Behavioural consequences of visual deprivation occurring at hatch or in the early life of chickens

Citation for published version:
Hocking, PM, Haldane, KA, Davidson, EM, Sandøe, P & Kristensen, HH 2015, 'Behavioural consequences of visual deprivation occurring at hatch or in the early life of chickens' Applied Animal Behaviour Science, vol. 172, pp. 33-43. DOI: 10.1016/j.applanim.2015.08.004

Digital Object Identifier (DOI):
10.1016/j.applanim.2015.08.004

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Peer reviewed version

Published In:
Applied Animal Behaviour Science

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.
Behavioural consequences of visual deprivation occurring early or later in the life of chickens

Paul M. Hocking\textsuperscript{a}, Kirsty-Anne Haldane\textsuperscript{b}, Emma M. Davidson\textsuperscript{c}, Peter Sandøe\textsuperscript{d} and Helle H. Kristensen\textsuperscript{e}

\textsuperscript{a} Roslin Institute and R(D)SVS, University of Edinburgh, Roslin, Easter Bush, Midlothian, EH25 9RG, Scotland, UK. Email: paul.hocking@roslin.ed.ac.uk

\textsuperscript{b} Roslin Institute and R(D)SVS, University of Edinburgh, Easter Bush, Midlothian, EH25 9RG, Scotland, UK. Email: kirstyhaldane@hotmail.com

\textsuperscript{c} Roslin Institute and R(D)SVS, University of Edinburgh, Easter Bush, Midlothian, EH25 9RG, Scotland, UK. Email: emma_marie_davidson@hotmail.co.uk

\textsuperscript{d} Department of Large Animal Sciences and Department of Food and Resource Economics, University of Copenhagen, Rolighedsvej 25, 1958 Frederiksberg C, Denmark. Email: pes@sund.ku.dk

\textsuperscript{e} Department of Large Animal Sciences, University of Copenhagen, Grønnegårdsvej 8, 1870 Frederiksberg, Denmark. Email: hek@sund.ku.dk

Corresponding author at: Dr P. M. Hocking, Roslin Institute and R(D)SVS, University of Edinburgh, Easter Bush, Midlothian, EH25 9RG, Scotland, UK. Phone: +44 (0) 131 651 9182. Fax: +44 (0) 131 9218. E-mail: paul.hocking@roslin.ed.ac.uk
The development of behaviour in a line of chickens that are born sighted (rdd) but turn blind after hatching was compared with a line that is blind at hatch (beg) and with sighted White Leghorn controls (WL) to test the hypothesis that birds that become blind later in their life will show intermediate behavioural disturbances compared to animals that are blind from hatch. Individual behaviour, group aggregation and behavioural synchrony were compared at 1, 5 and 9 weeks of age (experiment 1) and in the parents of these chicks at 9-13 months of age (experiment 2). Responses to visual and physical isolation were assessed at 1, 5 and 9 weeks of age.

Analyses of home-pen behaviour showed that rdd and beg had difficulty locating or consuming food. WL and rdd did not engage in abnormal behaviour at 1, 5 and 9 weeks of age whereas both beg and rdd adults did so. At 9 weeks of age beg and rdd birds showed decreased behavioural synchrony compared with WL, whereas group aggregation in rdd and WL was similar and higher than in beg. WL adults showed increased environmental pecking and higher rates of behavioural synchrony and group aggregation than both beg and rdd.

Under visual isolation from conspecifics rdd behaved like blind birds in some respects (e.g. decreased movement) and as sighted birds in others (e.g. peeping). Contrary to previous reports, the vision of rdd was apparently diminished compared with sighted controls (WL) at all ages.

It was concluded that abnormal behaviours are a response to a complete loss of vision regardless of initial sight. Birds that became blind during rearing (rdd) may be more active as adults than birds that were blind throughout life but in general the behaviour of blind birds was similar regardless of early sight.

