

HappyHens:

Final report, NFR project 178210:

“Behaviour, emotional expressions, stress and measures of brain function in relation to positive emotions in laying hens”

Background: Positive emotions, cue-induced anticipation and hen welfare

The experience of positive emotional states is increasingly recognised as important for animal welfare, in addition to absence of suffering. Accordingly, there is an increasing interest to develop methods to assess positive emotions in animals in order to evaluate and improve their welfare (Boissy et al., 2007). One major challenge is that emotions (i.e. what animals feel) cannot be assessed directly. However, the physiological and behavioural components of emotions may be studied as indirect indicators of emotional states.

Anticipation to a signalled positive reward induced by conditioned learning most likely elicits an appetitive “wanting” type of positive affect related to activity in dopaminergic pathways. Such dopaminergic “wanting” state is most likely experienced as a high-arousal positive affective state. Furthermore, consumption of a palatable reward most likely elicits a “liking” type of positive affect associated with opioid activation (Berridge, 1996). Thus, it has been argued that species-specific behavioural responses during anticipation and consumption of rewards may provide indirect information about appetitive and consummatory types of positive emotions in animals that is related to activity in brain reward circuits (Spruijt et al., 2001). Thus, animals can indicate their appraisal of stimuli by their behavioural responses during anticipation and consumption of rewards “here and now”. Furthermore, measuring the intensity of these behavioural responses has been proposed as a method to assess sensitivity of the animal’s brain reward system and, thus, the balance between past positive and negative experiences and emotional states. Such information is useful to assess animal welfare over a longer time period. Thus, animals can indicate their emotional state “retrospectively” through their behavior during anticipation of rewards (Spruijt et al., 2001). Exposure to repeated sessions of anticipation of a positive reward may also have a stress-reducing effect (van der Harst et al., 2003) and recent studies suggested a long-term impact on emotional reactivity and judgment biases in sheep.

Anticipatory behaviour induced by classical conditioning as an indirect measure of activity in the brain reward system was mostly studied in mammalian species. It is widely recognized that reward processing in mammalian brains depends on mesolimbic dopamine systems, comprising dopamine neurons in the ventral tegmental area (VTA) and their projections to the Nucleus accumbens (NAc) (Spruijt et al., 2001). Despite anatomical differences between the avian and mammalian brains, the dopaminergic system in the avian brain is involved in very much the same motor, emotional, cognitive and motivational functions. Importantly, avian brain homologues of VTA and NAc and the distribution and pharmacological properties of dopamine receptors have been described (Bálint E, Csillag 2007; Durstewitz et al., 1999). Thus, it may be proposed that anticipatory behaviour reflects activity in brain reward circuits also in avians and, therefore, could be a useful indicator of emotions also in laying hens. However, several questions regarding anticipation in hens needed to be clarified before being able to adapt the method as a valid measure of present and past emotional states.

In this project, the main objective was to develop a method based on anticipatory behaviour induced by classical conditioning to assess expressions of positive emotions in laying hens. In order to achieve this, the objectives of the project as listed in the project proposal were:

- To develop a model to identify conditions that create positive emotional states in laying hens by analysing the profile and quantitative aspects of anticipatory behaviour, and identifying potential emotional expressions during anticipation of positive rewards.
- To evaluate effects of positive or negative emotional states on physiological indicators of stress and emotions.
- To use antagonists of the opioid and dopaminergic system to test the involvement of brain reward circuits in association with anticipation of positive rewards.
- To investigate effects of reward incentive value and their physiological state on expression of anticipatory behaviours in hens
- To describe effects of domestication on the expression of cue-induced anticipatory behavior in fowl.

Briefly, the most important findings and conclusions are:

- Laying hens can be trained to anticipate a palatable reward and express a species-specific sequence of cue-induced anticipatory behavior which involves a high frequency of head movements
- The dopaminergic system is involved in the control of cue-induced anticipatory behaviour and appetitive foraging processes in laying hens
- Cue-induced anticipatory behaviour in laying hens is modulated by the incentive value of the announced feed reward and the hens physiological state
- Cue-induced anticipation is associated with an emotional arousal in hens as assessed by a peripheral temperature drop when exposed to a cue signalling an attractive reward
- Cue-induced appetitive processes are affected by domestication in fowl, i.e. standard laying hens express higher frequencies of appetitive behaviours in response to an attractive food reward compared to red jungle fowl.

Results and publications from the project are presented in the following overview:

- 1) A novel tool for the standardized study of anticipatory behaviour in laying hens

Background: In order to systematically test various hypotheses regarding the use of anticipatory behavior as an indicator of animal welfare in laying hens, there was a need to develop a standardized training and testing protocol, and to identify species-specific behavioural expressions during anticipation.

