Behavioural neurobiology of feather pecking
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Chapter 6

Chicks from a high and low feather pecking line of laying hens differ in apomorphine sensitivity

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Abstract

The dopaminergic (DA) system has been implicated in several behavioural pathologies in both human and animal species. Previously, chicks from a high feather pecking (HFP) line were found to display lower central DA activity than chicks from a low feather pecking (LFP) line. HFP and LFP birds have been suggested to display a proactive and reactive coping strategy, respectively. Proactive rodents show a larger behavioural response to apomorphine (APO) than reactive copers, suggesting a more sensitive DA (receptor) system in proactive individuals.

In the present study, it is investigated whether HFP chicks show a more enhanced behavioural response to acute APO treatment than LFP birds in an open field. Furthermore, it was determined whether behavioural variation between HFP and LFP birds, after APO injection, is reflected by variation of D₁ and D₂ receptor densities in the brain.

APO treatment (0.5 mg/kg BW) significantly more enhanced locomotor activity (i.e. total distance moved, mean and maximum velocity) in an open field in the HFP line compared to the LFP line (during the first 15 minutes). There were no significant line differences in D₁ and D₂ receptor densities in the brain.

The present study demonstrated that HFP birds have a higher sensitivity of the DA (receptor) system in the brain compared to LFP birds. Thus, confirming our hypothesis that HFP birds display a proactive and LFP birds a reactive coping strategy.

A possible relationship between the functioning of the DA system en feather pecking is discussed.
1. Introduction

Feather pecking behaviour in laying strains of domestic fowl is a long-standing welfare problem in the layer industry. It is characterised by rather stereotypic pecking (Kjaer and Vestergaard, 1999) and compulsive pulling (McAdie and Keeling, 2000) at feathers of conspecifics, ultimately leading to injury and death. Despite years of research, its complex aetiology remains hard to fathom. Feather pecking is usually performed by a limited number of individuals in a flock (Keeling, 1994). Specific interaction between a genetic predisposition for the development of feather pecking and environmental challenges is believed to underlie this behavioural pathology.

Previously (van Hierden et al., 2002a), we reported that birds from a high (HFP) and low feather pecking (LFP) line of laying hens displayed different physiological and neurobiological response patterns when challenged. More specifically, it was shown that in response to acute stress induced by manual restraint, HFP chicks had lower plasma corticosterone levels and lower dopamine (DA) and serotonin (5-HT) turnover levels in the forebrain than LFP chicks. The results from the study supported earlier findings (Korte et al., 1997; Korte et al., 1999) that the (physiological) characteristics of HFP and LFP birds show considerable analogy to the characteristics of respectively the proactive and reactive coping strategy, known to exist in other species like rodents and pigs. From this study (van Hierden et al., 2002a) we also postulated that the difference in feather pecking behaviour between both lines might be causally related to a difference in functioning of the 5-HT and DA system.

Recently (van Hierden et al., 2003a), we found evidence for a causal role of the 5-HT system in the development and performance of feather pecking. In the present study a first step is taken by investigating a possible role of the DA system in feather pecking behaviour.

It has been suggested that the neurobiological characteristics of ‘proactive’ individuals make them more vulnerable to develop (behavioural) pathologies than ‘reactive’ individuals (Cools et al., 1990; Degen et al., 2003; Koolhaas et al., 1999;
Sontag et al., 2003; Teunis et al., 2002). A difference in the functioning or sensitivity of the DA (receptor) system has been suggested to (partly) account for this difference in vulnerability (Cools et al., 1993). Apomorphine (APO), a full agonist of the dopaminergic D₁ and D₂ receptors, with similar intrinsic activity as DA, is often used to predict individual differences in the sensitivity of the (receptor) DA system (Lal, 1988; Surmann and Havemann-Reinecke, 1995). By stimulation of the postsynaptic D₁ and D₂ receptors, APO (dose-dependently) induces an increase of locomotor activity and stereotyped behaviour (Berridge and Aldridge, 2000), like stereotypic pecking in chickens (Osudo and Adejoh, 1973; Zarrindast and Amin, 1992). Proactive copers show a larger behavioural response to injection with APO than reactive copers (Benus et al., 1991a; Bolhuis et al., 2000), suggesting a more sensitive DA (receptor) system in proactive individuals.

