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**Title**

Where in the serotonergic system does it go wrong? Unravelling the route by which the serotonergic system affects feather pecking in chickens

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**Highlights**

- Feather pecking (FP) in chickens can be malfunctioning and maladaptive.
- Deficient 5-HT<sub>1A</sub> autoreceptor and MAO-A activity appear predisposing for FP.
- Young chicks with high FP tendency have low central 5-HT and DA activity.
- Central 5-HT and DA activity is affected in adult FP birds in specific brain regions
- Peripheral 5-HT, gut microbiota, immune functioning, are related to FP.

**Abstract**

A deficient serotonergic system is associated with psychopathological behaviors in various species, among which, feather pecking (FP) in chickens. Deficiency in the serotonergic system

can predispose birds to develop FP, while the serotonergic system has shown to be affected in birds that feather peck. Serotonin (5-HT) can further influence dopamine (DA) activity. Lines with high FP tendency generally have low central 5-HT and DA turnovers at a young age, but high turnovers at an adult age in brain areas involved in somato-motor regulation. Agonizing 5-HT<sub>1A</sub> and 5-HT<sub>1B</sub> receptors increases FP, while antagonizing D<sub>2</sub> receptor reduces FP. Genetic associations exist between FP, 5-HT<sub>1A</sub> and 5-HT<sub>1B</sub> receptor functioning and metabolism of 5-HT and DA. Birds with deficient functioning of the somatodendritic 5-HT<sub>1A</sub> autoreceptor and 5-HT metabolism appear predisposed to develop FP. Birds which feather peck often eat feathers, have low whole-blood 5-HT, different gut-microbiota composition and immune competence compared to non-peckers. FP and feather eating likely affect the interaction between gut microbiota, immune system and serotonergic system, but this needs further investigation.

### **Abbreviations**

3-MT: 3-methoxytyramine; 5-HIAA: 5-hydroxyindoleacetic acid; 5-HT: serotonin (5-hydroxytryptamine); 5-HTP: 5-hydroxy-L-tryptophan; Am: amygdala; Ap: arcopallium; Arch: archistriatum; ATD: acute tryptophan depletion; BBB: blood-brain-barrier; CL: control line of the LML; CNS: central nervous system; CORT: corticosterone; DA: dopamine (3,4-dihydroxyphenethylamine); DOPAC: 3,4-dihydroxyphenylacetic acid; dT: dorsal thalamus; DW: Dekalb White; DXL: Dekalb XL; EC: enterochromaffin; FP: feather pecking; GM: gut microbiota; HFP: high feather pecking; HGPS: high group productivity and survivability; HP: high pecking; Hpc: hippocampus; HVA: homovanillic acid; Hyp: hypothalamus; IL: interleukin; L-DOPA: L-3,4-di-hydroxyphenylalanine; LFP: low feather pecking; LGPS: low group productivity and survivability; LML: low mortality line; LNAA: large neutral amino acid; LP: low pecking; MAO: monoamine oxidase; mSt: medial striatum; NAc: nucleus

accumbens; NCC: cauda-central nidopallium; NCL: caudo-lateral nidopallium; NE: norepinephrine; OCD: obsessive compulsive disorder; PHE: phenylalanine; RIR: Rhode Island Red; RN: raphe nuclei; SCFA: short-chain fatty acids; SERT: serotonin transporter; SNP: single nucleotide polymorphism; SSRI: selective serotonin reuptake inhibitor; TH: tyrosine hydroxylase; TI: tonic immobility; TPH: tryptophan hydroxylase; TRP: tryptophan; TTM: trichotillomania; TYR: tyrosine; WL: White Leghorn.

**Keywords** feather pecking, laying hen chickens, serotonin, dopamine, 5-HT receptors, monoamine oxidase, tryptophan, malfunctioning, maladaptive, coping style, anxiety

## 1. Introduction

The serotonergic system contains the central neurotransmitter and peripheral signaling molecule serotonin (5-hydroxytryptamine: 5-HT). Central 5-HT is a neurotransmitter with diffuse afferent projections from the raphe nuclei (RN) to various brain areas, thereby having a role in many processes such as appetite, food intake, mood, reproduction, pain, sleep and growth (Angoa-Perez et al., 2012; Berger et al., 2009; Dalley and Roiser, 2012; de Boer, 2009; Gillette, 2006). Central 5-HT regulates, in part, also dopamine activity (3,4-dihydroxyphenethylamine: DA) in several brain areas (De Deurwaerdère and Di Giovanni, 2017; Matsunami et al., 2012; Navailles and De Deurwaerdère, 2011; Yadid et al., 1994). Central 5-HT hereby indirectly affects the motivation for reward related behaviors, a major function of DA (Kalenscher et al., 2005). Peripheral 5-HT can be found in several organs, thereby playing part in various physiological functions such as energy metabolism, pain perception, and bone remodeling (for review see Spohn and Mawe, 2017). The majority of 5-HT can be found peripherally in blood platelets and in enterochromaffin (EC) cells of the gastrointestinal tract (i.e. 95% of peripheral 5-HT is stored in EC cells (Mohammad-Zadeh et

al., 2008)). Here, gut 5-HT is released from EC cells upon mucosal stimulation, and acts as an important signaling molecule influencing gut motility, vasoconstriction/dilation, secretion, inflammation and immune regulation (Bornstein, 2012; Coates et al., 2017; Shajib and Khan, 2015). Central and peripheral 5-HT act independently, but can also interact via the bloodstream (as affected by hormones, cytokines, enzymes, short-chain fatty acids (SCFA)), gut microbiota and vagus nerve activation (Coates et al., 2017) (see Figure 1). They further share similar characteristics in their transporter, receptors, metabolite and are synthesized from their precursor tryptophan (TRP) (Berger et al., 2009). TRP is an essential amino acid, indicating that synthesis of both central and peripheral 5-HT is limited by the level of TRP in the diet (Young and Leyton, 2002).

Serotonin is essential to many homeostatic physiological processes while alterations, mainly in central 5-HT functioning, affect many pathological behaviors, directly or indirectly via its effects on DA. A vast amount of scientific evidence shows that dysfunctioning of central 5-HT and DA activity is key to a variety of mental disorders (Yildirim and Derksen, 2013). Deficient activity of central 5-HT is presumed causal in excessive aggression, impulsive and compulsive behavior (Fineberg et al., 2010; Potenza et al., 2009). Compulsive behavior is difficult to disrupt and often occurs under stress in predisposed subjects (Boulougouris et al., 2009). Acute tryptophan depletion (ATD) increases obsessive compulsive symptoms in predisposed subjects, while ATD does not affect healthy controls (Mendelsohn et al., 2009). Central 5-HT is reduced by ATD treatment (Crockett et al., 2012; Young and Leyton, 2002), linking motor disruptive behavioral effects of ATD to low central 5-HT. Compulsive hair pulling (trichotillomania: TTM) in humans is a motor impulse disorder (Fineberg et al., 2010) and has been associated with alterations in central and whole blood 5-HT (Johnson and El-Alfy, 2016). In many cases, symptoms of TTM are reduced with treatment of selective serotonin reuptake inhibitors (SSRIs) (McGuire et al., 2014). SSRIs block the serotonin transporter

(SERT), inhibiting 5-HT reuptake into the neural cell body and blood platelet, thereby extending extracellular 5-HT activity. By desensitizing the somatodendritic 5-HT<sub>1A</sub> autoreceptor in the RN, SSRIs increase levels of central and whole-blood 5-HT (Larsen et al., 2004; McGuire et al., 2014).

Self-directed feather picking in parrots (Bordnick et al., 1994; Garner et al., 2006; van Zeeland et al., 2009) and feather pecking (FP) of conspecifics in chickens (laying hens; Kops et al., 2014; van Hierden et al., 2002) have been suggested as avian models for TTM. Although many studies have highlighted the influence of 5-HT and DA on FP (Kops et al., 2014; Rodenburg et al., 2013; van Hierden et al., 2004a), it remains unclear which parts of the serotonergic system (see Figure 1) are affected in birds that are at risk to develop FP or that already have started to feather peck. Therefore, in this review, we have examined studies relating the serotonergic system with FP and behavioral modulations related to FP, such as feather eating, foraging, anxiety and activity. We specifically highlighted the route by which the serotonergic system was altered prior to and after FP developed. Although other neuroendocrine systems might play a role in the development of FP, we focused on the serotonergic system as to us crucial in the development of FP. We have also included studies on DA, as the serotonergic system and dopaminergic system interact, and both appear influential in FP.

>>>>>INSERT FIGURE 1

## **2. What is feather pecking?**

Feather pecking is the act of a bird plucking the feathers of other birds, which can be gentle or severe (Rodenburg et al., 2013; Savory, 1995). Gentle FP is a common behavior presumed to be related to social discrimination and exploration (Riedstra and Groothuis, 2002). Severe FP occurs in some birds and can lead to feather damage, denuded areas, injuries in victims and in extreme cases to cannibalistic pecking (Savory, 1995). In this review, we refer to severe FP as

FP. Cannibalistic pecking occurs also independently from FP and can occur during vent pecking. Vent pecking is the act of pecking at the vent of other birds, during the process of egg laying. Vent pecking causes tissue damage to the cloaca mucosa and can develop into cannibalistic pecking. Aggressive pecking is distinct from FP by having a different underlying motivation (i.e. establishing hierarchy) and is directed to the head and comb area rather than to the bird's back area as often occurs during FP (Rodenburg et al., 2013). Different forms of pecking can co-occur, but cannibalistic pecking, aggressive pecking, gentle and severe FP are presumed to have different underlying motivations (Buitenhuis and Kjaer, 2008; Savory et al., 1999).

### **2.1. Feather pecking; a redirected foraging behavior**

Feather pecking is considered a redirected foraging behavior (Rodenburg et al., 2013). Foraging is the act of searching for food, shown in chickens by active walking, pecking, scratching and ingesting edible items i.e. food intake. Food intake is regulated by central 5-HT and DA, with central 5-HT being involved in satiety and meal termination, and DA in meal initiation (Meguid et al., 2000). Central 5-HT and DA interact in controlling feeding behavior (Zendehdel et al., 2017). Increasing central 5-HT, its metabolite 5-hydroxyindoleacetic acid (5-HIAA) and 5-HT turnover (5-HIAA/5-HT) in the diencephalon of chicks by intracerebroventricular injection of TRP or 5-HT has been shown to inhibit food intake (Bungo et al., 2008; Zendehdel et al., 2017). Co-injection of 5-HT and Prazosin (an adrenergic receptor antagonist) weakens the 5-HT effect on food intake in chicks (Zendehdel et al., 2017, 2016). As 5-HT and DA are both involved in appetite and food intake in chickens, it is highly likely that these systems play part in foraging behavior in this species. Indeed, injection of S-15535 (somatodendritic 5-HT<sub>1A</sub> autoreceptor agonist and postsynaptic 5-HT<sub>1A</sub> receptor antagonist) decreased 5-HT turnover in hippocampus (Hpc), archistriatum (Arch) and remainder of forebrain, and increased foraging and FP in chicks (van Hierden et al., 2004a). Foraging is considered a highly motivated behavior

for chickens (Cooper and Albentosa, 2003; de Jong et al., 2007). DA likely stimulates food searching and feeding motivation via its influence on appetite and facilitating rewarding behavior (Ikemoto and Panksepp, 1996; Palmiter, 2007). Birds selected on high feather pecking (HFP) show not only more FP (Kjaer and Sørensen, 2002), but also more explorative pecking (de Haas et al., 2010) and they peck more in an operant conditioning test to obtain mealworms compared to birds selected on low feather pecking (LFP) (Hausler and Harlander-Matauschek, 2008). HFP birds are also highly active in various behavioral tests (Kops et al., 2017; van der Eijk et al., 2018) and in their home pen (Kjaer, 2009). These specific lines have been suggested as a hyperactivity disorder model (Kjaer, 2009), as supposedly mainly affected by DA dysfunctioning (Kjaer et al., 2015). The aforementioned studies give support to the assumption that FP is a form of redirected feeding/foraging behavior that is controlled by central 5-HT and DA.

## **2.2. Feather pecking; a maladaptive behavior**

Maladaptive behavior is a behavior that occurs in order to cope with a thwarted environment (Mills, 2003). When the environment lacks the ability for hens to forage (for example lack of floor substrate), this can lead to FP (reviewed by Nicol et al., 2013). When in a thwarted environment, a hen's foraging motivation cannot be fulfilled, foraging pecks can be redirected to the plumage of her group-mate(s). As such, fixed motor patterns of foraging and FP appear similar (Dixon et al., 2008). Feather pecking under suboptimal foraging conditions thus reflects a maladaptive behavior.