Aim: Developing an automated tool and standardized research protocol based on conditioned learning to induce anticipation in undisturbed *ad-libitum* fed laying hens freely moving in the home pen environment, in order to be able to test various hypotheses regarding anticipatory behaviour without external disturbance from the experimenter.

Experiment: An automated, computer controlled “learning device” was developed (Fig 1). This device emitted light signals and delivered rewards in a pre-programmed manner. Using this device, experimental hens were first trained to acquire the association between CS

(conditioned stimulus; a green light) and US (unconditioned stimulus; meal worms Fig. 2) using a trace interval of 3.5 s. After the hens had formed an association between CS and US, they were further trained to anticipate by gradually increasing the CS-US interval from the initial 3.5 s trace interval with a 1 s increment on every other CS+US presentation up to 22 s after three days and 32 s after five days of training. Finally, they were tested using a CS-US interval of 22 and 32 s. Control hens were exposed to CS and US in a random order, and tested as described for the experimental hens.

Results: Experimental hens responded to the CS with an increased level of anticipatory behaviour compared to the CO hens, and this difference between the groups was significant at a CS-US interval of 22 s. Anticipatory behavior consisted of interrupting previous behavioural priorities, and standing still or walking with slow steps, with legs, body and neck stretched upwards and eyes open in alert attention, and frequent head movements up and down and tilted (Fig 3).

Publications:

Moe RO, Nordgreen J, Janczak AM, Spruijt BM, Zanella AJ, Bakken M. Trace classical conditioning as an approach to the study of reward-related behaviour in laying hens: a methodological study. *Appl Anim Behav Sci* 2009;121:171-8.

Moe RO, Nordgreen J, Janczak AM, Zanella AJ, Bakken M. 2009. Forventningsatferd- en metode for å studere positive emosjonelle tilstander hos dyr. *Husdyrforsøksmøtet 2009*. Proceedings, 485-486.

Moe RO, Nordgreen J, Janczak AM, Spruijt BM, Zanella AJ, Bakken M. 2009. Automated induced reward-related behaviours in laying hens in the home pen environment. *World's Poultry Science Journal*, 8th European Symposium on Poultry Welfare, Cervia (Italia) 18-22 Mai 2009, Book of Abstracts, s.11.

Moe RO, Nordgreen J, Janczak AM, Spruijt BM, Zanella AJ, Bakken M. 2010. Pavlov's hens: using trace classical conditioning to study anticipatory behaviour. *Nordic ISAE 2010*, 20-22.1.2010, Siilinjärvi, Finland, Proceedings p. 12

2) Involvement of the brain reward system in the expression of anticipatory behavior in laying hens:

a. The role of dopamine

Background: Reward processing in mammalian brains depends on mesolimbic dopamine systems. Although avian brain reward system homologues are identified, the role of dopamine control in anticipatory behavior in hens has not yet been described.

Aim: To test the hypothesis that the dopaminergic system is involved in the behavioral control of anticipatory behavior in laying hens, using a dopamine D2 receptor antagonist. We predicted that haloperidol, a dopamine D2-like receptor antagonist, would lower the display of anticipatory behaviors.

Experiment: Hens were trained to anticipate using the automated learning device. After successful training, effects of four doses of haloperidol (0.3; 0.5; 1.0 and 2.0 mg kg⁻¹) administered 30 min before CS on anticipatory behavior in the CS-US interval was tested. Furthermore, intra- and interobserver reliability of the reward-related behaviors were calculated.

Results: Frequency of head movements and latency to initiate display of anticipatory behaviour were significantly affected by the lowest doses (0.3 and 0.5 mg kg⁻¹), consistent with the involvement of dopamine in control of reward-related behaviours in laying hens, as is the case in mammalian species. The highest doses had sedative effects. A high inter- and intraobserver agreement in the assessment of head movements together with their dopamine dependency suggested that this behaviour in the described classical conditioning paradigm represents an indicator of the state of the reward system in laying hens that can be assessed with good reliability.

Publications:

Moe RO, Nordgreen J, Janczak AM, Spruijt BM, Kostal L, Skjerve E, Zanella AJ, Bakken M. 2011. Effects of a dopamine receptor antagonist on reward-related behaviors in laying hens. *Physiology & Behavior* 102; 400-405.