From the above we postulate that birds from the (proactive) HFP line have a higher sensitivity of the DA (receptor) system, and will therefore show an enhanced behavioural response to acute APO treatment compared to (reactive) LFP birds. To test this hypothesis, the behaviour of LFP and HFP chicks in response to an APO injection was studied using an open field. Another objective of the present study was to determine whether behavioural variation (in an open field) between HFP and LFP birds, after APO injection, is also reflected by variation of D₁ and D₂ receptor densities in the brain. Therefore, receptor binding capacities were assessed by measuring specific binding of tritiated D₁ and D₂ receptor ligands in different regions of the brain of control HFP and LFP chicks.

2. Methods

2.1 Birds and housing

In this study 96 White Leghorn chicks were used: 48 LFP and 48 HFP chicks (for line specifications see Korte et al., 1997). All birds were female and non-beaktrimmed. Chicks arrived on the day of hatching and were kept in groups of
4 animals per line (12 groups per line) and housed in pens (0.75x1.0m) with woodshavings. The pens were placed in a climate controlled room. Individual pens were visually isolated by hardboard partitions. Chicks were individually marked on the back with waterproof markers (black, purple, blue and green) before housing.

The environmental temperature was gradually lowered from 34°C on day one to 22°C at 5 weeks of age. On days 1 and 2 of age the chicks received 24 hours of light. From 3 days to 5 weeks of age the light regime gradually decreased from an 18 h to a 10 h light period. All birds had access to three drinking cups and one square feeding trough placed along one of the walls of the pen. Water and commercial feed (mash) were provided ad libitum.

2.2 APO injection and open field test

Apomorphine hydrochloride (Sigma RBI, the Netherlands) was freshly dissolved in distilled water (vehicle) every day. APO was injected into the breast muscle at a dose of 0.5 mg/kg BW in a volume of 1 ml/kg BW. A pilot study showed that this dose was the most effective in eliciting a change in behaviour of the chicks (data not shown). This finding is in agreement with previous studies (Osuide and Adejoh, 1973; Zarrindast and Amin, 1992). The control chicks were injected (i.m.) with a volume of 1 ml distilled water/kg BW.

At either 29, 30 or 31 days of age each chick was individually tested in an open field. A chick was captured individually, taken to the test room and injected into the breast muscle with either APO or distilled water (control). In each pen two APO and two control chicks were randomly chosen. The open field was situated in a separate room, to ensure auditory isolation, and the ambient temperature and humidity were maintained at a similar level to that of the home environment. The open field consisted of a wooden box, measuring 1.5 x 1.5 x 1.5 m (LxWxH), with white solid walls and woodshavings on the floor.

Immediately following APO or vehicle injection, the chick was placed in the middle of the open field. The behaviour of the chicks was videotaped for 30 minutes using an overhead camera and scored afterwards using Ethovision® 2.1 software programme (Noldus, Wageningen, The Netherlands).
2.3 Dopamine D₁ and D₂ receptor binding

For analysis of D₁ and D₂ receptor binding in LFP and HFP chicks, only the control birds were used. At the age of 35 days the two control birds were captured from their homecage and killed by rapid decapitation. Their brains were removed immediately and dissected into 4 brain regions: telencephalic pallium, basal telencephalon, diencephalon and the mesencephalon (for details see Koštál et al., 1999). Dissected tissue samples were frozen in a mixture of isopentane and dry ice and stored at -70°C. The frozen samples were transported on dry ice from Lelystad (the Netherlands) to Ivanka pri Dunaji (Slovakia).

