [25] and had also increased locomotor activity in their home cage as compared to LFP [26].

The objective of the current study was to measure the release of monoamines in the extracellular synaptic cleft in the caudal nidopallium of adult HFP and LFP hens by *in vivo* microdialysis. The nidopallium is a large associative area in the chickens' forebrain with a potential role in the guiding of motor actions and decision making [27–29]. The caudolateral nidopallium (NCL) receives, more than the caudocentral nidopallium (NCC), input from monoaminergic systems and serves frontal-like executive functions [30]. The NCC displays a limbic connectivity [31,32]. Both NCL and NCC have reciprocal projections to the arcopallium intermedium, a somatosensory area, and the arcopallium mediale, a limbic region [31,33,34]. Both nidopallic regions contain serotonergic [35] and dopaminergic afferents [36] and receptors [37–39]. In contrast to taking samples of brain tissue [18] or measuring 5-HT blood concentrations (*e.g.* [40,41] *in vivo* microdialysis allows the measurement of the extracellular monoamine release within a short timeframe in a particular brain area of conscious freely moving animals. Appropriate central monoamine release is essential for stimulation of pre- and postsynaptic monoamine receptors corresponding with adequate stimulation of the second messenger systems or target organs (see review on 5-HT metabolism by [42]. Although microdialysis is used to study, for instance, the role of monoamines in imprinting [33,43,44] and feed intake [45] in young chickens, as far as we know, microdialysis has never been performed in adult chickens. A second objective was to compare blood plasma concentrations of tryptophan (precursor of brain 5-HT [46]) between the HFP and LFP lines to establish whether potential line differences in the release of monoamines in the brain might have a peripheral cause (*e.g.* by diet) or whether there is evidence for an altered synthesis and/or release in the brain. Here we investigate whether divergent selection for FP produces differences in serotonergic neurotransmission in the forebrain of adult laying hens.

2. Materials and methods

2.1. Ethical statement

All experimental procedures were approved by the Animal Care and Use Committee of Wageningen University, The Netherlands, and found to be 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. Animals and housing

White Leghorn hens from the 9th generation of divergently selected lines, the HFP and LFP, were used. Details regarding the selection procedure have been described previously [21,22]. Eggs of both HFP and LFP birds were brooded and after hatch, the one-day old female chicks received a health check followed by a neck tag with a color/number combination for identification. In total 84 female chicks were distributed over 12 pens (42 chicks/line; $n = 7$/pen). Birds were not beak-trimmed. The chicks were housed in pens with a concrete floor (1.9 by 1.2 m) covered with paper (first 7 weeks) or sawdust (after week 7). Water and a commercial mash diet were provided *ad libitum*: a starter diet (week 1–5), a grower diet (week 6–16) and a layer diet (from week 17 onwards). Each pen had a 50 cm high perch installed and a lower perch (a block of wood) in the first seven weeks. In week 8, each group was reduced by one chicken (used for another experiment). By that time, three chicks turned out to be male and 4 chicks had died within the first week. Therefore, the total group size was 65 animals (LFP: $n = 32$; HFP: $n = 33$). Continuous light was given the first week, and then 18 h of light (week 2) followed by 13 h (week 2–3), and 10 h of light (week 4–15). From 17 weeks of age onwards, the light period was extended by 1 h per week, until the birds had 16 h of light (2.00 am–6.00 pm) at 23 weeks of age.

2.3. HPLC-ECD determination of large neutral amino acids (LNAA) in blood plasma

At 17 weeks, blood taken from the wing vein was collected in a 4 ml EDTA tube and put on ice. Samples were centrifuged and 200 μl plasma was put in a 1 ml serum tube and stored at −70 °C until analysis. Large neutral amino acids (LNAA), such as tryptophan (TRP), L-valine (Val), I-methionine (Met), leucine (Leu), I-isoleucine (Ile), phenylalanine (Phe), tyrosine (Tyr), and the internal standard I-norleucine (NLeu) were detected simultaneously using an ultra-high performance liquid chromatography (UHPLC) with electrochemical detection using an Alexys 110 LC-EC analyzer (Antec, Zoeterwoude, The Netherlands). The system consisted of two pumps, one autosampler with a 1.5 μl loop, a column (Acquity UPLC HSS T3 1.0 mm × 50 mm, 1.8 μm particle size, Waters, Milford, USA), a μVT-03 detector flow cell with glassy carbon working electrode (potential setting +0.85 V *vs.* Ag/AgCl). The column and detector cell were kept at 40 °C in a column oven. Stock solutions of the amino acid were prepared in Milli-Q water and stored at −70 °C. To 20 μl of plasma 80 μl 100% methanol was added and subsequently vortex mixed. Then 20 μl 0.5 mM NLeu was added and vortex mixed. After 10 min on ice the samples were centrifuged during 10 min at 15,000 × *g*. Subsequently 20 μl of the supernatant was added to 60 μl of 0.05 M sodium borate buffer pH 10.4, mixed and pipetted into autosampler vials. During analysis the samples were kept at 4 °C in the autosampler. Primary amino acids in the sample were derivatized pre-column [47] using a reagent consisting of 37.5 mM o-Phtalaldehyde (OPA) (Pickering Laboratories, USA), 50 mM sodium sulphite, 90 mM sodium borate buffer pH 10.4. This reagent was prepared by mixing a 0.75 M OPA solution (prepared in methanol) with a 1 M sodium sulphite solution (in Milli-Q water) and a 0.1 M sodium borate buffer pH 10.4 (mixing ratio 1:1:18). The derivatization was performed automatically in-line using the autosampler. A 9 μl sample was mixed with 0.5 μl reagent just prior to the analysis. Separation was achieved using mobile phase A (50 mM phosphoric acid, 50 mM citric acid, 0.1 mM EDTA, pH 4.5, 8% acetonitril, 10% methanol). As soon as the compounds of interest were completely detected a step gradient using mobile phase B (50 mM phosphoric acid, 50 mM citric acid, 0.1 mM EDTA, pH 4.5, 60% acetonitril) was applied to rinse the column removing any late eluting compounds. The flow rate was set at