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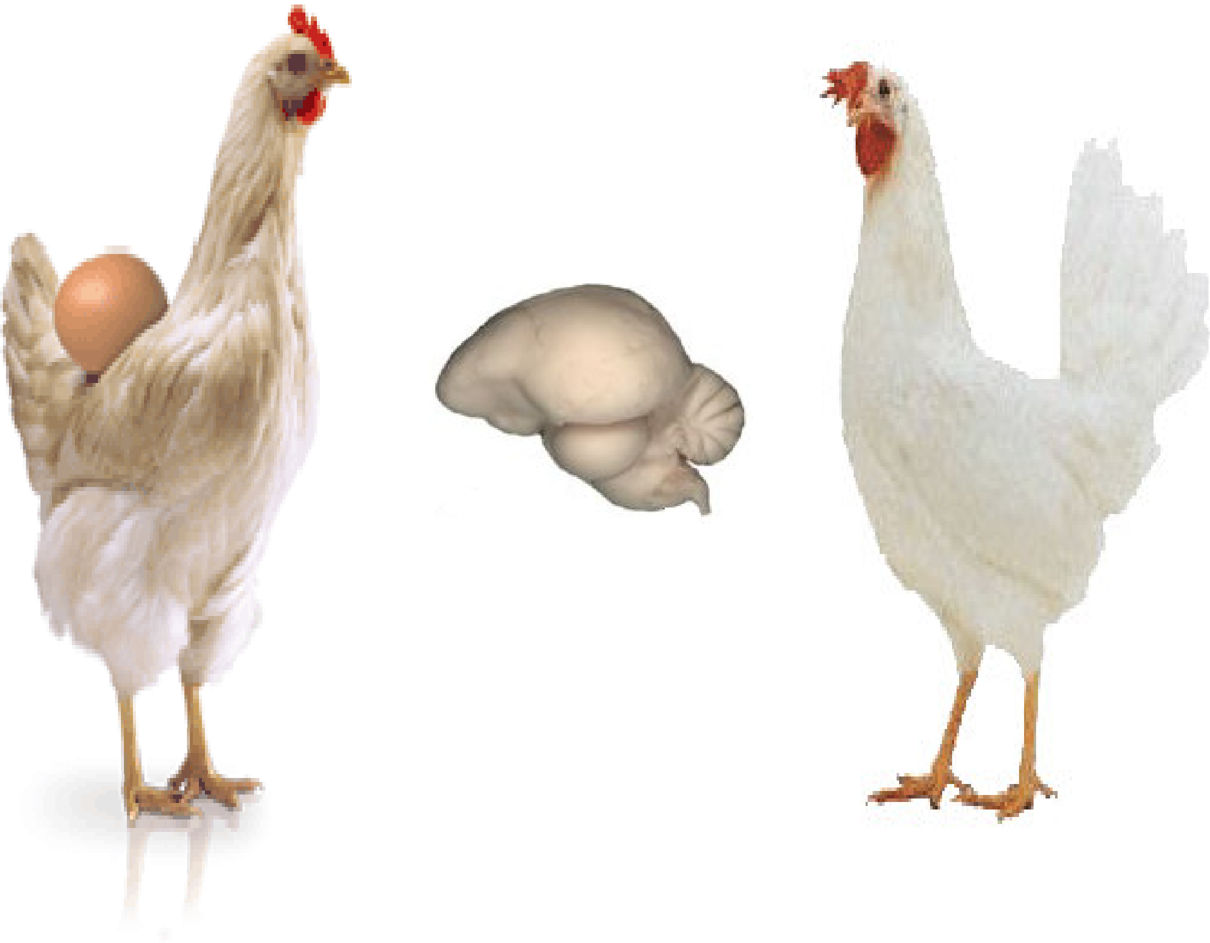

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A study of different feather pecking solutions and its effect on learning, memory and emotional reactivity in animals of the White Leghorn and Silver Nick laying hen strain

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Samenvatting

Het doel van deze studie is om de invloeden, van twee van de belangrijkste methoden voor het verminderen van verenpikken in de pluimveesector, op cognitie en gedragsaspecten te onderzoeken. Kippen hebben een sterk ontwikkeld zicht en gehoor maar smaak, reuk en tast zijn minder vertegenwoordigd. De enige plaats waar tast wel goed vertegenwoordigd is in de snavel. Kippen zijn zeer sociale dieren met uiteenlopende communicatiepatronen. Een van de meest belangrijke gedragspatronen is foerageren, dit kan in het wild tot 90% van de tijd innemen.

Verenpikken is een van de meest belangrijke welzijnsproblemen in de pluimvee sector. Twee van de belangrijkste methoden voor de bestrijding van dit probleem zijn genetische selectie en snavelbekapping. Het is van belang de invloeden van deze methoden op cognitie en gedrag te onderzoeken om te begrijpen hoe zij invloed hebben met betrekking tot het verenpikken probleem. Meer inzicht in de prestaties van cognitie en gedrag is van groot belang om vast te stellen wat kippen nodig hebben van een (productie) omgeving. Genetische selectie is een van de methoden die wordt gebruikt om verenpikken te bestrijden. Echter, de manier waarop de selectie invloed op de verenpik probleem uitoefent is nog niet geheel bekend. Dit is onderzocht door de resultaten van een vierde generatie Witte Leghorn ‘lage uitvalslijn’ te vergelijken met dat van een controlelijn. Het onderzoek toont aan dat kippen een sterk ontwikkeld werk- en een goed ontwikkeld referentie of lange termijn geheugen hebben. De kippen tonen een redelijk leereffect in de ruimtelijke ‘holeboard’ taak. Er werden geen significante verschillen gevonden in de prestaties van de twee lijnen, dit suggereert dat genetische selectie geen invloed heeft op geheugen, leren en ruimtelijke oriëntatie.

Snavelbekapping is een methode die al langere tijd wordt gebruikt met gevolgen voor de integriteit en het welzijn van het dier. Afhankelijk van de gebruikte methode en het tijdstip van bekappen kan deze methode langdurige effect hebben zoals sensorische afasie, langdurige pijnsensatie en verlies van functie in betrekking tot de snavel. De gevolgen van deze methode op gedragaspecten als angstigheid, socialiteit, voedselpreferentie en herkenning is onderzocht door een Silver Nick bekapte groep te vergelijken met een controlegroep. Als er pijnsensatie door bekapping aanwezig is kan dit een invloed hebben op cognitie, angstigheid en socialiteit. Socialiteit is getest in een T-doolhof en gaf geen significante groep verschillen. Dit laatste is niet geheel met zekerheid te zeggen omdat niet alle kippen hun soortgenootjes wisten te vinden. Angstigheid in de twee groepen werd onderzocht met een open veldtest. Dit geeft geen significante groepverschillen. De invloed van het snavel bekappen op voedsel manipulatie en voorkeur is getest door de kippen te laten kiezen tussen levend en dood aas. De test toonde geen significante verschillen tussen de twee groepen. Echter de snavel bekapte kippen besteedde meer tijd aan het eten van en pikte meer naar het levende aas. Dit suggereert dat zij meer moeite hadden met het manipuleren van het levende aas. Angst en herkenning is getest met het aanbieden van een beloning door een bekende en onbekende onderzoeker. De test toonde geen groep verschillen, maar heeft laten zien dat beide groepen het verband tussen de onderzoeker en de beloning geleerd hadden. Beide groepen benaderde de beloning sneller in de tweede sessie, ongeacht het feit dat dit was met een onbekende onderzoeker. In dit onderzoek lijkt genetische selectie geen invloed lijkt te hebben op geheugen en ruimtelijke oriëntatie. Ander onderzoek suggereert dat selectie wel invloed heeft op sociabiliteit, angst en stress response. Het snavel bekappen lijkt geen invloed te hebben op sociabiliteit en angstigheid, maar vertoonde wel verschil in voedsel manipulatie. De kippen in deze studie leken geen erge pijnsensatie te ervaren, maar dit onderzoek suggereert dat zij mogelijk lijden aan zintuig afasie.

Summary

The aim of this study is to investigate the influences of two of the main methods for reducing feather pecking in the poultry sector on cognition and behavioral aspects. Chickens have highly developed sense of sight and hearing, sense of taste, smell and touch are less represented. However touch is well represented in the beak. Chickens are a highly social species with varied communications patterns. One of the most important behavioral patterns is feeding, this can take op to 90% of the time in the wild.

Feather pecking is one of the most severe welfare implications in the poultry sector. Two of the main methods of fighting this problem are genetic selection and beak trimming. The influence of the methods on cognition is important in understanding how these methods relate to the feather pecking problem. Furthermore understanding the performance of cognition and behavioral aspects in chickens are of great importance to establish what chickens need from a (production) environment. Genetic selection is one of the methods used to control feather pecking. However, the way it influences the feather pecking problem is not yet fully known. This was researched by comparing the results of a fourth generation low mortality line with a control line of the white leghorn laying hen strain. The study revealed that chickens have a highly developed working memory and a well developed reference memory. The chickens showed good learning abilities in a spatial hole-board task. No differences were found in performance between the two genetic lines of hens, indicating no effect of this specific selection line on cognition. Beak trimming is a method which has been used for a long time, this method however has its implications on the integrity and welfare. Depending on the method used and the time of trimming, this method can have long lasting repercussions in the form of sensory aphasia, long lasting pain sensation and loss of function in regards to the beak. The effect of beak trimming on behavior was researched by comparing the results with a control line of Silver Nicks of the laying hen strain. If pain sensation as a result of the beak trimming is present this may have influence on sociability, fearfulness or cognition. Sociability tested in a T-maze apparatus revealed no significant differences. This however did not reveal the whole extend of the sociability between the two groups because not all the chicks fully participated in the apparatus. Fearfulness in the two groups was investigated with an open field test; this did not reveal any significant differences. The influence of beak trimming on food manipulation and preference was tested by letting the chickens choose between live and dead bait. The test showed no significant differences between the two groups. However the beak trimmed birds did marginally spent more time eating and pecking at live bait suggesting that they had more difficulty manipulating live bait. Fearfulness and recognition was tested by offering a reward by a familiar and unfamiliar researcher. The test showed no group differences but did show that both groups learned the connection between the researcher and the reward. Both groups approached faster in the second session regardless of the fact that this was with an unfamiliar researcher. This study revealed that genetic selection does not seem to have influence on cognitional aspects as memory and spatial orientation. Other studies suggested that selection does influence sociability, fearfulness and stress response. Beak trimming also did not seem to have influences on sociability and fearfulness but did show slight difference in food manipulation. The chickens in this study do not seem to experience major pain sensation but it is possible they experience sensory aphasia. The influences of genetic selection and beak trimming on cognition and other behavioral aspects need to be further investigated. The results were analyzed with repeated measures ANOVAs and the T-test procedure.

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Introduction

This study is carried out for INHolland University in Delft and the Faculty of Veterinary Medicine in Utrecht. This paper discusses the results of a cognitive experiment on a genetic selection line and a beak trimmed line in relation to one of the biggest welfare issues in today's poultry industry, feather pecking. This research focuses on the possible influences of genetic selection and beak trimming on different aspects of chicken cognition. In this introduction scientific information in relation to this subject and the major research statements of this study are revealed.

One of the most debated topics of applied ethology is the welfare of domestic animals. Welfare can for example be defined as a state of harmony between an individual and its environment. Any perceived deviation from this state results in a welfare deficit due to negative emotional experiences. The chicken is the most largely held production bird in the world. The housing of chickens is often designed to facilitate production needs and provide efficiency for the poultry keeper. This has its effect on the chicken's natural behavior, which some housing systems facilitate very poorly. An effect on the natural behavior leads to welfare problems, one of the major problems is feather pecking. The chicken as a species and its natural behavior is stated in appendix 1.

Feather pecking and cannibalism, regular treatment and alternatives

Feather pecking is one of the most severe health issues in today's poultry management. Feather pecking is a form of dysfunctional behavior which involves the pecking at and eating of feathers from other individuals. There are two types of feather pecking, soft and hard or severe feather pecking. Soft feather pecking is part of the natural behavior of chickens which is used to clean plumage. Severe feather pecking, which can also lead to cannibalism, is not part of normal behavior. The difference in those types of pecking is, during severe pecking the feather is pulled out and often eaten. Severe feather pecking is mostly directed at inactive individuals, while soft pecking is directed against dust bathing individuals [32]. This last statement goes along with the above mentioned fact that soft feather pecking is a natural behavior used for cleaning the plumage [26].

Feather pecking is a more widespread problem than often assumed by the public and the poultry farmers. Research shows that farmers come to the conclusion that around 30% of their flock has feather pecking issues. However investigators discovered a percentage of up to 70% at the end of the rearing period. The problem can be masked when the fathers are still re-growing and no physical damage can been seen at that time. However the older the hens get the higher the percentage of damaging feather pecking they deliver. By supplying adequate rearing conditions feather pecking can be positively influenced in early weeks and thereby greatly improve that plumage conditions in the laying period [24].

But how does feather pecking start? There are two hypotheses which have been supported by research. The first hypothesis states that feather pecking is a redirection of ground pecking related foraging behavior; this is stated be the research of *Blokhuis en Arkes* in 1984 en 1986. In 1989 *Blokhuis and van der Haar* determined that birds which have been raised on wired flooring show higher frequencies of feather pecking than bird reared on floor litter. This suggests that feather pecking is related to early

life experiences. Research in 1998 by *Huber-Eicher and Wechsler* also stated a relation between foraging behavior and feather pecking occurrence.
The second hypotheses originated from the research of *Vestergaard et al.* in 1993 and rests on the belief that feather pecking is a redirected behavior associated with pecking while dust bathing. Redirection most likely results from the absence or substrate for either activity. Other influential factors are group size, stocking density, housing en rearing types, lighting conditions, dietary factors and genetic predisposition [25].
Severe feather pecking can lead to cannibalism; cannibalism is the act of consuming tissues from other members of the same species whether living or dead and at any stage of the life cycle [29]. Cannibalistic pecks can be directed at feet or feathered area (especially the tail). If the latter is true it has a strong correlation with feather pecking. Tail feathers start to bleed if they are severed or broken during feather pecking, which can lead to cannibalistic reactions. Cannibalism can also be directed at the cloaca, which can lead to the removal of intestines, this is the deadliest form of cannibalism [29].

Beak trimming a treatment method with consequences

Beak trimming is done to minimize feather pecking and cannibalism by making the beak less sharp. This ensures that if the animals do peck at each other less damage is done in the process. A bird's beak is highly sensitive. It contains free nerve endings which are important for touch and temperature related sensory information.
In non organic production systems beak trimming is often used to control severe feather pecking and cannibalism. Beak trimming is the removal of the tip of the upper and lower part of the beak (see figure 1). This results in a deformed and less sharp beak which has a positive effect on the control of feather pecking because the animals some animal use their beak less. This procedure has an influence on the integrity of the animal by cutting away an important part of its physiology. It can also cause neurological discomfort due to the severed nerves which run through a chicken's beak; because of that pain sensation can occur. Long lasting pain sensation influences certain cognitive aspects as fearfulness [28].

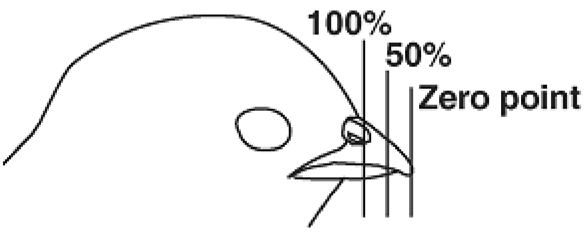

Figure 1: beak trimming procedure 50% and 100% [28]

Methods of beak trimming

<u>Heated blade</u>: the concept of the heated blade is similar to a (heated) guillotine, the device consists of a metallic bar to pun the beak on or a bar with a hole in which the beak is put trough. The blade comes down on the selected position on the beak and cuts and cauterizes at the same time. The blade is usually heated between 650 and 750 degrees Celsius. Two types of heated blade devices are available: the gas bases

version and the electric based version. The electric soldering instrument consists of a sharp edge of a brass/copper disk welded to an electric soldering iron. The other version, a gas based instrument, consists of a hot plate and a cutting bar. This model is easily portable and practical in use; however gas pressure and wind currents affect its efficiency. Heated blades are frequently used in beak trimming, but this is not the most recommended method. Cauterization can cause damage to the beak tissue and can result in a larger amputated area than was intended. Research has shown that the heat also increases the stress level of the bird in comparison the other methods of trimming [25].

Cold blade: cold cutting can be performed either with a sharp jackknife, a simple set of dog nail clippers or a secateurs, this procedure is less stressful than hot cutting. Removal in this method is said to be more precise than other methods, caution however should be taken on adequate removal of the beak tissue [25].

Electrical blade: the electrical blade provides a completely different method of trimming the beak, instead of just working with sharp edges, this method places two electrodes at the tip of the beak and the end of the section which should be removed. The electric current burns a hole in the upper beak, the beak will then die off in a week. This method is still painful but research by *Grigor et al.* in 1995 shows that beaks trimmed with this method will heal in less than three weeks. There is however a risk for damage if the electrodes are placed incorrectly or too far apart. If performed correctly however this method may be a more preferable experience than trimming with a heated or cold blade [25].

Laser blade: trimming with a laser blade is a relatively new procedure. However it shows great potential for future methods if commercial use is continued. Laser based cutting shows more uniformity and cauterization is not required [25].

The beak trimming procedure is controversial because of its positive effect on aggression/social disturbance and on the plumage of the birds and its possible negative effects. Researchers argue that less aggression is due to inactivity which relates to pain sensation. Furthermore research states that less pecking at each other can be related to a nerve imparity which alters the sensation in the beak. This altered sensation may also influence the ability to pick up food [28].
The procedure which is performed to trim the beak may cause acute pain, behavioral reactivity was seen when the procedure was performed: birds struggle and screech when the beak is cut away. There is evidence for chronic pain following beak trimming. Research statements say the following about the pain sensation of this procedure: 1) although the benefits of beak trimming contribute to its widespread use, the procedure is nonetheless traumatic *(Gentle, 1986a).* 2) Beak trimming is stressful and painful, as evidenced by bird vocalizations, especially when trimming is performed with a heated blade *(Grigor et al., 1995).*[28]
In many of the birds beak amputation can result in extensive neuroma formation, this is a growth or a tumor of nerve tissue. These neuromas can result in spontaneous neural activity in the trigeminal or facial nerve. Pain related behavior has been reported in research for up to 6 weeks after amputation. But much discussion still arises with the appearance of conflicting evidence regarding the nerve activity, food intake and welfare implications [28].

In any perspective it can be stated that the beak trimming procedure is painful and can lead to prolonged pain although evidence varies with respect to long term nerve activity. Furthermore beak trimming leads to changes in behavior indicative of pain. As explained before, beak trimming has a positive effect on aggression and social disturbance; this may be explainable by the inactivity of the trimmed birds in relation to non trimmed birds. Preening and feeding behavior also declines often in beak trimmed birds. In relation the food intake and body weight research is contradictive. Food intake en weight gain do quickly return to normal for some birds, but evidence in a pigeon study reveals that removing the feedback connection of mechanoreceptors can lead to sensory aphasia and a decline in body weight for a longer period of time [20, 25]. A great deal of research has been done on this subject and from that research a couple of major welfare implications of beak trimming can be concluded:

1) **Loss of normal function due to reduced ability to sense material**
Food and water intake reduces significantly during the first few weeks after trimming, this is not persistent. Trimming also affects preening, less pecking results in a better quality plumage of trimmed birds. When birds are trimmed at an age of 16 weeks or older the preening rates do not return to normal. In relation to loss of function more research is necessary to determine the amount of effort it takes trimmed birds to eat the same amount of food as before [28].
2) **Short term pain and debilitation**
Short term pain occurs when the tip of the beak is removed with a heat or infrared based instrument. Research shows a significant injury discharge from the intra mandibular nerve, this is a major nerve which runs thought the beak, and the discharge is short lasting. Research also shows on reduced pain period up to 24 hours after the initial injury discharge. After a 24 hour period, pecking behavior reduces, therefore there can be concluded that more pain is present at that time. This pain is recorded to persist for at least four weeks, after that pecking behavior can return to normal. Another indication of pain and stress is an increased heart rate. Research shows that this effect is less when the procedure is performed shortly after hatching [28]. Chickens in a research by Gentle in 1990 showed significantly less pecking at the environment and thereby displaying guarding behavior. They also showed less head shaking and beak wiping behavior when coming in to contact with waters at 45°C. This can be explained by hyperalgesia with additional pain induced by water at 45 °C and thereby increasing the guarding behavior. Although the pecking at water between 20 and 40 degrees Celsius did not differ in beak trimmed birds, the reluctance of dipping the beak in warm waters supports evidence for short and long term hyperalgasia [20].
3) **Tongue damage and burned nostrils**
This can only occur when more than 75% of the beak is removed. No research supports the need to remove such large portion of the beak, so any damage to the tongue or nostrils is unacceptable because cutting more than 75% of the beak is not necessary [28].
4) **Neuromas and scar tissue**
If chickens are trimmed very early after hatching, research shows they are less likely to devolved neuromas and scar tissue. Neuromas can cause a bird to suffer from long lasting pain. Research shows spontaneous burst of activity of the intramandibular nerve in some trimmed birds. The presence of both neuromas and nerve activity in the beak is presented by researchers as

evidence of long term pain and perhaps phantom limb pain that has been reported in mammals [28].

Chickens which received trimming at a later stage did show micro neuromas in week 10 after the procedure but not at the second investigation after 70 weeks. However birds which are trimmed at 1 day of age but had two- thirds of their beak removed did show persistent neuromas after week 70. Important factors are: age of trimming and amount of the beak which has been trimmed. If chicks are trimmed at day 1, the stress of the procedure during the important growing time is avoided and when birds are trimmed this young they seem to consume less without it having an effect on production levels. However trimming one day old chicks has its downsides, first if all chicks are still recuperating from al the experiences of hatching, vaccination and perhaps transport. Furthermore the anatomy of their beaks can be a risk; very small beaks may be hard to trim with absolute precision. Early trimming from 5 to 10 days or 7 to 10 days minimizes adverse effects [28]. Animals which are trimmed during this period do not seem to form neuromas or the neuromas are not persistent. If chickens are trimmed after this period it is easier to exercise absolute precision and the beak does not re-grow as much as when it was done in earlier stages. However these birds can more easily develop neuromas in the stump which could be a sign of chronic pain endurance according to *Gentle (1986).* Furthermore animals trimmed at 4 or 5 weeks of age are more prone to experience long term adverse effect on behavior, feeding and weight gain according to *Andrade and Carson (1975) en Duncan et al. (1989).*

Therefore it can be concluded that age of trimming and the amount of beak trimmed are factors which influence the presence of persistent neuromas at maturity [28].

What beak trimming does and what its welfare implications are is explained above but what can be concluded as to why this effect occurs in the behavioral traits of trimmed birds versus non trimmed birds. Several hypotheses are proposed to answer these questions;

1) Research of *Hughes and Michie (1982)* suggest that when beak trimming is performed before behavioral traits are established, learned feather-pecking behavior can be prevented.

2) Trimming may also reduce sensory perception as a result of damage to or removal of mechanoreceptors and nerve endings *(Hughes and Michie, 1982; Dubbeldam et al., 1995; Gentle et al., 1997).*

3) Consequently, sensory feedback that normally accompanies pecking does not occur and the behavior is not rewarded *(Hughes and Michie, 1982; Hughes and Gentle, 1995).*

4) Pecking activity may be further deterred by a chronic pain state arising from amputation of the beak *(Breward and Gentle, 1985; Gentle et al., 1997)* [20, 25].

The legislation on beak trimming in the poultry sector is stated in appendix 2.

Alternative options: management and genetic selection
Feather pecking and cannibalism are unnatural behavior patterns and are a sign of declined welfare. Since this behavior does not occur in wild chickens it is highly related to the ability to perform natural behavior. Poor management and rearing can cause severe problems in this department. For and foremost it is important to consider the ethological needs of laying hens:

- **Movement:** Dust bathing, running, fluttering and the ability to bask. Daylight stimulates activity.
- **Exploration:** A challenging and varied environment which can be explored. Foraging behavior must be encouraged with a proper substrate and access to a outdoor area.
- **Diet:** Water and food should be sufficiently available and offered in a variety of ways such as scattering of corn which lead the more foraging behavior.
- **Health:** The absence of injuries or illnesses.
- **Social:** The availability of conspecifics.
- **Thermoregulation:** A regular temperature and ventilation.
- **Self-care:** Sufficient room for comfort behavior.
- **Rest:** The availability if sufficient perches [10].