The densities of D₁ and D₂ receptors were determined according to the method described by Koštál et al. (1999). Each dissected brain region was weighed individually and homogenised in cold 50 mmol/l Tris-HCl (1:10 w/v) buffer (pH 7.8). The homogenate was diluted 1:4 with the same buffer and centrifuged at 48,000 g for 10 min at 4°C (Beckman L5-65, Ty-65 rotor). The supernatant was discarded, the pellet washed in the same buffer and recentrifuged as before. The final pellet was resuspended in 4ml of 50 mmol/l Tris-HCl incubation buffer (pH 7.4), containing 1 mmol/l MgCl₂, 2 mmol/l CaCl₂, 120 mmol/l NaCl and 5 mmol/l KCl and next diluted with the same buffer to give a concentration of 1 mg protein per ml, based on estimated protein content (Lowry et al., 1951).

Membrane suspensions were incubated in duplicate for 30 min at 37°C. For the estimation of specific binding to D₁ receptors each plastic tube contained 300 µl of membrane suspension (1 mg protein/ml), 100 µl of 0.59 nmol/l [³H]SCH 23390 (75.5 Ci/mmol, DuPont NEN, USA) and 100 µl of incubation buffer (total binding) or 100 µl of unlabelled SCH 23390 (1 µmol/l, RBI, USA; non-specific binding). For the estimation of specific binding to D₂ receptors each plastic tube contained 300 µl of membrane suspension, 100 µl 0.05 nmol/l [³H]spiperone (23 Ci/mmol, Amersham, Great Britain) and 100 µl of incubation buffer (total binding) or 100 µl of (+)-butaclamol (1 µmol/l, RBI, USA; non-specific binding). Following incubation, separation of free from bound ligand was achieved by centrifugation at 23,000 g for 6 min at 4°C. Supernatant was removed by aspiration, and the tips of
the tubes (containing unrinsed pellets) were cut off with a heated wire and placed in scintillation vials. After addition of scintillant, the vials were shaken for 2 h, allowed to equilibrate, and radioactivity (dpm) was counted (Beckman LS 6000SE, USA).

2.4 Statistical analysis

For the analysis of the open field behaviour, the 30-minutes observation period was divided into 6 periods of 5 minutes. Furthermore, the behaviour of the birds was divided into the states ‘moving’ and ‘not moving’ (i.e. ‘velocity’ = 0.5 cm/s in Ethovision) and statistical analysis was performed on these states separately. For the ‘moving state’ the mean and maximum velocity (cm/s), total distance moved (cm) and the total time spent ‘moving’ (% of time) were calculated. The sample rate used was 3 samples/second.

Open field behaviour was analysed per time period with a mixed analysis of variance model with main effect and interactions for the factors Age (day 29, 30 or 31), Open Field (1 or 2), Line (HFP/LFP) and Treatment (APO/Control). Pen was entered as a random effect in the analysis. The behavioural data were not normally distributed. Therefore, these data were analysed according to techniques described by Engel and Keen (1994) and Engel and Buist (1996), employing Genstat 5 procedure IRREML (Keen and Engel, 1998). The Wald test (Rao, 1973), was used for estimation of Line x Treatment, Line and Treatment effects. No Age or Open Field effects were found and therefore these factors were excluded from the analysis.

For the analyses of the levels of the D1 and D2 receptors in the four brain regions, components were estimated with Restricted Maximum Likelihood Model (REML) procedure (Patterson and Thompson, 1971). The Wald test (Rao, 1973) was used for estimation of the Line effect.

When used in the IRREML procedure, the Wald statistic (W) is an estimation of the F-statistic, when used in the REML procedure, the Wald statistic is equal to the F-statistic. Differences were considered significant if P < 0.05. For all calculations GenStat® 6 (2002) was used.
Figure 1. The behaviour of control and APO treated LFP and HFP chicks in an open field. Levels expressed as mean ± S.E.M. LFP-C = LFP-Control, HFP-C = HFP-Control, LFP-A = LFP-APO, HFP-A = HFP-APO
3. Results

3.1 Open field behaviour

Table 1 shows the Line x Treatment (L x T) interactions, Line and Treatment effects, per time period, for the parameters measured in the open field. No significant overall Line effects were found for open field behaviour. Significant Line x Treatment interactions were only observed during the first 3 time periods. Therefore, we restrict the discussion of the behavioural results of the LFP and HFP birds presented in Figure 1 to these first 3 time periods.