Moe RO, Nordgreen J, Janczak AJ, Zanella AJ, Bakken M. Dopamine regulation of reward-related behaviours in laying hens. ISAE August 2010 Uppsala, Sweden, Proceedings p. 18

Moe RO, Nordgreen J, Janczak AM, Hild S, Skjerve E, Bakken M. 2010. Intra- and interobserver reliability of reward-related behaviours in laying hens. European Poultry Conference EPC August 2010, Tours, France. Proceedings, p. 572.

Moe RO, Nordgreen J, Janczak AM, Bakken, M. 2011. Dopaminregulering av forventningsatferd hos verpehøner. Husdyrforsøksmøtet 2011. (Poster) Proceedings p. 596

b. The role of reward incentive value, physiological state, and opioid regulation

Background: Behaviour in unconditioned choice tests and in instrumental (operant) conditioning paradigms can be used to assess what animals like and the strength of motivation for incentives. Hens prefer mealworms over whole wheat in an unconditioned choice test, and work more for mealworms compared to other feed types in a conditioned operant test (Bruce et al. 2003), indicating that hens rank the incentive value of mealworms higher than that of whole wheat. Opioids are involved in palatability of rewards. Furthermore, the opioid and dopamine systems interact in the ventral tegmental area (VTA) and opioids may facilitate the activation of dopaminergic reward pathways and thereby stimulate the onset of motivated behaviors.

Aim: The aims of this study were to investigate control of cue-induced anticipatory behaviour in laying hens, by 1) investigating effects of incentive value of rewards on frequency of conditioned anticipatory behaviours, and 2) investigating the potential involvement of μ -opioid receptor transmission on the frequency of cue-induced anticipatory behaviours by systemic injections of the μ -opioid receptor antagonist naloxone before exposure to the conditioned stimulus.

Experiment: Hens were trained to anticipate two feed rewards differing in incentive value (whole wheat grains and meal worms) in response to two light signal colours (Conditioned stimulus; CS1 and CS2; green and red light, respectively) using the automated learning device. A third unrewarded CS (blue light) was applied as a control. Anticipatory behavior in

response to the different CS signaling feed rewards or non-reward was investigated in naloxone (5 mg/kg)- or saline treated laying hens that were either food sated or food restricted (24h fast).

Results: Incentive value of signalled reward was differentially reflected by frequency of cue-induced head movements. Reward type also affected frequency of steps and latency to display the first head movement, but there was no difference between the two rewarded cues. Hunger amplified number of head movements, and steps. No evidence for a role of naloxone in modulating the intensity of cue-induced appetitive behaviour was found as tested here.

Publications:

Moe RO, Nordgreen J, Janczak AM, Spruijt BM, Bakken M. Effects of signalled reward type, food status and a μ -opioid receptor antagonist on cue-induced anticipatory behaviour in laying hens (*Gallus domesticus*). Applied Animal Behaviour Science, accepted

Nordgreen J, **Moe RO**, Janczak AM, Spruijt BM, Bakken M. 2013. Hens know what they are waiting for: the effect of reward type on appetitive behaviour. IX European Symposium on Poultry Welfare. SLU Uppsala, 17-18 juni. Proceedings, p73.

3) Effects of cue-induced anticipation and access to a palatable reward on emotional state assessed by physiological indicators of stress

Background: A conditioned rise in body temperature (“emotional fever” or “stress-induced hyperthermia”) occurs in response to stimuli predictive of and during exposure to unpleasant events in all mammalian species that have been tested to date. This phenomenon was interpreted as an indicator of emotions, and has also been found in hens (Cabanac & Aizawa, 2000). However, although emotion is defined as “any mental experience with high intensity and high hedonic content (pleasure/displeasure)”, emotional fever has mostly been studied during anticipation of or exposure to negative events. Emotions may be regarded as linear combinations of two independent neurophysiological dimensions: valence (positive or negative) and arousal (high or low). Anticipation of positive rewarding stimuli in a conditioning paradigm could be said to influence (positive) affect and high arousal, whereas anticipation of a negative stimulus is highly arousing and induce negative affect. Thus, it

could be speculated that the emotional experience during anticipation or consumption of a highly valued food reward induce arousal and affects temperature regulation. If so, changes in body temperature could be indicative of a positive emotional state. However, physiological responses during anticipation and consumption of positive rewards have not yet been studied in hens.

Aim: To describe effects of anticipation and consumption of a highly palatable reward on peripheral comb temperature measured with infrared thermography in order to investigate potential physiological indicators of positive emotional states in laying hens.