Low stocking density can ensure that the animals are not crowded. If the density is too high the animals are hindered in all aspects of their behavior. The design of housing is important; it should be clearly dived in multiple functional sections. This ensures that the chickens which are roosting don't get interrupted by the chickens which want to eat for example. The functional areas should be adapted to the stocking density. An example of adaption to a higher stocking density is the use of two bars in front of the nests so that animals can pass each other easily without unnecessary disturbance. Perches should be high or low enough to avoid pecking from below. These perches should preferably be rectangular, round perches can cause bone anomalies in the chest [10]. Furthermore the possibilities of dust bathing should be available. In this birds prefer sand as a substrate. Bathing areas are active areas and should not be too close to other inactive areas for laying or roosting [25, 37].
The lighting is also important, more light means more activity. Rest orientated areas such as the nest should be kept darker; in nests where hens can see each other this is very important. There it has to be dark enough so that the animals cannot detect each others cloaca which is prominent after a laying session, this relates strongly to the deadliest form of cannibalism. The lighting should be adjusted to the functional areas of the stable. Furthermore the lighting needs to be uniform and suitable for chickens. This means that high frequency lamps should be used. Other types of mainly TL lighting are not suitable because chickens can detect the flickering of the lights and this results in stress/agitation [15].
Other management issues can arise in the form of stress from fear for the keeper, insufficient feeding management or an unsuitable daily rhythm. The presence of an outdoor area and daylight has a positive effect on chicken behavior. However there must be noted that too much lighting difference between outdoor and indoor areas should be avoided. This can be done by putting up sunscreens at the entrances of the stable [10]. To lower the parasitic pressure chickens can be let out in a different pasture every couple of months, if the vacant pastures are then managed, the parasitic pressure will drop. For hygienic purposes the walking in of filth can be limited by

putting grids in front of the stable entrance. If tan suitable outdoor area is not possible a *wintergarten* can be considered, this is an area cold free range area with a solid floor. The advantage of this type of outdoor area is that there is less parasitic pressure on the flock. Birds which are allowed to go outside from an early age show less feather pecking, however the farmer should try to get at least 75% of his birds to go outside for an optimal effect [10, 25].
Research states more important points which should be avoided especially to prevent the occurrence of cannibalism. First of all avoid early unset of lay, research by *Potzsch et al. in 2001* and *Newberry et al. in 2002* showed a correlation between the onset of lay before 20 weeks. Secondly the nutritional requirements of the birds must be met, research by *Cain et al. in 1984* and *Cooke in 1992* revealed a positive correlation between deficiency in minerals, proteins, energy and the occurrence of cannibalism. *Wahstrom et al. in 1998* reported a correlation between cannibalism and low sodium levels in the bloodstream. Since blood is salt flavored in would explain a greater appetite for blood, same has been proven in pigs by *Fraser in 1987.* However these results are not completely supported by other research [30].

Rearing, breeding and genetic selection are also major key elements in preventing and controlling feather pecking and cannibalism. Rearing is of great importance in preventing feather pecking and cannibalism. Chicks should be raised on a substrate which allows them to scratch. This ground directed behavior prevents the occurrence of redirected behavior such as feather pecking. Furthermore the chicks need to be able to access all of the stable elements such as perches and food/water attributes, which helps them to acclimate to a stable design and learn to roost on perches. Furthermore the ability to go outside during the rearing period reduces the change of feather pecking. Genetic selections for traits such as longevity and egg production have been shown to decrease mortality and improve welfare. Furthermore heritability studies on feather pecking and cannibalism have proved that these traits are heritable. Feather pecking is estimated to be heritable with a range between 0.05 and 0.56 and cannibalism with a range of 0.65. These figures are relatively high which means that genetic selection on the absence of these behavior patterns can make a significant difference in preventing these behavior patterns [29,30]. A study from *Hierden et al.* in 2002 showed that chickens of a high feather pecking line showed more soft feather pecking and preening in at an age of 14 till 28 days and showed significantly more severe feather pecking at 41 days of age than a line which was selected against feather pecking (low pecking line). It also showed that animals of the low pecking line spent significantly more time feeding and walking than animals of a high pecking line. High feathers pecking animals also pecked more at the comb of conspecifics [23]. Furthermore research by *Bolhuis* in 2009 states the success of genetic selection based on group survival. Research by *Ellen et al.* in 2007 stated the success of a group selection method of individually housed laying hens which was partly based on the performance of their relatives kept in family groups. This research provided a significant difference in mortality, 10 % less mortality after one generation. Fourth generation low mortality animals were used in the holeboard task of this study originated from the animals used in the study by *Ellen et al*. as mentioned above. Still largely unknown is what traits of the low mortality lines have changed due to selection. Identification of behavioral and physiological characteristics and cognitive abilities may help discover that difference [12].

Cognition and emotional reactivity

The term cognition or *cognoscere* translates in to three things: to know, to conceptualize or to recognize. It relates to the processing of information using knowledge and preferences. Emotions in (farm) animals are a controversial subject, but how do we classify emotions? According to *Disiree et al.* in 2002, an emotion is an intense but short lived response to an event and is materialized in specific body changes. Furthermore according to *Dantzer* in 1988 an emotion consists of a posture or activity, followed by an autonomic response (visceral and endocrine) and a subjective component (emotional experiences or feeling). According to *Scherer* in 2001, cognitive psychology states that emotions emerge form an appraisal process of the situation according to certain criteria as relevance, implication, coping potential and normative significance. Research has proven that farm animals at least to extend use some of these criteria as well. Examples are suddenness, familiarity and pleasantness for the criteria of relevance [14].

Cognitive abilities

Cognitive abilities in chickens is not a highly researched topic but there are some studies which have explored cognition in chickens in the form of spatial cognition and object permanence, time perception and self control, context sensitivity and interference and finally social learning [31].

Spatial cognition is a very important very important for understanding en navigating thought an animal's environment. These aspects and the question 'how chickens perceive' their surroundings are relevant to today's large scalded husbandry systems [31].

Understanding how spatial cognition works has inspired multiple researchers who were working with chickens. Research shows that chicks are able to locate hidden objects without any direct sensory inputs. They simply navigate to the place where the object was last seen, which suggests that chickens are capable of the concept of object permanence. Object permanence means the appreciation of an object continuing existing even thought not notable by direct perception at that time. Not clear is yet if chicks have the ability to predict the next appearance of an object when it is moved from one side to the other in a chosen direction. Chicks in research by *Freire and Nicol* in 1999 failed to do so but it was uncertain if the cognitive abilities in these artificially raised chicks were developed to the fullest. The imprinting of an artificial object is not the same as imprinting on a hen, furthermore that chicks in the research where imprinted on a stationary object in opposed to a moving object. This could explain why the chicks would only move to the positional cue and not the directional [17]. Spatial learning develops with age and experience [31].

Experience in the appearance and disappearance of objects in the sensitive parts of a chicks life have a strongly positive effect in spatial learning abilities. Another study by *Freire and Nicol* in 2004 also strongly emphasizes that sensitive period in a chick's life, tests showed that chicks which were able to experience occlusion around day 11 develop improved egocentric orientation of it s surroundings [18]. Research by *Tomassi and Vallortiga* in 2001 showed that the spatial processing occurs in the right hippocampus. This was concluded after it was apparent that chicks are betters at spatial learning when there are using their left eye and therefore their right brain hemisphere [30].

Another interesting aspect of chicken cognition is time perception, this reveals the question: 'do they live only in the present or do they contemplate the past and future?'

This is connected to the animals' ability to foresee the consequences of chosen actions. In this point of view a study has been done to see if chickens would decide for example to forgo an immediate reward in order to increase the reward in the future. This is also connected to a self control aspect, would chickens as proved in rats, humans and pigeons be abele to exert self control. The choice to take the less valuable reward after a short waiting time or the more valuable reward connected to a longer waiting time reveals that. Research by *Taylor et al.* in 2002 showed that chickens where indeed able to distinguish differences in waiting time. A study by *Abeyesinghe et al* in 2004 demonstrated that chickens where also able, in addition to predicting waiting time, to control their impulses to wait for the more valuable reward. The study proved that hens are cognitively capable of understanding the later consequences of food choices; it also proved that hens can perceive the reward as less valuable in respect to the delay time [2]. So the longer waiting time was connected to a much more valuable reward, the difference had to be significant [31], a difference seen in other species as well.

Another aspect of cognition is context sensitivity and interference, these factors where studied with laying hens. One of the examples that show chickens have context sensitivity is a study by *Moffatt and Hogan* in 1992, in which hens showed to give more intense and longer food calls in relation to the quality of the food items. Furthermore research by *Wauters et al.* in 1999 shows that a hen will vocalize longer and more when chicks are visible but physically separated. An interesting study on the ability of interference was performed by *Nicol and Pope* in 1996. This study shows the capability of hens to interfere when their chicks are exhibiting incorrect behavior in relation to what the hen has learned to be correct behavior [31].

A subject which connects to the latter is social learning; social learning means the ability the acquire knowledge or skills by observation of or interaction with a conspecific. In chickens this plays a major role in food preference and in the acquisition of new skills or behavior patterns [31].

Hens assist their chicks in learning to identify good food items. A study by *Gajdon* in 2001 showed that chicks learn food preference from their mother and maintain these preferences even when the hen is no longer present. Another study by *McQuoid and Galef* in 1992 and 1994 showed that chickens can show preference to a food source if they have observed conspecifics show a positive reaction to that food source earlier. Research by *Sherwin et al.* in 2002 showed that social learning may also be important in overcoming fear of objects, shapes, colors which signal things that would be avoided in the wild [31]. Social learning is also capable to help individuals to acquire new behavioral patterns and such for example accept novel food items. Social learning can be dependent on maturity, a study by *Sherwin et al*. in 2001 showed that social learning in respect to avoid unpalatable food sources does not always occur. The hens in this study did not avoid the unpalatable substances after have been given clear signs by a demonstrator. Social learning may become less important with maturity [36].

The acquisition of new behavior and skills is not only influenced by conspecifics demonstrating it but also by the relationship between the observer and the demonstrator. This was shown in a study by *Nicol and Pope* in 1994. This study let the observers see a task being performed by a dominant member, a subordinate member and unfamiliar birds. Most of the successful behavior came from the chickens which had observed the dominant demonstrators. Assumed was that this might be because of the superior confidence, body posture and striking appearance of the dominant animals. However this was not what was concluded in a later study provided by *Nicol and Pope* in 1999 with trained roosters as demonstrators [31]. Rooster are well respected, are dominant over the hens and have a striking appearance, however this did not lead to a better result in social learning. Researchers speculate that dominant figures may get more attention from the observers because they are more assertive and have shown success in the past. This theory is still not completely proven since another study by *Sherwin et al.* in 2002 showed that although birds pay selective attention to others which have been successful in foraging they don't necessarily pay more attention to these individuals in a differing foraging context. A final speculation is revealed as to why dominate hens are more effective demonstrators and dominant roosters are not, it is possible that because of the increased interaction from hen to hen in relation to rooster to hen, the subordinates pay more attention the dominant hen to avoid showing disobedience [31]. Furthermore research by Candace et al. in 2006 showed no difference in social dominance in relation to learning ability [13].

Neurobiology

Learning in general is a result of the gathering of information/experience which is then processed to different brain areas in terms of visual, olfactory, auditory and tactile inputs [21].

The processing of memory occurs in a sequence of physiologically determined stages and biochemical reactions in different locations inside the brain.

Neurotranmitters play a key role in memory and learning. There are two types of neurotranmitters in the brain. The classical neurotranmitters are involved with the computational aspect of the brain such as thinking, sensing and movement. The modulatory neurotransmitters are involved in the regulatory aspects of the brain in relation to behavior such as sleep cycle, mood stages and the flexibility in strength of synaptic connection involved in learning and memory [21].

The so-called *modulatory aminergic neurotransmitters* of the brain are involved in practically all important physiological systems within the brain and as mentioned above also play an important role in memory and learning and determining what aspects are remembered. The neuromodulatory transmitters are manufactured in the Central Nervous System or CNS. There are not many of these transmitters in present in the CNS but the extensive dendritic and axonal extensions reach trough almost all parts of the CNS [21].

Although excitatory and inhibitory neurotransmitters as glutamate and GABA are mainly responsible for the transfer of information and the communication between nerve cells, the modulation of synaptic activity by neurotransmitters as Noradrenaline regulates whether information in short term or labile memory is stored into permanent memory. Noradrenaline is a neurotransmitter which release is controlled by the LoC, plays an important part is aspects such as attention, arousal, anxiety, mood, cognition, reinforcement and memory consolidation. This transmitter has alternating effects in different parts of the brain; these effects can vary from consolidating synaptic connections in a brain area to acting as a global coordinator of memory consolidation. The regions of the brain in which Noradrenaline plays an important role are the cortex, the hippocampus and the basal ganglia [21].

Research by Gibbs in 2007 shows that memory processing over the first 60 minutes in relation to a discriminative task, is divided in to three stages, mainly short-term, intermediate and long-term memory. The transition between these stages was at 15 minutes and 55 minutes. The duration is specific for a task which is presented.

Gibbs states: *''the physiological representation of the information received by the brain travels to many sensory areas at the time of input, it is the role of the modulatory neurotransmitters to determine of memory proceeds in to the next stage''*. As mentioned above Noradrenergic input which is regulated from the *locus coeruleus* shows to control memory processing at two stages after training mainly during the acquisition (0 till 2.5 min after training) and consolidation (25 till 30 min after training). There is a difference between acquiring of information (working memory) and the processing of information which consolidates the memory form 30 minutes past training. Weakly reinforced memory lasts only up to 30 minutes after training. The research of Gibbs as explained above merges recent knowledge in to working and the origin of memory during specific tasks and the important role of modulatory neurotransmitters [21]. Finally also environmental factors in sensitive period of life may influence the learning ability as Krause et al. showed that chickens which grew up in an enriched environment perform better in certain tasks than others [27].

Memory testing apparatus

The holeboard is a well known apparatus for testing the working of memory and learning in a special discrimination task. The classical holeboard is an open field with 16 holes in the floor (see figure 2). More than a decade after its original introduction, the apparatus was used to access spatial learning and memory in rats.

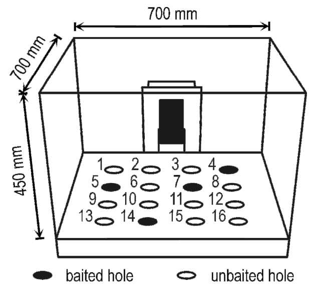

Figure 2: Classical Holeboard [38].

Today other variants of the holeboards are used including holeboard with no actual holes in the floor but with bowls or cups. This test gives access to evaluating the spatial learning and memory (working and reference memory) in a variety of species such as rats, pigs and fowl [38].

Research questions

This study consists of two parts, both investigating interventions in feather pecking behavior. The main research question addressed is: What are the cognitive effects of common interventions to prevent feather pecking?

In the first part of the study, working and reference memory in a low mortality and a control selection line are evaluated in a holeboard apparatus. The research questions addressed in part 1 are:

- How do chickens perform in a task which tests their short term or working memory as well as their long term or reference memory?
- Genetic selection is one method to reduce one of the major problems in the sector; does this influence the cognitive abilities of the chickens?

Another method of reducing the feather pecking problem is the beak trimming method. This study researches the effect of beak trimming in relation to food manipulation, social behavior and fearfulness.

In part 2, we address the following research questions:

- What are the cognitive capabilities of chickens in relation to food preference, social behavior and fearfulness?
- What effect does beak trimming have on food preference, social behavior and fearfulness?

Materials and Methods

This chapter summarizes the resources needed and the methods applied for this study. This study was dived in two separate parts. In the first part, animals from a low mortality line and a control line were tested in a holeboard task. In part two, the behaviors of beak trimmed animals were compared with that of animals with intact beaks in a T-maze task, a preference task for measuring food preference, an open field test and a Voluntary approach and Human recognition test. The protocol for these tests was approved by the Utrecht University Board (**D**ier**e**xperimenten **c**ommissie; DEC) for studies in experimental animals, following the Dutch law on animal experiments. This complies with the ETS123 (Council of Europe 1985) and the 86/609/EEC Directive.

Part One - Methods

Population of test animals

Two lines of laying hens where tested in the holeboard test. The first strain is a low mortality line derived from White Leghorns at Wageningen University (see figure 3). The other strain is a control strain of White Leghorns. Thirty fertilized eggs from the low mortality line and 30 fertilized eggs from the control line were obtained from ISA B.V. The low mortality animals were taken from the 4th generation of animals which were bred according to a sib selection scheme [16, 35]. The eggs were incubated and hatched at the Faculty of Veterinary Medicine of Utrecht University and after hatching they were moved to the Utrecht University farm animal facility 'De Tolakker'. The birds were vaccinated against New Castles Disease, Avian influenza and were continually free of disease.

All birds were first habituated and handled from week 1 till 3 of age. From week 3 till week 9 they were trained in the holeboard test. The chickens were weighed three times a week in the afternoon after the behavioral tests.

Figure 3: White Leghorn of the low mortality laying hen strain

Housing conditions

The chickens were kept in groups of 10. In total 20 chickens were housed in the testing facility. One of the chicks of the control group died soon after hatching and one of the low mortality animals turned out to be a rooster. Eventually 18 animals participated in the tests. These groups were housed separately in a pen 1.12m x 1.20m x 0.70m (w x l x h see figure 4). The floor was covered with wood chips to encourage scratching. A perch was available to roost on. Water and food were available ad libitum. A 400 Watt overhead heat provided warmth.

Figure 4: Housing conditions of the chickens during the holeboard experiment.

Welfare notification

During the testing period, the general health and weight of the chickens was monitored. All handling was reported in a welfare journal. This means that the birds were checked every day and were scored on health and weight three times a week. This welfare journal was kept using the DEC standards of judging general welfare. The following scores were awarded:

A) Appearance: 0 = normal, 1= unclean plumage, 2= edema and swelling of the head, eyelid or shank, 3= abnormal body posture

B) Behavior: 0=normal, 1= small changes, 2= decreased activity, 3= immobility.

C) Body weight: 0= normal, 1= lean (till 10% weight loss) 2= extremely lean (till 20% weight loss), 3= emaciated (more than 20% weight loss).

Holeboard

The holeboard apparatus was an open field measuring 2, 44 by 2, 44 meters. This apparatus contained 9 chalk circles with a diameter of 50 cm. A plywood surface (measuring 19 by 19 centimeters) with in its center a bright red cup (diameter 7 cm and height 5 cm) was positioned within each chalk circle. The distance between the cups was 70 cm. The animals were habituated to the red cups for one week and learned to associate the cups with a reward [11, 35] (see figure 5). The rewards in this test are mealworms obtained form a pet shop, in total 3 cups are rewarded for each animal. The bottom surface of the holeboard was made up of mats. The area was swept clean every day. The chalk circles were redrawn with a chalk and a piece of string of the right length in relation to the diameter of the circles, when necessary.

The animals were habituated in the holeboard for one week, in which they were placed in the holeboard for 5 minutes. Starting in week 4 the animals where individually tested in the apparatus (see figure 6).

During this test the animals learned, 1) which cups contain the rewards and 2) which cups they already visited. This test enables measuring the reference and working memory. Reference memory also known as long term memory relates to the learning curve the chickens show in learning which cups are rewarded for them from day to day. Reference memory was determined by dividing the number of visits to rewarded cups by the total number of visits to all cups. Working or short term memory relates to the visiting and revisiting of the baited cups within a single session. If the chickens only visited each baited cup once before completing the test, it had a perfect working memory. Working memory was determined by dividing the number of mealworms eaten by the total number of visits to baited cups.

Each session lasted a maximum of 5 minutes. Mealworms are places in three out of nine cups, the 'rewarded' cups. The test was terminated when the animals found the three mealworms or when 5 minutes have passed, whichever event occurred first. During each trial the latency to visit the first cup, number of cup visits, time spend in the test and number of mealworms eaten (figure 7) were registered. Two trials per day were run, separated by a 1-minute inter-trial interval, during which the animal was removed from the testing area to reset the short term memory. Later, one trail was first completed for all the animals before the second was performed. This was done to increase the motivation. Animals were more motivated when the sessions were separated by one hour than when they were separated by one minute pause. The chickens were tested randomly every day using the order determined by the statistical program SAS.

The cups which were rewarded are different for the animals of both groups. Animals L1/C1, L2/C2 and L3/C3 had to find their rewards in cups 2, 5 and 7. Animals L4/C4 and L5/C5 had to find their rewards in cups 1, 5 and 6. Furthermore animals L6/C6, L7/C7 and L8/C8 had to find their rewards in cups 3, 5 and 8. Finally animals L9/C9 and L10 had to find their rewards in cups 4, 5 and 9.

When the animals did not respond as well as expected a cued form of the holeboard instated for one week. This was done to highlight the pattern of rewarded cups to the animals with little keychain bicycle lights.

The animals were continually tested until they had sufficiently learned the task of finding the mealworm in the correct cups and ignoring the empty cups. At this point, the animals were tested during a reversal. This means that the normally rewarded cups are now kept empty and three different cups were rewarded. For animals 1,2 and 3 cups 1,4 and 6 were now rewarded, for animals 4 and 5 cups 2,3 and 7, for animals 6,7,8 cups 4,6,9 and finally for animals 9 and 10 cups 1,7 and 8 were rewarded.

This provides insight in flexibility of the learning ability. All sessions were recorded via a video camera above the apparatus. The learning curve, effects of changes in the testing conditions, and differences between the groups were analyzed using the SAS GLM repeated measures or ANOVA repeated measures procedure , where a P-value of < 0.05 was considered to be a significant effect/difference and a P-value between 0.05 and 0.10 was considered to indicate marginal effect/differences.

Figure 5: Model to access spatial working and reference memory [37]

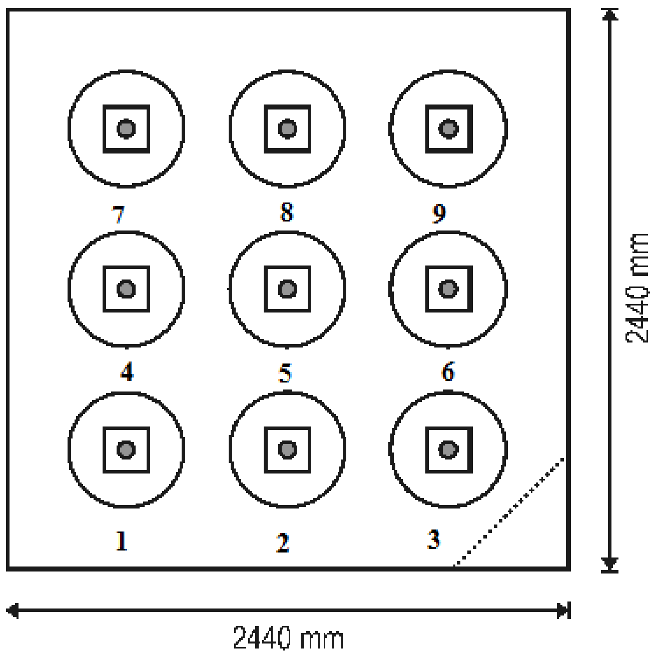

Figure 6: Chicken in the holeboard test.

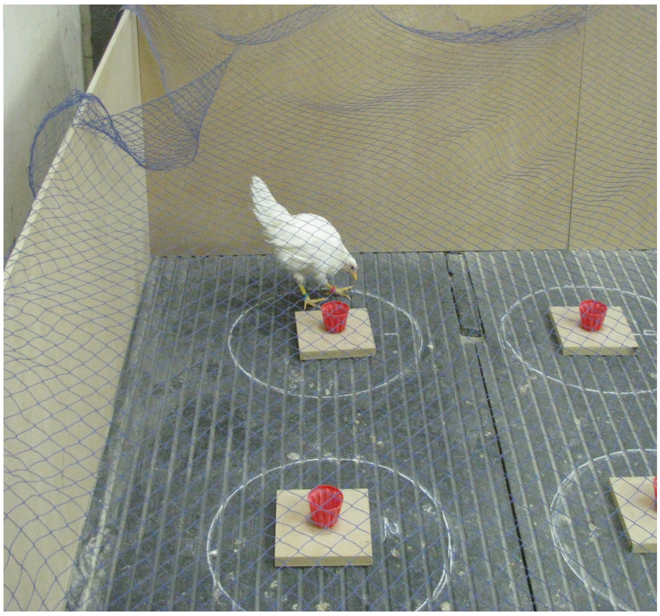

Figure 7: Measures of the holeboard test [11, 35]

Measure	Description
Visit of cup	When the hen is with both feet in the circle surrounding the cup.
Latency to visit first cup	Time elapsed between releasing the hen at the starting point and the first visit of a cup.
Trial duration	Time elapsed to find all baits, or if the chicken did not find all baits, the maximum trial duration.
Cups visited	Order and total number visited cups
Total number of visits	Sum of all visits and revisits
Frequency visits baited set	Total number of visits to baited set.
Frequency visits never baited set	Total number of visits to never baited set.
Frequency revisits baited set	Total number of revisits to the baited set.
Frequency revisits never baited set	Total number of revisits to the never baited set.
Number of mealworms eaten	Number of mealworms eaten at the end of a trial. Not to be confused with frequency of visits to baited set.
Working memory	Working memory is calculated as number of food rewarded cup visits divided by number of visits to baited set of cups (number of mealworms eaten) / (number of visits and revisits to baited set).
Reference memory	Reference memory is calculated as number of visits to the baited set of cups divided by total number of cup visits. (number of visits and revisits to the baited set) / (number of visits and revisits to all cups).

Part One – Materials

This part of the study required several materials, all of them are mentioned below.