Key words: chicken; blindness; sight; social isolation; behaviour
1. Introduction

Vision is important in birds and their highly specialised visual systems mediate the majority of their behaviours including feeding and drinking, navigation and social behaviour including dominance and courtship (Collins et al., 2011). Chickens have particularly well developed visual abilities and vision is thought to play a crucial role in the acquisition, development and maintenance of a number of normal behaviours through the use of visual cues (Mench and Keeling, 2001; Prescott et al., 2003). Lack of vision is therefore likely to compromise the development of their behaviour due to the absence of environmental sensory stimulation, and their ability to express key behaviours. A line of chickens with the genotype \textit{beg} (blindness, enlarged globe) are blind from hatch and show less group aggregation and behavioural synchrony than sighted controls (Collins et al., 2011). Furthermore the blind birds exhibited abnormal behaviours, grew less well than sighted counterparts and tended to have greater mortality (Collins et al., 2011).

Several issues regarding the importance of sight for the development of behaviour remain to be clarified: do the differences between the lines observed in young chicks, remain in adult birds? Can the results on abnormal and social behaviour be generalized to a genotype that develops blindness later in life? Can the problems of poor growth and higher mortality be ameliorated by the possession of sight at a young age and before becoming blind later in life?

The main aim of the current study was to investigate in detail the behaviour of chickens from the retinal dysplasia and degeneration (\textit{rdd}) genotype that were sighted at hatch and become progressively blind until there is complete loss of vision at sexual maturity (Randall et al., 1983), and to determine how their behaviour changed once they lost their sight, compared with chickens from a genotype that were blind from hatch (\textit{beg}) and normal sighted chickens. Our hypothesis is that birds that become blind later in their life will show intermediate behavioural disturbances compared to animals that are blind from hatch.
Chicks from the three lines were reared in separate pens to record behaviour in the home pen. Behavioural synchrony and group aggregation and a test of the effects of visual and physical isolation were also investigated. Body weight gain, mortality and heterophil-lymphocyte (HL) ratios were determined as proxy measures of essential functions behaviours and the welfare of blind birds. Stressors elevate the number of heterophils and depress the number of lymphocytes and HL ratios are less variable and longer lasting than corticosterone responses (Gross and Siegel, 1983). In a second experiment, adult females and males were moved from individual breeding cages into floor pens containing birds of the same line and gender. Behavioural observations, behavioural synchrony and social aggregation were recorded to compare the behaviour of the three lines when rdd were unequivocally blind.

2. Methods

2.1 Experiment 1: chicks 0 - 9 weeks of age

2.1.1 Animals and housing

A total of 72 White Leghorn-type birds of unknown gender were used for the experiment consisting of 24 White Leghorn (WL), 24 beg and 24 rdd. The WL chickens have normal sight and are genetically related to both beg and rdd by back crossing WL males into of the original lines.

The chicks were wing-banded and randomly allocated to one of 12 pens over two rooms after hatching. Each pen was 1.52 m wide and 2.10 m long with solid pen walls to 0.6 m and wire to 2.0 m. The aisle between the two blocks of pens was 1.60 m and there were two blocks of three pens in both rooms. Each pen contained 6 birds of one of the three lines. The birds were identified by wing-bands and given a unique symbol on the head and back.
with a black marker pen for ease of overhead identification during the observation period.

Birds were penned with those of the same genotype but the gender was not known until the completion of the experiment when the gender of the birds was determined retrospectively by visual inspection of size and color of the comb and wattles.

There were a number of spare birds of each line that were kept in room 1 in an extra pen, under the same conditions as the study birds, in case replacement was required. In the first 7 days, 7 **beg** chicks and 1 **rdd** chick died, and, in week 2, one **WL** chick died. These birds were replaced with randomly selected chicks of the same line from the stock of extra chicks.

The birds had *ad libitum* access to food and water in the home pens. They were fed a standard layer chick crumb from hatch to 6 weeks of age followed by a pelleted grower feed. The food was provided in large, shallow chick pans on the floor of each pen throughout the experiment to minimise the risk that feeding in the **beg** chicks might be compromised by the change to a hanging tube feeder. Water was available from bell drinkers placed on the floor and later from larger suspended drinkers. The temperature of the rooms was 29˚C at hatch and was gradually decreased to 22˚C at 4 weeks of age. Heat lamps were used to provide extra warmth until the chicks were 4 weeks of age. The photoperiod was 14 hours per day from 07:00 - 21:00 h. The average light intensity at chick eye level in room 1 was 77 lux, and 50 lux in room 2.