An animal's ability to cope with a thwarted environment depends on how it deals with various stressors (i.e. coping style: Koolhaas et al., 2007). The way in which animals cope with stressors (i.e. reactive or proactive) may influence FP. Behavior of hens of high FP lines have been suggested to reflect a proactive coping style (de Haas et al., 2010; Kops et al., 2017; Korte et al., 1997; van der Eijk et al., 2018), although behavior of individual birds which show FP

does not necessarily reflect proactive coping (Forkman et al., 2004). A proactive coping style has been linked to 5-HT and DA dysfunctioning (Coppens et al., 2010). Specifically, a proactive coping style relates to deficient 5-HT<sub>1A</sub> receptor functioning (Koolhaas et al., 2007; Puglisi-Allegra and Andolina, 2015; Vindas et al., 2017) and DA signaling (Giorgi et al., 2003; Höglund et al., 2017). Animals with a proactive coping style are more inclined to be aggressive, impulsive and to develop stereotypies under chronic stress (Geverink et al., 2003; Korte et al., 2005), whereas animals with a reactive coping style are more inclined to develop depression and anhedonia under chronic stress (de Boer et al., 2017; Koolhaas et al., 2007; Korte et al., 2005). The behaviors executed by animals with different coping styles may be equally effective in dealing with a stressor, but their success in coping varies per environment (Mendl et al., 2010). In humans, TTM under stressful conditions is seen as a way of proactive coping (Alexander et al., 2017). In summary, FP can be seen as a maladaptive behavior, i.e. as an attempt to cope with a thwarted environment, which has been linked to deficiencies in 5-HT functioning.

### *2.2.1 Interaction stress, central 5-HT and maladaptive feather pecking*

Stress can affect central 5-HT activity (Korte et al., 2005). In rodents, elevation of glucocorticoid hormones (i.e. corticosterone: CORT) can decrease tryptophan hydroxylase activity (TPH; enzyme that converts TRP to 5-hydroxy-L-tryptophan (5-HTP)) and central 5-HT tone (Chen et al., 2017). Furthermore, CORT can affect serotonergic activity by increasing sensitivity of 5-HT<sub>1A</sub> receptors (Meijer and de Kloet, 1998) and by decreasing SLC6A4 expression (gene coding for SERT) (Borue et al., 2007). Stress factors, such as social stress, can further influence peripheral 5-HT in chickens. For example, within group-aggression causes stress in hens (de Haas et al., 2012) and influences hens' peripheral TRP and TRP/(PHE + TYR) ratio (PHE: phenylalanine and TYR: tyrosine)(Birkl et al., 2017a), thereby potentially affecting 5-HT synthesis. In response to social stress, low group productivity and survivability (LGPS)

birds had higher plasma DA and CORT compared to high group productivity and survivability (HGPS) birds (Cheng et al., 2002). These lines also differ in central 5-HT and DA functioning (Dennis et al., 2008; Dennis and Cheng, 2012). Further, Lutz et al. (2016) showed that genetic correlations between FP, locomotion and feather eating is strongly affected by environmental effects. Being successful in coping with (social) stress may thus limit the risk of developing FP as a maladaptive behavior in hens.

### **2.3. Feather pecking; a malfunctioning behavior**

A malfunctioning behavior is a pathological behavior that occurs relatively irrespective of the animal's environment, and is strongly related to an individual's predisposition. An individual's predisposition to develop FP as a malfunctioning behavior could be caused by the individual's (epi) genetic set-up. FP is moderately heritable (Kjaer and Sørensen, 2002) and epigenetic modulation is likely involved (Rodenburg and de Haas, 2016). Epigenetic alterations can influence various behavior patterns in chickens (Jensen, 2014) and malfunctioning stereotypic behavior in birds (Bateson et al., 2004). Stress in the maternal hen can lead to epigenetic modifications, which affects behavior and stress responses in the offspring (Henriksen et al., 2011). In young chickens, exposure to elevated CORT during incubation resulted in lower whole-blood 5-HT and higher platelet 5-HT uptake as compared to saline treated chicks (Ahmed et al., 2014). On a flock level, maternal birds with high whole-blood 5-HT and high plasma CORT are found to have offspring which are more anxious and perform more FP already at one week of age compared to offspring from birds with low whole-blood 5-HT and low plasma CORT levels (de Haas et al., 2014b). On an individual level, it is known that stress in parental birds can affect gene methylation, gene expression and behavior of the offspring and grand-offspring in chickens and quail (Goerlich et al., 2012; Guibert et al., 2012; Lindqvist et al., 2007; Nätt et al., 2009). Preliminary results show differences in methylated genes in the hypothalamus (Hyp) between HFP and LFP birds (De Haas et al. unpublished results), which

suggest epigenetic effects on FP. However, more evidence is needed to unravel whether and how epigenetic modulation, via egg hormones, and other forms of epigenetic modifications play a role in FP (Rodenburg and de Haas, 2016).

Alterations in functioning of 5-HT system may lead to FP or associated malfunctioning behaviors, such as excessive aggression and anxiety. For example, in-ovo injection of 5-HT during incubation reduces chick aggression and increases tissue level of 5-HIAA, DA and DA turnover in the Hyp (Dennis et al., 2013a). Excessive aggression in rodents is a form of malfunctioning behavior related to deficient 5-HT system and a proactive coping style (Koolhaas et al., 1999). Although FP and aggression in chickens appear different in origin, some studies have also found consistent evidence for alterations in 5-HT<sub>1A</sub> and 5-HT<sub>1B</sub> receptor functioning in aggression and FP, but only in a highly aggressive line (Dennis and Cheng, 2012). FP may be similarly affected by alterations in the serotonergic system, as shown in extremely aggressive rodents and chickens. Thereby FP reflects a pathology influenced by a dysregulated 5-HT system, similar to aggression.

Genetic and phenotypic associations have also been found between high anxiety at a young age and FP at an adult age (Rodenburg et al., 2004a). Anxiety is a sustained high tendency to be fearful (Clement and Chapouthier, 1998; Korte, 2001; Korte et al., 2009; Reiss et al., 1986). Birds of a line which are more likely to peck conspecifics and perform cannibalism are more anxious at a young age than birds less likely to peck conspecifics (de Haas et al., 2012; Rodenburg et al., 2009b). Low central 5-HT activity appears predisposing for anxiety (Lucki, 1998). In humans, alterations in TPH, SERT, monoamine oxidase (MAO; enzyme that metabolizes 5-HT to 5-HIAA) and somatodendritic 5-HT<sub>1A</sub> autoreceptor functioning have been related to anxiety (Borue et al., 2007). Broiler chickens selected on long tonic immobility (TI; death feigning fear response) show lower hypothalamic expression of the gene *SLA6C* (encoding SERT) and the *HTR1A* gene (encoding the somatodendritic 5-HT<sub>1A</sub> autoreceptor)

than chickens selected on short TI (Wang et al., 2014). Furthermore, layers exhibit longer TI when exposed to 5-MT (5-methoxytryptamine; a 5-HT<sub>1</sub>, 2, 4, 6, 7 receptor agonist) (Dennis et al., 2013b), and high fear roosters have lower levels of plasma 5-HT than low fear roosters (Agnvall et al., 2015). Animals with high anxiety can develop a psychopathology such as FP (Korte et al., 2009; Pryce and Feldon, 2003) irrespective of their environment.

In summary, the tendency to develop FP appears pre-set by a chicken's (epi) genetic background which can further create a (epi) genotype\*environment sensitivity. Certain lines of commercial hybrids seem more sensitive to develop FP as a maladaptive behavior, while others are more sensitive to develop FP as a malfunctioning behavior (see Figure 2). For example, ISA Brown hens are strongly influenced by social factors (group composition, (Uitdehaag et al., 2011, 2009); group size, (de Haas et al., 2013), and litter disruption (de Haas et al., 2014a)). These environmental challenges create a risk for the development of FP as a maladaptive behavior. Dekalb White (DW) hens have a high (epi)genetic predisposition for development of FP as a malfunctioning behavior, relating to anxiety and low whole-blood 5-HT, central 5-HT and DA compared to ISA Brown (de Haas et al., 2014b, 2013; Uitdehaag et al., 2011). FP can thus derive as a form of maladaptive behavior due to a thwarted or stressful environment as well as a form of malfunctioning behavior due to (epi) genetic predisposition.

>>>INSERT FIGURE 2<<<<<

### **3. Genetic evidence for involvement of the serotonergic system in feather pecking**

Birds differing in pecking tendency have shown to differ in gene expression and in genetic background considering genes important for functionality of the 5-HT system.

#### **3.1. Gene-expression related to the 5-HT system in birds with diverging pecking tendency**

The most recent study on FP identified eight genes differently expressed in the whole brain of HFP and LFP birds (Lutz et al., 2017). Potential candidate genes were SLC12A9 (encode

electroneutral cation – chloride cotransporters) and GNG2 (G protein subunit gamma 2 gene) related to high aggression and FP, respectively. Family of SLC12 genes control transporters which cause a reduction of postsynaptic GABA-A receptor current (Medina et al., 2014). This effect is similarly caused by 5-HT via 5-HT<sub>2C</sub> receptors (Feng et al., 2001). The GNG2 gene encodes gamma subunits of a guanine nucleotide-binding protein, which are important for signaling across membranes. According to Lutz et al. (2017), the GNG2 gene is involved in postsynaptic signaling at 5-HT synapses, and emphasized as candidate gene for FP. Wysocki et al. (2013) found that the HTR1B gene was upregulated and the MAO-A gene was downregulated in the whole brain of HFP birds compared to LFP birds. The HTR1B gene encodes the G-protein coupled 5-HT<sub>1B</sub> receptor. HTR1B manages release of 5-HT (Olivier and Van Oorschot, 2005). The MAO-A gene encodes MAO-A enzymes catalyzing deamination of amines as 5-HT, DA and norepinephrine (NE). Hughes and Buitenhuis (2010) also compared gene-expression in the whole brain of HFP and LFP birds, but did not find differences in expression in genes related to 5-HT or DA system. They found that the PDZD2 gene (encode endoplasmatic protein) was positively correlated to severe FP in the HFP line. Although not specific to the serotonergic system, PDZD2 gene is similar in sequence and protein encoding as the pro-interleukin-16 gene. Interleukins (IL) are cytokines, which are key signaling molecules of the immune system. In feather peckers genes involved in the immune system (LAG3 (encoding immuno regulatory receptors); MAPK8 and TNFSF15 (tumor necrosis factor regulating genes) were upregulated in the Hyp compared to victims (Brunberg et al., 2011). These genes are involved in intestinal inflammation (Yuan et al., 2017). Intestinal inflammation is said to be regulated by gut 5-HT (Bornstein, 2012; Coates et al., 2017; Shajib and Khan, 2015). Although no direct link between FP and genes encoding central 5-HT was found in this study, it also showed that feather peckers had higher expression of genes associated with glucose metabolism and obsessive-compulsive disorder (OCD) compared to victims and

neutrals (Brunberg et al., 2011). OCD is related to deficiency in 5-HT functioning in mammals (Boulougouris et al., 2009; Joel, 2006). Hereby, upregulation of OCD related genes in feather peckers indirectly links to deficiency in 5-HT functioning.

### **3.2. Genetics related to the 5-HT system in birds with diverging pecking tendency**

Birds which differ in pecking tendency also have a different genetic makeup. For example, see the animal genome database for QTLs identified for FP at <https://www.animalgenome.org>. When comparing the HFP and LFP line, two sub-haplotypes were found for the DRD4 gene (encoding the dopamine D4 receptor) and DEAF-1 (encoding deformed epidermal autoregulatory factor 1 homolog) (Flisikowski et al., 2009). These sub-haplotypes were linked to FP. The DEAF-1 protein represses the gene encoding the somatodendritic 5-HT<sub>1A</sub> autoreceptor promoter (Albert et al., 2011). Feather damage (as a proxy for FP, Bilcik and Keeling, 1999) has been associated with thirteen clusters of single nucleotide polymorphisms (SNPs) in a F<sub>2</sub> cross of the HFP and LFP lines (Lutz et al., 2017), but SNPs were not related to the 5-HT system. In 9 commercial lines, however, SNPs were found in regions of the HTR<sub>2C</sub> (encoding 5-HT<sub>2C</sub> receptor), MAO-A and IL-19 genes in relation to feather damage (Biscarini et al., 2010). Another study in the FP selection lines showed that most SNPs in relation to FP were found on chromosome 4 where the HTR<sub>2C</sub> gene is located, followed by chromosome 3 where the HTR<sub>1B</sub> gene is located (Grams et al., 2015). Feather damage was also associated with an unfavorable high allele frequency for the HTR<sub>2C</sub> gene in birds being severely feather pecked and cannibalized. Similar results were obtained by Yao et al. (2017) where SNP and enhanced gene expression for HTR<sub>2C</sub> were related to high feather damage in a Chinese layer line. Although these results were not supported by a recent genome wide association study of Lutz et al. (2017) in relation to performing FP. The results on the 5-HT<sub>2C</sub> receptor appear to reflect a predisposition for being feather pecked rather than on performing FP. Overall, these genetic studies show that FP is associated with changes in sequence and expression of genes

encoding receptors and metabolic enzyme of 5-HT, and the immune system in selection lines as well as in commercial hybrids.