- Bright cups
- Ply wood
- Crayon
- Camera
- Bird net
- Mealworms
- Registration protocol
- 18 chickens (9 low mortality and 9 control)
- Chicken pen
- Broom
- Hammer
- String
- Nails
- Wood chips (substrate)
- Stopwatch
- Laying hen food
- Drinking cups
- Heating lamp (40W)
- Perches

Part Two - Methods

Population of test animals

Chickens of the Silver Nick laying hen strain were used for this test. The animals were purchased at 1 day of age from Verbeek B.V location The Netherlands.
50% had an intact beak, whereas the other 50% had trimmed beaks. The chickens where trimmed with laser technique. Beak trimmed chickens and chickens with intact beaks were kept in separate groups. The chickens were weighted two to three times a week in the afternoon after the behavioral tests. The animals were given the opportunity to habituate to their new environment during 7 days. The animals were habituated to being handled for 5 days. The T-maze test was started on the 19^{th} day after arrival. This test was performed on 8 working days with one weekend in between. Habituation took 3 days and the testing was done on 5 successive days. Next the chickens were tested on the 26^{th} day in the open field test in on single session. Finally the preference test was performed for three days and the human approach test on the 30^{th} day after arrival for one day.

Housing conditions

These groups were housed separately with 10 animals per pen measuring 1.12m x 1.20m x 0.70m (w x l x h). The floor was covered with wood chips to encourage scratching. A perch was available to roost on. Water and food were available ad libitum. A 400 Watt overhead provided warmth.

Preference test
The preference test was performed in an open field measuring 1.22 x 1.22 x 0.74m (width x length x height) of Medium-density fiberboard (MDF). There were two different reward types. The amount of each reward eaten and the time spend in the vicinity of the rewards were measured. The rewards where live mealworms and dead mealworms. The animals were placed in the centre of the four quadrants. The latency to move in a quadrant, the time spent in each quadrant of the reward type and the latency and time of eating the reward was measured. The test lasted 10 minutes. A GLM procedure is used to analyze the data, a P value > 0, 05 was considered to be a statistical difference.

Sociability test
A T-maze according to Marin and Jones (2000) is used to measure the sociability (see figure 8). The T-maze consisted of a starting chamber, a corridor and two arms. At the end of one arm conspecifics (taken from the group to which the tested chicken belonged) were confined in a caged area. The other arm ended in a blind wall. Each chicken was placed in a starting position at the beginning of the maze. At the intersection the chickens was presented with two choices: to choose the arm where contact with other chickens was possible or to choose the empty arm.

For each tested animals conspecifics of her own group are placed in the right or left arm. This is determined forehand. Each bird was habituated to the maze for 5 minutes per day during a three consecutive days. The initial experiment started when the bird was caught gently and stress free and put in the starting chamber of the maze. After 30 seconds the separation was removed and the chick could leave the start box. During a single session that lasted 10 minutes the latency of entering the different compartments and the duration of time spend in each compartment were recorded. Immediately after the session birds were returned to their pen. The order in which the birds were tested was randomly determined using the procedure PLAN of the statistical software SAS.

Sociability is defined as an above chance level (50%) choice to enter the arm which allows social interaction with other members of the group. To test if the animals had a conditioned preference for the arm with conspecifics, a test without conspecifics was performed (conditioned place preference). The results were analyzed with the help of a T-test analysis and a GLM repeated measures or ANOVAs repeated measures procedure. Repeated measure was done with the repeated measures factor Days (day 1 to 4, because on day 5 no conspecifics were present). A P value > 0, 05 was considered to be a statistical difference.

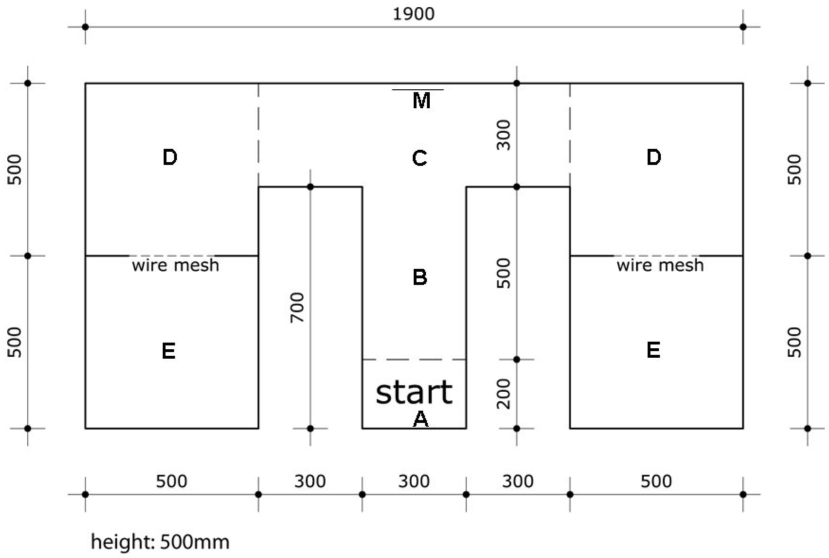

Fig. 3 T-maze outline. Starting chamber (A), corridor (B), perpendicular arm (C), compartment were companions were visible if present (D), compartments containing companions (E), mirror (M).

Figure 8: Design of the T-maze [11, 35].

Open field test

The open field test measuring 1.22 x 1.22 x 0.74m (width x length x height) of Medium-density fibreboard (MDF). The floor was divided equally in 5x5 squares (of 24 cm x 24 cm each) by white markings. The chicken was placed in the centre of the field and the latency to walk and vocalize was measured. Also the total number of sections walked, the amount of time spent walking and the frequency of defecating was measured. The chickens where tested individually during 10 minutes in the field test. A GLM or T-test procedure is used to analyze the data, a P value > 0, 05 was considered to be a statistical difference [11, 35].

Human Approach test

The human approach test was performed in the open field test as mentioned above. The chickens were tested individually and placed in one corner of the open field apparatus. The researcher stood in the other corner with a reward in one hand. The time it took to approach the researcher, the reward en the latency to consume the reward were measured. The test was performed twice on the same day. During the first test, animals were confronted with a familiar human. During the second test, animals were confronted with an unfamiliar human. A GLM repeated measures or ANOVA repeated measure procedure was used to analyze the data, a P value > 0, 05 was considered to be a statistical difference.

Part Two – Materials

- Ply wood plates (open field)
- Chickens (10 trimmed/ 10 non trimmed)
- Wood chips (substrate)
- Laying hen food
- Heating lamp (40W)
- Perches
- Chicken pen
- Camera
- T-maze structure made of ply wood plates
- Wire mesh
- Mealworms
- Earthworms
- Drill set
- Nails
- Hammer
- Stopwatch

Results

Genetic selection lines – Holeboard task

Working memory
16 chickens where evaluated in the holeboard test and where scored on their working memory. The methods used for these results are summarized in Material and Methods. Figure 9 illustrates the low mortality line as black or LML and the control line as white or CL.

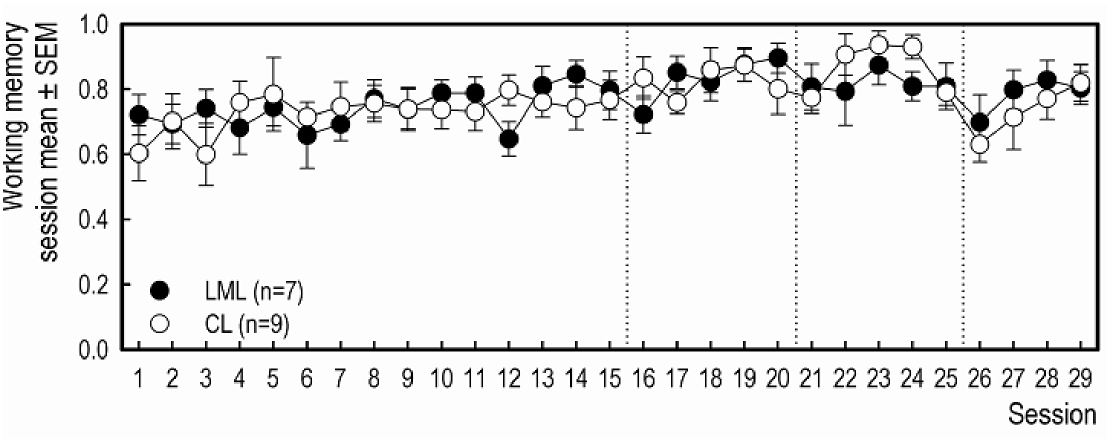

Figure 9: Working Memory of the low mortality and control animals

<u>The first 15 sessions:</u>
Group effect: $F_{1,14}$ = 010, P= 0,7584 which means that no significant difference between the average performance of the two lines within the first 15 sessions have been detected.
Session effect during the first 15 session indicates whether or not there is a learning curve. Session effect: $F_{14,196}$ = 0,92, P= 0,5397, no session effect was detected during the first 15 sessions in relation to working memory.
Session by group interaction: $F_{14,196}$ = 0,80, P= 0,6638. There is no group interaction within the session effect during the first 15 sessions.
The Polynomial analysis indicates P= 0, 0046 within the first degree. P < 0,05 which means that statistically, despite the fact that no learning curve has been proven, the curve as shown in figure 8 is most similar to that of a linear formula
(Appendix 3, table 1a and 1b).

<u>Cued sessions:</u> The holeboard was cued with lights to emphasize the pattern of rewarded cups for all of the animals. The cued sessions involves session 16 till 20 (see figure 8).
Group effect: $F_{1,14}$ = 0,03, P = 0,8713, this indicates that no significant difference was found between the average performances of the two lines during the cued session of the holeboard in relation to their working memory.
Session effect: $F_{4,56}$ = 1,14, P= 0,3494, this indicates that no session effect or learning curve has been detected in the cued sessions in relation to their working memory.

Session by group interaction: $F_{4,56}$ = 1,54, P= 0,2035. This indicates that no difference within the session effect of the two lines has been detected within the cued session in relation to their working memory (Appendix 3, tables 1c and 1d).

Over training sessions: The overtraining sessions involves session 21 till 25.
Group effect: $F_{1,14}$= 0,58, P = 0,4605, this indicates that no difference has been found in average performance between the two lines within the overtraining sessions in relation to their working memory.
Session effect: $F_{4,56}$ = 1,67, P= 0,1689. No session effect has been detected in the overtraining sessions. This means that no learning curve was found within these sessions in relation to their working memory.
Session by group interaction: $F_{4,56}$ = 0,93, P = 0,4542. This indicates that no group interaction was found within the session effect of the overtraining sessions (Appendix 3, tables 1e and 1f).

Reversal sessions: the reversal involves session 26 till 29. Group effect: $F_{1,14}$ = 0,63, P = 0,4391. This indicates that no difference was found between the average performances of the two lines within the reversal sessions in relation to their working memory.
Session effect: $F_{3,42}$ = 2,38, P = 0,0835, this indicates a marginal difference within the reversal sessions. This means that there is a learning curve trend in relation to their working memory within the reversal.
Session by group interaction: $F_{3,42}$ = 0,26, P= 0,8550. This indicates that there is no group interaction within the sessions. The two lines do not differ within the session effect.
The polynomial analysis also indicates with P= 0, 0106 that the reversal session are most similar to a linear trend (Appendix 3, tables 1g and 1h).

Complete view of all the sessions: the complete view of results involve session 1 till 29. Group effect: $F_{1,14}$ = 0,00, P = 0,9734, this indicates that the two lines did not differ in average performance in all session in relation to their working memory.
Session effect: $F_{24,336}$ = 2,22, P = 0,0011. This indicates a significant difference within working memory performance in the sequence of session 1 till 25. This means that a learning curve exists in relation to working memory performance.
Session by group interaction: $F_{24,336}$ = 0,84, P = 0,6832. This indicates that there is no difference between the two groups within the session effect.
The polynomial analysis with P= 0, 0012 indicates that the sequence of all sessions is most similar to that of a linear trend. This indicates the existence of a learning curve in relation to the working memory of the two lines (Appendix 3, tables 1i and 1j).

Reference memory

16 chickens where evaluated in the holeboard test and where scored on their reference memory. The methods used for these results are summarized in Material and Methods. Figure 10 illustrates the low mortality line as black or LML and the control line as white or CL.

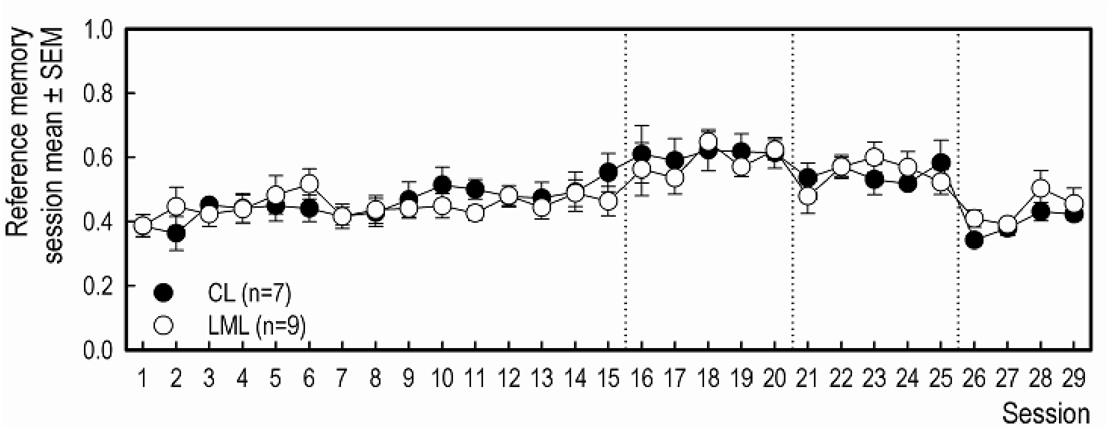

Figure 10: Reference memory of the low mortality and control animals

<u>The first 15 sessions:</u> The first 15 sessions show no between subjects difference, there is no group effect. Group effect: $F_{1,14}$ = 0,04, P = 0,8365, this means that there is no significant difference between the average performance of the two lines in relation to their reference memory.
Session effect: $F_{14,196}$ = 1, 63, P = 0, 0727, this indicates a marginal difference because 0, 05 < P > 0, 10. There is a learning curve trend in within the sessions in relation to the working memory of the two lines. Session by group interaction: $F_{14,196}$ = 0, 85, P = 0, 6100. This indicates that the session 'effect' does not differ between the two lines. The polynomial analysis shows with P = 0, 0231 that the first 15 sessions most resemble a linear trend (Appendix 3, tables 2a and 2b).

<u>Cued sessions:</u> the cued sessions involve session 16 till 20.
Group effect: $F_{1,14}$ = 0,11, P = 0,7420, this indicates that there is no difference between the average performance of the two lines in relation to their reference memory within these sessions.
Session effect: $F_{4, 56}$ = 0, 83, P = 0, 5102, this indicates that there is no significant session effect or learning curve present within these sessions in relation to reference memory.
Session by group interaction: $F_{4, 56}$ = 0, 36, P = 0, 8332. This indicates that the session 'effect' also does not differ between the two lines (Appendix 2c and 2d).

<u>Overtraining sessions:</u> the overtraining sessions are the session after the cues had been removes, session 21 till 25. Group effect: $F_{1,14}$ = 0,00, P = 0,9949, this indicates that there is no group effect or difference in the average performance of the two lines in relation to their reference memory during these sessions.
Session effect: $F_{4, 56}$ = 0, 81, P = 0, 5253, this means that no significant session effect or learning curve is present during these sessions. Session by group interaction: $F_{4, 56}$ = 1, 14, P = 0, 3483, this indicates that the session 'effect' does not differ between the two lines (Appendix 2e and 2f).

Reversal sessions: the reversal session involve session 26 till 29 in which the reward patterns are altered.
Group effect: $F_{1,14}$ = 4,41, P = 0,0543, this indicates a marginal difference since 0,05 < P > 0,10. There is a marginal difference in average performance between the two lines in relation to their reference memory during these sessions.
Session effect: $F_{3, 42}$ = 4, 21, P = 0, 0109, this indicates a significant session effect or (re)learning curve during these sessions.
Session by group interaction: $F_{3, 42}$ = 0, 44, P = 0, 7228, this indicates that this session effect does not differ between the two lines.
The polynomial analysis shows P = 0, 0286 that the reversal session most relate to that of a linear trend (Appendix 3, tables 2g and 2h).

Complete view of all the sessions: involves session 1 till 25.
Group effect: $F_{1,14}$ = 0,07, P = 0,8020. This indicates that there is no difference between the average performances of the two lines in relation to their reference memory in all the sessions.
Session effect: $F_{24, 336}$ = 5, 23, P = < 0, 0001, this indicates a strongly significant session effect or learning curve of the two lines in relation to their reference memory.
Session by group interaction: $F_{24, 336}$ = 0, 62, P = 0, 9206, this indicates that the session effect does not differ between the two lines.
The polynomial analysis shows P = < 0, 0001 that session 1 till 25 are most similar to that of a linear trend (Appendix 3, tables 2i and 2j).

Trial Duration

16 chickens where evaluated in the holeboard test and where scored on their trial duration to find all rewards. The methods used for these results are summarized in Material and Methods. Figure 11 illustrates the low mortality line as black or LML and the control line as white or CL.

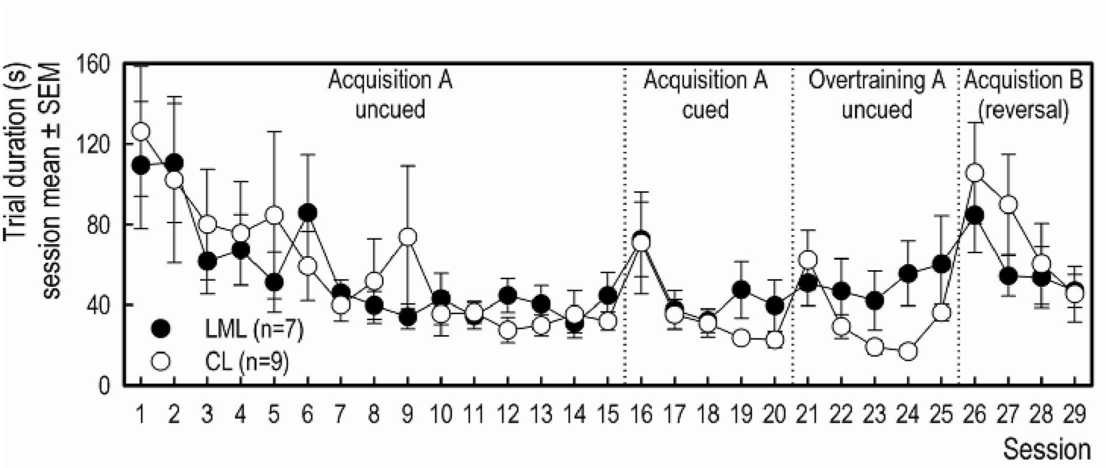

Figure 11: Trial duration of the low mortality and control animals

<u>The first 15 sessions:</u>

Group effect: $F_{1,14}$ = 0,03, P = 0,8567, this indicates that there is no significant difference in the average duration of the session between the two lines within the first 15 sessions.

Session effect: $F_{14,196}$ = 5, 15, P = <,0001, this indicates that there is session effect in relation to the duration of completing each session. The sessions take less time and less time to complete during this period.

Session by group interaction: $F_{14,196}$ = 0, 63, P = 0,8409, this indicates that this session effect does not differ between the two lines.

The polynomial analysis showed with P = 0, 0029 that the first 15 sessions show the most resemblance to that of a linear trend. Animals spent less time recovering al the rewards during the 15 sessions (Appendix 3, tables 3a and 3b).

<u>Cued sessions:</u>

Group effect: $F_{1, 14}$ = 0, 57, P = 0, 4617, this indicates that there is no difference between the average performances of the two lines in relation to the duration of the session within the cued period.

Session effect: $F_{4, 56}$ = 5, 14, P = 0, 0013, this indicates that there is a session effect in relation to the duration of completing each session within the cued period.

Session by group interaction: $F_{4, 56}$ = 0, 48, P = 0, 7468, this indicates that this session effect does not differ between the two lines.

The polynomial analysis shows with P = 0, 0352, that the results of this period show the most resemblance to that of a linear trend (Appendix 3, tables 3c and 3d).

<u>Overtraining sessions:</u>

Group effect: $F_{1,14}$ = 1,59, P = 0,2282. This indicates that there is no difference between the average performances of the two lines in relation to the duration of the session within the overtraining period.

Session effect: $F_{4,56}$ = 1,63, P = 0,1787. This indicates that there is no session effect in relation to the duration of completing each session within the overtraining period.
Session by group interaction: $F_{4, 56}$ = 0, 2860, P = 0, 2912. This indicates that this session effect does not differ between the two lines (Appendix 3, tables 3e and 3f).

Reversal sessions:
Group effect: $F_{1,14}$ = 1,10, P = 0,3119, this indicates that there is no difference between the average performances of the two lines in relation to the duration of the session within the reversal period.
Session effect: $F_{3, 42}$ = 3, 51, P = 0, 0233, this indicates a significant session effect in relation to the duration of the sessions in the reversal period. As the reversal is first introduced, the duration goes up and goes down again during the following sessions.
Session by group interaction: $F_{3,42}$ = 0,52, P = 0,6730 , this indicates that the session effect does not differ between the two lines.
The polynomial analysis shows with P = 0, 0094 that the results of the reversal sessions are most similar to that of a linear trend (Appendix 3, tables 3g and 3h).

Complete view of all the sessions:
Group effect: $F_{1, 14}$ = 0, 11, P = 0, 7475, this indicates that there is no significant difference in the average performance of the two lines in relation to the duration of the all sessions.
Session effect: $F_{23, 336}$ = 4, 22, P = <, 0001, this indicates that there is a significant session effect present in relation to the duration of all the sessions. The sessions take less time to complete each session.
Session by group interaction: $F_{23, 336}$ = 0, 69, P = 0, 8615, this indicates that there is no difference in sessions effect between the two lines (Appendix 3, tables 3i and 3j).

Effect of transference situations on working memory

Switch from non-cued to cued (task A)*
*Comparing the last two sessions before the switch and first two sessions after the switch.

Group effect: $F_{1,14}$ = 0,33, P = 0,5768, this indicated that there is no significant difference in the average performance of the groups in relation to working memory.
Block effect: $F_{1,14}$ = 0,01, P = 0,9312, this indicates that there is no significant block effect in switching from non-cued to cued in relation to working memory.
Block by group interaction: $F_{1, 14}$ = 0, 77, P = 0, 3949, this indicates that there is no significant difference between the performance of the two group within the block effect in relation to working memory (Appendix 3, table 4a and 4b).

Switch from cued to overtraining (task A)
*Comparing the last two sessions before the switch and first two sessions after the switch.

Group effect: $F_{1,14}$ = 0,00, P = 0,9463, this indicates that there is no significant difference in the average performance of the two groups in relation to working memory.
Block effect: $F_{1,14}$ = 0,52, P = 0,4840, this indicates that there is no significant block effect in switching from cued to non-cued overtraining sessions in relation to working memory.

Block by group interaction: $F_{1,14}$ = 0,59, P = 0,4549, this indicates that there is no significant difference between the two group within the block effect in relation to working memory (Appendix 3, tables 4c and 4d).

Switch from task A to task B (reversal)
*Comparing the last two sessions before the switch and first two sessions after the switch.

Group effect: $F_{1,14}$ = 0,05, P = 0,8299, this indicates that there is no significant difference between the average performance of the two groups switching from overtraining to reversal in relation to working memory performance.
Block effect: $F_{1,14}$ = 7,33, P = 0,0170, this indicates that there is a significant difference in switching from task A to task B in relation to working memory. The performances are lower at the beginning of the reversal than before.
Block by group interaction: $F_{1,14}$ = 1,94, P = 0,1859, this indicates that there is no significant difference between the two groups in the block effect in switching from task A till task B (Appendix 3, tables 4e and 4f).

Effect of transference situations on reference memory

Switch from non-cued to cued (task A)
*Comparing the last two sessions before the switch and first two sessions after the switch.

Group effect: $F_{1,14}$ = 0,37, P = 0,5552, this indicates that there is no significant difference between the average performance of the two groups switching from non cued to cued in relation to reference memory performance.
Block effect: $F_{1,14}$ = 4,15, P = 0,0609, this indicates a marginal block effect in switching from non-cued to cued in relation to reference memory performance in relation to reference memory performance. The results do slightly increase compared with the non cued situation.
Block by group interaction: F 1,14 = 0,00, P = 0,9593, this indicates that there is no difference between the two groups within the block effect when switching from non cued to cued in relation to reference memory performance (Appendix 3, tables 5a and 5b).

Switch from cued to overtraining (task A)
*Comparing the last two sessions before the switch and first two sessions after the switch.

Group effect: $F_{1,14}$ = 0,36, P = 0,5568, this indicates that there is no significant difference between the average performance of the two groups switching from cued to overtraining in relation to reference memory performance.
Block effect: $F_{1,14}$ = 3,56, P = 0,0800, this indicates that there is a marginal block effect in switching from cued to overtraining in relation to reference memory performance. The results slightly decrease when the cues are removed.
Block by group interaction: $F_{1,14}$ = 0,01, P = 0,9049, this indicates that there is no significant difference between the two groups within the block effect in relation to reference memory performance (Appendix 3, tables 5c and 5d).

Switch from task A to task B (reversal)
*Comparing the last two sessions before the switch and first two sessions after the switch.