For 'total distance moved' and 'mean velocity' (Figure 1A and 1B), significant Line x Treatment effects were found. During the first 15 minutes, APO treatment enhanced the 'total distance moved' and 'mean velocity', in the HFP birds significantly more than in the LFP birds. In fact no significant differences were found between APO treated and control chicks of the LFP line during this period of time.

The 'maximum velocity' is shown in Figure 1C. In the first time period, a significant Treatment effect was found. Both APO-treated HFP and LFP chicks showed a significantly higher 'maximum velocity' than control HFP and LFP chicks. Furthermore, a significant Line x Treatment interaction was found for the first two time period. APO treatment enhanced the 'maximum velocity' in the HFP birds significantly more than in the LFP birds.

For 'total duration of moving' (Figure 1D), no significant Line x Treatment effects were found. Except for time period 3, significant Treatment effects were found. Control birds spent more time moving during time period 1 and 2, compared to APO treated birds.

3.2 D₁ and D₂ receptor binding

There were no significant Line differences in the specific binding to dopamine D₁ and D₂ receptors in the four brain regions studied (Figure 2).
4. Discussion

The purpose of the present study was to investigate whether HFP and LFP birds differ in the sensitivity of the DA (receptor) system and whether differences in D1 and D2 receptor densities in the brain are correlated with APO-induced behavioural differences between the lines.

4.1 Behavioural effects of APO

APO treatment was effective in inducing a behavioural change in LFP and HFP birds in an open field environment. The behaviour of the APO birds was characterised by an increased locomotor activity, i.e. bouts of inactivity were alternately followed by bouts of hyperactivity (i.e. running behaviour). This finding is in agreement with other APO studies in chicks (Bilčík, 2000; Osuide and Adejoh,
1973) and rodents (Benus et al., 1991a; Surmann and Havemann-Reinecke, 1995). However, in our experiment no stereotypic pecking behaviour, like pecking at the wall or pecking its own toes, was observed, as is often reported to occur with APO treatment in chicks (Bilčík, 2000; Machlis, 1980; Osuide and Adejoh, 1973). Possibly, the interaction between APO treatment and the novel environment, is responsible for the absence of stereotypic behaviour in both lines. Harkin and his colleagues (2000) showed that in mice, APO produced qualitatively different responses under novel conditions when compared to those behaviours elicited in the home cage. In their study APO-induced locomotion was more prominent in the novel exploratory box than in the home cage. The absence of stereotypic pecking may also be an age-related effect. Osuide and Adejoh (1973) showed that APO induced more stereotypic pecking in very young chicks than in older birds.

The results of the present study clearly demonstrated a quantitative difference in the behavioural response to acute APO treatment between HFP and LFP birds in an open field. HFP chicks showed a higher increase in locomotor activity in response to APO than birds of the LFP line. This is in agreement with the previous findings that proactive rodents and proactive pigs show a higher sensitivity to the effects of APO (Benus et al., 1991a; Bolhuis et al., 2000; Cools et al., 1993) than their reactive counterparts. Thus, our data are consistent with the concept of coping strategies, confirming our hypothesis that HFP birds display a proactive and LFP birds a reactive coping strategy.

Recently (van Hierden et al., 2002a), we showed that HFP chicks display a lower level of DA turnover compared to LFP birds. A less active DA system may be accompanied by compensatory upregulation of DA receptors (Griffin and Ojeda, 1992). Therefore, we may have expected higher DA receptor densities in the HFP line compared to the LFP line. However, the present study showed that the different behavioural effects of APO in HFP and LFP birds, cannot be explained from a difference in D₁ and D₂ receptor densities in both lines. This suggests that other mechanisms account for the more enhanced behavioural effect of HFP chicks to APO treatment compared to LFP chicks. Cools and his colleagues (1990) also found that individuals with a functionally high activity of the DA system show a weak response to APO and vice versa. They suggested that APO is poorly
effective at high levels of fractional occupancy of the receptors by DA, but strongly effective at low levels of fractional occupancy of the receptors by DA. It can be speculated that, apart from a difference in receptor occupancy, differences in functioning or sensitivity of intracellular signal transduction pathways may also account for the more enhanced behavioural effect of HFP chicks to APO treatment (Griffin and Ojeda, 1992). However, the exact mechanisms should be examined in future studies.

