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Research report

Brain monoamine levels and behaviour of young and adult chickens genetically selected on feather pecking

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ABSTRACT

Severe feather pecking (SFP) in chickens is a detrimental behaviour with possibly neurochemical deficits at its base. Recent neurological studies depicted conflicting results on the role of serotonin (5-hydroxytryptamine, 5-HT) and dopamine (DA) in the development and display of feather pecking. We studied brain monoamine levels and behaviour in domestic chickens divergently genetically selected on feather pecking behaviour, the Low Feather Pecking (LFP) and High Feather Pecking (HFP) lines, both at a young age and when adult, to elucidate the role of 5-HT and DA in feather pecking. Also pecking behaviour and the behavioural response to challenging test situations was determined. At 8 weeks of age, HFP had lower 5-HT and DA turnover in several brain areas than LFP, whereas these differences had disappeared or were even reversed at 25 weeks of age. Line differences in central monoamine activity were found both in emotion-regulating and motor-regulating areas. As expected from previous generations, HFP exceeded LFP in most types of pecking at other birds, including severe feather pecking. Furthermore, HFP responded more actively in most behavioural tests conducted, and seem more impulsive or (hyper)active in their way of coping with challenges. This paper shows different developmental trajectories of the neurochemical systems (5-HT and DA) for chickens divergently selected on feather pecking behaviour, and a remarkable reversion of differences in monoamine activity at a later stage of life. Whether this is a cause or consequence of SFP needs further investigation.

1. Introduction

Severe feather pecking (SFP), i.e. the pecking at and pulling out of feathers of group mates, is a detrimental behaviour in birds [1]. SFP can easily evolve to skin pecking and cannibalism resulting in mortality of recipients and is therefore a considerable welfare problem in laying hens on commercial poultry farms. SFP in chickens has multiple risk factors, many of which are related to the environment of the birds [2,3] and their social setting [4–6]. In addition, (genetically influenced) individual characteristics that determine how animals respond to their environment influence the tendency to develop SFP [7]. Characteristics that have been associated with SFP are high fearfulness and anxiety [4,8–11], (hyper)activity [12], and a proactive coping style [13]. Although either of these characteristics (fearfulness, proactive coping or hyperactivity) may predispose chickens to develop SFP, the underlying neural mechanisms for SFP are not clear.

Neurochemical deficits might lie at the base of the SFP problem, and several genetic studies point to a role of the brain monoamines serotonin (5-hydroytryptamine, 5-HT) and dopamine (DA) (e.g. [14,15]). Pharmaceutical studies have shown that haloperidol–a dopamine D2 receptor agonist known to increase dopamine release when acutely administered [16] – reduces SFP in adult chickens [17]. Moreover, chronic dietary supplementation with the 5-HT precursor tryptophan, leading to enhanced 5-HT neurotransmission, decreased feather pecking (FP) in young chickens [18], whereas the 5-HT_{1A} autoreceptor agonist S-15535, inhibiting 5-HT release, increased the incidence of FP in young chicks [19] and adult hens [20]. Initial brain analyses pointed out that young chickens displaying high FP incidences had lowered serotonergic and dopaminergic turnover compared to chickens with low FP [19,21,22].

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Recent brain analyses in adult hens, however, provided seemingly conflicting results: phenotypically and genotypically selected adult high feather peckers had a higher 5-HT turnover [6,23] and a higher DA metabolism [24] than low feather peckers. Thus, relationships between FP tendencies and brain neurochemical levels may vary between ages, but also SFP behaviour itself is not constantly present in chickens. Young chickens predominantly perform gentle feather pecking (GFP), whereas most SFP incidences are recorded when laying hens reach reproductive maturity [3,6,25]. Moreover, GFP in young chicks is generally not a good predictor of SFP in adult hens and not all animals will perform SFP [3,26,27]. Importantly, with one exception [28], previous brain studies have all been performed in lines not directly selected on SFP but on related traits such as productivity [21], or mortality [24].

Genetic selection on SFP behaviour itself has resulted in divergent feather pecking lines, called the high feather pecking (HFP) and low feather pecking (LFP) lines [29]. These lines do not only differ in SFP, but also in other behavioural and physiological characteristics such as general (hyper)activity [12,30] and heart rate variability in response to stress [31]. In order to more clearly study the role of 5-HT and DA in SFP, this study aimed to compare these monoamines in brain areas involved in both emotional regulation and motor control between HFP and LFP hens, both when young and early into lay. This was combined with behavioural tests and observations to learn more on the animals' behavioural characteristics. During observations, the prevalence of gentle, severe, aggressive and toe pecking was recorded.