Group effect: $F_{1,14}$ = 0,46, P = 0,5077, this indicates that there is no significant difference between the average performance of the two groups switching from task A to B in relation to reference memory performance.
Block effect: $F_{1,14}$ = 24,52, P = 0,0002, this indicates a significant block effect in switching from task A to task B in relation to reference memory performance. The performances in reference memory drop significantly when the configuration of the rewards was changed.
Block by group interaction: $F_{1,14}$ = 0,44, P = 0,5198, this indicates that there is no significant difference between the two groups within the block effect in relation to reference memory performance (Appendix 3, tables 5e and 5f).

Effect of transference situations on trail duration

Switch from non-cued to cued (task A)
*Comparing the last two sessions before the switch and first two sessions after the switch.

Group effect: $F_{1,14}$ = 0,05, P = 0,8257, this indicates that there is no significant difference in the average performance of the two lines in relation to their response in switching to the cued sessions.
Block effect: $F_{1,14}$ = 4,55, P = 0,0511, there is a marginal block effect from switching to the cued task in relation to the duration of a session. First the duration goes up and than quickly down again in the first two cued sessions.
Block by group interaction: $F_{1,14}$ = 0,02, P = 0,9038, this indicates that the block effect does not differ between the two lines (Appendix 3, tables 6a and 6b).

Switch from cued to overtraining (task A)
*Comparing the last two sessions before the switch and first two sessions after the switch.

Group effect: $F_{1,14}$ = 0,72, P = 0,4093, this indicates that there is no difference in the average performance of the two lines when switching from cued to non cued in the overtraining period. Block effect: $F_{1,14}$ = 6,79, P = 0,0208, this indicates that there is a significant block effect when switching from the cued to the non cued sessions in relations to the duration in the overtraining period. First the duration goes up and than quickly down again in the first two overtraining sessions when the cues are removed again. Block by group interaction: $F_{1,14}$ = 2,65, P = 0,1258, this indicates that the block effect does not differ between the two lines (Appendix 3, tables 6c and 6d).

Switch from task A to task B (reversal)
*Comparing the last two sessions before the switch and first two sessions after the switch.

Group effect: $F_{1,14}$ = 0,01, P = 0,9256, this indicates that there is no significant difference in the average performance of the two lines in switching from test A to B in relation to the trail duration.
Block effect: $F_{1,14}$ = 10,17, P = 0,0066, this indicates that there is a significant block effect in relation to the trail duration in the reversal period. The trail duration goes up when the new task is introduced in relation to the last two session of the overtraining period. Block effect by group interaction: $F_{1,14}$ = 5,26, P = 0,0378, this indicates that the block effect does differ between the two lines (Appendix 3, tables 6e and 6f).

Beak trimmed line – T-maze test

Habituation
During the 3 day habituation prior to the testing period, the amount of stress calls were measured. The chick was placed in the apparatus for a period of 10 minutes (Appendix 3, table 7a and 7b).

Comparing performance of groups per day (t-test)

Latency to visit the corridor (compartment B)
Day 5 showed no significant difference in the performance of the two groups (Appendix 3, table 8a till 8k).

Time spent in compartment B (corridor):
Day 5 showed no significant difference in the performance of the two groups (Appendix 3, table 9a till 9k).

Latency to visit compartment C
Day 5 showed no significant difference in the performance of the two groups (Appendix 3, table 12a till 12k).

Time spent in compartment C:
Day 5 showed no significant difference in the performance of the two groups (Appendix 3, table 13a till 13k).

Latency to visit compartment D with conspecifics
Day 5 showed no significant difference in the performance of the two groups (Appendix 3, table 16a till 16k).

Time spent in compartment D with conspecifics:
Day 5 showed no significant difference in the performance of the two groups (Appendix 3, table 17a till 17k).

Latency to visit compartment D without conspecifics
Day 5 showed no significant difference in the performance of the two groups (Appendix 3, table 20a till 20k).

Time spent in compartment D without conspecifics:
Day 5 showed no significant difference in the performance of the two groups (Appendix 3, table 21a till 21k).

Comparing average performance and development of the 5 testing days between groups (GLM - repeated measures)

<u>Latency to visit the corridor (compartment B) (figure 12):</u>
Group effect (between subject analysis): $F_{1,18}$ = 0,07, P = 0,7935, this indicates that there is no significant difference in the average performance of the two groups.
Session effect (within subject analysis): $F_{3, 54}$ = 4, 37, P = 0, 0079, this indicates a session effect relating to the latency of first visiting compartment B.
Session by group interaction: $F_{3,54}$ = 2,52, P = 0,0673, this indicates that the session effect marginally differs between the two groups (Appendix 3, table 10a and 10b).

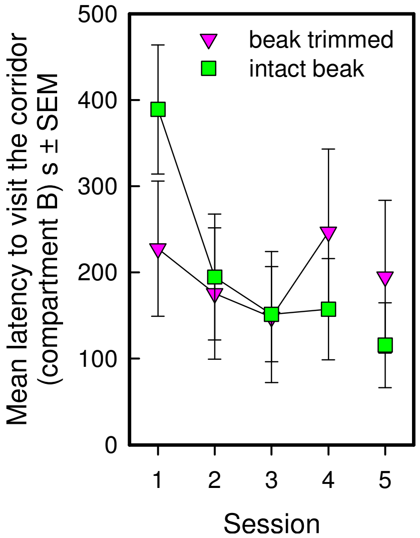

Figure 12: mean latency to compartment B

<u>Time spent in compartment B (corridor) (figure 13):</u>
Group effect (between subject analysis): $F_{1,18}$ = 0,31, P = 0,5819, this indicates that there is no difference in the average performance of the two groups.
Session effect (within subject analysis): $F_{3,54}$ = 2,27, P = 0,0908, this indicates that there is a marginal session effect present 0, 05 > P < 0, 10 in the time spent in compartment B.
Session by group interaction: $F_{3,54}$ = 0,49, P = 0,6941, this indicates that session effect does not differ between the two groups (Appendix 3, table 11a and 11b).

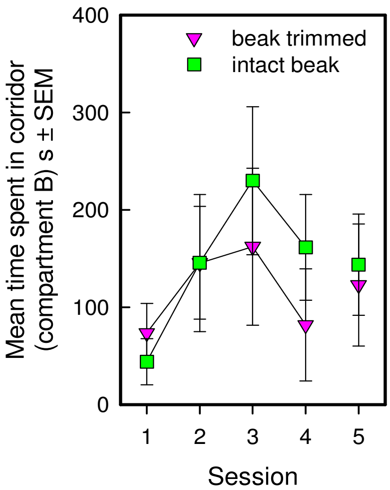

Figure 13: mean time spent in compartment B

Latency to visit compartment C:
Group effect (between subject analysis): $F_{1,18}$ = 0,39, P = 0,5381, this indicates that there is no significant difference in the average performance of the two groups. Session effect (within subject analysis): $F_{3,54}$ = 0,85, P = 0,4719, this indicates that there is no significant session effect present.
Session by group interaction: $F_{3,54}$ = 1,83, P = 0,1534 ,this indicates that session 'effect' does not differ between the two groups (Appendix 3, table 14a and 14b).

Time spent in compartment C:
Group effect (between subject analysis): $F_{1,18}$ = 2,08, P = 0,1667, this indicates that there is no significant difference in the average performance of the two groups. Session effect (within subject analysis): $F_{3,54}$ = 0,29, P = 0,8320, this indicates that there is no significant session effect present.
Session by group interaction: $F_{3,54}$ = 0,36, P = 0,7786. This indicates that session 'effect' does not differ between the two groups (Appendix 3, table 15a and 15b).

Latency to visit compartment D with conspecifics:
Group effect (between subject analysis): $F_{1,18}$ = 0,01, P = 0,9048., this indicates that there is no significant difference in the average performance of the two groups. Session effect (within subject analysis): $F_{3,54}$ = 1,36, P = 0,2642, this indicates that there is no significant session effect present.
Session by group interaction: $F_{3,54}$ = 1,08, P = 0,3650, this indicates that session 'effect' does not differ between the two groups (Appendix 3, table 18a and 18b).

Time spent in compartment D with conspecifics:
Group effect (between subject analysis): $F_{1,18}$ = 0,77, P = 0,3910, this indicates that there is no significant difference in the average performance of the two groups. Session effect (within subject analysis): $F_{3,54}$ = 1,03, P = 0,3866, this indicates that there is no significant session effect present.
Session by group interaction: $F_{3,54}$ = 0,65, P = 0,5874. This indicates that session 'effect' does not differ between the two groups (Appendix 3, table 19a and 19b).

Latency to visit compartment D without conspecifics:
Group effect (between subject analysis): $F_{1,18}$ = 0,44, P = 0,5150, this indicates that there is no significant difference in the average performance of the two groups. Session effect (within subject analysis): $F_{3, 54}$ = 0, 74, P = 0, 5311, this indicates that there is no significant session effect present.
Session by group interaction: $F_{3,54}$ = 1,26, P = 0,2984, this indicates that session 'effect' does not differ between the two groups (Appendix 3, table 22a and 22b).

Time spent in compartment D without conspecifics:
Group effect (between subject analysis): $F_{1,18}$ = 0,69, P = 0,4162, this indicates that there is no significant difference in the average performance of the two groups. Session effect (within subject analysis): $F_{3,54}$ = 0,88, P = 0,4574, this indicates that there is no significant session effect present.
Session by group interaction: $F_{3,54}$ = 1,12, P = 0,3490, this indicates that session 'effect' does not differ between the two groups (Appendix 3, table 23a and 23b).

Beak trimmed line – Open field test

Comparing performance of groups (t-test)

Means variances: (Appendix 3, table 24a and 24b)

Latency walking:
$t_{12,6}$ = 1.10, P = 0,2910, this indicates that there is no significant difference in the performance of the two groups (Appendix 3, table 24c and 24d). Group 1 with trimmed beaks showed an average latency of 27,700 seconds and group 2 with intact beaks showed an average latency of 17,400 seconds (Appendix 3, table 24a and 24b).

Number of squares walked in the inner circle:
t_{18} = - 0, 44, P = 0, 6656, this indicates that there is no significant difference in the performance of the two groups (Appendix 3, table 24e and 24f). Group 1 with trimmed beaks showed an average of 25 squares walked and group 2 with intact beaks showed an average of 29,9 squares (Appendix 3, table 24a and 24b).

Number of squares walked in the outer circle:
t_{18} = - 0,78, P = 0,4465, this indicates that there is no significant difference in the performance of the two groups (Appendix 3, table 24g and 24h). Group 1 with trimmed beaks showed an average of 27 and group 2 with intact beaks showed an average of 34, 8 squares walked (Appendix 3, table 24a and 24b).

Time spent walking:
t_{18} = -1,26, P = 0,2252, this indicates that there is no significant difference in the performance of the two groups (Appendix 3, table 24i and 24j). Group 1 with trimmed beaks showed an average time spend walking of 219,7 seconds and group 2 with intact beaks showed an average of 301,9 seconds (Appendix 3, table 24a and 24b).

Total squares walked:
t_{18} = - 0,65, P = 0,5237, this indicates that there is no significant difference in the performance of the two groups (Appendix 3, table 24k and 24l). Group 1 with trimmed beaks showed an average of 52 squares walked in total and group 2 with intact beaks showed an average of 64, 7 squares walked in total (Appendix 3, table 24a and 24b).

Number of stress calls:
t_{18} = 0,68, P = 0,5051, this indicates that there is no significant difference in the performance of the two groups (Appendix 3, table 24m and 24n). Group 1 with trimmed beaks showed an average of 182, 9 stress calls and group 2 with intact beaks showed an average of 153, 5 stress calls (Appendix 3, table 24a and 24b).

Beak trimmed line – Preference test

Comparing performance of groups per day (t-test)
* Day 1 had 1 item of every reward type; day 2 and 3 had 2 items of every reward type.

Reward A:

Latency to visit square A (live bait) (Appendix 3, table 25a):
The latency to visit the square with the live bait was measured in 3 consecutive days. Day 1 till 3 showed no significant difference in the performance of the two groups during each day (Appendix 3, table 25b till 25g).

Time spent eating reward A (live bait) (Appendix 3, table 27a):
The time spent eating reward A was measured in 3 consecutive days. Day 1 till 3 showed no significant difference in the performance of the two groups during each day (Appendix 3, table 27b till 27g).

Worms eaten reward A (live bait) (Appendix 3, table 29a):
The amount of worms eaten of reward A live bait was measured in 3 consecutive days. Day 1 till 3 showed no significant difference in the performance of the two groups during each day (Appendix 3, table 29b till 29g).

Pecking frequency toward eating A (Appendix 3, table 31a):
The times a chick pecked at reward A until it was eventually eaten was measured in 3 consecutive days. Day 1 till 3 showed no significant difference in the performance of the two groups (Appendix 3, table 31b till 31g).

Time spent near reward A (live bait) (Appendix 3, table 33a):
Day 1 till 3 showed no significant difference in performance of the two groups during each day (Appendix 3, table 33b till 33g).

Reward B:

Latency to visit square B (dead bait) (Appendix 3, table 35a):
The latency to visit the square with the dead bait was measured in 3 consecutive days. Day 1 till 3 showed no significant difference in the performance of the two groups during each day (Appendix 3, table 35b till 35g).

Time spent eating reward B (dead bait) (Appendix 3, table 37a):
The time spent eating reward B was measured in 3 consecutive days. Day 1 till 3 showed no significant difference in the performance of the two groups during each day (Appendix 3, table 37b till 37g).

Worms eaten reward B (dead bait) (Appendix 3, table 39a):
The amount of worms eaten of reward B dead bait was measured in 3 consecutive days. Day 1 till 3 showed no significant difference in the performance of the two groups during each day (Appendix 3, table 39b till 39g).

Pecking frequency toward eating B (dead bait) (Appendix 3, table 41a):
The times a chick pecked at reward B until it was eventually eaten was measured in 3 consecutive days. Day 1 till 3 showed no significant difference in the performance of the two groups (Appendix 3, table 41b till 41g).

Time spent near reward B (dead bait) (Appendix 3, table 43a):
Day 1 and 2 showed no significant difference in the performance of the two groups during each day.
Day 3: t_{18} = -1, 96, P = 0, 0659. This indicates a marginal difference in the performance of the two groups. Group 2 spent more marginally more time in the vicinity of the reward B than group 1 (Appendix 3, table 43b till 43g).

Empty squares:

Time spent in empty squares (Appendix 3, table 45a):
The time spent in the empty squares was measured in 3 consecutive days. Day 1 till 3 showed no significant difference in the performance of the two groups during each day (Appendix 3, table 45b till 45g).

Comparing average performance and development of the 5 testing days between groups (GLM - repeated measures)

Reward A:

Latency to visit square A (live bait)**:**
Group effect: $F_{1,18}$ = 0,02, P = 0,885, this indicates that there is no significant difference in the average performance of the two groups in relation to latency of approaching square A.
Session effect: $F_{2,36}$ = 0,71, P = 0,4977, this indicates that no significant session effect is present in relation to the latency of approaching square A.
Session by group interaction: $F_{2,36}$ = 0,48, P = 0,6252, this indicates that the session 'effect' does not differ between the two groups (Appendix 3, table 26a and 26b).

Time spent eating reward A (live bait):
Group effect: $F_{1,18}$ = 3,05, P = 0,0978, this indicates a marginal difference in the average performance of the two groups in relation to the time they spent eating reward A. Group 1 (beak trimmed) spends more time eating reward A than group 2 (intact beaks).
Session effect: $F_{2,36}$ = 1,98, P = 0,1535, this indicates that no significant session effect is present in relation to the time spent eating reward A.
Session by group interaction: $F_{2,36}$ = 0,76, P = 0,4750, this indicates that the session 'effect' does not differ between the two groups (Appendix 3, table 28a and 28b).

Worms eaten of reward A (live bait):
Group effect: $F_{1,18}$ = 0,36, P = 0,5535, this indicates that the average performance of the two groups does no differ in relation to the amount of worms eaten of reward A.
Session effect: $F_{2,36}$ = 7,555, P = 0,0018, this indicates that there is a significant session effect in relation to the amount of worms eaten of reward A. Both groups consume more worms over the period of three days.
Session by group interaction: $F_{2,36}$ = 0,27, P = 0,7620, this indicates that the session effect does not differ between the two groups (Appendix 3, table 30a and 30b).

Pecking frequency toward eating A (live bait) (figure 14a):
Group effect: $F_{1,18}$ = 2,13, P = 0,1615, this indicates that there is no difference in the average performance of the two groups in relation to pecking frequency toward eating reward A.
Session effect: $F_{2,36}$ = 2,25, P = 0,1200, this indicates that there is no significant session effect present.
Session by group interaction: $F_{2,36}$ = 1,30, P = 0,2857, this indicates that the session 'effect' does no differ between the two groups (Appendix 3, table 32a and 32b).

Time spent near reward A (live bait):
Group effect: $F_{1,18}$ = 0,96, P = 0,3410, this indicates that there is no difference in the average performance of the two groups in relation to the amount of time spent in square A.
Session effect: $F_{2,36}$ = 1,49, P = 0,2395, this indicates that there is no significant session effect present.
Session by group interaction: $F_{2,36}$ = 0,12, P = 0,8548, this indicates that the session 'effect' does no differ between the two groups (Appendix 3, table 34a and 34b).

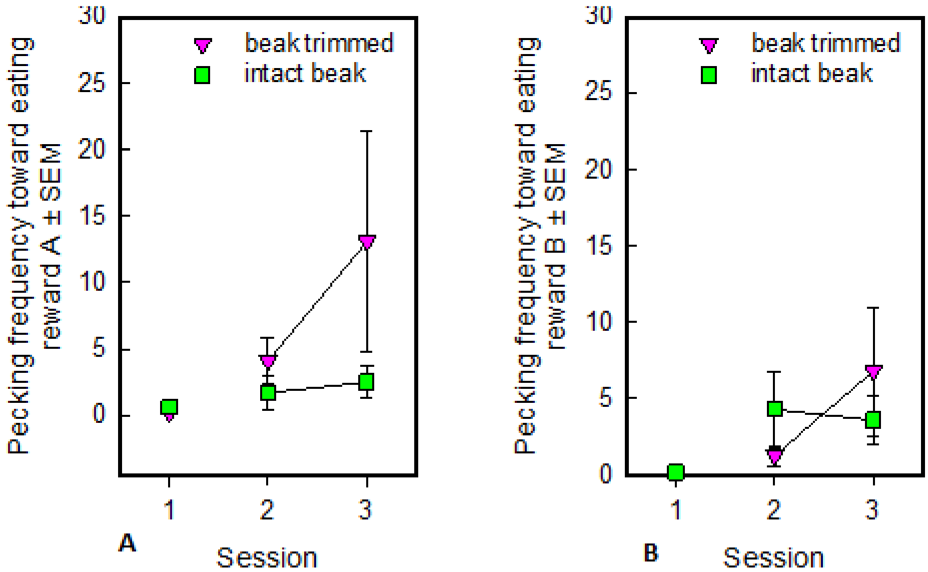

Figure 14a,b: Mean pecking frequency toward eating reward A and B

Reward B:

Latency to visit square B (dead bait):
Group effect: $F_{1,18} = 0,03$, P = 0,8665, this indicates that there is no difference in the average performance of the two groups in relation to latency of approaching square B.
Session effect: $F_{2,36} = 1,34$, P = 0,2749, this indicates that no significant session effect is present in relation to the latency of approaching square B.
Session by group interaction: $F_{2,36} = 0,46$, P = 0,331, this indicates that the session 'effect' does not differ between the two groups (Appendix 3, table 36a and 36b).

Time spent eating reward B (dead bait):
Group effect: $F_{1,18} = 0,53$, P = 0,4751, this indicates that there is no difference in the average performance of the two group in relation to the time spent eating reward B.
Session effect: $F_{2,36} = 3,40$, P = 0,0442, this indicates that there is a significant session effect in relation to the time spent eating reward B. Both groups spent more time eating reward B during the three testing days.
Session by group interaction: $F_{2,36} = 0,78$, P = 0,4844, this indicates that this session effect does not differ between the two groups (Appendix 3, table 38a and 38b.

Worms eaten reward B (dead bait):
Group effect: $F_{1,18} = 0,13$, P = 0,7187, this indicates that there is no difference in the average performance of the two groups in relation to the amount of worms eaten of reward B.
Session effect: $F_{2,36} = 7,81$, P = 0,015, this indicates that there is a significant session effect present in relation to the amount of worms eaten of reward A. Both groups consume more worms of reward B over the period of three days.
Session by group interaction: $F_{2,36} = 0,11$, P = 0,9005, this indicates that the session effect does not differ between the two groups (Appendix 3, table 40a and 40b).

Pecking frequency toward eating B (dead bait) (figure 14b):
Group effect: $F_{1,18} = 0,00$, P = 0,9711, this indicates that there is no difference in the average performance of the two groups in relation to pecking frequency toward eating reward B.
Session effect: $F_{2,36} = 3,00$, P = 0,0623, this indicates a marginal session effect in relation to the pecking frequency toward eating reward B. This does show that Group 1 pecks more from session 1 till 3 and group 2 pecks less from session 1 till 3.
Session by group interaction: $F_{2,36} = 1,15$, P = 0,3268, this indicates that the session effect does no differ between the two groups (Appendix 3, table 42a and 42b).

Time spent near reward B (dead bait):
Group effect: $F_{1,18} = 0,92$, P = 0,3511, this indicates that there is no difference in the average performance of the two groups in relation to the amount of time spent in square B.
Session effect: $F_{2,36} = 0,48$, P = 0,6215, this indicates that there is no significant session effect present.
Session by group interaction: $F_{2,36} = 1,26$, P = 0,2962, this indicates that the session 'effect' does no differ between the two groups (Appendix 3, table 44a and 44b).

Empty squares:

Time spent in empty squares:
Group effect: $F_{1,18} = 0{,}04$, $P = 0{,}8461$, this indicates that there is no difference between the average performance of the two groups in relation to the time they spent in the empty squares.
Session effect: $F_{2,36} = 7{,}59$, $P = 0{,}018$, this indicates that a significant session effect is present in relation to the time spent in the empty squares. Both groups spent less time in the empty squares of the 3 testing days.
Session by group interaction: $F_{2,36} = 1{,}43$, $P = 0{,}2536$, this indicates that the session effect does not differ between the two groups (Appendix 3, 46a and 46b).

Beak trimmed line – Voluntary Approach and Human Recognition Test

Researcher 1 is a familiar person to the chicks. Researcher 2 is an unfamiliar researcher to the chicks.

Latency to enter square with researcher with reward (figure 15a):
Group 1, the beak trimmed birds showed an average 25.9 seconds to approach the familiar researcher in the first session and 5,4 seconds to approach the unfamiliar researcher in the second session. Group 2, with intact beaks showed an average 42, 0 seconds to approach the familiar researcher in the first session and 18, 2 seconds to approach the unfamiliar researcher (Appendix 3, table 47a and 47b).

Group effect (between subject analysis): $F_{1,18}$ = 2,37, P = 0,1413. This indicates that there is no significant difference between the average performances of the two groups in relation to their latency to enter the square containing the researcher with the reward.
Session effect (within subject analysis): $F_{1,18}$ = 4,34, P = 0,0517, this indicates that there is a significant session effect within this variable. The time to enter the square with the researcher and the reward is significantly lower during the second sessions.
Session by group interaction: $F_{1,18}$ = 0,04, P = 0,8487, this indicates that the session effect does not differ between the two groups (Appendix 3, table 47c and 47d).

Time spent in square with researcher with reward (figure 15b):
Group 1, the beak trimmed birds showed an average 115,7 spent with the familiar researcher in the first session and 135,4 seconds spent with the unfamiliar researcher in the second session. Group 2, with intact beaks showed an average 81, 1 seconds spent with the familiar researcher in the first session and 125, 6 seconds spent with the the unfamiliar researcher in the second session (Appendix 3, table 47a and 47b).

Group effect (between subject analysis): $F_{1,18}$ = 1,15, P = 0,2985, this indicates that there is no significant difference in the average performance of the two groups in relation to the time spent in the square containing the researcher with reward.
Session effect (within subject analysis): $F_{1,18}$ = 21,31, P = 0,0002, this indicates that a significant session effect is present. The chicks spend relatively more time with the researcher in the second sessions.
Session by group interaction: $F_{1,18}$ = 3,18, P = 0,0915, this indicates that the session effect marginally differs between the two groups (Appendix 3, table 47e and 47f).

Latency approach of the reward (figure 15c):
Group 1, the beak trimmed birds showed an average 95.8 seconds to approach the reward offered by the familiar researcher in the first sessions and 23,2 seconds to approach reward in the second session. Group 2, with intact beaks showed an average 114.3 seconds to approach the reward offered by the familiar researcher in the first session and 33, 7 seconds in the second session (Appendix 3, table 47a and 47b).

Group effect (between subject analysis): $F_{1,18} = 0{,}08$, $P = 0{,}7788$. This indicates that there is no significant difference in the average performance of the two groups in relation to their latency to approach the reward.
Session effect (within subject analysis): $F_{1,18} = 6{,}33$, $P = 0{,}0216$. This indicates that a significant session effect is present. The chicks are significantly faster in approaching the reward during the second session.
Session by group interaction: $F_{1,18} = 0{,}02$, $P = 0{,}8969$. This indicates that the session effect does not differ between the two groups (Appendix 3, table 47g and 47h).