#### **4. Central levels, metabolite and turnover of 5-HT in birds with diverging pecking tendency**

When identifying differences in the central serotonergic system between birds with diverging pecking tendency, studies measure central 5-HT, 5-HIAA and 5-HT turnover (5-HIAA/5-HT). 5-HIAA is used to reflect MAO-A activity (Wolf et al., 1985) and 5-HT turnover is used as an indicator for activity of the central serotonergic system (Kops, 2014). 5-HT derived from punched tissue are said to reflect metabolic activity of afferent raphe 5-HT fibers and thereby parallels fiber density (Hale and Lowry, 2011), and gives some indication on the storage level of 5-HT. However, levels of 5-HT derived from punched tissue do not provide specific information where in the neuronal system (cell body or synaptic cleft) these levels were measured and it provides no information on the actual release of 5-HT. 5-HT from dialysate gives more information on 5-HT tone and release. All studies discussed here were based on the punched method, except Kops et al. (2014) who, as first, used microdialysis in HFP and LFP birds. In general, lines with a high tendency to develop FP show low central 5-HT turnover in multiple brain areas at young age, but at adult age central 5-HT turnover was high in specific brain areas compared to lines with a low tendency to develop FP (see Table 1). Levels of 5-HT, 5-HIAA and 5-HT turnover in relation to FP at adult age appear brain area specific. Several specific brain areas were studied to identify differences in the central serotonergic system between birds with diverging pecking tendency, namely the dorsal thalamus (dT) involved in goal-directed behavior (van den Heuvel et al., 2010), medial striatum (mSt) involved in reward (Aoki et al., 2006b), hippocampus (Hpc) involved in memory and learning (Colombo and Broadbent, 2000), hypothalamus (Hyp) involved in the stress response (Puglisi-Allegra and Andolina, 2015), archistriatum (Arch) which includes the arcopallium (Ap) and amygdala (Am)

(Reiner et al., 2004) both involved in emotional processes mainly including fear-related behavior (Am) and somatomotor regulation (Ap) (Saint-Dizier et al., 2009), caudo-lateral nidopallium (NCL) and caudo-central nidopallium (NCC) involved in motor actions and decision making (Güntürkün, 2005; Jarvis et al., 2013; Reiner et al., 2004) and the raphe nuclei (RN) a portal for afferent 5-HT neurons (Adell et al., 2002).

#### **4.1. Central levels, metabolite and turnover of 5-HT in young birds**

Only three studies compared lines which diverge in FP tendency at a young age. van Hierden et al. (2002) examined differences in frontal 5-HT in young birds from a line selected on egg production traits which diverged in FP behavior, resulting in high (HP) and low pecking (LP) lines (Korte et al., 1997). At 4 weeks of age, HP chicks had lower 5-HT turnover and tended to have lower 5-HIAA, but higher 5-HT in the rostral brain compared to LP chicks (van Hierden et al., 2002). At 7 weeks of age, HP chicks had lower 5-HIAA (Arch) and tended to have lower 5-HT turnover (forebrain and Arch) and 5-HT (forebrain) compared to LP chicks (van Hierden et al., 2004b). Kops et al. (2017) studied HFP and LFP lines at 8 weeks of age in seven brain areas. HFP chicks had lower 5-HT turnover (Ap, mSt and NCC), 5-HIAA (Am, dT, mSt, and NCC) and 5-HT (dT), and compared to LFP chicks, but no line differences were found in levels of 5-HT turnover, 5-HIAA, 5-HT in the NCL and Hpc (Kops et al., 2017).

#### **4.2. Central levels, metabolite and turnover of 5-HT in adult birds**

At an adult age, multiple studies compared birds which diverge in pecking behavior. Namely, comparing HFP with LFP line (Kops et al., 2017), feather peckers with non-peckers (Kops et al., 2013a), a control line (CL) with a line selected on low group mortality due to cannibalism (LML; low mortality line) (Kops et al., 2013b), White leghorn (WL) with Rhode Island Red (RIR) (Uitdehaag et al., 2011), and LGPS, HGPS with the commercial hybrid Dekalb XL (DXL) (Cheng and Fahey, 2009; Dennis et al., 2008; Dennis and Cheng, 2014).

Overall, line or phenotype comparisons did not show differences in 5-HT levels in any brain area measured, except for WL birds that tended to have lower 5-HT in the rostral brain compared to RIR birds (Uitdehaag et al., 2011), and for HFP birds which had higher extracellular 5-HT in the NCC and NCL compared to LFP birds (Kops et al., 2014). For 5-HIAA, birds with high pecking tendency generally had higher levels in dT (feather pecker vs. non-pecker), mSt (feather pecker vs. victim (Kops et al., 2013a)), Ap (HFP vs. LFP and CL vs. LML (Kops et al., 2017, 2013b)) and the rostral brain (WL vs. RIR (Uitdehaag et al., 2011)) compared to birds with low pecking tendency. 5-HT turnover was higher for birds with high pecking tendency in NCC (HFP vs. LFP (Kops et al., 2017)), dT (feather pecker vs. non-pecker (Kops et al., 2013a)), and in the rostral brain (WL vs. RIR (Uitdehaag et al., 2011)) compared to birds with low pecking tendency. Apart from DXL birds which had lower 5-HT turnover in RN compared to HGPS birds (Cheng and Fahey, 2009).

In the FP selection lines, results regarding FP and 5-HT at an adult age seem opposite to what was found at a young age (see Table 1). All differences between the HFP and LFP lines at a young age regarding 5-HT, 5-HIAA or 5-HT turnover in the Am, Ap, dT, mSt and NCC were not found or were opposite at an adult age (Kops et al., 2017). This contradiction in results between young and adult age can be due to the performance of FP at adult age. For example, in the HFP and LFP lines, FP started at 11 weeks of age, after measurements at young age (i.e. 8 weeks of age) but prior to measurements at adult age (i.e. 25 weeks of age) (Kops et al., 2017). Feather pecking as well as receiving FP can influence levels of 5-HIAA and 5-HT turnover, especially in dT and mSt. Namely, feather peckers have higher 5-HIAA and 5-HT turnover in the dT compared to non-peckers, and higher 5-HIAA in the mSt compared to victims. While victims had higher 5-HIAA and 5-HT turnover compared to non-peckers in dT, but lower 5-HT and 5-HIAA in mSt (Kops et al., 2013a).

When measuring extracellular 5-HT and 5-HIAA in the NCC and NCL, HFP birds had higher 5-HT tone, but with no difference in 5-HIAA compared to LFP birds at 23 weeks of age (Kops et al., 2014). In birds that developed FP higher levels of basal extracellular 5-HT indicate changes within the neuronal cell, which could be due to a change in sensitivity of the somatodendritic 5-HT<sub>1A</sub> autoreceptor influencing the release of 5-HT. Differences between dialysate and punched tissue measurements in these lines can further direct to extracellular or intracellular mechanisms, respectively. Punched tissue measurements more likely gives information on intracellular processes, while microdialysis gives more information on extracellular processes. Measuring efflux of extracellular 5-HT in specific brain areas (as done by Kops et al., 2014) prior to and during FP could indicate consequences of performing FP on central 5-HT activity. However, ethical considerations and practical feasibility of microdialysis in a social setting where FP could occur has limited this research venue so far. Pharmacological treatments that affect intra- or extracellular processes could be used as an approach to identify where in the synapse, or system alterations occur during and after FP developed.

In summary, at a young age laying hen lines with a high FP tendency had low levels of 5-HT, 5-HIAA or 5-HT turnover in several brain areas (Am, Ap, Arch, dT, forebrain, mSt, NCC and rostral brain) compared to lines with a low FP tendency (Kops et al., 2017; van Hierden et al., 2004b, 2002). The brain areas where low activity was seen are part of the network involved in regulating motor actions and decision making by integrating sensorimotor, motivational and emotional information (Güntürkün, 2005; Jarvis et al., 2013; Reiner et al., 2004). A deficient serotonergic system in these specific brain areas at a young age appears predisposing in the development of FP.

At an adult age, hens originating from lines with a high tendency to develop pecking directed to group mates (FP, toe pecking, cannibalism) show increased levels of 5-HT, 5-HIAA or 5-HT turnover in the Ap, NCC, NCL and rostral brain compared to hens from lines with a

low tendency to develop pecking directed to group mates (Kops et al., 2017, 2014, 2013b; Uitdehaag et al., 2011). These brain areas are involved in motor actions, decision making and fear-related behavior (Güntürkün, 2005; Jarvis et al., 2013; Reiner et al., 2004; Saint-Dizier et al., 2009). Many studies have linked FP to fearfulness (Rodenburg et al., 2004b) and motor activity (Kjaer, 2009), indicating that high 5-HIAA or 5-HT turnover in these specific brain areas at adult age supports FP behavior. Interestingly, feather peckers were found to have a high activity of the serotonergic system in the dT and mSt compared to non-peckers and victims (Kops et al., 2013a), indicating that a high activity of the serotonergic system in these brain areas could be related to the actual performance of FP. The dT and mSt are involved in goal-directed behavior and reward (Aoki et al., 2006b; van den Heuvel et al., 2010), suggesting that the performance of FP could be rewarding. The changes in 5-HT activity (mainly shown by metabolism and turnover) in FP birds (young vs. adult) indicate brain region specific alteration in 5-HT activity. For both young and adult FP chickens, 5-HT metabolism and turnover were affected in the Ap and Mst, and are considered areas as pathways for executive control (Matsushima et al., 2008). Interestingly, in chickens, lesions of the VSt (ventral striatum; containing Mst and nucleus accumbens (NAc)) do not affect cost-dependent foraging choices, while lesions of the Ap do (Aoki et al., 2006a, 2006b). Further, in chickens' lesions in the Vst cause impulsive choices after a learned paradigm while in lesions in the chicks' Ap did not affect impulsivity (Izawa et al., 2003; Matsushima et al., 2008). This indicates disassociated functions between Ap and VSt, and a potential role for site-specific 5-HT influence on impulsivity and foraging in chicken. In chickens, Ap is an important modulator for reward anticipation and foraging choices (Aoki et al., 2003), while the VSt facilitates executive control of behavior dependent on memory-based decision making (Matsushima et al., 2008). All brain regions where 5-HT activity was affected (either 5-HT levels, metabolites or turnover) in FP birds (Am-Ap-Mst-dT-Hpc) are part of a neural network involved in executive cognitive

control of foraging behavior (Izawa et al., 2003) and social decision making (O'Connell and Hofmann, 2011). Alterations in 5-HT activity in this network can thus influence different behaviors, as seen in FP birds, such as anxiety, motor activity, stress response and foraging decisions.

>>>> INSERT TABLE 1

## **5. Where in the serotonergic system of feather pecking birds does it go wrong?**

### **5.1. Serotonin synthesis**

Tryptophan is used for synthesis of central 5-HT in the RN and for the synthesis of peripheral 5-HT in EC cells (Coates et al., 2017). Serotonin levels, both central and peripheral, can be influenced by TRP availability and enzymatic activity of TPH. TRP availability is influenced by TRP metabolism via the kynurenine pathway (O'Mahony et al., 2015). Transportation of TRP across the blood-brain-barrier (BBB) via the large amino acid transporter can further influence central 5-HT synthesis. Here, TRP competes with other large neutral amino acids (LNAA). Thus, the TRP/LNAA ratio reflects TRP availability for transportation across the BBB (Fernstrom and Wurtman, 1972). TRP levels and TRP/LNAA ratios in plasma did not differ between lines with high or low FP tendency (HP vs. LP: (7 weeks) van Hierden et al. (2004b); HFP vs. LFP: (17 weeks) Kops et al. (2014); (31 weeks) Buitenhuis et al. (2006)). These findings suggest that TRP availability and availability of TRP for transport across the BBB might not be factors that dysregulate the serotonergic system and thus might not affect FP. However, Kops (2014) found that HFP birds tended to have lower TRP levels compared to LFP birds. This indicates that HFP birds had less TRP available for 5-HT synthesis which could explain the lower central 5-HT activity found in several brain regions compared to LFP birds at a young age (Kops et al., 2017, 2014).