2. Materials and 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. Animals were visually checked daily for signs of wounds as a consequence of SFP to react immediately when animal welfare was compromised.

2.2. Birds and housing

White leghorn hens from the 9th generation of divergently selected lines for high feather pecking (HFP) and low feather pecking (LFP) were used (see [32,33] for details on the selection procedure). Eggs of both HFP and LFP birds were brooded and after hatch the one-day old female chicks received 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 floor pens (1.9×1.2 m) covered with paper (first seven 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. Continuous light was given the first week, 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 between 2.00 am–6.00 pm at 23 weeks of age, in line with commercial practice. In the first two weeks, three chicks turned out to be male and four chicks had died. In week 8, each group was reduced by one chicken and the brains of these chickens were dissected and stored ($n = 6$/line) (referred to as young). The group size was now 65 animals ($n = 32$ LFP; $n = 33$ HFP, $n = 5$ or 6 per pen). At an age of 10 weeks, the pullets were moved to a new animal facility. In week 23, two hens per pen were selected for microdialysis, as described elsewhere [28]. In week 25, 25 animals ($n = 12$ LFP; $n = 13$ HFP) were culled and the brains were dissected and stored (referred to as adult).

2.3. Behavioural observations and tests

Birds were individually subjected to six behavioural tests, which are described below. In addition, pecking behaviours were scored between two and 16 weeks of age. Order of testing and observations was always balanced for lines and pens. The experimenter was blind to the allocation of lines to the different pens.

2.3.1. Pecking observations

Pecking behaviour of each individual bird was weekly observed from week 2 till week 16 with exception of week 9 and 10. Each observation lasted 25 min. At the start of each observation, the experimenter sat in front of the pen and waited for 5 min until starting with behavioural recordings. Frequencies of gentle feather pecking (GFP), severe feather pecking (SFP), toe pecking, and aggressive pecking were recorded at individual level. GFP was defined as light pecks given at the feathers; SFP was vigorous pecking and/or the pulling of feathers resulting in feather damage and/or removal; toe pecking was pecking directed at toes of others with risk of damaging the skin; and aggressive pecking consisted of forceful pecks at the head [34]. Pecking behaviours were averaged over weeks 2–8 for young birds, and weeks 10–16 for older birds, and expressed as frequencies per hour.

2.3.2. Behavioural tests

Isolation test. At an age of 8–9 days, each chick was subjected to an isolation test, carried out on two consecutive days. The chick was put in a round bucket (diameter 28 cm) outside the home pen, but in the same room. For 2 min, the latency to move and to vocalize, the number of vocalizations and escape attempts were recorded.

Runway test. At an age of 15–16 days, each chick was subjected to a runway test, carried out on two consecutive days. The wooden runway of 160×20 cm was 25 cm high and had a start box (20×20 cm) and a goal box (20×20 cm) at both ends of the runway closed with a steel mesh door. Three female stimulus chickens of similar age, which were not part of the experiment, were placed in the goal box. The tested chick was placed in the start box, and after 1 min, the mesh door was removed and the chick was given 5 min to get to its conspecifics. Latency to move, time to reach the goal box, frequency and latency of vocalizing and number of defecations were recorded.

Novel object test. At 23 days of age, the response to a novel object was tested. The novel object was a wooden block ($5 \times 5 \times 2$ cm) wrapped with colored tape (red, yellow, white, and green) which was placed on the floor of the pen. The experimenter stood in front of the pen and recorded the latency of each bird to approach the object at a distance (radius) of 25 cm. Birds that did not approach the object within the maximum test time of 5 min, were given the maximum time score. As many birds did not approach the object within 25 cm or closer during the test, approaching at 25 cm was also scored as a binary variable (yes/no).

Human approach test. At 31 days of age, a human approach test was conducted. One experimenter squatted in front of the opened door of the pen and stretched her arm inside the pen while the gaze was averted (head turned away) (Welfare Quality: Assessment Protocol for Poultry). Another observer stood aside and recorded the latency of each animal to approach the person's hand at a distance (radius) of 25 cm. Birds that did not approach the person within the maximum test time of 5 min, were given the maximum time score. Also, the binary variable of approaching the person at 25 cm (yes/no) within the total observation time of 5 min was scored.