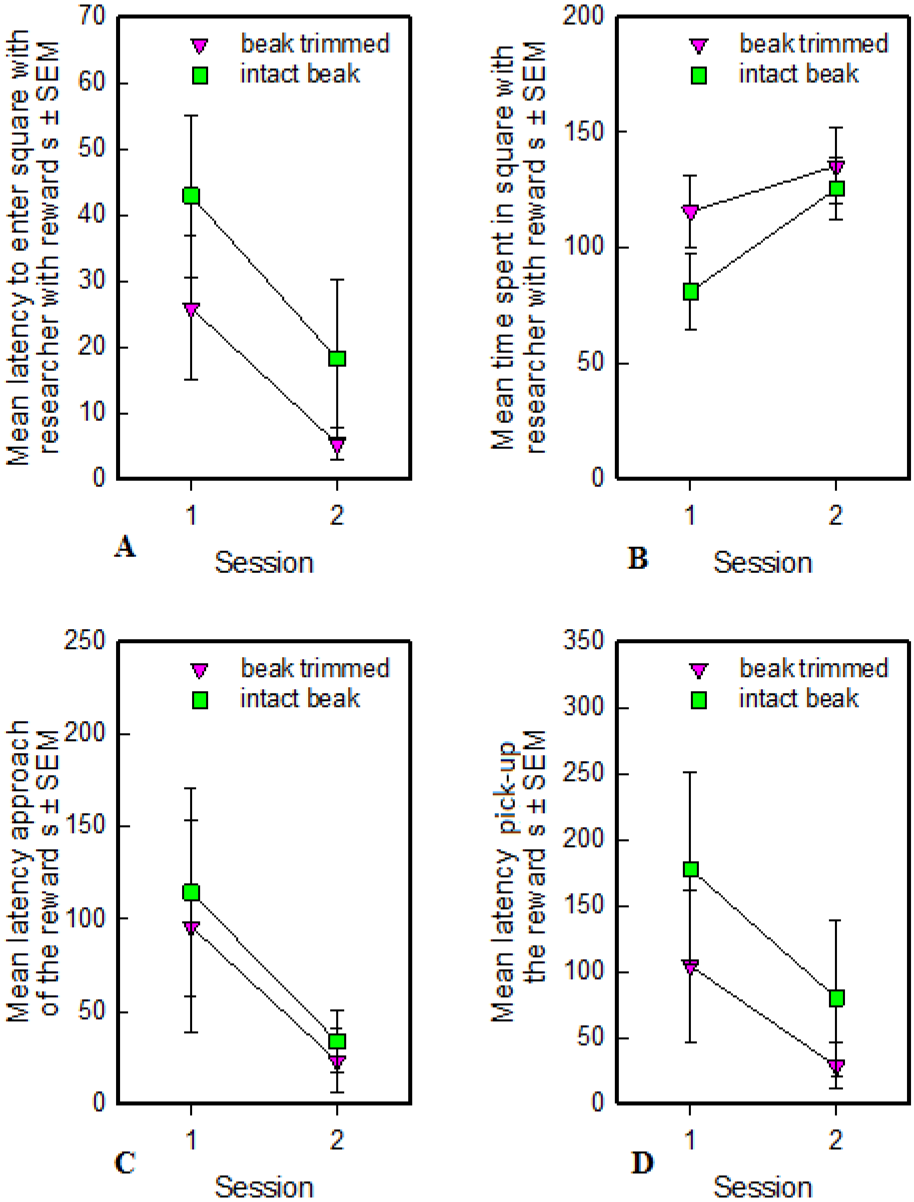

Figure 15a, b, and c, d (from left to right) : Results Voluntary approach and human recognition test

Latency pick-up reward (removed from the hand of the researcher) (figure 15d):
Group 1, beak trimmed birds showed an average 104 seconds remove the reward from the hand of the familiar in the first session and 28,6 seconds in the second session. Group 2, with intact beaks showed an average 177,8 seconds to remove the reward from the hand of the familiar researcher in the first session and 80,2 seconds in the second session (Appendix 3, table 47a and 47b).

Group effect (between subject analysis): $F_{1,18}$ = 0,75, P = 0,3986, this indicates that there is no significant difference in the average performance of the two groups in relation to their latency to pick-up the reward from the researchers hand (Appendix). Session effect (within subject analysis): $F_{1,18}$ = 7,87, P = 0,0117, this indicates that a significant session effect is present. The chicks are significantly faster at picking up the reward during the second session.
Session by group interaction: $F_{1,18}$ = 0,12, P = 0,7298, this indicates that the session effect does not differ between the two groups (Appendix 3, table 47i and 47j).

Weight gain during the testing period:

The chicks were weighed during the whole testing period from 7 days of age. The results of these measurements show that the beak trimmed chicks are averagely lighter than their counterparts with intact beaks (Appendix 3, table 48a and 48b).

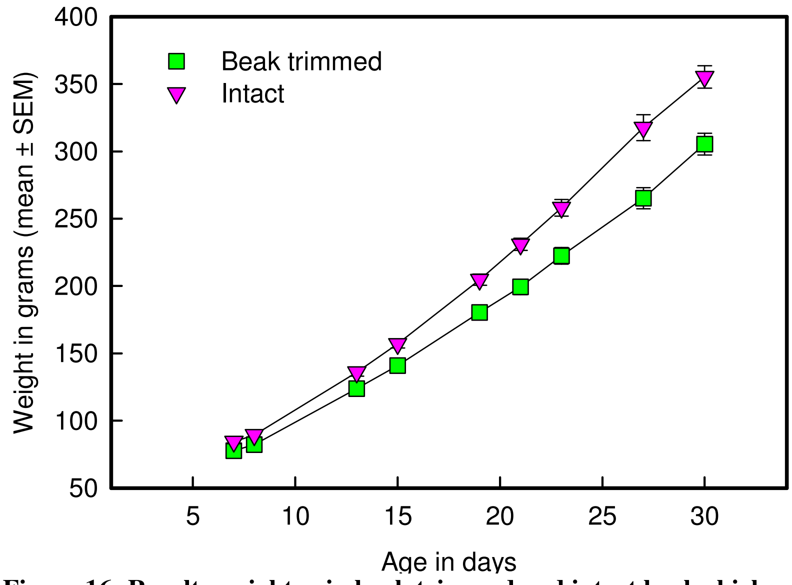

Figure 16: Results weight gain beak trimmed and intact beak chickens

Group effect: $F_{1,18}$ = 18,65, P= 0,0004, this is a strongly significant group effect which indicates a significant difference between the average weight of the two groups. Session effect: $F_{8,144}$ = 1382,66, P= < 0.0001, the chickens do significantly gain weight during each weighing. Session effect by group interaction: $F_{8,144}$ = 13,68, P = < 0,0001 , this indicates a significant difference. Chickens of group 2 (intact) are not only on average heavier they also increase more between each weighing than the birds of group 1 (trimmed) (Appendix 3, tables 49a and 49b).

Discussion

Part I: Low mortality and control line in the spatial holeboard task
The working memory aspect in this study was addressed in the holeboard task. The chickens showed a score between the 0,6 and 0,9 at the end of the experiment. The first fifteen sessions showed no significant differences between the two lines and no session effect or group interaction. The first session however did show a linear trend in relation to the development of the working memory performance. The cued period and overtraining period showed no significant lines differences and no session effect or group interaction on that point. The reversal sessions however did show a significant session effect and thus a learning curve after the test configuration had changed. Furthermore a linear trend was significantly present within the reversal period. When looking at the complete sessions in relation to working memory no line difference is present but there is a significant session effect and a linear trend present. The working memory did develop significantly when looking at the reversal test and the complete testing period as a whole. The chickens showed a highly developed working memory, however this does not seem to differ between the two lines.

The reference memory was also addressed in the holeboard task were the first fifteen sessions showed marginal session effects which indicates a learning curve, also suggested by the significant linear trend indicated by the significant polynomial analysis. The results did not differ between the two lines. The cued and overtraining sessions also revealed no session effect in any of the two lines and showed no difference in average group performance or group interaction within the session performances. The change of configuration in the reversal period showed a significant drop in performance followed by a significant session effect which indicates a clear learning curve. The reversal period also revealed a marginal group effect which indicates a marginal difference in the performance of the two lines. The low mortality line did seem to pick up the change in configuration faster than the control group, suggesting that this line maybe more adaptable to changing situations. Looking at the total number of sessions, the reference memory results showed a significant session effect or learning curve but no line differences. The results vary from 0.4 till 0.6 indicating a reference memory performance well above chance level but not as highly developed as the working memory. In comparison rats in a study by *Van der Staay et al.* in 1990, performed with an reference memory between 6 and 6,5 after 80 trials [39]. The rats seem to perform better and with a faster learning curve. Furthermore pigs in a study by *Arts et al.* in 2009 performed with a reference memory between the 0,6 and 0,7 in a holeboard with sixteen possibilities and thus a lower change level [1]. Pigs perform three times chance level and learn faster than the chickens in this study. This maybe because the birds needed more time to evaluated that pattern which they were exposed to.
The time it took for the chicks to find al rewards was also analyzed. The first fifteen sessions and the cued sessions showed a significant session effect in which the time it took the chicks to find the rewards decreases. During the overtraining sessions no effects were present. When the configuration was changed during the reversal sessions that time it took to find the rewards went down quickly after the first session. This is also an indication of a strong learning curve in the previous configuration. The durations did not differ between the two lines.
This study exposed the chickens to three variations in the set up of the apparatus. The first is switching from non cued to cued. This had no effect on the working memory

when the last two session of the non cued configuration and the first two session of the cued variation where compared. This did have a marginal effect on the trial duration and references memory but no differences were between the two lines. Suggesting that the cues did have an effect, mostly on trial duration but also on reference memory even tough reference memory did not improve significantly during this period it did improve in comparison to the un cued variation.
The second variation was switching from cued to non cued again (overtraining). This showed no switching effect, no session effect or line differences in working, reference memory of trail durations. The last variation, the reversal showed no lines differences in any of the variables but showed a marginal session effect in working memory and significant session effect in reference memory and trial duration. This also suggest that the chicks learned the task, the performance collapsed when the configuration changed but improved faster after the first session than in the beginning of the testing period. Working and reference memory in chickens need to be investigated further to give an accurate picture of their capabilities, a different set-up in which choices have consequences may be able to shed light on the limits of the memory and spatial learning capabilities. As stated in a study by *Abeyesinghe et al* in 2004, chickens are able to access the consequences of actions on future outcomes [2]. Furthermore the way the birds orient themselves within the space is of importance. This study did not reveal the way the chickens learned to visit the right cups. As a study by *Freire and Nicol* in 2004 suggested that chickens may use egocentric evaluation of the environment in spatial learning tasks [18]. Further testing with the holeboard apparatus in which chicks start in different locations may reveal just how the chickens orient themselves in specific spatial learning tasks such as memory based tasks. No other studies have been done on spatial holeboard tasks, it can be concluded that this study suggests that genetic selection does not seem to have an influence on learning, memory and spatial orientation
However genetic selection did have influence on activity in the open field test as stated in a study by *Jones et al*. in 1995 [34]. A study by *Bolhuis et al.* in 2009 states that low mortality line also show a decrease in corticosterone response to being handled, which means lower stress levels. This study also stated that low mortality animals are less fearful in new situations and less sensitive to stressors [34]. A study by *Heerkens* in 2010 however is contradictory to the last statement since no difference were found between low mortality and control animals in the fearfulness related open field test. However this study did state a significant difference in the voluntary approach test, the low mortality animals approached the researcher with the reward significantly faster than the control animals [22]. The study by *Heerkens* also stated a significant difference in sociability in a T-maze apparatus, low mortality animals were more motivated to find conspecifics and spent more time with them [22].

Part II: Beak trimmed and intact birds compared in sociality, fearfulness, food preference/manipulation and voluntary approach and human recognition
In this study the T-maze apparatus was used to evaluate the capacity of the social aspects and fearfulness of the two groups of chicks. Group 1 was beak trimmed and Group 2 had intact beaks. The latency to visit the different compartments were measured, the first compartment the chicks encountered was compartment B or the corridor. No differences were found in between the average performances of the two groups, but session effect was significantly present. This means that it took the chicks less time to approach the corridor during the sessions The beak trimmed birds although not significantly did seem to approach the first compartment slightly faster

than the other group. No difference in average performance of the two groups or performance over the sessions was found in the latency of approaching the other compartments. The beak trimmed birds were also slightly more prone to entering the compartment C, than the intact beaks group although the results did not give a significant results on this.

The time that was spent in the segments of the maze showed no significant differences, although the time spent with the conspecifics was higher in group 2 with intact beaks. However this is not statistically significant. One explanation for that is the high variance of the results. Not all birds reached the conspecifics during the task. This could indicate fearfulness for a new environment or incomplete habituation the apparatus. This could also explain why the stress calls during the habituation did not go down during the three days of habituation.

No difference was found in average group performances on day 5 when no conspecifics were present in the T-maze apparatus. No preference for one compartment was detected.

The open field test was used to evaluate the fearfulness the two groups. The test showed no differences between the two groups in relation to their latency to walk, amount of squares walked and amount of stress calls. Also no significant differences were found between the amount of squares walked in the inner circle or outer circle. The results however, although not statistically, did show that group 2 with intact beaks spent more time walking and walked more squares in total. This indicates that there does not seem to be an effect of beak trimming on behavior in relation to the fearfulness of a new environment.

The preference test was used to evaluate food preferences. The test revealed that the time spent in the empty squares decreased in the three day period suggesting that the animals were getting used to the test. The rewards were significantly more eaten in the three days. Also more time was spent in the vicinity of the rewards, in which group 1 with trimmed beaks spent more time eating reward A (live bait) than group 2 with intact beaks. This may suggest that group 1 had more difficulty with eating reward A. This is also suggested by the pecking frequency toward reward A although not statistically significant because of the high variances, group 1 averagely peck more at reward A (live bait) than group 2. The beak trimmed chicks seem the peck more before they are able to pick-up and eat the reward, this could indicate decreased sensory input as stated by *Hughes and Michi* in 1982*, Dubbeldam et al* in 1999 and *Gentle et al.* in *1997* [20,25].

Also group 1 pecked on average more at reward B (dead bait) than group 2 although this was not statistically proven in this study it does indicate signs of a difference between the groups. More pecking could indicates that the chicks experience little to no pain from the beak trimming this could support research by *Kuenzel* in 2007 [28]. Since these chicks were trimmed at 1 day of age using a laser blade *Kuenzel* states that birds are less likely to experience long lasting pain. This study also seems supports that theory because no real group differences were found in relation to sociability or fearfulness [28]. Stated must be however that the sociability was not tested fully due to that fact the animals of both groups did not all seem to navigate through the maze, this could relate to fear or incomplete habituation.

No significant preference was demonstrated in this study, there are signs however that the ability the manipulate food may difference between the two groups as stated in a study by *Kuenzel* in 2007 [28]. Further testing is necessary to evaluate this.

The Voluntary approach and Human recognition test was used to evaluated fearfulness to familiar and unfamiliar humans. The latencies to approach the square

with the researcher, approach the reward and pick up the reward all showed a significant session effect. The chicks were faster to approach the unfamiliar human in the second session. Also the time spent in the square with the researcher showed a session effect, the chicks spent more time in the square with the unfamiliar human in session 2. This last variable also showed a marginal group interaction which seemed to indicate that group 2 was less prone to spent time with the researcher in the first session but caught up quickly during the second session. Beak trimming seems to have no influence on cognition in relation to fearfulness of humans/new situations in this study. The results also suggest that the chicks do not react to particular humans because the chicks quickly found the connection between approaching the researcher and the reward, therefore they were more prone to do so in sessions 2 whether or not it was a familiar of unfamiliar researcher. Although no group differences where significantly proven group 1 did seem the approach the new situation faster than group 2. The effect of familiarity and the effect or session cannot be clearly separated. Testing the birds is different order were the familiar researcher goes first followed by the unfamiliar researcher and the other way around per chick.
During the whole testing period the weight of the two groups of birds were compared. Results show that birds of group 1 (trimmed) are averagely lighter than birds of group 2 (intact beaks).
The differences in weight are not getting smaller at an age of 30 days, there is actually a larger difference than earlier. This could support conclusions form *Gentle et al.* in 1991 and *Jendral et al*. in 2004 that sensory aphasia can lead to a decline in body weight over a longer period of time [20,25].

Conclusion

Part I: Low mortality and control line in the spatial holeboard task

Working memory: The first fifteen sessions showed no significant lines differences or session effects. It did show a linear tend (P = 0, 0046). The cued and overtraining sessions did not show any lines differences or session effects and interaction. The reversal sessions reveal a marginal session effect (P = 0, 0835). Looking at all sessions the results conclude a strongly significant (P = 0,001) session effect but no lines differences or interaction in the sessions. The cognitive capabilities of chicken do not differ between the lines can vary between 0.7 and 0.9 which concludes a highly developed working memory. Switching from situations has no significant session or group effect except for the reversal period. The switch in this period, from one task to another, reveals a session effect and relearning period.

Reference memory: The first fifteen session show a marginal (P = 0, 0727) session effect or learning curve, also supported by the significant linear trend (P = 0, 0231). The cued and overtraining sessions show no session effect or relevant learning curve within these periods. The reversal period reveals a strong significant session effect (P = 0, 0109) or re-learning curve after the situation is altered. No line differences were found in the first sessions, the cued sessions or the overtraining sessions. However a almost significant (P = 0, 0543) lines difference or group effect was found in the reversal sessions. It can be concluded can that the low mortality animals seem to adapt faster to a changing situation. Looking at all session a strong sessions effect can be concluded (P = < 0, 0001), this is supported by the significant linear trend (P = < 0, 0001). No lines differences were found by looking at all the sessions. It can be concluded that the capabilities of the chickens to learn the task do no differ between the lines, however the LML does seem to be more adaptable. The capabilities vary between 0,4 and 0,6 indicating adequate reference memory. Results also conclude that chickens show a strong learning curve within a spatial task in relation to reference memory. Switching from situations gives marginal block effects but no lines differences. The reversal switch gives a significant block effect (P= 0, 0002).

Trail duration: the trial durations reveal significant session effect in the first fifteen sessions (P = < 0, 0001), no lines differences or interaction. The cued sessions reveal a significant session effect (P = 0, 0013), no lines differences or interaction. No effects were detected in the overtraining sessions. A significant session effect was also detected in the reversal sessions (P= 0, 0233), no lines differences or interaction. Looking at all session a significant session effect is present (P = < 0, 0001) but no lines differences or interaction. Switching from situation reveals a significant block effect when switching from cued to overtraining (P = 0, 0208) and switching from task A to B in the reversal (P = 0, 0066). The reversal switch also revealed a significant block interaction (P= 0,0378) concluding that the block effect differs between the two lines in which the low mortality animals are faster to adapt to the new configuration which lead to lower trail durations.

Part II: Beak trimmed and intact birds compared in sociality, fearfulness, food preference/manipulation and voluntary approach and human recognition

In relation to sociality and fearfulness in the T-maze no significant group differences or interactions were found. Significant session effects was detected in the latency to visit the first compartment (P= 0, 0079) and marginal effects in the time spent there (P = 0, 0908). A marginal interaction was found in approaching the first compartment, the sessions effect curve of the intact beaks was different from the beak trimmed birds. No significant group differences were found in relation to sociability or fearfulness.

In relation to fearfulness, the open field showed no significant group differences.

In relation to food preferences , the results conclude that the chicks spent less time in the empty squares in the 3 day period (session effect) (P = 0,018). A marginal difference was found in the time spent eating reward A (live bait) (P = 0, 0908) where group 1 spent more time eating reward A (live bait) than group 2. It also reveals a significant session effect (P = 0, 0018) in the worms eaten of live bait A increases for both groups in the three days, no group differences. Also a significant session effect (P= 0,015) was revealed in worms eaten of dead bait B for both groups, but no group differences.

In relation to fearfulness in the Voluntary approach and Human recognition test no group differences were found. Significant session effects were revealed, in which the latency to approach the square with researcher (P = 0, 0517), the reward (P = 0, 0216) and the latency of picking up the reward (P = 0, 0117) were lower and the time spent with researcher (P = 0, 0517) higher in the second session with an unfamiliar human.

The weight gain of the trimmed and non trimmed birds differ over a period of 30 days. Group 1 with trimmed beaks is on average lighter than birds of group 2 with intact beaks. Statistics show (P= 0,0004) a strongly significant group effect which indicates a significant difference between the average weight of the two groups. Group 2 is significantly heavier on day 7 till day 30. Also a significant session effect was found (P= < 0.0001), the chickens do gain weight during each weighing. Furthermore this session effect differ between the two groups is session by group interaction shows (P = < 0,0001). Chickens of group 2 (intact) are not only on average heavier they also increase more between each weighing than the birds of group 1 (trimmed).

Recommendations

Memory testing: The holeboard is a useful apparatus for testing the limits of memory in chickens. The cued sessions had a significant effect when compared to the sessions without cues. It also had a positive effect on the time it took the chicks to find all three rewards. Starting with cued sessions may help the chicks to see the pattern better en therefore learn the task faster. Within the holeboard the chick should be tested when starting at different starting positions, the way the chickens orient in the spatial task can than become more clear.
Also other apparatuses than the holeboard can be useful in testing the possibilities of memory based spatial task in chickens. Chickens can evaluate the consequences of their choices as proven in other studies. A apparatus which binds every choice to a consequence of a reward or no reward could shed more light on the extend of the memory.

Sociability: The T-maze is a suitable apparatus for testing sociability however not all chicks in this study felt compelled to investigate the maze. This may have to do with the structure of the apparatus, the chick has to turn several corers to navigate trough. A simpler apparatus such as a Y-maze can provide some advantages in testing the chicks for sociability.

Food preference and manipulation: The preference test needs to be developed further. The manipulation of food did show some differences in the beak trimmed birds. It would be interesting to investigate this further. A possibility is finding out with different reward types how they are manipulated or test the accuracy of pecking. This can be done by lining up several rewards of the same type and measure the time it takes in contrast with birds with an intact beak.

Voluntary approach and recognition: The way this test was performed is suitable for establishing voluntary approach differences but the recognition aspects needs to be investigated further. Switching with unfamiliar and familiar researcher sequences, gives the opportunity to evaluate the effect of familiarity more separately from the learning effect.

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Appendix 1 Species information of Gallus Gallus Domesticus

Anatomy and brain functions

All birds are vertebrates; over 8500 species are known today. Believed is that all of these species have evolved 150 million years ago from the Archaeopteryx. Birds are a unique class of vertebrates. The possession of feathers which enables them to fly, gives them a high number of survival opportunities [9].

A birds' skeleton

The skeleton of birds has evolved to serve flight; this has resulted in a reduction of the number of bones and a fusion of many of the joints. The bones are light with a hollow cortex, which keeps the skeleton light enough to allow the anima to be airborne with the upward power the wings provide. The longer bones in a bird's body are usually completely pneumatic which means that they are filled with air. Another aspect which is important to the ability of flight is the sternum of a bird. A bird's sternum is flattened into a keel which provides the perfect area for attachment of the major flight or pectoral muscles [9]. Not only the number and nature of the bones and joints differ from mammals also the cervical vertebrae in the neck are different. Depending on the type of bird, a bird's neck can contain up to 25 cervical vertebrae, compared to the seven found in mammals. This enables the bird to create a complete vision of its world, which is important for survival, food gathering and preening behavior. The skull of a bird contains a light but powerful beak and a special bone known as the quadrate bone which makes dislocation of the jaw almost impossible [9].

The legs of a bird are controlled by long tendons, a digital flexor tendon which runs behind the intertarsel joint serves one of the most important aspects in a birds feet (figure 17).

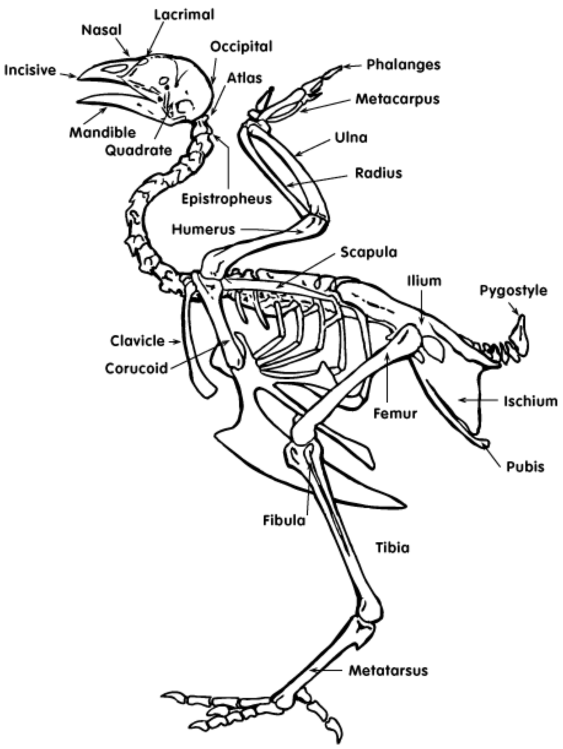

Figure 17: Anatomy of the chicken (Gallus Gallus) [3]

This tendon ensures the perching reflex which is the reflex seen when a bird lands on a branch or perch. The feet of most birds consist of three toes facing forward and one facing backwards. The wings of birds are a unique design of bones with a separate humerus, an ulna, a radius, fused carpal and metacarpal bones and finally also two digits. These digits are important for different aspects or the wing: the main digit, digit 3 is attached to the fused metacarpal bones and carries the major flight feathers, digit 1 carries only a few feathers and forms a bastard wing which is responsible for controlling the landing procedure [9].