Since it is known that newly hatched **beg** chicks often have trouble locating food (Pollock et al., 1982), for the first week, the **beg** chicks were assisted to find feed and water and some were fed and watered by hand. Curved hardboard surrounds were also placed at the back corners of the pens to encourage the chicks towards the food and water.

*2.1.2. Home pen behaviour*
Video cameras were positioned above each pen to record the birds’ behaviour. There were 6 VCRs and video recording was conducted over two days of one block in each room followed by the other block on the subsequent day at 1, 5 and 9 weeks of age. On each day, the pens were recorded for three 1-hour periods from 09:00-10:00 h, 13:00-14:00 h and 17:00-18:00 h. From the video tapes, 5-minute instantaneous scan sampling (Martin and Bateson, 1993, pp 85-86) was used to record the frequency of behaviours (%) in the pens by one person, who was blind to the treatment, using the ethogram in Table 1. To quantify group aggregation, mean nearest neighbour distance (NND; (2009)) was recorded at successive 5-minute intervals using digital callipers to measure the distance (mm) between each bird and the nearest part of its closest pen mate on the video monitor screen. Behavioural synchrony (Bs) was calculated at each interval using Simpson’s Diversity Index (King and Cowlishaw, 2009) as follows for each behavioural scan:

\[ Bs = \frac{\sum n_i(n_i - 1)}{N(N - 1)} \]

where \( n_i \) is the number of individuals exhibiting the \( i \)th behaviour and \( N \) is the total number of individuals visible in the scan.

Since some of the behaviours were observed to be performed by the chicks very infrequently, the behavioural categories were merged into 9 classes: feeding, drinking, sitting, standing, walking and running, environmental pecking, preening, abnormal (circle walking, air pecking, star gazing), and other (lie, gentle feather peck, severe feather peck, aggressive peck, dust bathe, stretch, chase and display). Mean time spent carrying out each behaviour was calculated for each pen, time period and week, and expressed as a proportion of the birds in view.

2.1.3 Social Isolation
The social isolation tests were performed as described by Collins et al., (2011). Briefly, a testing arena was divided into a ‘test side’ and a ‘companion side’ by a divider that was either a wire mesh (physical isolation) or a wooden panel (visual isolation). The subject was placed in the test side, while its pen mates remained in the companion side. The test side contained a wooden ‘start box’ that was removed by the observer pulling on a rope and pulley system. The start box was placed in the centre of the test side with one wall of wire mesh facing the companion side. The chicks could not see the experimenter at any time during the recording period and behavioural recording was conducted through a camera placed directly above the test area.

Testing occurred between 10:00 and 18:00 h. The pens were selected in a predefined random order from each block and all six birds were placed in the companion side of the arena for 1 min to habituate to the surroundings. The birds were then tested in a predefined random order. Each bird was given 1 min to habituate to the start box and the test lasted for 2 min. When all 6 birds in the pen had been tested, they were returned to the home room and the next group was collected. All chicks were tested under both physical and visual isolation treatments over two days, and to avoid potential order effects, genotype and isolation treatment were balanced over these two days. To estimate the importance of a carryover effect, the trials from day 2 were repeated on day 3. The same predefined random order of pens and birds was used at weeks 1, 5 and 9.

For each chick, the 2 min videos were used to record the latency to move (s), number of pecks, proximity to pen mates (all putative measures of sight), number of peeps (a potential measure of frustration) and number of lines crossed (a measure of activity). The lines crossed refer to a 25 cm x 25 cm grid on the test side floor that was videoed and transferred to an acetate sheet placed over the video monitor screen. Proximity to pen mates was recorded by 10 s instantaneous sampling (Martin and Bateson, 1993, pp 87-88); test chicks were recorded as ‘near’ to pen mates if located in one of the 4 grid squares closest to
the divider and as ‘far’ if in one of the other 4 squares. If the chick was positioned in more
than one square of the grid, the square that its beak was in was recorded. All observations
were conducted by one person who was blind to the treatment of the birds.