4.3 Possible (causal) role of DA in feather pecking

Recently, we suggested that feather pecking might be a suitable animal model for OCD (van Hierden et al., 2003a). Like the 5-HT system, dysfunction of the DA system has been implicated in the pathophysiology of behavioural disorders, like animal stereotypies (de Lanerolle, 1980; Nistico and Stephenson, 1979; Pitman, 1989; Schoenecker and Heller, 2001; Sharman, 1978) and obsessive compulsive disorders (OCDs) (Jenkins, 2001; McDougle et al., 1994; van Ameringen et al., 1999). Feather pecking has stereotypic as well as compulsive characteristics. Once birds start feather pecking, they tend to do so successively and it is almost impossible to discourage their feather pecking behaviour. In two recent experiments we found evidence for a causal role of the 5-HT system in the performance and development of feather pecking. An acute decrease in 5-HT turnover in the forebrain increased feather pecking, while a chronic increase in 5-HT turnover decreased feather pecking in HFP and LFP chicks (van Hierden et al., 2003a; van Hierden et al., 2003b).

From the present study it can be postulated that sensitivity of the DA system, is indicative for the development of feather pecking behaviour and that the DA system may also play a (causal) role in the development and performance of feather pecking behaviour. Bilčík (2000) found no evidence that differences in DA sensitivity of young chicks (i.e. response to APO injection) can be used for prediction of susceptibility to feather pecking. However, Kjaer et al. (2002) showed that acute haloperidol treatment, significantly reduced feather pecking behaviour in adult laying hens. Haloperidol, a drug with anti-OCD effects, is a D<sub>2</sub> receptor
antagonist which, with acute administration, increases DA-turnover by blocking the presynaptic DA autoreceptor (McElvain and Schenk, 1992). This suggests that low DA neurotransmission might be involved in the performance of feather pecking behaviour.

4.4 Summary and Conclusion

In the present study, a first step was taken in investigating a possible role of the DA system in feather pecking behaviour. However, feather pecking behaviour of the birds was not measured in this experiment. Therefore, in future experiments direct effects of manipulation of the DA system on feather pecking behaviour in HFP and LFP birds should be investigated, by applying DA (ant)agonists like haloperidol. Furthermore, an extensive body of data supports the existence of a functional interaction between central 5-HT and DA. For instance, DA receptor stimulation, with receptor agonists, was found to increase 5-HT efflux in several forebrain regions (Mendlin et al., 1998), suggesting a facilitatory DA modulation of 5-HT neurotransmission. The interaction between the DA and the 5-HT system, in relation to feather pecking behaviour should also be examined, in pharmacological studies.

In summary, the present study demonstrated that HFP birds have a higher sensitivity of the DA (receptor) system in the brain, compared to LFP birds, as reflected by a more enhanced behavioural response to APO. This difference in response cannot be explained from a difference in D₁ and D₂ receptor densities between both lines. In the future, more pharmacological experiments, studying the (interacting) role of the 5-HT, DA or other neurobiological systems, are necessary to reveal the exact mechanisms underlying feather pecking behaviour.
Table 1. Wald statistics (\(W\)) and P-values (\(P\)) for open field behaviour, per time period (\(df=1\)). ns = non-significant, if \(P > 0.05\)

<table>
<thead>
<tr>
<th>Timeperiod</th>
<th>Effect</th>
<th>Total distance moved</th>
<th>Mean velocity</th>
<th>Maximum velocity</th>
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<td>(W = 4.20; P = 0.04)</td>
<td>(W = 6.55; P = 0.01)</td>
<td>(W = 6.46; P = 0.01)</td>
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<td></td>
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<td>T</td>
<td>(W = 8.10; P = 0.004)</td>
<td>(W = 16.30; P &lt; 0.001)</td>
<td>(W = 59.25; P &lt; 0.001)</td>
<td>(W = 15.28; P &lt; 0.001)</td>
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<td>2</td>
<td>L x T</td>
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<td>(W = 6.72; P = 0.01)</td>
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<td>T</td>
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