Senses and interpretation

An avian brain (figure 18), responsible for interpreting all the data supplied by the bird's senses is larger in birds in proportion to their body than in mammals. The avian brain is divided in to three main areas: the fore-, mid- and hindbrain. The hind- and midbrain are very similar to that in mammals but the forebrain differs. In the brain the optic lobes control the sense of sight, the cerebellum controls hearing and the olfactory bulbs control taste, smell and touch [9].

Figure 18. Avian brain model [4]

The sense of sight is very well developed in birds. Sight is vital for flight, finding food and avoiding predators. The optic lobes in the brain are well developed and the skull is adapted for housing large eyes. The eyes take up so much room in the eye sockets that there is little room for much muscular definition. This means that birds have to turn their head rather than move their eyes around. The eyes of a bird are similar to those in mammals but with a few distinctive differences. One of the differences is the *nicitating mebrane* which is similar to the third eyelid in dogs or cats. In birds however the *nicitating membrane* is supplied with striated muscle which means the bird can move this membrane voluntarily. The same is true for the iris. In contrast to the smooth muscle in mammals, the iris of birds is surrounded by striated muscle which allows voluntary adjustment. Finally the retina contains rods for night vision and cones for day and colored vision [9]. Chickens are sensitive to the 3 basic colors, as are humans, mainly blue, yellow and red. In addition to these basic colors chickens are also sensitive for ultraviolet light [15].

Hearing in birds is slightly different than mammals, first of all there is no external ear and the structure of the inner ear is simpler than that in mammals. The external ear collects sound and summits it to the middle ear by a special bone called the *columma.* The inner ear is similar of that in mammals in which the membranous canals correspond to balance and the *cochlea* corresponds to hearing. The senses of the olfactory bulbes mainly taste, smell and touch are less developed. The taste buds in the tongue and palate of a bird are low in numbers and the ability to distinguish bitter, salt and sour is species related [9].

Smell is one of the senses of which little is known in avian species, thought it is known that some species of birds can locate food by sense of smell.

Touch is represented in the skin, in which sensory nerve ending are sensitive to heat, cold, pain and touch. Touch sensitive nerve ending are found around the beak and at the base of the feathers [9].

Ancestry and development

The chicken is the most common domestic fowl breed in the world. The domesticated chicken or *Gallus Gallus Domesticus* is derived from his wild ancestor the red jungle fowl or *Gallus Gallus*. This species is still found in parts of South-east Asia and was turned in to a domesticated form over 8000 years ago. The usefulness of the domestic chicken at that time revolved around its ceremonial value due to its beautiful plumage and its entertainment value in cockfights. The Roman Empire was the first civilization which used the chicken for massive egg production. After the decline of the Roman civilization egg production did not return until the 19th century. From which point the selection of breeds to specialize in either egg or meat production started [18]. The modern laying hens are usually hybrids which are bred for optimal egg production, modern day breeds can produce up to 300 eggs in a single year. The commercial broiler chickens which are bred for fast growth en fat production are now a lot heavier than their wild ancestor. Commercially bred broiler can reach a weight up to 2, 5 kilograms which is three times heavier than its wild ancestor. This weight is usually reached in 42-45 days [26]

Social behavior and interaction

Many species of fowl including the chicken live in highly social groups. This group consists of a dominant cock, hens of all ages and a few submissive/adolescent cocks. This group dynamic functions on the base of a strict social hierarchy. Observation made in chickens resulted in the first mention of a so called pecking order which is now known in most of social species. Aggression in this pecking order can take the form of subtle threats and avoidances as well as pecks, fights and chases. In social clashes (usually amongst unfamiliar males) the birds rear up at each other using the spurs on their feet and their beaks to conflict damage. In less severe fights one peck is usually enough to establish dominance. Hierarchy related pecks are almost always directed at the head. Dominance statures involve rearing of the head, submissive statures involve lowering of the head or turning away [26].

Communication systems

The chicken as a social species has a highly developed communication system which consists of a variety of visual, auditory and physical contact related cues. Visual and acoustic signals are the most important. Vision is needed to interact with the environment, including for protection as the chicken is a prey species, and to interact with other members of the same species. The eye of the chickens is different from a human eye. First chickens have but a 26 degrees binocular vision, but very good monocular vision. Secondly the eye of a chicken is based on four different types of photo reactive pigments in opposed to three in a human eye. As a result the chicken eye is able to detect ultraviolet light. Vision is important in the identification of individuals; research has shown that the characteristics of the head are the most important means of recognition. Comb size, color and shape play an important role in these distinctive features of the head. Furthermore body and comb seize relates to dominance. Next to visual cues, acoustic cues are also frequently used and start before a chicken even hatches out of his egg. Inside the egg chickens produce peeping sounds. Research indicates that this may coordinate the hatching of the clutch and help the hen organize her breeding time. Newly hatched chicks can identify their mother by her call [26].

Foraging and feeding

Feeding behavior consists of visual, olfactory and tactile elements required for picking up small particles of food from the ground. It consist of five separate stages mainly 1) food recognition, 2) orientation of head and beak toward the food particles, 3) actual grasping of the food particles, 4) mandibulation or the movement of particles from the beak towards the throat and 5) swallowing reflex which moves the particles from to throat to the esophagus [28].

Foraging and feeding can take up to 40% of the time each day, dependent of the genotype, age and environmental aspects. Poultry species as chickens use a series of well coordinated pecks to regulate their intake of food. The beak plays an essential part in this process. Like most domesticated fowl the chicken is an omnivore and its diet can consist of seeds to small invertebrates. In natural conditions the search for compiling such a diet would take up to 90% of the day. Birds which are used in commercial conditions are offered food in troughs but still spent a lot of time scratching and pecking at the ground for food. This is probably related to the need to investigate and gather information about the environment. Some animals in domesticated conditions will even perform a task to get to food even if food is freely offered elsewhere; this can be explained by the need to gather information about additional food sources. Chickens are selective eaters which can result in a problem for some animals in a domesticated or commercial environment. Lower animals or animals with less access to the food can end up with an imbalanced diet because of the preference eating of their neighbors [26].

Ontogeny

In commercial systems, chicks hatch in an incubator and are reared in age related groups. Chicks can survive in commercials systems without the guidance of a hen but this can still cause behavioral problems. The hen has an important role; the hen teaches the chicks to discriminate between different types of food and maintains a daily rhythm. The hen also instructs the chicks how and where to roost in the first few weeks. Another important aspect of their behavior is dust bathing to maintain and clean their plumage. Recognition of suitable substrate is also learned by guidance of the hen in the first days. Social dominance in groups in usually established in weeks 9 till 10. Chickens are sexually mature at an age of 16 till 18 weeks and hen can start to lay between 18 and 20 weeks [26].

Appendix 2 Legislation of welfare in the poultry sector

This chapter explains the rules of animal welfare in research or laboratory animals and explains the legislation on a very important welfare aspect as beak trimming.

DEC and experimental design

The DEC or animal experiments committee debates every presented experiment in relation to education and research using legislation and interest of the test in relation to discomfort for the animals of each experiment. If a committee is convinced that the benefit of a test does not conform to the amount of discomfort experienced by the animals it will give a negative evaluation. In this case the experiment may not be carried out. Each DEC of any research facility conforms to the fact that animal testing is illegal except if no other options exist to gather the insight and knowledge. An approval by a DEC committee is always according to European law and therefore ETS 123 (Council of Europe 1985) and Directive 86/609/EEC on the protection of animals used in experiments which is currently being revised. The ETS 123 states the rules of housing test animals, methods used in experiments and when animal experiments are permitted.
Article 2 of ETS 123 states the circumstances in which animals are allowed to be used in an experiment:

a) Avoidance or prevention of disease, ill-health or other abnormalities and their effects.
b) Detection, assessment, regulation or modification of physiological conditions.
c) Protection of the environment
d) Scientific research
e) Education and training
f) Forensic inquiries

The directive 86/609/EEC further states all important aspects of animal experiments in order to protect laboratory or test animals. This directive however has not been revised science its adoption in 1986. Due to the developments in non-animals methods an increased understanding of animals in term of the ability to suffer and experience pain, the directive is now undergoing revision [5].

Legislation Beak trimming intervention

EU legislation

The European legislation is not very specific on the topic of interventions such as beak trimming in poultry. There is a directive statement in the form of *the European Directive 1999/74/EC of 19 July 1999*. This regulation states the minimum standards for the protection of laying hens. The regulation states that beak trimming is legal in animals which are intended as laying hens before the age of 10 days, this procedure should be done by a qualified beak trimmer [8].

Dutch Legislation
The so called 'Gezondheid en Welzijn Wet Dieren' or the Health and Welfare Legislation instated in the Netherlands provides a large number of regulations in relation to the handling, housing, transporting and overall management of animals. One of the paragraphs of this law sates the rules of physical manipulation of animals (Department 2, Article 40).

1. It is forbidden to exercise one or multiple physical manipulation acts on to an animal in which a part or parts of the body are removed.
2. The above mentioned paragraph is not applied when:

a) When a physical manipulation consist of the spading or neutering of an animal.
b) When the manipulation has a medical necessity.
c) When it is designated by general administrative procedures
d) By or under any other statutory provision required or permitted interventions.

3. For general administrative rules can be made out how and where physical interventions, referred to in the second paragraph, pars c and d, may be carried out [8].

About the physical operation of the beaks in chickens the Intervention decision exists. This describes the operation as a shortening of the upper and lower beak in chickens. This is permitted as stated by Article 40, second paragraph, parts c and d.
This intervention legislation states the definition of beak trimming in article 2, first paragraph section g. It also states the following in article 4 section b:
Notwithstanding the first paragraph, the procedures referred to in article 2, sections g, h and s, assigned till September 1, 2011, provided they are performed in kept animals or animals which are intended to be held in a housing system, in which the user can demonstrate that it already existed on September 1, 2001 and was not rebuilt [6].

Beak trimming and legislation within the poultry sectors
The poultry industry of laying hens can be divided in different production processes and lines. This ranges from caged birds, to free ranging birds and birds from organic production lines.

Caged birds
The animals are held closely together in cages and have a space requirement of 550 square centimeters per chicken. This system has very large welfare impairment issues, the chickens are cramped together with no adequate substrate or space for movement. At least 5 of the 8 ethological aspects of normal behavior in laying hens are completely impossible or seriously impaired. Since 2003 it is no longer permitted to build a stable according to this design. Exciting stables have to reform to enriched cages by 2012. From 2012 the chickens will have to have al least 750 square centimeters per chicken and 15 centimeter long perches, nests and litter must be available. Beak trimming is permitted in this system [7].

Free range birds (without outdoor area)
These animals can range free in a stable and have more individual space mainly 1111 square centimeter per chicken. The floor space is for at least one third covered with litter. Perches of 15 centimeters and 1 nest per 7 chickens should be available and the birds should be allowed daylight. Beak trimming is also permitted within this system [7].

Voliere birds
This system is similar to an indoor free range area except this stable is divided in two different levels. These levels accommodate the different needs of the chickens, there must be enough nests, 15 centimeter perches, the floor should allow 45 centimeters of free space above and litter must be present at the ground floor. Animals in this system can suffer from multiple fractures due to the altitude of the levels. Beak trimming is permitted [7].

Free range birds (With outdoor area)
The regulations are the same as the free range systems without an outdoor area, except for the fact that chickens in this housing type must have at least 4 square meters of space in an outdoor area. The outdoor area must be overgrown for the larger part. Beak trimming is permitted [7].

Organic production systems
Organic production is regulated by European legislation states a stocking density of 6 laying hens per square meters. That stable must contain a floor which is cover with litter for at least one third of the total amount of ground space. Nests and 20 centimeter perches need to be provided for all animals. Lighting must contain a proportion of natural daylight. Furthermore 70-80% of the nutritional aspects need to be organic. Birds also need to have at least 4 square meters of outside area per individual. Beak trimming is not allowed in this sector, behavioral disturbances are minimized by optimizing the living conditions and welfare. The compliance of the EU legislation on organic farming in the Netherlands is the responsibility of Skal [7, 10]

Appendix 3 Statistics

Appendix 1: tables of statistical analysis

The Hole-board test

Working memory

Table 1a: Group effect (between Subjects analysis) first 15 sessions WM

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	0.00838542	0.00838542	0.10	0.7584
Error	14	1.19361291	0.08525807		

Table 1b: Session effect and group interaction (within Subjects analysis) first 15 sessions WM

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	14	0.39669434	0.02833531	0.92	0.5397
Session*Group	14	0.34740267	0.02481448	0.80	0.6638
Error (Session)	196	6.04594899	0.03084668		

Table 1c: Group effect (between subjects analysis) cued sessions WM

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	0.00132071	0.00132071	0.03	0.8713
Error	14	0.67951429	0.04853673		

Table 1d: Session effect and group interaction (within Subjects analysis) cued sessions WM

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	4	0.08996341	0.02249085	1.14	0.3494
Session*Group	4	0.12196341	0.03049085	1.54	0.2035
Error (Session)	56	1.10950159	0.01981253		

Table 1e: Group effect (between subjects analysis) overtraining sessions WM

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	0.04754571	0.04754571	0.58	0.4605
Error	14	1.15595429	0.08256816		

Table 1f: Session effect and group interaction (within subject analysis) overtraining sessions WM

Source	DF	Type III SS	Mean square	F value	Pr > F
Session	4	0.14538306	0.03634576	1.67	0.1689
Session*Group	4	0.08063306	0.02015826	0.93	0.4542
Error (Session)	56	1.21600444	0.02171437		

Table 1g: Group effect (between subject analysis) reversal sessions WM

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	0.03697522	0.03697522	0.63	0.4391
Error	14	0.81628571	0.05830612		

Table 1h: Session effect and group interaction (within subject analysis) reversal sessions WM

Source	DF	Type III SS	Mean square	F value	Pr > F
Session	3	0.21101257	0.07033752	2.38	0.0835
Session*Group	3	0.02292507	0.00764169	0.26	0.8550
Error (Session)	42	1.24289524	0.02959274		

Table 1i: Group effect (between subject analysis) all sessions WM

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	0.00010673	0.00010673	0.00	0.9734
Error	14	1.29428927	0.09244923		

Table 1j: Session effect and group interaction (within subject analysis) all sessions WM

Source	DF	Type III SS	Mean square	F value	Pr > F
Session	24	160.111.726	0.06671322	2.22	0.0011
Session*Group	24	0.60714426	0.02529768	0.84	0.6832
Error (Session)	336	1010624724	0.03007812		

<u>Reference memory</u>

Table 2a: Group effect (between subject analysis) first 15 sessions RM

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	0.00374011	0.00374011	0.04	0.8365
Error	14	1.18420656	0.08458618		

Table 2b: Session effect and group interaction (within subject analysis) first 15 sessions RM

Source	DF	Ttpe III SS	Mean square	F value	Pr > F
Session	14	0.25290652	0.01806475	1.63	0.0727
Session*Group	14	0.13208985	0.00943499	0.85	0.6100
Error (Session)	196	2.16587598	0.01105039		

Table 2c: Group effect (between subjects analysis) cued sessions RM

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	0.01031431	0.01031431	0.11	0.7420
Error	14	1.28052444	0.09146603		

Table 2d: Session effect and group interaction (within subjects analysis) cued sessions RM

Source	DF	Type III SS	Mean square	F value	Pr > F
Session	4	0.05040575	0.01260144	0.83	0.5102
Session*Group	4	0.02204075	0.00551019	0.36	0.8332
Error (Session)	56	0.84755175	0.01513485		

Table 2e: Group effect (between subjects analysis) overtraining sessions RM

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	0.00000161	0.00000161	0.00	0.9949
Error	14	0.52385714	0.03741837		

Table 2f: Session effect and group interaction (within subjects analysis) overtraining sessions RM

Source	DF	Type III SS	Mean square	F value	Pr > F
Session	4	0.03904218	0.00976055	0.81	0.5253
Session*Group	4	0.05496218	0.01374055	1.14	0.3483
Error (Session)	56	0.67646032	0.01207965		

Table 2g: Group effect (between subjects analysis) reversal sessions RM

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	0.03348819	0.03348819	4.41	0.0543
Error	14	0.10630556	0.00759325		

Table 2h: Session effect and group interaction (within subjects analysis) reversal session RM

Source	DF	Type III SS	Mean square	F value	Pr > F
Session	3	0.09170169	0.03056723	4.21	0.0109
Session*Group	3	0.00967669	0.00322556	0.44	0.7228
Error (Session)	42	0.30514206	0.00726529		

Table 2i: Group effect (between subjects analysis) all sessions RM

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	0.00850514	0.00850514	0.07	0.8020
Error	14	1.82324686	0.13023192		

Table 2j: Session effect and group interaction (within subjects analysis) all sessions RM

Source	DF	Type III SS	Mean square	F value	Pr > F
Session	24	181.339.167	0.07555799	5.23	<.0001
Session*Group	24	0.21464367	0.00894349	0.62	0.9206
Error (Session)	336	4.85522933	0.01445009		

Trial Duration

Table 3a: Group effect (between subject analysis) trail duration first 15 sessions

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	545.7180	545.7180	0.03	0.8567
Error	14	22567.76143	16119.8296		

Table 3b: Session effect and group interaction (within subjects analysis) trail duration first 15 sessions

Source	DF	Type III SS	Mean square	F value	Pr > F
Session	14	1.54950.7342	11067.9096	5.14	<.0001
Session*Group	14	18912.7842	1350.9132	0.63	0.8409
Error	196	4.22425.7429	2155.2334		

Table 3c: Group effect (between subject analysis) trail duration cued sessions

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	1701.60.957	1701.60957	0.57	0.4617
Error	14	41592.18730	2970.87052		

Table 3d: Session effect and group interaction (within subjects analysis) trial duration cued sessions

Source	DF	Type III SS	Mean square	F value	Pr > F
Session	4	18584.36528	4646.09132	5.14	0.0013
Session*Group	4	1753.87778	438.46944	0.48	0.7468
Error	56	50651.62222	904.49325		

Table 3e: Group effect (between subject analysis) trail duration overtraining sessions

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	6661.95045	6661.95045	1.59	0.2282
Error	14	5873.447143	4195.31.\939		

Table 3f: Session effect and group interaction (within subjects analysis) trial duration overtraining sessions

Source	DF	Type III SS	Mean square	F value	Pr > F
Session	4	6789.45704	1697.36426	1.63	0.1787
Session*Group	4	5350.95704	1337.73926	1.29	0.2860
Error	56	58198.44921	1039.25802		

Table 3g: Group effect (between subject analysis) trail duration reversal sessions

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	3695.33730	3695.33730	1.10	0.3119
Error	14	47000.53770	3357.18126		

Table 3h: Session effect and group interaction (within subjects analysis) trial duration reversal sessions

Source	DF	Type III SS	Mean square	F value	Pr > F
Session	4	21224.32465	7074.77488	3.51	0.0233
Session*Group	4	3123.43403	1041.14468	0.52	0.6730
Error	56	84626.09722	2014.90708		

Table 3i: Group effect (between subject analysis) trail duration all sessions

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	1358.2679	1358.2679	0.11	0.7475
Error	14	176349.0165	12596.3583		

Table 3j: Session effect and group interaction (within subjects analysis) trial duration all sessions

Source	DF	Type III SS	Mean square	F value	Pr > F
Session	4	205122.9167	8546.7882	4.22	<.0001
Session*Group	4	33568.6292	1398.6929	0.69	0.8615
Error	56	680931.0708	2026.5806		

Effect of transference situations on working memory

Table 4a: Group effect (between subjects analysis) Switch from non-cued to cued (task A)

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	0.00612858	0.00612858	0.33	0.5768
Error	14	0.26274563	0.01876755		

Table 4b: Session effect and group interaction (within subjects analysis) Switch from non-cued to cued (task A)

Source	DF	Type III SS	Mean square	F value	Pr > F
Block	1	0.00011192	0.00011192	0.01	0.9312
Block*Group	1	0.01116817	0.01116817	0.77	0.3949
Error (Block)	14	0.20291230	0.01449374		

Table 4c: Group effect (between subjects analysis) Switch from cued to overtraining (task A)

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	0.00013934	0.00013934	0.00	0.9463
Error	14	0.41440754	0.02960054		

Table 4d: Session effect and group interaction (within subjects analysis) Switch from cued to overtraining (task A)

Source	DF	Type III SS	Mean square	F value	Pr > F
Block	1	0.01346434	0.01346434	0.52	0.4840
Block*Group	1	0.01538934	0.01538934	0.59	0.4549
Error (Block)	14	0.36465754	0.02604697		

Table 4e: Group effect (between subjects analysis) Switch from task A to task B (reversal)

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	0.00119942	0.00119942	0.05	0.8299
Error	14	0.35066230	0.02504731		

Table 4f: Session effect and group interaction (within subjects analysis) Switch from task A to task B (reversal)

Source	DF	Type III SS	Mean square	F value	Pr > F
Block	1	0.12218900	0.12218900	7.33	0.0170
Block*Group	1	0.03226400	0.03226400	1.94	0.1859
Error (Block)	14	0.23342897	0.01667350		

Effect of transference situations on reference memory

Table 5a: Group effect (between subjects analysis) Switch from non-cued to cued (task A)

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	0.01876116	0.01876116	0.37	0.5552
Error	14	0.71876071	0.05134005		

Table 5b: Session effect and group interaction (within subjects analysis) Switch from non-cued to cued (task A)

Source	DF	Type III SS	Mean square	F value	Pr > F
Block	1	0.04401607	0.04401607	4.15	0.0609
Block*Group	1	0.00002857	0.00002857	0.00	0.9593
Error (Block)	14	0.14839643	0.01059974		

Table 5c: Group effect (between subjects analysis) Switch from cued to overtraining (task A)

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	0.00453900	0.00453900	0.36	0.5568
Error	14	0.17532897	0.01252350		

Table 5d: Session effect and group interaction (within subjects analysis) Switch from cued to overtraining (task A)

Source	DF	Type III SS	Mean square	F value	Pr > F
Block	1	0.03545983	0.03545983	3.56	0.0800
Block*Group	1	0.00014733	0.00014733	0.01	0.9049
Error (Block)	14	0.13929563	0.00994969		

Table 5e: Group effect (between subjects analysis) Switch from task A to task B (reversal)

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	0.00228225	0.00228225	0.46	0.5077
Error	14	0.06913571	0.00493827		

Table 5f: Session effect and group interaction (within subjects analysis) Switch from task A to task B (reversal)

Source	DF	Type III SS	Mean square	F value	Pr > F
Block	1	0.22262114	0.22262114	24.52	0.0002
Block*Group	1	0.00395864	0.00395864	0.44	0.5198
Error (Block)	14	0.12709683	0.00907834		

Effect of transference situations on trail duration

Table 6a: Group effect (between subjects analysis) Switch from non-cued to cued (task A)

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	74.57840	74.57840	0.05	0.05
Error	14	20739.91964	1481.42283		

Table 6b: Session effect and group interaction (within subjects analysis) Switch from non-cued to cued (task A)

Source	DF	Type III SS	Mean square	F value	Pr > F
Block	1	2647.447948	2647.447948	4.55	0.0511
Block*Group	1	8.807323	8.807323	0.02	0.9038
Error (Block)	14	8142.268849	581.590632		

Table 6c: Group effect (between subjects analysis) Switch from cued to overtraining (task A)

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	1094.59834	1094.59834	0.72	0.4093
Error	14	21177.33135	1512.66652		

Table 6d: Session effect and group interaction (within subjects analysis) Switch from cued to overtraining (task A)

Source	DF	Type III SS	Mean square	F value	Pr > F
Block	1	1543.500000	1543.500000	6.79	0.0208
Block*Group	1	602.929688	602.929688	2.65	0.1258
Error (Block)	14	3184.125000	227.437500		

Table 6e: Group effect (between subjects analysis) Switch from task A to task B (reversal)

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	21.04960	21.04960	0.01	0.9256
Error	14	32635.38790	23310.9914		

Table 6f: Session effect and group interaction (within subjects analysis) Switch from task A to task B (reversal)

Source	DF	Type III SS	Mean square	F value	Pr > F
Block	1	13482.43167	13482.43167	10.17	0.0066
Block*Group	1	6977.30667	6977.30667	5.26	0.0378
Error (Block)	14	18565.31052	1326.09361		

The T - maze (latency and time spent)

Habituation

Table 7a : Means stress calls group 1 habituation t-maze day 3 days

Variable	Mean	Std Error	Std Dev	N
Stress calls day 1	254.20	32.08	101.45	10
Stress calls day 2	221.10	22.26	70.40	10
Stress calls day 3	216.70	38.42	121.48	10

Table 7b : Means stress calls group 2 habituation t-maze 3 days

Variable	Mean	Std Error	Std Dev	N
Stress calls day 1	216.00	30.37	96.05	10
Stress calls day 2	180.60	27.15	85.86	10
Stress calls day 3	177.00	25.44	80.45	10