Two ways to identify whether 5-HT synthesis is affected are via ATD or TRP supplementation. Up to now, effects of ATD on FP are still unpublished, although research is ongoing. Birkl et al. (2017b) showed that ATD can decrease TRP levels by 50% and TRP/(PHE + TYR) ratios by 73% in the plasma of adult HFP birds. Further, feather peckers of the HFP and LFP lines are said to react more strongly on ATD treatment with increase in impulsive action and FP compared to victims and neutrals (Birkl, personal communication). These findings indicate that the availability of TRP could play a role in FP and impulsivity, highlighting the importance of 5-HT synthesis in FP. Further research is needed to assess whether ATD affects central TRP and 5-HT levels. Supplementing TRP in the diet of HP and LP chicks from 5 weeks of age onwards increased TRP/LNAA ratio in plasma and 5-HT turnover in the Hpc and Arch at 7 weeks of age (van Hierden et al., 2004b). For HP chicks this led to an increase in 5-HT turnover in Arch and forebrain, while in LP chicks TRP supplementation increased 5-HT turnover in the Hpc. HP chicks which received TRP supplementation showed less gentle FP but more feeding and walking compared to control HP chicks. TRP-supplemented LP chicks showed more foraging but less feeding compared to control LP chicks (van Hierden et al., 2004b). TRP supplementation, via its effect on the serotonergic system in specific brain regions, appears to have affected the behavior of HP and LP chicks differently. This could indicate that birds with high FP tendency may have a more sensitive serotonergic system, especially in brain regions that regulate somato-motor and motivational responses (see Table 1). Overall, TRP supplementation seems to reduce FP behavior, likely via increasing 5-HT levels in these specific brain regions. Savory et al. (1999) showed similar findings in bantams where TRP supplementation resulted in less pecking damage compared to the control treatment, indicating reduced FP in the TRP treated group.

The activity of the rate limiting enzyme TPH could further affect 5-HT synthesis (Walther et al., 2003) and thereby influence FP. Indeed, Wysocki (2007) found that Lohmann

Selected Leghorns (high incidence of FP) had lower TPH2 gene expression (encoding tryptophan hydroxylase 2) compared to Lohmann Browns (low incidence of FP). This could indicate reduced TPH activity in lines with a high FP tendency. For instance, mice lacking the gene encoding for brain TPH2, were depleted of brain 5-HT and exhibited an excess of compulsive, aggressive and motor-impulsive behaviors (Angoa-Perez et al., 2012), supporting a potential role for TPH in FP.

In summary, a reduced 5-HT synthesis, potentially due to low TRP availability and/or reduced TPH activity may influence FP. Whether a low TRP availability and/or low TPH activity lies at the heart of FP remains elusive as there are discrepancies between studies and no studies have identified differences between FP phenotypes in relation to TRP availability or TPH activity yet. Research is needed to identify whether feather peckers differ from victims and neutrals in TRP levels and in TPH activity (both central and peripheral).

## **5.2. Serotonin transporter**

In the central nervous system (CNS), reuptake of 5-HT from the synaptic cleft is mediated by SERT. Inhibiting SERT by Fluvoxamine, a SSRI, can increase extracellular 5-HT in chicks (Matsunami et al., 2012). Lines with high pecking tendency had higher platelet 5-HT uptake than lines with low pecking tendency (CL vs. LML, Bolhuis et al. (2009); Rodenburg et al. (2009a); WL vs. RIR, Uitdehaag et al. (2011)). Platelet 5-HT uptake is controlled by peripheral SERT (Coates et al., 2017). In mammals, peripheral and central SERT show biochemical, pharmacological and genetic similarities (Yubero-Lahoz et al., 2013). Furthermore, inhibition of SERT functioning by SSRIs or psychostimulants modulates 5-HT levels in platelets and in neurons in a similar way (Larsen et al., 2004). However, caution is needed when using platelet 5-HT uptake as a peripheral measurement for central SERT activity. Neurons contain MAO-A and MAO-B, while platelets only contain MAO-B (Yubero-Lahoz et al., 2013). SERT in the gut also appears to be regulated differently from SERT in neurons (Coates et al., 2017).

Unfortunately, there is no direct measurement of SERT in relation to FP. SERT activity can be measured in rodents (Yubero-Lahoz et al., 2013). Kops et al. (2014) injected D-fenfluramine, which induces 5-HT release and blocks 5-HT reuptake by reversing SERT-mediated 5-HT transport (Skelin et al., 2010). Post-injection of D-fenfluramine, HFP and LFP birds had similar 5-HT and 5-HIAA in the NCC and NCL. The lack of difference in extracellular 5-HT and 5-HIAA post D-fenfluramine injection rules out SERT involvement in causing a deficient serotonergic activity in HFP birds in the NCC and NCL. Thus, to date there is no clear evidence that SERT plays a role in FP.

In pigs, a polymorphism in the SERT gene (SLC6A4) has been related to aggression (Terenina et al., 2012), but it is unknown if SERT is related to tail biting, a behavior similar to FP (Brunberg et al., 2016). Addressing the influence of SERT directly by use of SSRIs could help to better understand its role in FP. However, despite the finding that human and chicken SERT are comparable in kinetics and amino acid profile, the affinity of chicken SERT to specific SSRIs is much lower (Larsen et al., 2004). Larsen et al. (2004) showed that the SSRI Fluvoxamine and DASB have high pharmacological affinity in chicken and could thus be used to disentangle the role of SERT in FP.

### **5.3. Serotonin metabolism**

Metabolism of 5-HT can take place in cell bodies, terminals, astrocytes and glial cells by MAO-A and MAO-B. In the terminals, metabolism takes place exclusively via MAO-B, while in cell bodies, astrocytes and glial cells MAO-A and MAO-B are expressed. 5-HT metabolism mostly takes place via MAO-A. MAO-A activity is reflected by metabolite 5-HIAA levels. In the HFP line the MAO-A gene was found to be downregulated compared to the LFP line (Wysocki et al., 2013), indicating that MAO-A activity is reduced in the HFP line. However, adult HFP and LFP birds do not differ in extracellular 5-HIAA post D-fenfluramine injection indicates similar metabolite activity in the NCC and NCL (Kops et al., 2014). But, in two lines predisposed to

develop FP lower 5-HIAA was found at a young age (Kops et al., 2017; van Hierden et al., 2002). These findings can point to a low activity of MAO-A. Yet, at adult age the opposite was found. When comparing HFP vs. LFP (Kops et al., 2017), LML vs. CL (Kops et al., 2013b), WL vs. RIR (Uitdehaag et al., 2011) and feather peckers vs. non-peckers (Kops et al., 2013a), birds with a high tendency to develop FP (HFP, CL, WL and feather peckers) show higher 5-HIAA in several brain areas (Ap, dT, Mst, rostral brain).

In sum, lower MAO-A activity seems to be predisposing in young chicks, while higher MAO activity occurs in birds that already peck. Similarly, tail biting pigs had higher 5-HIAA in their prefrontal cortex compared to victims (Valros et al., 2015). These results further corroborate our suggestion that 5-HT metabolism is involved in damaging behaviors that are directed to conspecifics, such as FP in chickens and tail biting in pigs.

#### **5.4. Serotonin receptors**

The 14 families of 5-HT receptors can be found centrally as well as peripherally (for distribution in CNS and peripheral, see Figure 1, for functions see Coates et al. (2017); Nichols and Nichols (2008)). Pharmacological treatments that act on central 5-HT system can induce or reduce FP and associated behaviors (see Table 2). Most effects on FP have been related to the somatodendritic 5-HT<sub>1A</sub> autoreceptor. The somatodendritic 5-HT<sub>1A</sub> autoreceptor has an inhibitory effect on release of 5-HT at the synapse terminals, while other receptors are mainly excitatory (Meguid et al., 2000). Activity of the somatodendritic 5-HT<sub>1A</sub> autoreceptor controls firing and excitability of 5-HT neurons (Rojas and Fiedler, 2016), and is key to 5-HT tone modulation in 5-HT neurons. At terminals of 5-HT neurons where somatodendritic 5-HT<sub>1A</sub> autoreceptor are not present, other receptors such as 5-HT<sub>1B</sub> inhibit 5-HT release. Especially in the mSt 5-HT<sub>1B</sub> functions as an autoreceptor located at the axon terminal, while in the Hyp 5-HT<sub>1B</sub> functions as postsynaptic receptor (Adell et al., 2002). Furthermore, agonists of 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> reduce electrical activity of 5-HT neurons, as indicated by reduction of

extracellular 5-HT levels at terminals in the RN and also via other neurons (Fink and Göthert, 2007). The chicken brain contains a high density of 5-HT fibers in the Ap and Am and moderate density of 5-HT fibers in the basal ganglia and Hpc (Metzger et al., 2002). The Mst (together with the NAc) previously named the lobus parolfactorius (LPO; Reiner et al., (2004)) also shows extremely high innervation of 5-HT, while the NAc is mentioned as having near absence of 5-HT fibers (Metzger et al., 2002). The NAc is involved in reward (Aoki et al., 2006b) and highly DA innervated as well (Kuenzel et al., 2011; Mezey and Csillag, 2002). The NAc described by Metzger et al. (2002) however likely refers to the BST (bed nucleus of the stria terminalis) as shown by recent anterograde pathway tracing study in chickens (Bálint et al., 2011; Kuenzel et al., 2011). Further, the NAc can be divided in shell and core (Bálint and Csillag, 2007) with different efferent and afferent projections (Bálint et al., 2011). Now looking at the results of the study by Metzger et al. (2002) the NAc core and shell are more likely highly 5-HT innervated. Further, the defined chick' NAc by (Puelles et al., 2007) is disputed by (Matsushima et al., 2008) and could likely contain a larger caudal area of the MSt (Bálint and Csillag, 2007). Quantification of 5-HT-receptor density in different brain areas, and peripherally in relation to FP is unknown.

>> INSERT TABLE 2

#### *5.4.1. Pharmacological treatments acting on 5-HT receptors in relation to feather pecking*

S-15535 and NAN-190 are drugs with agonistic action on the somatodendritic 5-HT<sub>1A</sub> autoreceptor and thereby reduce 5-HT release and turnover. They further have antagonistic actions on the postsynaptic 5-HT<sub>1A</sub> receptor, hereby they block or dampen the binding of 5-HT to the postsynaptic 5-HT<sub>1A</sub> receptor. S-15535 increased FP in HP chicks compared to saline treated HP chicks and reduced 5-HT turnover in the Arch of HP chicks (van Hierden et al., 2004a). NAN-190 induced FP and aggression (Dennis et al., 2008) and decreased 5-HIAA in the RN of the LGPS line, a highly aggressive chicken line (Dennis and Cheng, 2012). In

adult hens, GR-127935 (antagonist for 5-HT<sub>1B</sub> receptor) increased FP and aggression also in a less aggressive line (HGPS) but did not affect 5-HT or 5-HIAA levels (Dennis et al., 2008; Dennis and Cheng, 2012). Treatment with 8-OH-DPAT (full agonist for somatodendritic 5-HT<sub>1A</sub> autoreceptor) at a young age increased fearfulness (Dennis et al., 2013a), but did not affect FP (Korte, personal communication). In other species, agonizing somatodendritic 5-HT<sub>1A</sub> autoreceptor can aggravate compulsive behaviors (Zohar and Insel, 1987), while inhibiting the somatodendritic 5-HT<sub>1A</sub> receptor can reduce self-inflicted feather picking in parrots (Bordnick et al., 1994) and stereotypic behavior in voles (Schoenecker and Heller, 2003). These studies show a direct link between the somatodendritic 5-HT<sub>1A</sub> autoreceptor and malfunctioning motor disruptive behavior (FP and feather picking) as well as maladaptive stereotypic behavior.

#### *5.4.2. Evidence for involvement of the 5-HT<sub>1A</sub> receptor in feather pecking*

There is strong evidence that somatodendritic 5-HT<sub>1A</sub> autoreceptor functioning is related to FP (pharmacological treatment (van Hierden et al., 2004a) and genetic polymorphisms of DEAF1 (Flisikowski et al., 2009)). Deficient functioning of the somatodendritic 5-HT<sub>1A</sub> autoreceptor and 5-HT<sub>2C</sub> are associated with impulsivity in rodents (Dalley et al., 2011). Especially action inhibition, the inability to stop an action when it is initiated, has been associated with reduced somatodendritic 5-HT<sub>1A</sub> autoreceptor functioning (Korte et al., 2017). SSRI treatment, which desensitizes the somatodendritic 5-HT<sub>1A</sub> autoreceptor and blocks SERT, decreased impulsivity and increased extracellular 5-HT and DA in the mSt of chicks (Matsunami et al., 2012). There are indications that birds with low levels of whole-blood 5-HT (de Haas and Rodenburg, 2016) and HFP birds (Birkl et al., 2016) lack action inhibition in impulsivity tests. HFP birds also react faster and more active to various behavioral tests at young and adult ages (Kops et al., 2017; Pichova et al., 2017; van der Eijk et al., 2018). These findings together suggest that FP

birds appear more impulsive which could be due to a deficient somatodendritic 5-HT<sub>1A</sub> autoreceptor.