2.1.4. Body weight, mortality and heterophil:lymphocyte (HL) ratio

All birds were weighed to the nearest gram (g) on the day of hatch, and every week
thereafter. The date of death of any bird was recorded and overall mortality of the three
genotypes was assessed. A drop of blood was obtained at 5 and 9 weeks of age by inserting a
needle (orange 25G) into a wing vein. A blood smear was immediately prepared on a glass
slide, air dried and subsequently stained using May-Grünwald’s solution as previously
described (Robertson and Maxwell, 1990). The numbers of heterophils and lymphocytes in
100 cells were counted and the ratio (HL) calculated.

2.1.5. Tests for blindness

During week 3, a basic test was carried out to assess if the birds could see or not. A hand was
moved at a distance of 10-20 cm from each eye and recording whether or not it reacted by
moving its head or walking away. At the start of week 6, all birds were again tested on their
ability to see. Three different assessments were carried out; i) the box test, ii) the “hawk” test,
and iii) the laser test. In the box test, for each pen all 6 birds were placed in a cardboard
transport box. They were left in the box for 1 min to habituate. After 1 min, the box was
opened and a stopwatch was started. The following were recorded: the time (latency) to exit
the box for the first and last bird and the number of birds that had not left the box after 3 min.
In the “hawk” test, a black cardboard outline of a hawk attached to a wooden pole was
“swooped” over each pen. The number of birds that initially responded (either by freezing or
running away) was recorded. The third test was conducted with a red laser presentation
pointer by the observer standing outside of the pen. The light was shone on the ground near
each bird and moved around; the number of birds that responded by either pecking or running
away in each pen was recorded. At the end of week 9, the laser test was repeated since it
appeared to be the most accurate for assessing blindness.

2.2. Experiment 2: adult chickens

2.2.1. Animals and housing

The birds for this experiment were reared under similar conditions as experiment 1 and were
housed in individual laying cages (800 x 450 mm) at 16 weeks of age for pedigree breeding.
They were rehoused to floor pens (1.5 x 2.2 m) when they were between 9 and 13 months of
age. The birds were allowed to familiarise themselves with the pen environment for 6 weeks
before behaviour recording. A standard layer ration and water were available at all times and
the photoperiod was 14L:10D (0730-2130h). Each pen was provided with a set of 3 nest
boxes.

There were originally 8 males and 8 females in each line housed at random in each of
12 pens containing 4 males or 4 females of the same genotype. Line and sex were randomised
to pen in a 3 x 2 completely randomised design with 2 replicates (blocks). The blindness of
rdd was assessed by the red laser test as described in section 2.1.4.

2.2.2. Home pen behaviour

Video cameras were placed above the centre of each pen and the birds were video recorded
for 1 h periods starting at 09:00h, 13:00h and 17:00h during 2 consecutive weeks. One
replicate from each breed-sex group was videotaped on day one and the second on day 2 in each week and again on the following week so that each pen was recorded for a total of 6 hours. The frequency of different behaviours, behavioural synchrony and group aggregation in the home pens was assessed from the video tapes as described in section 2.1.2.

2.3 Statistical analysis

The numbers of observations of each behaviour in every pen and hour were summed and analysed as a proportion of the total number of observations assuming binomial errors in the GLMM procedure of the Genstat statistical package (http://www.vsni.co.uk/software/genstat). For each category of behaviour, a nested analysis of a model including effects for block (4 rows) and pen within block as random factors and line, week, time of day and their interactions as fixed effects was conducted. Line x week x time interactions were not significant for any behaviour and the data were reanalysed with two-way interactions only. Line x time and time x week interactions were also not significant and a final model with line x week and time was analysed. The residuals for mean Bs and NND were normally distributed with approximately equal variance and the untransformed data for both measures were analysed using the same statistical model as behavioural frequency in the ANOVA procedure.