Comparing average performance and development of the 3 testing days between groups (GLM - repeated measures)

Table 7c: group effect (between subjects analysis) stress calls 3 days

Source	DS	Type III SS	Mean square	F value	Pr > F
Group	1	23364.2.667	23364.2667	1.27	1.27 0.2751
Error	18	331945.4667	18441.4148		

Table 7d: Session effect and group interaction (within subjects analysis) stress calls 3 days

Source	DS	Type III SS	Mean square	F value	Pr > F
Session	2	17680.8333	8840.4167	2.18	0.1273
Session*group	2	13.6333	6.8167	0.00	0.9983
Error (session)	36	145729.5333	4048.0426		

Compartment B

Comparing performance of groups per day (t-test)

Table 8a: Means latency compartment B day 1- 5

Variable	Mean	Std. error	Std. deviatie	N
Lat. B day 1_Group 1	227.60	78.39	247.90	10
Lat. B day 2_Group 1	175.50	76.30	241.29	10
Lat. B day 3_Group 1	148.10	76.09	240.61	10
Lat. B day 4_Group 1	246.90	96.17	304.11	10
Lat. B day 5_Group 1	195.00	88.69	280.47	10
Lat. B day 1_Group 2	389.10	74.88	236.78	10
Lat. B day 2_Group 2	194.70	72.89	230.48	10
Lat. B day 3_Group 2	151.50	55.34	175.00	10
Lat. B day 4_Group 2	157.30	58.62	185.36	10
Lat. B day 5_Group 2	115.60	49.34	156.02	10

Table 8b: Equality of variances latency compartment B day 1

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	1.10	0.8935

Table 8c: T-test latency compartment B day 1

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	-1.49	0.1536
Satterthwaite	Unequel	17.962	-1.49	0.1536

Table 8d: Equality of variances latency compartment B day 2

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	1.10	0.8936

Table 8e: T-test latency compartment B day 2

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	-0.18	0.8577
Satterthwaite	Unequel	17.962	-0.18	0.8577

Table 8f: Equality of variances latency compartment B day 3

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	1.89	0.3568

Table 8g: T-test latency compartment B day 3

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	-0.04	0.9716
Satterthwaite	Unequel	16.44	-0.04	0.9716

Table 8h: Equality of variances latency compartment B day 4

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	2.69	0.1563

Table 8i: T-test latency compartment B day 4

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	0.80	0.4367
Satterthwaite	Unequel	14.876	0.80	0.4388

Table 8j: Equality of variances latency compartment B day 5

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	3.23	0.0955

Table 8k: T-test latency compartment B day 5

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	0.78	0.4442
Satterthwaite	Unequel	14.083	0.78	0.4470

Table 9a: Means time spent in compartment B day 1-5

Variable	Mean	Std. error	Std. deviatie	N
Time spent in B day 1_Group 1	73.70	30.11	95.22	10
Time spent in B day 2_Group 1	145.50	70.42	222.67	10
Time spent in B day 3_Group 1	162.30	80.59	254.85	10
Time spent in B day 4_Group 1	81.90	57.50	181.82	10
Time spent in B day 5_Group 1	122.90	62.68	198.23	10
Time spent in B day 1_Group 2	44.20	23.68	74.87	10
Time spent in B day 2_Group 2	145.70	57.73	182.56	10
Time spent in B day 3_Group 2	230.10	75.95	240.19	10
Time spent in B day 4_Group 2	161.60	54.32	171.78	10
Time spent in B day 5_Group 2	143.80	52.00	164.44	10

Table 9b: Equality of variances time spent in compartment B day 1

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	1.62	0.4850

Table 9c: T-test time spent in compartment B day 1

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	0.77	0.4512
Satterthwaite	Unequel	17.051	0.77	0.4518

Table 9d: Equality of variances time spent in compartment B day 2

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	1.49	0.5634

Table 9e: T-test time spent in compartment B day 2

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	-0.00	0.9983
Satterthwaite	Unequel	17.334	-0.00	0.9983

Table 9f: Equality of variances time spent in compartment B day 3

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	1.13	0.8628

Table 9g: T-test time spent in compartment B day 3

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	-0.61	0.5480
Satterthwaite	Unequel	17.937	-0.61	0.5481

Table 9h: Equality of variances time spent in compartment B day 4

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	1.12	0.8684

Table 9i: T-test time spent in compartment B day 4

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	-1.01	0.3270
Satterthwaite	Unequel	17.937	-1.01	0.3270

Table 9j: Equality of variances time spent in compartment B day 5

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	1.45	0.5866

Table 9k: T-test time spent in compartment B day 5

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	-0.26	0.8004
Satterthwaite	Unequel	17.937	-0.26	0.8005

Comparing average performance and development of the 5 testing days between groups (GLM - repeated measures)

Table 10a: Group effect (between subjects analysis) latency compartment B day 1-4

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	11162.813	11162.813	0.07	0.7935
Error	18	2847290.825	158182.824		

Table 10b: Session effect and group interaction (within subjects analysis) latency compartment B day 1-4

Source	DF	Type III SS	Mean square	F value	Pr > F
Session	3	279440.538	93146.846	4.37	0.0079
Session*Group	3	161290.237	53763.412	2.52	0.0673
Error	54	1150741.475	21310.027		

Table 11a: Group effect (between subjects analysis) time spent in compartment B day 1-4

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	17.464.050	17.464.050	0.31	0.5819
Error	18	1.000.022.700	55.556.817		

Table 11b: Session effect and group interaction (within subjects analysis) time spent in compartment B day 1-4

Source	DF	Type III SS	Mean square	F value	Pr > F
Session	3	194.808.050	64.936.017	2.27	0.0908
Session*Group	3	41.632.050	13.877.350	0.49	0.6941
Error	54	1.545.053.900	28.612.109		

Compartment C

Table 12a: Means latency to compartment C day 1-5

Variable	Mean	Std. error	Std. deviatie	N
Lat. C day 1_Group 1	283.10	88.52	279.92	10
Lat. C day 2_Group 1	319.30	90.29	285.53	10
Lat. C day 3_Group 1	309.50	97.03	306.83	10
Lat. C day 4_Group 1	308.90	97.22	307.43	10
Lat. C day 5_Group 1	289.00	96.33	304.63	10
Lat. C day 1_Group 2	482.80	60.39	190.98	10
Lat. C day 2_Group 2	339.60	77.78	245.96	10
Lat. C day 3_Group 2	346.30	77.30	244.44	10
Lat. C day 4_Group 2	317.40	85.73	271.10	10
Lat. C day 5_Group 2	245.90	82.40	260.58	10

Comparing performance of groups per day (t-test)

Table 12b: Equality of variances latency compartment C day 1

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	2.15	0.2701

Table 12c: T-test latency compartment C day 1

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	-1.86	0.0788
Satterthwaite	Unequel	15.887	-1.86	0.0810

Table 12d: Equality of variances latency compartment C day 2

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	1.35	0.6639

Table 12e: T-test latency compartment C day 2

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	-0.17	0.8666
Satterthwaite	Unequel	17.614	-0.17	0.8667

Table 12f: Equality of variances latency compartment C day 3

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	1.58	0.5089

Table 12g: T-test latency compartment C day 3

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	-0.30	0.7701
Satterthwaite	Unequel	17.144	-0.30	0.7703

Table 12h: Equality of variances latency compartment C day 4

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	1.29	0.7140

Table 12i: T-test latency compartment C day 4

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	-0.07	0.9484
Satterthwaite	Unequel	17.723	-0.07	0.9484

Table 12j: Equality of variances latency compartment C day 5

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	1.37	0.6492

Table 12k: T-test latency compartment C day 5

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	0.34	0.7378
Satterthwaite	Unequel	17.578	0.34	0.7379

Table 13a: Means time spent in compartment C day 1-5

Variable	Mean	Std. error	Std. deviatie	N
Time spent in C day 1_Group 1	217.10	63.18	199.78	10
Time spent in C day 2_Group 1	224.00	74.91	236.88	10
Time spent in C day 3_Group 1	218.70	84.08	265.88	10
Time spent in C day 4_Group 1	187.50	74.72	236.28	10
Time spent in C day 5_Group 1	252.30	81.16	256.64	10
Time spent in C day 1_Group 2	87.60	45.10	142.62	10
Time spent in C day 2_Group 2	150.20	67.21	212.53	10
Time spent in C day 3_Group 2	78.50	33.98	107.45	10
Time spent in C day 4_Group 2	126.10	50.29	159.04	10
Time spent in C day 5_Group 2	288.20	70.42	222.68	10

Table 13b: Equality of variances time spent in compartment C day 1

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	1.96	0.3298

Table 13c: T-test time spent in compartment C day 1

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	1.67	0.1126
Satterthwaite	Unequel	16.282	1.67	0.1144

Table 13d: Equality of variances time spent in compartment C day 2

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	1.24	0.7519

Table 13e: T-test time spent in compartment C day 2

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	0.73	0.4728
Satterthwaite	Unequel	17.792	0.73	0.4729

Table 13f: Equality of variances time spent in compartment C day 3

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	6.12	0.0126

Table 13g: T-test time spent in compartment C day 3

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	1.55	0.1395
Satterthwaite	Unequel	11.864	1.55	0.1483

Table 13h: Equality of variances time spent in compartment C day 4

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	2.21	0.2539

Table 13i: T-test time spent in compartment C day 4

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	0.68	0.5041
Satterthwaite	Unequel	15.766	0.68	0.5053

Table 13j: Equality of variances time spent in compartment C day 5

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	1.33	0.6792

Table 13k: T-test time spent in compartment C day 5

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	-0.33	0.7422
Satterthwaite	Unequel	17.649	-0.33	0.7422

Comparing average performance and development of the 5 testing days between groups (GLM - repeated measures)

Table 14a: Group effect (between subjects analysis) latency compartment C day 1-4

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	87980.113	87980.113	0.39	0.5381
Error	18	4019730.625	223318.368		

Table 14b: Session effect and group interaction (within subjects analysis) latency compartment C day 1-4

Source	DF	Type III SS	Mean square	F value	Pr > F
Session	3	56252.237	18750.746	0.85	0.4719
Session*Group	3	120613.238	40204.413	1.83	0.1534
Error	54	1189148.275	22021.264		

Table 15a: Group effect (between subjects analysis) Time spent in compartment C day 1-4

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	204.930.013	204.930.013	2.08	0.1667
Error	18	1.775.550.625	98.641.701		

Table 15b: Session effect and group interaction(within subjects analysis time spent in compartment C day 1-4

Source	DF	Type III SS	Mean square	F value	Pr > F
Session	3	18.545.037	6.181.679	0.29	0.8320
Session*Group	3	23.283.437	7.761.146	0.36	0.7786
Error	54	1.148.482.275	21.268.190		

Compartment D (with conspecifics)

Table 16a: Means latency compartment D (with conspecifics) day 1-5

Variable	Mean	Std. error	Std. deviatie	N
Lat. DWC day 1_Group 1	476.90	63.41	200.53	10
Lat. DWC day 2_Group 1	488.40	74.43	235.35	10
Lat. DWC day 3_Group 1	461.00	76.52	241.98	10
Lat. DWC day 4_Group 1	474.50	67.36	213.01	10
Lat. DWC day 5_Group 1	440.40	81.69	258.32	10
Lat. DWC day 1_Group 2	561.60	25.68	81.22	10
Lat. DWC day 2_Group 2	480.80	64.55	204.14	10
Lat. DWC day 3_Group 2	427.80	73.18	231.40	10
Lat. DWC day 4_Group 2	392.90	86.88	274.73	10
Lat. DWC day 5_Group 2	396.30	84.49	267.19	10

Comparing performance of groups per day (t-test)

Table 16b: Equality of variances latency compartment DWC day 1

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	6.10	0.0128

Table 16c: T-test latency compartment DWC day 1

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	-1.24	0.2316
Satterthwaite	Unequel	11.876	-1.24	0.2396

Table 16d: Equality of variances latency compartment DWC day 2

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	1.33	0.6785

Table 16e: T-test latency compartment DWC day 2

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	0.08	0.9394
Satterthwaite	Unequel	17.647	0.08	0.9394

Table 16f: Equality of variances latency compartment DWC day 3

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	0.31	0.7575
Satterthwaite	Unequel	17.964	0.31	0.7575

Table 16g: T-test latency compartment DWC day 3

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	1.09	0.8962

Table 16h: Equality of variances latency compartment DWC day 4

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	1.66	0.4601

Table 16i: T-test latency compartment DWC day 4

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	0.74	0.4675
Satterthwaite	Unequel	16.948	0.74	0.4681

Table 16j: Equality of variances latency compartment DWC day 5

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	1.07	0.9216

Table 16k: T-test latency compartment DWC day 5

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	0.38	0.7119
Satterthwaite	Unequel	17.98	0.38	0.7119

Table 17a: Means time spent in compartment D (with conspecifics) day 1-5

Variable	Mean	Std. error	Std. deviatie	N
Time spent in DWC day 1_Group 1	61.40	34.16	108.02	10
Time spent in DWC day 2_Group 1	53.10	35.59	112.54	10
Time spent in DWC day 3_Group 1	69.00	47.72	150.89	10
Time spent in DWC day 4_Group 1	77.40	45.06	142.51	10
Time spent in DWC day 5_Group 1	16.00	10.38	32.81	10
Time spent in DWC day 1_Group 2	39.70	26.64	84.25	10
Time spent in DWC day 2_Group 2	107.70	58.65	185.46	10
Time spent in DWC day 3_Group 2	141.50	60.99	192.87	10
Time spent in DWC day 4_Group 2	147.40	66.86	211.42	10
Time spent in DWC day 5_Group 2	47.40	24.43	77.24	10

Table 17b: Equality of variances time spent in compartment DWC day 1

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	1.64	0.4705

Table 17c: T-test time spent in compartment DWC day 1

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	0.50	0.6225
Satterthwaite	Unequel	16.992	0.50	0.6229

Table 17d: Equality of variances time spent in compartment DWC day 2

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	2.72	0.1528

Table 17e: T-test time spent in compartment DWC day 2

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	-0.80	0.4365
Satterthwaite	Unequel	14.837	-0.80	0.4386

Table 17f: Equality of variances time spent in compartment DWC day 3

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	1.63	0.4760

Table 17g: T-test time spent in compartment DWC day 3

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	-0.94	0.3615
Satterthwaite	Unequel	17.015	-0.94	0.3622

Table 17h: Equality of variances time spent in compartment DWC day 4

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	2.20	0.2555

Table 17i: T-test time spent in compartment DWC day 4

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	-0.87	0.3967
Satterthwaite	Unequel	15.779	-0.87	0.3983

Table 17j: Equality of variances time spent in compartment DWC day 5

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	5.54	0.0178

Table 17k: T-test time spent in compartment DWC day 5

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	-1.18	0.2521
Satterthwaite	Unequel	12.145	-1.18	0.2594

Comparing average performance and development of the 5 testing days between groups (GLM - repeated measures)

Table 18a: Group effect (between subjects analysis) latency compartment D (with conspecifics) day 1-4

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	1776.613	1776.613	0.01	0.9048
Error	18	2172.705.625	120705.868		

Table 18b: Session effect and group interaction (within subjects analysis) latency compartment D (with conspecifics) day 1-4

Source	DF	Type III SS	Mean square	F value	Pr > F
Session	3	92216.437	30738.812	1.36	0.2642
Session*Group	3	73186.638	24395.546	1.08	0.3650
Error	54	1218732.675	22569.124		

Table 19a: Group effect (between subjects analysis) time spent in compartment D (with conspecifics) day 1-4

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	384.564.500	384.564.500	0.77	0.3910
Error	18	8.959.017.500	497.723.194		

Table 19b: Session effect and group interaction (within subjects analysisitime spent in compartment D (with conspecifics) day 1-4

Source	DF	Type III SS	Mean square	F value	Pr > F
Session	3	470.059.000	156.686.333	1.03	0.3866
Session*Group	3	295.850.500	98.616.833	0.65	0.5874
Error	54	8.212.930.500	152.091.306		

<u>Compartment D (without conspecifics)</u>

Table 20a: Means latency compartment D (without conspecifics) day 1-5

Variable	Mean	Std. error	Std. deviatie	N
Lat. DNC day 1_Group 1	582.00	18.00	56.92	10
Lat. DNC day 2_Group 1	600.00	0.00	0.00	10
Lat. DNC day 3_Group 1	592.70	7.30	23.08	10
Lat. DNC day 4_Group 1	600.00	0.00	0.00	10
Lat. DNC day 5_Group 1	583.10	16.90	53.44	10
Lat. DNC day 1_Group 2	600.00	0.00	0.00	10
Lat. DNC day 2_Group 2	600.00	0.00	0.00	10
Lat. DNC day 3_Group 2	562.90	37.10	117.32	10
Lat. DNC day 4_Group 2	554.30	45.70	144.52	10
Lat. DNC day 5_Group 2	548.40	51.60	163.17	10

Comparing performance of groups per day (t-test)

Table 20b:Equality of variances latency compartment DNC day 1

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	.	<.0001

Table 20c: T-test latency compartment DNC day 1

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	-1.00	0.3306
Satterthwaite	Unequel	9	-1.00	0.3434

Table 20d:Equality of variances latency compartment DNC day 2

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	.	.

Table 20e: T-test latency compartment DNC day 2

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	.	.
Satterthwaite	Unequel	18	.	.

Table 20f:Equality of variances latency compartment DNC day 3

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	25.83	<.0001

Table 20g: T-test latency compartment DNC day 3

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	0.79	0.4409
Satterthwaite	Unequel	9.69	0.79	0.4495

Table 20h:Equality of variances latency compartment DNC day 4

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	Infty	<.0001

Table 20i: T-test latency compartment DNC day 4

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	1.00	0.3306
Satterthwaite	Unequel	9	1.00	0.3434

Table 20j:Equality of variances latency compartment DNC day 5

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	9.32	0.0027

Table 20k: T-test latency compartment DNC day 5

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	0.64	0.5308
Satterthwaite	Unequel	10.909	0.64	0.5360

Table 21a: Means time spent in compartment D (without conspecifics) day 1-5

Variable	Mean	Std. error	Std. deviatie	N
Time spent in DNC day 1_Group 1	1.40	1.40	4.43	10
Time spent in DNC day 2_Group 1	0.00	0.00	0.00	10
Time spent in DNC day 3_Group 1	0.90	0.90	2.85	10
Time spent in DNC day 4_Group 1	0.00	0.00	0.00	10
Time spent in DNC day 5_Group 1	3.20	3.20	10.12	10
Time spent in DNC day 1_Group 2	0.00	0.00	0.00	10
Time spent in DNC day 2_Group 2	0.00	0.00	0.00	10
Time spent in DNC day 3_Group 2	2.20	2.20	6.96	10
Time spent in DNC day 4_Group 2	12.40	12.40	39.21	10
Time spent in DNC day 5_Group 2	1.40	1.40	4.43	10

Table 21b: Equality of variances time spent in compartment DNC day 1

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	Infty	<.0001

Table 21c: T-test time spent in compartment DNC day 1

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	1.00	0.3306
Satterthwaite	Unequel	9	1.00	0.3434

Table 21d: Equality of variances time spent in compartment DNC day 2

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	.	.

Table 21e: T-test time spent in compartment DNC day 2

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	.	.
Satterthwaite	Unequel	18	.	.

Table 21f: Equality of variances time spent in compartment DNC day 3

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	5.98	0.0137

Table 21g: T-test time spent in compartment DNC day 3

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	-0.55	0.5912
Satterthwaite	Unequel	11.93	-0.55	0.5945

Table 21h: Equality of variances time spent in compartment DNC day 4

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	Infty	<.0001

Table 21i: T-test time spent in compartment DNC day 4

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	-1.00	0.3306
Satterthwaite	Unequel	9	-1.00	0.3434

Table 21j: Equality of variances time spent in compartment DNC day 5

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	5.22	0.0217

Table 21k: T-test time spent in compartment DNC day 5

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	0.52	0.6126
Satterthwaite	Unequel	12.324	0.52	0.6154

Comparing average performance and development of the 5 testing days between groups (GLM - repeated measures)

Table 22a: Group effect (between subjects analysis) latency compartment D (without conspecifics) day 1-4

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	41.328.125	41.328.125	0.44	0.5150
Error	18	1.686.584.250	93.699.125		

Table 22b: Session effect and group interaction (within subjects analysis) latency compartment D (without conspecifics) day 1-4

Source	DF	Type III SS	Mean square	F value	Pr > F
Session	3	73.122.375	24.374.125	0.74	0.5311
Session*Group	3	123.698.375	41.232.792	1.26	0.2984
Error	54	1.771.386.750	32.803.458		

Table 23a: Group effect (between subjects analysis) time spent in compartment D (without conspecifics) day 1-4

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	189.112.500	189.112.500	0.69	0.4162
Error	18	4.915.125.000	273.062.500		

Table 23b: Session effect and group interaction (within subjects analysis) time spent in compartment D (without conspecifics) day 1-4

Source	DF	Type III SS	Mean square	F value	Pr > F
Session	3	469.637.500	156.545.833	0.88	0.4574
Session*Group	3	597.937.500	199.312.500	1.12	0.3490
Error	54	9.608.175.000	177.929.167		

Open field test

Table 24a: Means open field group 1

Variable	Mean	Std error	Std deviatie	N
Latency_walk	27.700	8.501	26.883	10
Walked_squares_IC	25.000	8.575	27.117	10
Walked_squares_OC	27.000	7.643	24.171	10
Time spent walking	219.700	49.547	156.682	10
Tot. squares walked	52.000	15.912	50.317	10
Stresscalls	182.900	37.830	119.629	10

Table 24b: Means open field group 2

Variable	Mean	Std error	Std deviatie	N
Latency_walk	17.400	3.879	12.267	10
Walked_squares_IC	29.900	7.182	22.541	10
Walked_squares_OC	34.800	6.482	20.498	10
Time spent walking	301.900	42.754	135.201	10
Tot. squares walked	64.700	11.322	35.802	10
Stresscalls	153.500	20.931	66.190	10

Table 24c: Equality of variances latency walking

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	4.80	0.0286

Table 24d: T-test latency walking

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	1.10	0.2849
Satterthwaite	Unequel	12.6	1.10	0.2910

Table 24e: Equality of variances walked squares inner circle

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	1.45	0.5907

Table 24f: T-test walked squares inner circle

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	-0.44	0.6656
Satterthwaite	Unequel	17.4	-0.44	0.6658

Table 24g: Equality of variances walked squares outer circle

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	1.39	0.6313

Table 24h: T-test walked squares outer circle

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	-0.78	0.4465
Satterthwaite	Unequel	17.5	-0.78	0.4468

Table 24i: Equality of variances walking time

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	1.34	0.6675

Table 24j: T-test walking time

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	-1.26	0.2252
Satterthwaite	Unequel	17.6	-1.26	0.2255

Table 24k: Equality of variances squares walked

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	1.98	0.3251

Table 24l: T-test squares walked

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	-0.65	0.5237
Satterthwaite	Unequel	16.3	-0.65	0.5246

Table 24m: Equality of variances stress calls

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	3.27	0.0926

Table 24n: T-test squares stress calls

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	0.68	0.5051
Satterthwaite	Unequel	14	0.68	0.5076

Preference test

Reward A (live bait)

Latency to square A

Table 25a: Means latency square A day 1-3

Variable	Mean	Std. error	Std. deviatie	N
Lat. Sq. A_ day 1_ Group 1	172.80	62.88	198.84	10
Lat. Sq. A_ day 2_ Group 1	176.10	61.91	195.78	10
Lat. Sq. A_ day 3_ Group 1	161.30	74.82	236.61	10
Lat. Sq. A_ day 1_ Group 2	155.10	52.55	166.17	10
Lat. Sq. A_ day 2_ Group 2	248.50	62.71	198.30	10
Lat. Sq. A_ day 3_ Group 2	134.40	60.41	191.03	10

Comparing performance of groups per day (t-test)

Table 25b: Equality of variances latency square A day 1

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	0.22	0.8314
Satterthwaite	Unequel	17.45	0.22	0.8315

Table 25c: T-test latency square A day 1

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	1.43	0.6014

Table 25d: Equality of variances latency square A day 2

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	1.03	0.9702

Table 25e: T-test latency square A day 2

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	-0.82	0.4221
Satterthwaite	Unequel	17.997	-0.82	0.4221

Table 25f: Equality of variances latency square A day 3

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	1.53	0.5339

Table 25g: T-test latency square A day 3

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	0.28	0.7829
Satterthwaite	Unequel	17.234	0.28	0.7830

Comparing average performance and development of the 5 testing days between groups (GLM - repeated measures)

Table 26a: Group effect (between subject analysis) latency to square A

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	1288.0667	12880.667	0.02	0.8805
Error	18	996789.2000	55377.1778		

Table 26b: Session effect and group interaction (within subjects analysis) latency to square A

Source	DF	Type III SS	Mean square	F value	Pr > F
Session	2	45004.900	22502.450	0.71	0.4977
Session*Group	2	30105.233	15052.617	0.48	0.6252
Error	36	1138743.200	31631.756		