Further, a tendency for low plasma TRP levels in HFP birds can point to deficient functioning of the somatodendritic 5-HT<sub>1A</sub> autoreceptor (Cahir et al., 2007). Synthesis of 5-HT from TRP is regulated, in part, by the somatodendritic 5-HT<sub>1A</sub> autoreceptor as it can inhibit 5-HT synthesis via its action on TPH (Best et al., 2010). This receptor might affect 5-HT levels and FP via its effects on TRP availability (as shown by van Hierden et al. (2002)) and injecting NAN-190 in chickens increased plasma TRP (Dennis et al., 2008). Moreover, binding of somatodendritic 5-HT<sub>1A</sub> autoreceptor in the dorsal raphe of rats is reduced by ATD (Cahir et al., 2007). Therefore, lower levels of TRP in lines with a high pecking tendency could represent a dysregulated somatodendritic 5-HT<sub>1A</sub> autoreceptor. This dysregulation can reduce 5-HT synthesis from TRP and the termination of 5-HT activity. However, it remains unknown whether alterations in the number, sensitivity and/or density of the somatodendritic 5-HT<sub>1A</sub> autoreceptor is causing these effects.

### **5.5. Peripheral serotonin**

Peripheral 5-HT plays a role in various physiological functions, such as gut motility, immune regulation, and energy metabolism (Coates et al., 2017). Lines that are prone to peck conspecifics or already developed FP generally have lower whole-blood 5-HT levels compared to lines less prone to peck conspecifics (see Table 3). Namely, DW vs. ISA Brown at 15 and 40 weeks of age (de Haas et al., 2014a, 2013), CL vs LML at 29 and 33 weeks of age (Bolhuis et al., 2009; Rodenburg et al., 2009a), WL vs. RIR at 47 weeks (Uitdehaag et al., 2011) and HFP vs. LFP at 24 weeks of age but not at 14 weeks of age (van der Eijk et al., 2017, unpublished results). Feather pecker-victims and victims of a White shaver line also had lower whole-blood 5-HT levels compared to neutrals but only at 21 weeks of age and not at 24, 27, 32 or 37 weeks of age (Daigle et al., 2015). A previous study in the FP selection lines found

that HFP birds had higher plasma 5-HT levels compared to LFP birds at 31 weeks of age (Buitenhuis et al., 2006). Plasma 5-HT derives mainly from gut 5-HT which has not yet been transported back into platelets or EC cells by SERT (Shajib and Khan, 2015). Whole-blood 5-HT more likely reflects the storage concentration of 5-HT, while plasma 5-HT reflects unbound free 5-HT (Shajib and Khan, 2015). Free 5-HT is namely bound by SERT on the platelet membrane and stored or metabolized (Coates et al., 2017). But platelets have a biphasic saturated mechanism, that is when plasma 5-HT increases generally also 5-HT in platelets increase, but when plasma 5-HT continues to increase this limits uptake and downregulates SERT (Shajib and Khan, 2015). Furthermore, unbounded 5-HT can be taken up by immune cells or certain microbiota species thereby limiting the uptake in blood platelets (Coates et al., 2017). Whole blood 5-HT level is thus affected by number and sensitivity of SERT, and direct comparison between plasma and whole blood 5-HT levels needs precaution. In rats and humans, platelet 5-HT and plasma 5-HT levels are correlated, but platelet 5-HT levels were more correlated with central 5-HT levels than plasma 5-HT levels (Audhya et al., 2012).

Measurements of 5-HT uptake can provide indications of peripheral SERT activity. Platelet 5-HT uptake was found to be higher for CL vs. LML hens (Bolhuis et al., 2009; Rodenburg et al., 2009a) and WL vs. RIR birds (Uitdehaag et al., 2011), but no studies on platelet 5-HT uptake in relation to FP phenotypes have been conducted. In adult hens, a positive correlation between whole-blood 5-HT and central 5-HT in the rostral brain have been found irrespective of line or social environment, and between platelet 5-HT turnover and central 5-HT turnover (rostral brain) within the WL line (Uitdehaag et al., 2011). These findings give rise to the suggestion that platelet 5-HT activity is related to central 5-HT activity, although research is needed to assess these relations by taking into account brain area and age at measurement. As in tail-biting pigs the correlation between whole-blood and central 5-HT measurements are dependent on age (Ursinus et al., 2013) and brain area (Valros et al., 2015). Up to now, it is

unknown whether peripheral 5-HT (plasma, whole blood or platelet uptake) is affected in young birds predisposed to develop FP. It would be interesting to identify peripheral 5-HT in birds prior to and after the development of FP. With this information it would be possible to assess cause and effect of peripheral 5-HT on FP. Although central and peripheral 5-HT act independently, they can also interact via the bloodstream as affected by the immune system and gut microbiota (GM; microflora of the intestine).

>>>>>INSERT TABLE 3

#### *5.5.1. Interaction between immune system, 5-HT and feather pecking*

Serotonin receptors and the 5-HT transporter are distributed on various immune cells. Peripheral 5-HT has several functional roles in the immune system for example, it is required for optimal functioning of monocytes and macrophages, it is important for natural immunity delivered by macrophages (Mössner and Lesch, 1998), and plays an important role in the gut inflammation response (O'Mahony et al., 2015). For example, in broilers, TRP supplementation helped to maintain the intestinal barrier, increased plasma 5-HT and reduced TPH expression (Yue et al., 2017). In turn, the immune system can influence the serotonergic system as well. Cytokines, the key signaling molecules of the immune system (Kaiser and Stäheli, 2014), influence the synthesis, re-uptake and release of 5-HT (Anisman and Merali, 2002; Karrenbauer et al., 2011; Miller et al., 2013). For example, inflammatory cytokines activate indoleamine 2,3 dioxygenase the enzyme that converts TRP into kynurenine, hereby reducing TRP availability for 5-HT synthesis (Dantzer et al., 2008). Further, Tumour Necrosis Factor and IL-1 increase SERT activity in the midbrain and striatum of mice (Zhu et al., 2006). The immune system might affect FP via its influence on central and peripheral 5-HT. Indeed, several studies found a relation between the immune system and FP; by associations between feather damage and cytokine genes (IL-9, IL-4) (Biscarini et al., 2010), by upregulation and downregulation of genes involved in immune responses in feather pecking birds (Brunberg et al., 2011), by genetic

associations between FP and total antibody response (Buitenhuis et al., 2003), and by a specific immune challenge as young and increased feather damage as adult (Parmentier et al., 2009). In the FP selection lines, HFP birds showed a higher antibody response to Infectious Bursal Disease Virus vaccination, while LFP birds showed more leukocytes and higher expression of MHC class I molecules on T (CD4, CD8) and B cells (Buitenhuis et al., 2006). Indicating that HFP and LFP birds differ in immune competence. Whether the relation between FP and the immune system occurs via, or affects the serotonergic system remains to be proven.

#### *5.5.2. Interaction between gut microbiota, 5-HT and feather pecking*

Gut 5-HT is an important signaling molecule regulating gastrointestinal motor, secretory and sensory functions (Keszthelyi et al., 2009; Lesurtel et al., 2008). Another peripheral factor which can influence both peripheral and central 5-HT is the GM. GM can activate cytokines (Bauer et al., 2006; Sommer and Bäckhed, 2013), which can influence central 5-HT release, as described above. Specific GM utilize or produce TRP (Zhang and Davies, 2016) and thereby influence TRP availability for 5-HT synthesis. Plasma TRP is increased in *Bifidobacterium infantis* treated rats (Desbonnet et al., 2008). Also 5-HT turnover (striatum) was higher in germ-free mice which lack a GM (Heijtz et al., 2011) and 5-HIAA (frontal cortex) was reduced in probiotics treated mice (Desbonnet et al., 2008). GM also produce SCFA involved in neurotransmitter synthesis and release (DeCastro et al., 2005; Shah et al., 2006), such as butyrate, acetate and propionate that can cross the BBB (Oldendorf, 1973). Oral treatment with propionate shows to lower 5-HT in the entire rat brain (El-Ansary et al., 2012). GM can further influence central 5-HT release via the vagus nerve (Bercik et al., 2011; Bravo et al., 2011). In rodents, GM composition has been shown to influence various types of behaviors (Collins et al., 2012; Cryan and Dinan, 2012). GM composition could potentially influence FP via its effects on central and peripheral 5-HT. HFP and LFP lines differ in microbial metabolites, the products of GM, with HFP birds having more propionate and butyrate compared to LFP birds

(Meyer et al., 2013). Preliminary results show that these FP selection lines also tended to differ in GM composition at 30 weeks of age (van der Eijk et al., 2017b). However, whether a specific GM composition is causal in FP remains to be proven. It is plausible that the differences in GM composition and metabolites in the FP selection lines is caused by eating feathers. Adding feathers to diet has been shown to alter GM and increases propionate and butyrate in hens (Meyer et al., 2012). HFP birds often ingest feathers, work to obtain freely available feathers (Harlander-Matauschek et al., 2007) and like foraging in feathers (McKeegan and Savory, 1999). Eating loose feathers also often precedes FP (de Haas et al., 2014a). Some authors have suggested that FP and eating feathers may be a way to compensate for the lack of (central) 5-HT (Kops et al., 2017). Ingestion of feathers may cause 5-HT release in the gut. Gut 5-HT namely reacts on sensory perception of the mucosal layer (Coates et al., 2017). As a consequence of ingesting fibers, large particles or feathers, gut 5-HT can stimulate secretion of signaling mediators. These mediators can potentiate neurons, making them more likely to respond, and influence neuronal action potential (Coates et al., 2017). Peripheral 5-HT may thereby influence neuroplasticity of serotonergic neurons. When chicks are kept on paper remnants and wood shavings (i.e. large particles) in the first weeks of life whole-blood 5-HT at 15 weeks of age was higher compared to chicks that did not have access to substrate (de Haas et al., 2014a). As these birds have the ability to ingest large particles, this could influence gut 5-HT and GM. Interestingly, feeding a high fiber diet tended to increase plasma 5-HT levels compared to feeding a low fiber diet in horses (Alberghina et al., 2006). Eating fibers or feathers might thus increase peripheral 5-HT levels in chickens. Eating feathers may derive from a need for satiety sensation, and thereby playing a causal role in FP (Harlander-Matauschek and Bessei, 2005). For example, feathers in the diet reduced FP and improved plumage condition in hens (Kriegseis et al., 2012). How and if eating feathers affects the serotonergic system is unknown.

>> INSERT TABLE 4

## **6. The dopaminergic system in relation to feather pecking**

Central DA activity is affected in birds with a high FP tendency (see Table 4). As mentioned, central 5-HT is involved in appetite, food intake and satiety. Central DA facilitates the motivation to perform rewarding behavior (De Deurwaerdère and Di Giovanni, 2017). Through their functions, 5-HT and DA could facilitate FP, as a redirected feeding related behavior. Central 5-HT could initiate FP and feather eating and DA could further reinforce this behavior. Rewarding effects of several stimuli (such as feeding behavior) are regulated via DA in the mesolimbic system (De Deurwaerdère and Di Giovanni, 2017). Mesolimbic DA regulates acquisition, control and maintenance of goal-directed behavior (Kiyatkin, 1995). Thus, it is plausible that DA regulates the continuation of FP.

### *6.1. Genetic associations*

Genetic polymorphisms of genes encoding the D4 receptor (DRD4) and influencing transcription of HTR1A via DEAF1 were associated with FP (Flisikowski et al., 2009). The DEAF1 gene also modulates the proenkephalin gene (Huggenvik et al., 1998). Proenkephalin is a component in the dopamine-reward cascade associated with compulsivity (Blum et al., 2000; Bordnick et al., 1994). Compulsive animals appear to be unable to stop their behavior and reflect a stick-in-set perseverance. Stick-in-set perseverance is reflected by impulsivity and compulsivity, and associated with behavioral pathologies in rodents (Garner et al., 2006), TTM in humans (Chamberlain et al., 2006; Fineberg et al., 2010) and self-inflicted feather picking and plucking in parrots (van Zeeland et al., 2009). A recent study, however, found that HFP birds have similar perseverance as LFP birds in a pecking specific task (Kjaer et al., 2015), indicating that FP in these lines might not be related to compulsivity but more to hyperactivity (Kjaer, 2009). Whether continuing to perform FP is related to DA and compulsivity is unknown.