Results: A substantial decrease (1.5°C) in comb temperature was found the first two minutes during anticipation and consumption of a reward (Fig 4). Such temperature drop indicates a peripheral vasoconstriction and has clear resemblances to emotional fever as seen during negative emotional states. Thus, we propose that a drop in comb temperature reflects emotional arousal more than emotional valence.

Publications:

Moe RO, Stubsjøen SM, Bohlin J, Flø A, Bakken M. 2012. Peripheral temperature drop in response to anticipation and consumption of a signaled palatable reward in laying hens (*Gallus domesticus*). *Physiology & Behavior* 106; 527–533

Moe RO, Stubsjøen SM, Bohlin J, Flø A, Bakken M. 2012. Drop in peripheral comb temperature during positive anticipation. ISAE August 2012 Vienna, Austria, Uppsala, Proceedings p. 203

Moe RO, Stubsjøen SM, Bohlin J, Flø A, Bakken M. 2013. Forventning om en positiv belønning fører til endringer i kammens overflatetemperatur hos verpehøner.

Husdyrforsøksmøtet 2013.

4) Effects of genotype on appetitive processes in fowl

Background: The red jungle fowl (RJF), which is regarded as the wild ancestor of the domestic hen, spends more time on feeding activities, prefer to work for food even if freely available, and perform more exploratory behaviour compared to domestic hens (Schütz & Jensen, 2001). Thus, selection processes (for reduced fearfulness, high production, etc.) have

affected some aspects of feeding behaviour in hens, in particular behaviours seen during foraging which is an appetitive phase. However, only standard diets were used in these studies, and effects of selection processes on feeding behaviour and cue-induced appetitive behaviours in response to an attractive food reward have not been investigated in fowl. Furthermore, the role of dopamine regulation of foraging behaviour in response to an attractive food reward has not been investigated in fowl.

Experiment: Two breeds with different selection history were included in the experiment; RJF and Lohmann White Selected Leghorns (LSL). Eggs were hatched in the same brooder and chicken were later reared together in a large floor pen (Fig 5). During the experiment, hens were housed in pairs (i.e. RJF + RJF and LSL + LSL) (Fig 6). Hens were exposed to the standardized training and anticipation protocol as described. Foraging behaviours, comfort behaviours and locomotor activity were studied in RJF and LSL with or without pre-treatment with a dopamine D2 antagonist.

Publications:

Moe RO, Nordgreen J, Janczak AM, Bakken M, Spruijt BM, Jensen P. 2013. Appetitive and consummatory behaviours in relation to attractive feed rewards and their dopaminergic control: A window to understand positive emotions in fowl. IX European Symposium on Poultry Welfare. SLU Uppsala, 17-18 juni. Proceedings, p. 151

Moe RO, Nordgreen J, Janczak AM, Bakken M, Spruijt BM, Jensen P. Domestication effects on behaviour in response to an attractive food reward: a comparison between the red junglefowl (*Gallus gallus*) and White Leghorn layers. Manuscript in preparation.

Complete list of publications resulting from the project

Scientific journals:

Moe RO, Nordgreen J, Janczak AM, Spruijt BM, Zanella AJ, Bakken M. Trace classical conditioning as an approach to the study of reward-related behaviour in laying hens: a methodological study. *Appl Anim Behav Sci* 2009;121:171-8.

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Moe RO, Stubbsjøen SM, Bohlin J, Flø A, Bakken M. 2012. Peripheral temperature drop in response to anticipation and consumption of a signaled palatable reward in laying hens (*Gallus domesticus*). *Physiology & Behavior* 106; 527–533

Moe RO, Nordgreen J, Janczak AM, Spruijt BM, Bakken M. Effects of signalled reward type, food status and a μ -opioid receptor antagonist on cue-induced anticipatory behaviour in laying hens (*Gallus domesticus*). *Applied Animal Behaviour Science*, accepted (2013)

Moe RO, Nordgreen J, Janczak AM, Bakken M, Spruijt BM, Jensen P. Domestication effects on behaviour in response to an attractive food reward: a comparison between the red junglefowl (*Gallus gallus*) and White Leghorn layers. (Manuscript in preparation).

Conferences proceedings:

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Nordgreen J, **Moe RO**, Janczak AM, Spruijt BM, Bakken M. 2013. Hens know what they are waiting for: the effect of reward type on appetitive behaviour. IX European Symposium on Poultry Welfare. SLU Uppsala, 17-18 juni. Proceedings, p73.

Boissy A, **Moe RO**, Lidfors L. 2013. Cognitive processes as the links between positive emotions and welfare to achieve a better quality of life in livestock. Behaviour, IEC 2013 Newcastle, 4-8.8.13, Newcastle, UK, Proceedings

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