<u>Time spent eating reward A</u>

Table 27a: Means time spent eating reward A

Variable	Mean	Std. error	Std. deviatie	N
Time spent eating reward A_ day 1_ Group 1	0.20	0.20	0.63	10
Time spent eating reward A_ day 2_ Group 1	13.60	6.08	19.24	10
Time spent eating reward A_ day 3_ Group 1	22.60	15.13	47.84	10
Time spent eating reward A_ day 1_ Group 2	0.00	0.00	0.00	10
Time spent eating reward A_ day 2_ Group 2	3.50	2.53	7.99	10
Time spent eating reward A_ day 3_ Group 2	5.20	2.77	8.77	10

Comparing performance of groups per day (t-test)

Table 27b: Equality of variances time spent eating reward A day 1

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	Infty	<.0001

Table 27c: T-test time spent eating reward A day 1

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	1.00	0.3306
Satterthwaite	Unequel	9	1.00	0.3434

Table 27d: Equality of variances time spent eating reward A day 2

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	5.80	0.0152

Table 27e: T-test time spent eating reward A day 2

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	1.53	0.1427
Satterthwaite	Unequel	12.014	1.53	0.1512

Table 27f: Equality of variances time spent eating reward A day 3

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	29.78	<.0001

Table 27g: T-test time spent eating reward A day 3

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	1.13	0.2727
Satterthwaite	Unequel	96.037	1.13	0.2854

Comparing average performance and development of the 3 testing days between groups (GLM - repeated measures)

Table 28a: Group effect (between subject analysis) time spent eating reward A

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	1278.816667	1278.816667	3.05	0.0978
Error	18	75501.66667	419.453704		

Table 28b: Session effect and group interaction (within subject analysis) time spent eating reward A

Source	DF	Type III SS	Mean square	F value	Pr > F
Session	2	1936.43333	968.21667	1.98	0.1535
Session*Group	2	745.23333	372.61667	0.76	0.4750
Error	36	17648.33333	490.23148		

Worms eaten reward A

Table 29a: Means worms eaten reward A (day 1 = 1 worm, day 2 and 3 = 2 worms)

Variable	Mean	Std. error	Std. deviatie	N
Worms eaten reward A_ day 1_ Group 1	0.10	0.10	0.32	10
Worms eaten reward A_ day 2_ Group 1	0.70	0.30	0.95	10
Worms eaten reward A_ day 3_ Group 1	1.00	0.33	1.05	10
Worms eaten reward A_ day 1_ Group 2	0.10	0.10	0.32	10
Worms eaten reward A_ day 2_ Group 2	0.40	0.27	0.84	10
Worms eaten reward A_ day 3_ Group 2	0.80	0.33	1.03	10

Comparing performance of groups per day (t-test)

Table 29b: Equality of variances worms eaten reward A day 1

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	1.00	1.00

Table 29c: T-test worms eaten reward A day 1

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	0.00	1.00
Satterthwaite	Unequel	18	0.00	1.00

Table 29d: Equality of variances worms eaten reward A day 2

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	1.27	0.7314

Table 29e: T-test worms eaten reward A day 2

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	0.75	0.4645
Satterthwaite	Unequel	17.756	0.75	0.4646

Table 29f: Equality of variances worms eaten reward A day 3

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	1.04	0.9525

Table 29g: T-test worms eaten reward A day 3

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	0.43	0.6733
Satterthwaite	Unequel	17.993	0.43	0.6733

Comparing average performance and development of the 3 testing days between groups (GLM - repeated measures)

Table 30a: Group effect (between subject analysis) worms eaten reward A day 1-3

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	0.41666667	0.41666667	0.36	0.5535
Error	18	20.56666667	1.14259259		

Table 30b: Session effect and group interaction (within subject analysis) worms eaten reward A day 1-3

Source	DF	Type III SS	Mean square	F value	Pr > F
Session	2	6.43333333	3.21666667	7.55	0.0018
Session*Group	2	0.23333333	0.11666667	0.27	0.7620
Error	36	15.33333333	0.42592593		

Pecking frequency toward eating reward A

Table 31a: Means pecking frequency toward eating A

Variable	Mean	Std. error	Std. deviatie	N
Pecking frequency toward eating A_ day 1_ Group 1	0.20	0.13	0.42	10
Pecking frequency toward eating A_ day 2_ Group 1	4.10	1.75	5.55	10
Pecking frequency toward eating A_ day 3_ Group 1	13.10	8.29	26.21	10
Pecking frequency toward eating A_ day 1_ Group 2	0.60	0.50	1.58	10
Pecking frequency toward eating A_ day 2_ Group 2	1.70	1.32	4.16	10
Pecking frequency toward eating A_ day 3_ Group 2	2.50	1.19	3.78	10

Comparing performance of groups per day (t-test)

Table 31b: Equality of variances pecking frequency toward eating reward A day 1

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	14.00	0.0006

Table 31c: T-test frequency toward eating reward A day 1

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	-0.77	0.4486
Satterthwaite	Unequel	10.279	-0.77	0.4560

Table 31d: Equality of variances pecking frequency toward eating reward A day 2

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	1.77	0.4061

Table 31e: T-test frequency toward eating reward A day 2

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	1.09	0.2883
Satterthwaite	Unequel	16.7	1.09	0.2894

Table 31f: Equality of variances pecking frequency toward eating reward A day 3

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	48.12	<.0001

Table 31g: T-test frequency toward eating reward A day 3

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	1.27	0.2217
Satterthwaite	Unequel	93.739	1.27	0.2361

Comparing average performance and development of the 3 testing days between groups (GLM - repeated measures)

Table 32a: Group effect (between subject analysis) pecking frequency toward eating reward A day 1-3

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	264.600000	264.600000	2.13	0.1615
Error	18	2234.666667	124.148148		

Table 32b: Session effect and group interaction (within subject analysis) pecking frequency toward eating reward A day 1-3

Source	DF	Type III SS	Mean square	F value	Pr > F
Session	2	566.800000	283.400.000	2.25	0.1200
Session*Group	2	326.800000	163.400.000	1.30	0.2857
Error	36	4533.733333	125.937037		

Time spent in square A

Table 33a: Means time spent in square A day 1-3

Variable	Mean	Std. error	Std. deviatie	N
Time spent in square A_ day 1_ Group 1	98.30	29.20	92.33	10
Time spent in square A_ day 2_ Group 1	115.30	42.37	133.99	10
Time spent in square A_ day 3_ Group 1	143.40	32.90	104.03	10
Time spent in square A_ day 1_ Group 2	65.20	17.07	53.99	10
Time spent in square A_ day 2_ Group 2	99.50	25.16	79.57	10
Time spent in square A_ day 3_ Group 2	104.10	21.60	68.32	10

Comparing performance of groups per day (t-test)

Table 33b: Equality of variances time spent in square A day 1

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	2.93	0.1256

Table 33c: T-test time spent in square A day 1

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	0.98	0.3407
Satterthwaite	Unequel	14.51	0.98	0.3438

Table 33d: Equality of variances time spent in square A day 2

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	2.84	0.1364

Table 33e: T-test time spent in square A day 2

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	0.32	0.7522
Satterthwaite	Unequel	14.645	0.32	0.7530

Table 33f: Equality of variances time spent in square A day 3

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	2.32	0.2262

Table 33g: T-test time spent in square A day 3

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	1.00	0.3312
Satterthwaite	Unequel	15.545	1.00	0.3333

Comparing average performance and development of the 3 testing days between groups (GLM - repeated measures)

Table 34a: Group effect (between subject analysis) time spent in square A

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	12965.4000	12965.4000	0.96	0.3410
Error	18	243989.8667	135549.926		

Table 34b: Session effect and group interaction (within subjects analysis) time spent in square A

Source	DF	Type III SS	Mean square	F value	Pr > F
Session	2	17928.3000	89641.500	1.49	0.2395
Session*Group	2	14833.000	741.6500	0.12	0.8548
Error	36	216927.7333	6025.7704		

<u>Reward B (dead bait)</u>

<u>Latency square B</u>

Table 35a: Means latency square B day 1-3

Variable	Mean	Std. error	Std. deviatie	N
Lat. Sq. B_ day 1_ Group 1	112.80	48.97	154.86	10
Lat. Sq. B_ day 2_ Group 1	180.20	77.92	246.41	10
Lat. Sq. B_ day 3_ Group 1	180.60	79.15	250.30	10
Lat. Sq. B_ day 1_ Group 2	104.10	55.83	176.53	10
Lat. Sq. B_ day 2_ Group 2	220.90	82.70	261.51	10
Lat. Sq. B_ day 3_ Group 2	112.60	54.43	172.12	10

Comparing performance of groups per day (t-test)

Table 35b: Equality of variances latency square B day 1

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	1.30	0.7027

Table 35c: T-test latency square B day 1

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	0.12	0.9080
Satterthwaite	Unequel	17.7	0.12	0.9081

Table 35d: Equality of variances latency square B day 2

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	1.13	0.8622

Table 35e: T-test latency square B day 2

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	-0.36	0.7244
Satterthwaite	Unequel	17.937	-0.36	0.7244

Table 35f: Equality of variances latency square B day 3

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	2.11	0.2799

Table 35g: T-test latency square B day 3

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	0.71	0.4881
Satterthwaite	Unequel	15.956	0.71	0.4892

Comparing average performance and development of the 3 testing days between groups (GLM - repeated measures)

Table 36a: Group effect (between subjects analysis) latency square B day 1-3

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	2160.000	2160.000	0.03	0.8665
Error	18	1337221.600	74290.089		

Table 36b: Session effect and group interaction (within subjects analysis) latency square B day 1-3

Source	DF	Type III SS	Mean square	F value	Pr > F
Session	2	85656.233	42828.117	1.34	0.2749
Session*Group	2	29620.900	14810.450	0.46	0.6331
Error	36	1151544.200	31987.339		

Time spent eating reward B

Table 37a: Means time spent eating reward B

Variable	Mean	Std. error	Std. deviatie	N
Time spent eating reward B_ day 1_ Group 1	0.40	0.40	1.26	10
Time spent eating reward B_ day 2_ Group 1	4.90	3.11	9.85	10
Time spent eating reward B_ day 3_ Group 1	19.20	11.51	36.39	10
Time spent eating reward B_ day 1_ Group 2	0.00	0.00	0.00	10
Time spent eating reward B_ day 2_ Group 2	6.20	3.56	11.25	10
Time spent eating reward B_ day 3_ Group 2	8.60	4.00	12.65	10

Comparing performance of groups per day (t-test)

Table 37b: Equality of variances time spent eating reward B day 1

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	Infty	<.0001

Table 37c: T-test time spent eating reward B day 1

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	1.00	0.3306
Satterthwaite	Unequel	9	1.00	0.3434

Table 37d: Equality of variances time spent eating reward B day 2

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	1.31	0.6977

Table 37e: T-test time spent eating reward B day 2

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	-0.27	0.7865
Satterthwaite	Unequel	17.689	-0.27	0.7866

Table 37f: Equality of variances time spent eating reward B day 3

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	8.27	0.0043

Table 37g: T-test time spent eating reward B day 3

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	0.87	0.3957
Satterthwaite	Unequel	11.144	0.87	0.4026

Comparing average performance and development of the 3 testing days between groups (GLM - repeated measures)

Table 38a: Group effect (between subject analysis) time spent eating reward B

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	156.816667	156.816667	0.53	0.4751
Error	18	5303.366667	294.631481		

Table 38b: Session effect and group interaction (within subjects analysis) time spent eating reward B

Source	DF	Type III SS	Mean square	F value	Pr > F
Session	2	1906.90.000	953.45000	3.40	0.0442
Session*Group	2	414.23333	207.11667	0.74	0.4844
Error	36	10081.53333	280.04259		

Worms eaten reward B

Table 39a: Means worms eaten reward B day 1-3

Variable	Mean	Std. error	Std. deviatie	N
Worms eaten reward B_ day 1_ Group 1	0.10	0.10	0.32	10
Worms eaten reward B_ day 2_ Group 1	0.60	0.31	0.97	10
Worms eaten reward B_ day 3_ Group 1	1.00	0.33	1.05	10
Worms eaten reward B_ day 1_ Group 2	0.00	0.00	0.00	10
Worms eaten reward B_ day 2_ Group 2	0.60	0.31	0.97	10
Worms eaten reward B_ day 3_ Group 2	0.80	0.33	1.03	10

Comparing performance of groups per day (t-test)

Table 39b: Equality of variances worms eaten reward B day 1

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	Infty	<.0001

Table 39c: T-test worms eaten reward B day 1

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	1.00	0.3306
Satterthwaite	Unequel	9	1.00	0.3434

Table 39d: Equality of variances worms eaten reward B day 2

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	1.00	10.000

Table 39e: T-test worms eaten reward B day 2

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	0.00	1.00
Satterthwaite	Unequel	18	0.00	1.00

Table 39f: Equality of variances worms eaten reward B day 3

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	1.04	0.9525

Table 39g: T-test worms eaten reward B day 3

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	0.43	0.6733
Satterthwaite	Unequel	17.993	0.43	0.6733

Comparing average performance and development of the 3 testing days between groups (GLM - repeated measures)

Table 40a: Group effect (between subjects analysis) worms eaten reward B day 1-3

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	0.15000000	0.15000000	0.13	0.7187
Error	18	20.16666667	1.12037037		

Table 40b: Session effect and group interaction (within subjects analysis) worms eaten reward B day 1-3

Source	DF	Type III SS	Mean square	F value	Pr > F
Session	2	7.43333.333	3.71666667	7.81	0.0015
Session*Group	2	0.10000000	0.05000000	0.11	0.9005
Error	36	17.13333333	0.47592593		

Pecking frequency toward eating reward B

Table 41a: Means pecking frequency toward eating reward B

Variable	Mean	Std. error	Std. deviatie	N
Pecking frequency toward eating B_ day 1_ Group 1	0.20	0.13	0.42	10
Pecking frequency toward eating B_ day 2_ Group 1	1.30	0.67	2.11	10
Pecking frequency toward eating B_ day 3_ Group 1	6.80	4.17	13.18	10
Pecking frequency toward eating B_ day 1_ Group 2	0.20	0.20	0.63	10
Pecking frequency toward eating B_ day 2_ Group 2	4.30	2.46	7.79	10
Pecking frequency toward eating B_ day 3_ Group 2	3.60	1.61	5.08	10

Comparing performance of groups per day (t-test)

Table 41b: Equality of variances pecking frequency toward eating reward B day 1

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	2.25	0.2428

Table 41c: T-test pecking frequency toward eating reward B day 1

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	0.00	1.00
Satterthwaite	Unequel	15.68	0.00	1.00

Table 41d: Equality of variances pecking frequency toward eating reward B day 2

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	13.62	0.0006

Table 41e: T-test pecking frequency toward eating reward B day 2

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	-1.18	0.2551
Satterthwaite	Unequel	10.315	-1.18	0.2662

Table 41f: Equality of variances pecking frequency toward eating reward B day 3

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	6.73	0.0090

Table 41g: T-test pecking frequency toward eating reward B day 3

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	0.72	0.4830
Satterthwaite	Unequel	11.618	0.72	0.4879

Comparing average performance and development of the 3 testing days between groups (GLM - repeated measures)

Table 42a: Group effect (between subject analysis) Pecking frequency toward eating reward B day 1-3

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	0.0666667	0.0666667	0.00	0.9711
Error	18	887.6666667	49.3148148		

Table 42b: Session effect and group interaction (within subject analysis) pecking frequency toward eating reward B day 1-3

Source	DF	Type III SS	Mean square	F value	Pr > F
Session	2	250.133.333	125.066667	3.00	0.0623
Session*Group	2	96.133.333	48.066667	1.15	0.3268
Error	36	149.9733333	41.659259		

Time spent in square B

Table 43a: Means time spent in square B

Variable	Mean	Std. error	Std. deviatie	N
Time spent in square B_ day 1_ Group 1	85.80	22.78	72.03	10
Time spent in square B_ day 2_ Group 1	106.00	38.28	121.05	10
Time spent in square B_ day 3_ Group 1	76.10	22.44	70.97	10
Time spent in square B_ day 1_ Group 2	95.30	23.08	72.99	10
Time spent in square B_ day 2_ Group 2	111.20	33.66	106.44	10
Time spent in square B_ day 3_ Group 2	148.60	29.45	93.13	10

Comparing performance of groups per day (t-test)

Table 43b: Equality of variances time spent in square B day 1

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	1.03	0.9691

Table 43c: T-test time spent in square B day 1

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	-0.29	0.7729
Satterthwaite	Unequel	17.997	-0.29	0.7729

Table 43d: Equality of variances time spent in square B day 2

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	1.29	0.7077

Table 43e: T-test time spent in square B day 2

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	-0.10	0.9199
Satterthwaite	Unequel	17.71	-0.10	0.9199

Table 43f: Equality of variances time spent in square B day 3

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	1.72	0.4305

Table 43g: T-test time spent in square B day 3

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	-1.96	0.0659
Satterthwaite	Unequel	16.816	-1.96	0.0670

Comparing average performance and development of the 3 testing days between groups (GLM - repeated measures)

Table 44a: Group effect (between subject analysis) time spent in square B day 1-3

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	12673.0667	12673.0667	0.92	0.3511
Error	18	248898.6000	13827.7000		

Table 44b: Session effect and group interaction (within subjects analysis) time spent in square B day 1-3

Source	DF	Type III SS	Mean square	F value	Pr > F
Session	2	5434.0333	2717.0167	0.48	0.6215
Session*Group	2	14194.6333	7097.3167	1.26	0.2962
Error	36	202970.0000	5638.0556		

<u>Time spent in empty squares</u>

Table 45a: Means time spent in empty squares day 1-3

Variable	Mean	Std. error	Std. deviatie	N
Time spent in empty squares_ day 1_ Group 1	359.30	56.23	177.81	10
Time spent in empty squares_ day 2_ Group 1	271.60	54.80	173.29	10
Time spent in empty squares_ day 3_ Group 1	176.60	31.16	98.52	10
Time spent in empty squares_ day 1_ Group 2	348.00	38.93	123.12	10
Time spent in empty squares_ day 2_ Group 2	234.00	52.58	166.26	10
Time spent in empty squares_ day 3_ Group 2	256.40	46.03	145.55	10

Comparing performance of groups per day (t-test)

Table 45b: Equality of variances time spent in the empty squares day 1

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	2.09	0.2885

Table 45c: T-test time spent in the empty squares day 1

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	0.17	0.8706
Satterthwaite	Unequel	16.017	0.17	0.8708

Table 45d: Equality of variances time spent in the empty squares day 2

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	1.09	0.9039

Table 45e: T-test time spent in the empty squares day 2

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	0.50	0.6265
Satterthwaite	Unequel	17.969	0.50	0.6265

Table 45f: Equality of variances time spent in the empty squares day 3

Method	Num DF	Den DF	F value	Pr > F
Folded F	9	9	2.18	0.2606

Table 45g : T-test time spent in the empty squares day 3

Method	Variances	DF	t value	Pr > |t|
Pooled	Equal	18	-1.44	0.1682
Satterthwaite	Unequel	15.817	-1.44	0.1705

Comparing average performance and development of the 3 testing days between groups (GLM - repeated measures)

Table 46a: Group effect (between subject analysis) time spent in empty squares day 1-3

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	1591.3500	1591.3500	0.04	0.8461
Error	18	738855.6333	41047.5352		

Table 46b: Session effect and group interaction (within subjects analysis) time spent in empty squares day 1-3

Source	DF	Type III SS	Mean square	F value	Pr > F
Session	2	201990.2333	100995.1167	7.59	0.0018
Session*Group	2	37956.1000	18978.0500	1.43	0.2536
Error	36	479179.6667	13310.5463		

Voluntary approach and Human recognition test

Table 47a: Means variables voluntary approach and recognition test group 1 (beak trimmed)

Variable	Mean	Std error	Std deviatie	N
Latency square_researcher 1	25.900	10.965	34.674	10
Latency square_researcher 2	5.400	2.300	7.275	10
Time spent in square_researcher 1	115.700	15.851	50.126	10
Time spent in square_researcher 2	135.400	16.522	52.247	10
Latency worm approach_researcher 1	95.800	57.305	181.215	10
Latency worm approach_researcher 2	23.200	17.467	55.238	10
Latency pick up worm_ reseacher 1	104.000	57.361	181.393	10
Latency pick up worm_ researcher 2	28.600	17.456	55.201	10

Table 47b: Means variables voluntary approach and recognition test group 2 (intact beaks)

Variable	Mean	Std error	Std deviatie	N
Latency square_researcher 1	42.900	12.252	38.745	10
Latency square_researcher 2	18.200	12.133	38.368	10
Time spent in square_researcher 1	81.100	16.077	50.840	10
Time spent in square_researcher 2	125.600	13.187	41.703	10
Latency worm approach_researcher 1	114.300	56.298	178.031	10
Latency worm approach_researcher 2	33.700	16.341	51.676	10
Latency pick up worm_ reseacher 1	177.800	72.769	230.115	10
Latency pick up worm_ researcher 2	80.200	58.778	185.874	10

Table 47c: Group effect (between subjects analysis) latency to enter square with researcher and reward

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	2220.10000	2220.10000	2.37	0.1413
Error	18	16880.50000	937.80556		

Table 47d: Session effect and group interaction (within subjects analysis) latency to enter square with researcher and reward

Source	DF	Type III SS	Mean square	F value	Pr > F
Session	1	5107.60000	5107.60000	4.34	0.0517
Session*Group	1	44.10000	44.10000	0.04	0.8487
Error	18	21177.30000	1176.51667		

Table 47e: Group effect (between subjects analysis) time spent in square with researcher

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	4928.40000	4928.40000	1.15	0.2985
Error	18	77395.50000	4299.75000		

Table 47f: Session effect and group interaction (within subjects analysis) time spent in square with researcher

Source	DF	Type III SS	Mean square	F value	Pr > F
Session	1	10304.10.000	10304.10000	21.31	0.0002
Session*Group	1	1537.60000	1537.60000	3.18	0.0914
Error	18	8702.30000	483.46111		

Table 47g: Group effect (between subjects analysis) latency approaching reward

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	2102.5000	2102.5000	0.08	0.7788
Error	18	465412.0000	25856.2222		

Table 47h: Session effect and group interaction (within subjects analysis) latency approaching reward

Source	DF	Type III SS	Mean square	F value	Pr > F
Session	1	58675.6000	58675.6000	6.33	0.0216
Session*Group	1	160.0000	160.0000	0.02	0.8969
Error	18	16689.34000	9271.8556		

Table 47i: Group effect (between subjects analysis) latency approaching reward

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	39000.0250	39000.0250	0.75	0.3986
Error	18	938941.4500	52163.4139		

Table 47j: Session effect and group interaction (within subjects analysis) latency approaching reward

Source	DF	Type III SS	Mean square	F value	Pr > F
Session	1	75255.6250	75255.6250	7.87	0.0117
Session*Group	1	1177.2250	1177.2250	0.12	0.7298
Error	18	172138.6500	9563.2583		

Weight gain:

Table 48a: Group 1 (trimmed beaks)

Animal Group 1	Age in days (+date) 7 (31-03-10)	8 (01-04-10)	13 (06-04-10)	15 (08-04-10)	19 (12-04-10)	21 (14-04-10)	23 (16-04-10)	27 (20-04-10)	30 (23-04-10)
1	84	90	134	153	198	218	243	288	322
2	77	83	129	150	193	214	243	287	323
3	83	90	132	148	179	187	202	255	300
4	70	73	117	136	169	182	212	262	291
5	66	70	100	116	149	167	188	222	254
6	87	87	124	141	174	202	221	267	301
7	75	80	126	141	194	220	245	295	342
8	78	84	127	148	196	216	238	287	329
9	85	87	131	142	175	194	215	255	283
10	73	78	118	134	176	194	217	233	308
AVE	78	82	124	141	180	199	222	265	305
SEM	2,2	2,2	3,2	3,4	4,8	5,6	6,1	7,8	8,1

Table 48b: Group 2 (intact beaks)

Animal Group 2	Age in days (+date) 7 (31-03-10)	8 (01-04-10)	13 (06-04-10)	15 (08-04-10)	19 (12-04-10)	21 (14-04-10)	23 (16-04-10)	27 (20-04-10)	30 (23-04-10)
1	83	87	137	159	204	236	261	318	364
2	78	86	128	152	198	222	243	300	346
3	89	95	139	162	208	235	257	311	359
4	76	81	120	143	187	215	238	284	321
5	82	88	135	154	203	231	255	304	342
6	86	92	136	158	202	226	260	375	349
7	90	98	155	182	238	268	308	368	420
8	89	90	139	157	206	230	257	310	359
9	83	87	137	154	202	226	259	313	361
10	89	90	134	150	199	221	243	293	332
AVE	85	89	136	157	205	231	258	318	355
SEM	1,6	1,5	2,8	3,2	4,1	4,6	6,1	9,5	8,4

Table 49a: Group effect (between subjects analysis) weight gain

Source	DF	Type III SS	Mean square	F value	Pr > F
Group	1	31073.47222	31073.47222	18.65	0.0004
Error	18	29993.74444	1666.31914		

Table 49b: Session effect and group interaction (within subjects analysis) weight gain

Source	DF	Type III SS	Mean square	F value	Pr > F
Age	8	1222358.744	152794.843	1382.66	<.0001
Age*group	8	12089.878	1511.235	13.68	<.0001
Error	144	15913.156	110.508		