### 6.2. Dopamine synthesis

Dopamine is synthesized from its precursor TYR. Dopamine synthesis appears reduced in HFP birds compared to LFP birds, as indicated by lower TYR and PHE in HFP birds (Kops, 2014; Kops et al., 2014). In CL birds aggression was related to DA synthesis as peripheral TRP/(PHE + TYR) ratio was reduced (Birkel et al., 2017a). Furthermore, CL birds had a higher innervation of tyrosine hydroxylase (TH; the rate limiting enzyme for DA synthesis) in the NCC than LML birds (Nordquist et al., 2013). TH innervation reflects development of DA neurons. Thus CL birds, with higher aggression and cannibalistic pecking show increased innervation of DA system, and higher availability of DA precursors. However, whether the direct precursor of DA, L-3,4-dihydroxyphenylalanine (L-DOPA,) is affected in birds which are prone to develop FP or perform FP is unknown.

### 6.3. Central levels, metabolites and turnover of DA in young birds

At a young age, no differences were found in DA levels between HP vs. LP and HFP vs. LFP (see Table 4). Also, no differences were found between HFP and LFP birds in DA metabolites (i.e. 3,4-dihydroxyphenylacetic acid: DOPAC and homovanillic acid: HVA) in any of the brain areas measured (Am, Ap, dT, Hpc, mSt, NCC or NCL) (Kops et al., 2017). Yet, HP chicks had lower DOPAC and HVA levels compared to LP chicks in the rostral brain at 4 weeks of age (van Hierden et al., 2002). HP and HFP chicks had lower DA turnover in dT, mSt and rostral brain compared to LP and LFP chicks (Kops et al., 2017; van Hierden et al., 2002).

### 6.4. Central levels, metabolites and turnover of DA in adult birds

At an adult age, birds with a high pecking tendency had lower DA in rostral brain (WL vs. RIR (Uitdehaag et al., 2011)), NCC (HFP vs. LFP (Kops et al., 2017)) and RN (DXL vs. HGPS (Dennis and Cheng, 2012)) than birds with a low pecking tendency (see Table 4). Apart from CL birds which tended to have higher DA levels in the Ap compared to LML birds (Kops et

al., 2013b). Birds with a high pecking tendency had higher metabolite levels of DA in Ap (DOPAC: HFP vs. LFP and CL vs. LML; HVA: feather pecker vs. neutral and CL vs. LML), dT (DOPAC: CL vs. LML), rostral brain (DOPAC: WL vs. RIR) compared to birds with a low pecking tendency (Kops et al., 2017, 2013a, 2013b; Uitdehaag et al., 2011). Apart from HFP birds which had lower DOPAC in the NCC compared to LFP birds (Kops et al., 2017). DA turnover was higher in the NCC for HFP vs. LFP birds and in the rostral brain for WL vs. RIR birds (Kops et al., 2017; Uitdehaag et al., 2011), but lower in the Hpc for CL vs. LML birds (Kops et al., 2013b). Discrepancies between studies might be caused by the calculation of DA turnover - either by DOPAC + HVA/DA (Kops et al., 2017; van Hierden et al., 2002), DOPAC + HVA + 3-methoxytyramine (3-MT)/DA (Kops et al., 2013b) or DOPAC/DA (Uitdehaag et al., 2011) and by inclusion of phenotypes in analysis of genotypes.

In summary, chicks with high FP tendency not only show deficiency in the serotonergic system, but also in the dopaminergic system. Although less brain areas were affected compared to the serotonergic system, lower DA activity was found in brain areas involved in somato-motor responses (mSt) and in goal-directed behavior (dT) in chicks with a high FP tendency. These findings suggest that a low activity of the dopaminergic system in these brain areas at a young age is involved in the development of FP. At an adult age, DA activity was higher in a brain region involved in motor actions and decision making (NCC) in birds with a high pecking tendency. This indicates high activity of the dopaminergic system in this specific brain area in the performance of pecking behavior. However, precaution is needed when interpreting these results as findings for the DA system showed more inconsistencies than those for the 5-HT system (see Table 1 & 4), especially at an adult age. Discrepancies seen between the mortality selection lines, group productivity and survivability lines and FP selection lines might further be explained by the selection criteria. Within the LML and CL lines hardly any FP or feather damage due to FP has been found (Bolhuis et al., 2009; de Haas et al., 2012), but CL birds

performed more cannibalistic toe pecking than LML birds (Rodenburg et al., 2009b). Similarly, the HGPS, LGPS and DXL lines did not differ in FP (Dennis et al., 2008), but HGPS birds had less feather damage, performed less cannibalistic and aggressive pecking compared to DXL and LGPS birds (Craig and Muir, 1996a, 1996b). Aggressive pecking and FP may be both influenced by DA activity, but specific for the different genetic lines (i.e. having a tendency for more FP, more cannibalism, or more aggression).

>>>>>INSERT TABLE 5

## **6.5. Interaction between 5-HT and DA and the influence on feather pecking**

### *6.5.1. Pharmacological treatments acting on 5-HT receptors influencing the DA system*

The serotonergic system can influence brain region-specific DA tone and release by various routes via the 5-HT receptors (De Deurwaerdère and Di Giovanni, 2017). It is known that 5-HT receptors can influence DA release from mesolimbic dopaminergic neurons in mammals (Fink and Göthert, 2007). The postsynaptic 5-HT<sub>1A</sub> receptor is a major modulator for striatal DA activity (De Deurwaerdère and Di Giovanni, 2017), but also 5-HT<sub>2</sub> receptors play an important role in DA release in the NAc (De Deurwaerdère and Spampinato, 2001). Also in chicken, explicit roles for the somatodendritic 5-HT<sub>1A</sub> autoreceptor and postsynaptic 5-HT<sub>1B</sub> receptor in DA activity have been found (Dennis et al., 2013a). Treatment with NAN-190 decreased tissue-level DA in the RN of HGPS birds but not in DXL birds (Dennis and Cheng, 2012). When given GR-127935 (5-HT<sub>1B</sub> and 5-HT<sub>1D</sub> receptor antagonist) tissue-level DA decreased and DA turnover increased in the RN of HGPS birds but not in DXL birds (Dennis and Cheng, 2012). In HP chicks the highest dosage of S-15535 enhanced tissue-level DA turnover in the Hpc and Arch, but this effect was not seen in LP chicks (van Hierden et al., 2004a) (see Table 2). Another 5-HT<sub>1A</sub> agonist (8-OH-DPAT) shows biphasic actions on 5-HT<sub>1A</sub> receptors in the Hyp of rats. A low dose inhibits 5-HT release by acting on the

somatodendritic 5-HT<sub>1A</sub> autoreceptor and increased DA release, while a high dose also acts strongly on postsynaptic 5-HT receptors and inhibits DA release (Montgomery et al., 1991).

#### *6.5.2. Pharmacological treatments acting on DA receptors in relation to feather pecking*

Pharmacological treatments that affect the dopaminergic system either reduce or induce pecking behavior (see Table 5). Treatment with a D1 agonist (SKF-38393) increased FP, aggression, tissue-level 5-HT and 5-HIAA levels in RN of subordinate adult hens, while treatment with D1 or D2 antagonist (SCH-23390 and Raclopride, respectively) decreased FP, aggression and increased tissue-level 5-HT in RN of dominant hens (Dennis and Cheng, 2011). A D2 antagonist (Raclopride) also reduced aggression in highly aggressive birds (Dennis et al., 2006). Thus, D1 and D2 receptors play a role in aggression, likely via their influence on 5-HT levels in the RN. Interestingly, D2 receptor activity is also involved in FP, namely in adult HFP hens, a D2 antagonist (Haloperidol) decreased FP, foraging, walking and eating, but not aggression (Kjaer et al., 2004). These results are likely caused by Haloperidol blocking the effects of DA and increasing frontal and NAc' DA (Bonaccorso et al., 2002; Li et al., 2005) and increase in overall behavior inhibition (Logemann et al., 2017). When given a D2 receptor antagonist (Haloperidol or Raclopride), all types of pecking behaviors are reduced (Dennis et al., 2006; Goodman, 1981) and specifically FP and not aggressive pecking in HFP birds (Kjaer et al., 2004). Haloperidol also tends to decrease the frequency of foraging when anticipating a food reward (Moe et al., 2014). Further, van Hierden et al. (2005) showed that Apomorphine (full D1 and D2 agonist) in HP chicks increased walking in a novel environment, indicating reduced fear and a higher sensitivity of both DA receptors in HP chicks compared to LP chicks. Also stereotyped pecking is affected by Apomorphine treatment in domestic fowl (Nistico' and Stephenson, 1979) and pigeons (Cheng and Long, 1974; Goodman, 1981). Density of DA receptors could play a role in FP. D1 and D2 receptor density has been found higher in the

striatum and NAc of high frequency pecking birds compared to low frequency pecking birds (Sedlackova et al., unpublished results).

## 7. Conclusion

With this review we highlighted the routes by which the serotonergic system is involved in and influenced by feather pecking (FP) in laying hen chickens. Feather pecking, a redirected foraging behavior, can develop as a maladaptive behavior where the animal attempts to cope with a thwarted stressful environment, but FP can also develop as a malfunctioning behavior. Genetic associations have been found between FP or feather damage and genes encoding 5-HT and DA receptors, MAO-A and immune regulatory factors. The activity of the serotonergic system appears deficient in young birds with a high FP tendency, as indicated by reduced 5-HT metabolism and turnover homogeneously in brain areas important for executive control of foraging behavior, and in regulating emotional responses. A deficient serotonergic system in executive control pathway regions seems predisposing for FP. Serotonin activity in FP birds shows strongly influenced by 1) dysregulation of the 5-HT<sub>1A</sub> and 5-HT<sub>1B</sub> receptors reducing 5-HT synthesis and 5-HT tone, and influencing DA release, and 2) deficient MAO-A functioning decreasing 5-HT metabolism. The dopaminergic system also plays a role in pecking behavior, either directly via DA receptors, or as influenced by 5-HT receptors. Partly similarly to 5-HT, young birds with a high FP tendency also show low DA activity in specific brain areas regulating somato-motor responses and goal-directed behavior. When FP is apparent, 5-HT and DA activity is increased in specific brain areas involved in motor actions, decision making and reward. These results indicate that in FP birds the neurobiological network influencing goal-directed behavior is affected by altered 5-HT and DA activity. However, inconsistencies in central 5-HT and DA activity were found between studies likely due to inclusion of FP phenotypes (feather peckers and victims) when comparing genotypes and differences in calculation of the DA turnover. It is important to include FP phenotypes in the analysis, as they

show to differ in peripheral and central 5-HT and central DA. Peripheral 5-HT is involved in gut health, and interestingly, birds with a high pecking tendency had low peripheral 5-HT, different gut microbiota composition and immune competence as compared to birds with low pecking tendency. Peripheral 5-HT, GM, and the immune system likely interact with central 5-HT and DA activity and could thereby influence FP and feather eating.

### **8. Future perspectives for research**

Based on this review some aspects of the serotonergic system in relation to FP remain unknown. The 5-HT<sub>1A</sub> and 5-HT<sub>1B</sub> receptors appear important in FP, but it is unknown whether the number, sensitivity and/or density of these receptors (and other 5-HT and DA receptors) are affected in FP birds. Pharmacological treatments can further inform on intra- or extracellular mechanisms, such as SERT involvement in FP. Also, what happens during the act of FP in relation to release of 5-HT and DA is unclear. Similarly, it is unknown which changes occur in peripheral 5-HT and GM before and after the development of FP and feather eating. The role of peripheral 5-HT on gut functioning, immune system and GM seem influential when birds feather peck and when feather eating takes place. How these systems interact with central 5-HT, and if these factors are predisposing in FP remains to be further studied.

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Figure 1. The serotonergic system in chicken

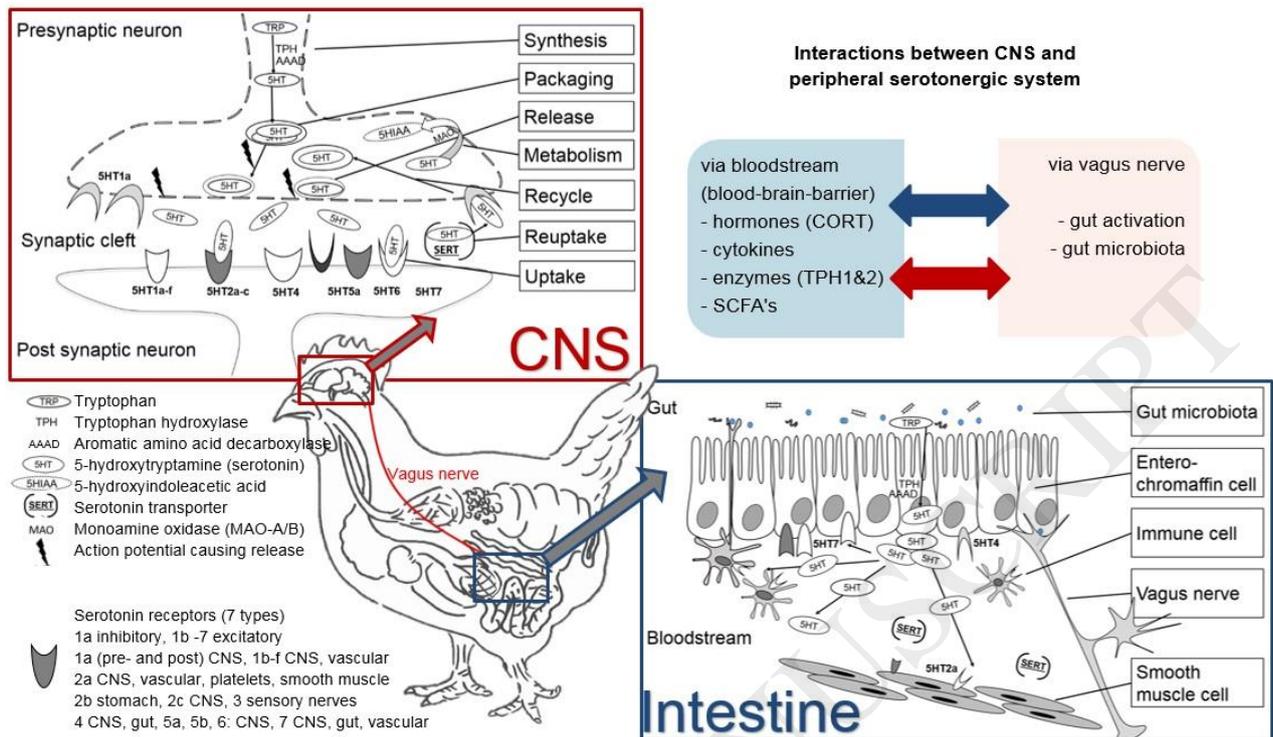


Figure 1 footnote

In the CNS (central nervous system) synthesis of central serotonin (5-HT) takes place in the nerve cell body. After tryptophan (TRP) is transported across the blood-brain-barrier (BBB) it is converted to 5-HT by the enzymes tryptophan hydroxylase (TPH) and aromatic amino acid decarboxylase (AAAD). Serotonin is then stored in presynaptic vesicles. When an action potential reaches the presynaptic neuron this causes release of 5-HT into the synaptic cleft. In the synaptic cleft, 5-HT can bind to various 5-HT receptors (uptake). Reuptake of 5-HT is mediated by the serotonin transporter (SERT), which transports 5-HT back into the presynaptic neuron where it can be recycled in presynaptic vesicles. Serotonin is metabolized by monoamine oxidase (MAO) into 5-hydroxyindoleacetic acid (5-HIAA). In the intestine, synthesis of 5-HT takes place in enterochromaffin (EC) cells in a similar way as central 5-HT (by TPH and AAAD). Serotonin receptors are found on EC cells, immune cells, platelets and smooth muscle cells. The gut microbiota can affect the serotonergic system in various ways: 1) by influencing TRP availability, 2) by activating the immune system which results in activation of cytokines, 3) by production of short-chain fatty acids (SCFA) that can cross the BBB and 4) by directly acting on the vagus nerve. The CNS and peripheral serotonergic systems act independently but can interact via the blood stream, as affected by hormones (corticosterone (CORT)), cytokines, enzymes and SCFA, and activation via the vagus nerve.

Figure 2. Explanation of how feather pecking in white and brown egg laying chicken can develop as a maladaptive behavior or a malfunctioning behavior

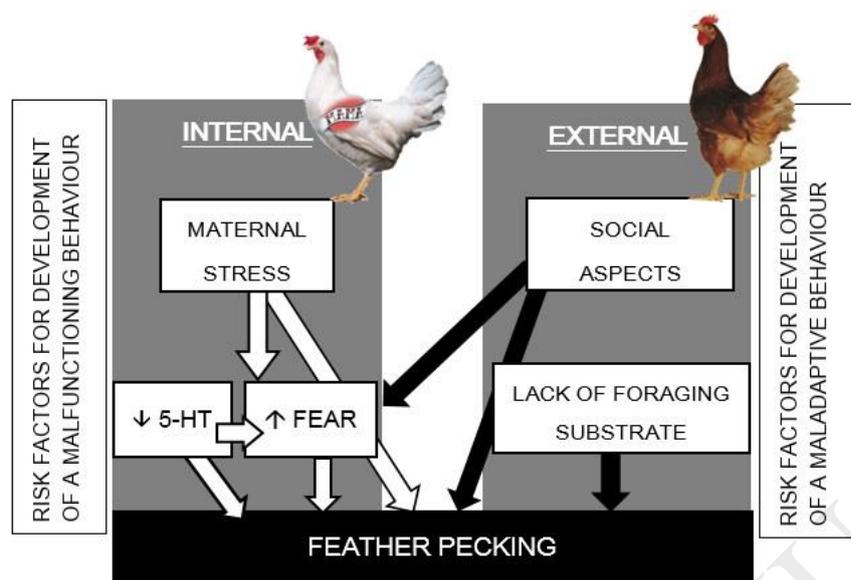


Figure 2 footnote

Adapted from De Haas (2014). 5-HT = 5-hydroxytryptamine, serotonin. Internal refers to internal predisposition. External refers to sensitivity for external conditions. White hens (Dekalb White) show to have a high (epi)genetic predisposition (see "MAMA" tattoo on white hen) for low central and peripheral 5-HT levels and high fear levels, which creates at risk for development of feather pecking as a malfunctioning behavior. Brown hens (ISA Brown) are strongly influenced by social factors and lack of foraging substrate, which creates a risk for development of feather pecking as a maladaptive behavior. Solid black arrows show external risk factors, solid white arrows show internal risk factors.

## Headings for Figures and Tables

Table 1. Central 5-HT, 5-HIAA and 5-HT turnover in birds with diverging pecking tendency

Comparison	Age (weeks)	Brain area	5-HT	5-HIAA	5-HT turnover	Reference
HP vs. LP	4	Rostral brain	↑	↓ <sup>^</sup>	↓	van Hierden et al., 2002
HP vs. LP	7	Arch	=	↓	↓ <sup>^</sup>	van Hierden et al., 2004b
		Forebrain	↓ <sup>^</sup>	=	↓ <sup>^</sup>	
		Hpc	=	=	=	
HFP vs. LFP	8	Am	=	↓	=	Kops et al., 2017
		Ap	=	=	↓	
		dT	↓	↓	=	
		Hpc	=	=	=	
		mSt	=	↓	↓	
		NCC	=	↓	↓	
		NCL	=	=	=	
HFP vs. LFP	23	NCC + NCL	↑*	=*	N.D. <sup>#</sup>	Kops, 2014; Kops et al., 2014
HFP vs. LFP	25	Am	=	=	=	Kops et al., 2017
		Ap	=	↑	=	
		dT	=	=	=	
		Hpc	=	=	=	
		mSt	=	=	=	
		NCC	=	=	↑	
		NCL	=	=	=	
Severe feather pecker vs. non-pecker	33	Ap	=	=	=	Kops et al., 2013a
		dT	=	↑	↑	
		Hpc	=	=	=	
		mSt	=	=	=	
Severe feather pecker vs. victim	33	Ap	=	=	=	Kops et al., 2013a
		dT	=	=	=	
		Hpc	=	=	=	
		mSt	=	↑	=	
Victim vs. non-pecker	33	Ap	=	=	=	Kops et al., 2013a
		dT	=	↑	↑	
		Hpc	=	=	=	
		mSt	↓	↓	=	
CL vs. LML	33	Ap	=	↑ <sup>^</sup>	=	Kops et al., 2013b
		dT	=	=	=	
		Hpc	=	=	=	
		mSt	=	=	=	
WL vs. RIR	47	Rostral brain	↓ <sup>^</sup>	↑	↑	Uitdehaag et al., 2011
DXL vs. HGPS	58	Hyp	=	=	=	Cheng and Fahey, 2009
		RN	=	=	↓	

Abbreviations for neurotransmitters: 5-HT : 5-hydroxytryptamine, serotonin; 5-HIAA : 5-hydroxyindoleacetic acid; 5-HT turnover : 5-HIAA /5-HT; = : similar levels i.e. no difference; ↑ : increase in levels; ↓ : decrease in levels; \* : extracellular concentration measured with microdialysis, <sup>#</sup> extracellular turnover was determined in the thesis of Kops, 2014, but not used in the published paper Kops et al., 2014; <sup>^</sup> : tendency 0.1 < P > 0.05. Cells in grey : represents birds younger than 10 weeks of age. Cells in white : represents birds older than 20 weeks of age. Abbreviations for comparison: HP : high pecking line; LP : low pecking line (selection on egg production, Korte et al., 1998); HFP : high feather pecking line; LFP : low feather pecking line (selection on feather pecking, Kjaer and Sørensen, 2002); CL : control line for LML; LML: low mortality line (selection on low group mortality, Ellen et al., 2008); WL: white leghorn; RIR : Rhode Island Red; DXL : DeKalb XL; HGPS: high group productivity and survivability line (selection on group productivity and survivability, Cheng and Fahey, 2009). Abbreviations for brain areas (and functions): Am : amygdala (emotion); Ap : arcopallium (somato-motor); Arch : archistriatum (includes Am and Ap); dT : dorsal thalamus (goal-directed behavior); Hpc : hippocampus (memory and learning); Hyp : hypothalamus (stress response); mSt : medial striatum (reward);

NCC : caudocentral nidopallium (motor actions and decision making); NCL: caudolateral nidopallium (executive functions and planning); RN : raphe nuclei (afferent); rostral brain and forebrain (various).

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Table 2. Pharmacological treatments acting on 5-HT receptors and the effects on behavior, central 5-HT and DA, metabolites and turnovers in chickens

Treatment	Action	Lines	Age at treatment	Age at measurement	Behavior effect	Brain area	5-HT	5-HIAA	5-HT turnover	DA	DOPAC	HVA	DA turnover	Reference
5-HT	Elevate 5-HT	WL	incubation day 1	1d, 15d, 35d, 9w, 18w	↓aggr (9w) ↑TI (18w)	Hyp RN	↑ <sup>^</sup> 1d ↑18w	↓9w	↓9w	↓9w	↓9w	↑15d	↑15d	Dennis et al., 2013a
8-OH-DPAT	5-HT1A agonist				=	Hyp RN	=	↓9w	↓9w	=	=	=	=	
5-MT	5-HT1, 2, 4, 6, 7 agonist	WL	first 2 days	10w	↑aggr ↑walk	Hyp RN	=	=	=	=	=	=	=	Dennis et al., 2013b
TRP	Elevate TRP	Local breed	3d	3d + 30min 3d + 60min	↓eat (30min)	ND	ND	ND	ND	ND	ND	ND	ND	Bungo et al., 2008
			5d	5d + 15min	ND	Dph	↑	↑	↑	=	=	↑	↑	
5-HT	Elevate 5-HT	Local breed	5d	5d	↓eat	ND	ND	ND	ND	ND	ND	ND	ND	Zende del et al., 2017
Fluvoxamine	SSRI	WL	8d	8-11d	↓impulsivity ↓walk	mSt	↑*	ND	ND	↑*	ND	ND	ND	Matsunami et al., 2012
8-OH-DPAT	5-HT1A agonist	Local breed	3w	3w for 4h	=	ND	ND	ND	ND	ND	ND	ND	ND	Zende del et al., 2012
SB 242084	5-HT2C antagonist				↑eat	ND	ND	ND	ND	ND	ND	ND	ND	
S-15535	Auto 5-HT1A agonist, Post 5-HT1A antagonist	HP, LP	4w	4w	↑ <sup>^</sup> FP, HP ↑ <sup>^</sup> foraging, HP ↑ <sup>^</sup> walk, HP	Hpc Arch Forebrain	ND ND ND	ND ND ND	↓ ↓HP ↓	ND ND ND	ND ND ND	ND ND ND	↑HP ↑ <sup>^</sup> HP ↑HP	van Hierden et al., 2004a
TRP	Elevate TRP	HP, LP	5w	7w	↓FP ↓ <sup>^</sup> foraging ↑eat	Hpc Arch Forebrain	ND ND ND	ND ND ND	↑ ↑ ↑ <sup>^</sup>	ND ND ND	ND ND ND	ND ND ND	ND ND ND	van Hierden et al., 2004b
D-fenfluramine	Serotonin releasing agent	HFP, LFP	23w	23w	ND	NCC NCL	↑*	=	=	ND	ND	ND	ND	Kops et al., 2014
NAN-190	Auto 5-HT1A agonist, Post 5-HT1A antagonist	LGPS, DXL, HGPS	24w for 5d	24w for 5d	↑FP, LGPS ↑aggr, LGPS & DXL	Hyp RN	= =	= ↓HGPS	ND ND	= ↓HGPS	= =	= =	= =	Dennis et al., 2008; Dennis and Cheng, 2012
GR-127935	5-HT1B antagonist				↑FP, HGPS ↑aggr, HGPS	Hyp RN	= =	= =	ND ND	= ↓HGPS	= =	= ↑HGPS	= ↓HGPS	

Abbreviation for treatment effects: 5-HT : 5-hydroxytryptamine, serotonin; 5-HIAA : 5-hydroxyindoleacetic acid; 5-HT turnover : 5-HIAA /5-HT; DA : dopamine; DOPAC : 3,4-dihydroxyphenylacetic acid; HVA : homovanillic acid; DA turnover : metabolites (HVA, DOPAC)/DA ; = : similar levels i.e. no treatment effect; ↑ : increase after treatment; ↓ : decrease after treatment; when lines are expressed :

indicates treatment effect within specific line; ND : not determined; \* : extracellular concentration measured with micro dialysis; ^ : tendency  $0.1 < P > 0.05$ . Cells in grey : represents birds younger than 10 weeks of age. Cells in white : represents birds older than 20 weeks of age. Abbreviations for action: auto 5-HT<sub>1A</sub> : somatodendritic 5-HT<sub>1A</sub> autoreceptor; post-5-HT<sub>1A</sub> : postsynaptic 5-HT<sub>1A</sub> receptor; 5-HT : 5-hydroxytryptamine, serotonin; 5-HIAA : 5-hydroxyindoleacetic acid; 5-MT : 5-Methoxytryptamine; HVA : homovanillic acid; SSRI : Selective Serotonin Reuptake Inhibitor. Abbreviations for lines: WL : White Leghorn; HP : high pecking line; LP : low pecking line (selection on egg production, Korte et al., 1998); DXL : DeKalb XL; HGPS : high group productivity and survivability line; LGPS : low group productivity and survivability line (selection on group productivity and survival, Cheng and Fahey, 2009); HFP : high feather pecking line; LFP : low feather pecking line (selection on feather pecking, Kjaer and Sørensen, 2002). Abbreviations for ages: d : days; w : weeks; h : hours; min : minutes. Abbreviations for behaviors: aggr : aggression; TI : tonic immobility duration (fear response); FP : feather pecking. Abbreviations for brain areas: Arch : archistriatum; Dph : Diencephalon; Hpc : hippocampus; Hyp : hypothalamus; mSt : medial striatum; NCC : caudocentral nidopallium; NCL : caudolateral nidopallium; RN : raphe nuclei.

Table 3. Peripheral serotonin in birds with diverging pecking tendency

Comparison	Age (weeks)	Whole-blood 5-HT	5-HT uptake	Reference
HFP and LFP vs. CON	14	↓	ND	van der Eijk et al., unpublished results
DW vs. ISA Brown	15	↓	ND	de Haas et al., 2014a
Feather pecker vs. neutral	21	=	ND	Daigle et al., 2015
Feather pecker vs. victim		=		
Feather pecker vs. feather pecker-victim		=		
Feather pecker-victim vs. victim		=		
Feather pecker-victim vs. neutral		↓		
Victim vs. neutral		↓		
Feather pecker vs. neutral	24, 27, 32, 37	=	ND	Daigle et al., 2015
Feather pecker vs. victim		=		
Feather pecker vs. feather pecker-victim		=		
Feather pecker-victim vs. victim		=		
Feather pecker-victim vs. neutral		=		
Victim vs. neutral		=		
HFP vs. LFP and CON	24	↓	ND	van der Eijk et al., 2017a
HFP vs. LFP	31	↑ <sup>p</sup>	ND	Buitenhuis et al., 2006
CL vs. LML	29	↓	↑	Bolhuis et al., 2009
	33	↓*	↑	Rodenburg et al., 2009a
DW vs. ISA Brown	40	↓	ND	de Haas et al., 2013
WL vs. RIR	47	↓	↑	Uitdehaag et al., 2011
DXL vs. HGPS	58	=	ND	Cheng and Fahey, 2009

Abbreviations for measurements: 5-HT : 5-hydroxytryptamine, serotonin; = : similar levels i.e. no difference; ↑ : increase in levels; ↓ : decrease in levels; p : measured in plasma; \* : interaction brooding by surrogate hen. Cells in grey : represents birds younger than 20 weeks of age. Cells in white : represents birds older than 20 weeks of age. Abbreviations for comparison: DW : Dekalb White, commercial cross of White leghorn; ISA Brown: commercial cross of Rhode Island Red; HFP : high feather pecking line; LFP : low feather pecking line; CON : unselected control line of the FP selection lines (selection on feather pecking, Kjaer and Sørensen, 2002); CL : control line for LML; LML: low mortality line (selection on low group mortality, Ellen et al., 2008); WL: white leghorn; RIR : Rhode Island Red; DXL : DeKalb XL; HGPS: high group productivity and survivability line (selection on group productivity and survival, Cheng and Fahey, 2009).

Table 4. Central DA, metabolites and DA turnover in birds with diverging pecking tendency

Comparison	Age (weeks)	Brain area	DA	DOPAC	HVA	3-MT	DA turnover	Reference
HP vs. LP	4	Rostral brain	=	↓	↓	ND	↓	van Hierden et al., 2002
HFP vs. LFP	8	Am	=	=	=	ND	=	Kops et al., 2017
		Ap	=	=	=	ND	=	
		dT	=	=	=	ND	↓	
		Hpc	=	=	=	ND	=	
		mSt	=	=	=	ND	↓	
		NCC	=	=	=	ND	=	
		NCL	=	=	=	ND	=	
DXL vs. HGPS	24	Hyp	=	=	=	ND	=	Dennis and Cheng, 2012
		RN	↓	=	↑	ND	=	
HFP vs. LFP	25	Am	=	=	=	ND	=	Kops et al., 2017
		Ap	=	↑	=	ND	=	
		dT	=	=	=	ND	=	
		Hpc	=	=	=	ND	=	
		mSt	=	=	=	ND	=	
		NCC	↓	↓	=	ND	↑	
		NCL	=	=	=	ND	=	
Severe feather pecker vs. non-pecker	33	Ap	=	=	↑	=	=	Kops et al., 2013a
		dT	=	=	=	=	=	
		Hpc	bdl	bdl	bdl	bdl	bdl	
		mSt	=	=	=	=	=	
Severe feather pecker vs. victim	33	Ap	=	=	=	=	=	Kops et al., 2013a
		dT	=	=	=	=	=	
		Hpc	bdl	Bdl	bdl	bdl	bdl	
		mSt	=	=	=	=	=	
Victim vs. non-pecker	33	Ap	=	=	↑	=	=	Kops et al., 2013a
		dT	=	=	=	=	=	
		Hpc	bdl	Bdl	bdl	bdl	bdl	
		mSt	=	=	=	=	=	
CL vs. LML	33	Ap	↑ <sup>^</sup>	↑	↑ <sup>^</sup>	=	=	Kops et al., 2013b
		dT	=	↑	=	=	=	
		Hpc	=	=	=	=	↓	
		mSt	=	=	=	=	=	
WL vs. RIR	47	Rostral brain	↓	↑	ND	ND	↑	Uitdehaag et al., 2011

Abbreviations for neurotransmitters: DA : dopamine; DOPAC : 3,4-dihydroxyphenylacetic acid; HVA : homovanillic acid; 3-MT : 3-methoxytyramine; DA turnover : DOPAC + HVA / DA (for Kops et al., 2017; van Hierden et al., 2002); DOPAC + HVA + 3-MT / DA (for Kops et al., 2013a, 2013b); DOPAC / DA (for Uitdehaag et al., 2011); = : similar levels i.e. no difference; ↑ : increase in levels; ↓ : decrease in levels; bdl : below detection level; ND : not determined; <sup>^</sup> : tendency 0.1 < P > 0.05.

Cells in grey: represents birds younger than 10 weeks of age. Cells in white: represents birds older than 20 weeks of age.

Abbreviations for comparison: HP : high pecking line; LP : low pecking line (selection on egg production, Korte et al., 1998); HFP : high feather pecking line; LFP : low feather pecking line (selection on feather pecking, Kjaer and Sørensen, 2002); DXL : DeKalb XL; HGPS: high group productivity and survivability line (selection on group productivity and survivability, Cheng and Fahey, 2009); CL : control line for LML; LML: low mortality line (selection on low group mortality, Ellen et al., 2008); WL: white leghorn; RIR : Rhode Island Red. Abbreviations for brain areas (functions): Am : amygdala (emotion); Ap : arcopallium (somato-motor); dT : dorsal thalamus (goal-directed behavior); Hpc : hippocampus (memory and learning); Hyp : hypothalamus (stress response); mSt : medial striatum (reward); NCC : caudocentral nidopallium (motor actions and decision making); NCL : caudolateral nidopallium (executive functions and planning); RN : raphe nuclei (afferent); rostral brain (various).

Table 5. Pharmacological treatments acting on DA receptors and the effects on behavior, central 5-HT and DA, metabolites and turnovers in chickens

Treatment	Action	Lines	Age at treatment	Age at measurement	Behavior effect	Brain area	5-HT	5-HIAA	5-HT turnover	DA	DOPAC	HVA	DA turnover	Reference
SCH-23390	D1 antagonist	Local breed	5d	5d + 30min, 5d + 1h,	= eat	ND	ND	ND	ND	ND	ND	ND	ND	Zendehdel et al., 2016
Sulpride	D2 and D3 antagonist			5d + 2h, 5d + 3h	= eat	ND	ND	ND	ND	ND	ND	ND	ND	
Apomorphine	D1 and D2 agonist, 5-HT2 antagonist	HP, LP	29-31d	29-31d	↑ walk in novel environment	ND	ND	ND	ND	ND	ND	ND	ND	van Hierden et al., 2005
SKF-38393	D1 and D5 agonist	LGPS, DXL, HGPS	21w for 5d	21w + 5d	↑ aggr ↑ FP, DXL & HGPS	Hyp RN	= =	= =	= =	= =	= =	= =	= =	Dennis and Cheng, 2011
Quinpirole	D2 and D3 agonist	(sub)			↑aggr, HGPS =FP	Hyp RN	= =	= =	= =	= =	= =	= =	= =	
SCH-23390	D1 antagonist	LGPS, DXL, HGPS	21w for 5d	21w + 5d	↓aggr, LGPS & DXL ↓^FP	Hyp RN	↑ DXL =	↑HGPS =	= =	= =	= =	= =	= =	Dennis and Cheng, 2011
Raclopride	D2 antagonist	(dom)			↓aggr ↓^FP	Hyp RN	↑ DXL, HGPS =	= =	= =	= =	= =	= =	= =	
Raclopride	D2 antagonist	LGPS, DXL, HGPS	66w for 10d	66w + 5d, +10d	↓aggr, dom (5d) ↑aggr, sub (12d)	ND	ND	ND	ND	ND	ND	ND	ND	Dennis et al., 2006
Haloperidol	D2 antagonist	ISA	118w	+15min	=aggr ↓FP	ND	ND	ND	ND	ND	ND	ND	ND	Kjaer et al., 2004

Abbreviations for treatment effects: 5-HT : 5-hydroxytryptamine, serotonin; 5-HIAA : 5-hydroxyindoleacetic acid; 5-HT turnover : 5-HIAA /5-HT; DA : dopamine; DOPAC : 3,4-dihydroxyphenylacetic acid; HVA : homovanillic acid; DA turnover : metabolites/DA (DOPAC, HVA)/DA ; = : similar levels i.e. no treatment effect; ↑ : increase after treatment; ↓ : decrease after treatment; when lines are expressed noted this indicates treatment effect within that specific line; ND : not determined; ^: tendency 0.1 < P > 0.05. Cells in grey : represents birds younger than 10 weeks of age; Cells in white : represents birds older than 20 weeks of age. Abbreviations for lines: HP : high pecking line; LP : low pecking line (selection on egg production, Korte et al., 1998); DXL : DeKalb XL; HGPS: high group productivity and survivability line; LGPS : low group productivity and survivability line (selection on group productivity and survival, Cheng and Fahey, 2009) dom: dominant; sub: subordinate; ISA : ISA Brown. Abbreviations for ages: d : days; w : weeks; h : hours; min : minutes. Abbreviations for behaviors: aggr : aggression; FP : feather pecking. Abbreviations for brain areas: Hyp : hypothalamus; RN : raphe nuclei.