Mean pen latency to move (s), number of peeps, frequency of pecks, number of lines crossed and % of scans near pen mates were calculated for each pen in the isolation experiment for each combination of treatment, day and week. Latency to move was transformed by taking the logarithm \((x + 0.1)\), the number of peeps and pecks by the square root \((x + 0.2)\), the number of lines crossed by the square root \((x)\) and the percentage \((p)\) time near pen mates as the empirical logistic (logit). A nested analysis of variance was carried out
for each trait, with line, isolation, week day (1, 2, or 3), carryover from visual isolation on the
previous day, and their interactions as the treatment effects.

The overall number of birds responding or not responding in the evaluation of sight in
each line in experiment 1 was evaluated by an overall Chi-square test for each test and age.

Analysis of the weekly weights was performed by a REML analysis of a model with
fixed effects for line, gender and their interaction. Mortalities only occurred in weeks 1 and 2.
Pen mean mortality (number dead in first 2 weeks/total at start) was analysed by analysis of
deviance assuming a binomial distribution of errors. A nested analysis of variance of the HL
ratio was performed with treatment effects of line, time and their interaction.

For the analysis of behaviour in experiment 2, the GLMM procedure in Genstat was
used to analyse a model with breed, gender, time and their 2- and 3-way interactions as fixed
terms with block/pen/day as nested random effects. Data assumptions and transformations
were as described for experiment 1 except that natural logarithms of NND were taken to
ensure equality of the residual variance across treatments.

All differences between effects were supported by a level of statistical significance of
at least $P \leq 0.05$.

2.4. Ethical note

The birds were kept at The Roslin Institute in Edinburgh under a Home Office licence
(PPL/60/3815 protocol 2) after ethical review. The birds were from a breeding stock that is
used for the study of potential animal models for human conditions and for basic research
into the development of sight. The minimum numbers of breeding birds are kept to maintain
the genetic lines without incurring unacceptable levels of inbreeding.

3. Results
3.1. Social behaviour

3.1.1. Frequency of behaviours in young chicks and adult chickens

Results for time of day were comparable with the well-known effects of time of day on the behaviour of chickens and are ignored for clarity of presentation. Relatively few interactions of main effects and time of day were significant and were also ignored as the effects were relatively small and conclusions for line and age were not affected. Back transformed means for each line by week are plotted in Fig. 1. Levels of statistical significance are indicated above the relevant column for tests of beg vs rdd and rdd vs WL. Means and SED on the transformed scale and statistical tests of significance for line, week and the line x week interaction are presented in Supplementary Table 1.

Observations of drinking were low in beg compared with rdd in week 1 and week 5 but were similar to WL at week 9. Feeding was relatively infrequent in week 1 in beg compared with rdd and was lower in rdd compared with WL but the means were similar at weeks 5 and 9. Abnormal behaviour was relatively high in beg at 5 and 9 weeks and rare in both rdd and WL. The ranking of the three lines for pecking at the environment was similar at 1, 5 and 9 weeks: differences between beg and rdd were significant at 5 and 9 weeks and rdd was different from WL at 9 weeks. Sitting behaviour was the most frequently observed behaviour in beg and rdd at ages. However at 9 weeks the frequency of sitting in beg was greater that in rdd and WL which were similar. Walking was relatively high in rdd at 9 weeks compared with both beg and WL.

Means and SED on the transformed scale and statistical tests of significance for line, gender and line x gender interaction for adult birds are presented in Supplementary Table 2. The effects of time of day were ignored as described above for the younger birds.
Backtransformed means for line and gender are plotted in Fig. 2 with tests for beg vs rdd and rdd vs WL above the relevant columns.

The frequency of drinking was relatively high in female rdd adults but there were no line differences in males. Feeding has higher in WL male adults compared with beg and rdd which were similar and there were no differences in females. Abnormal behaviours were observed only in beg and rdd in both sexes and differences between the blind birds were not significant. Preening was observed more frequently in male rdd compared with beg and WL which were similar. Standing and pecking the environment were the most frequently observed behaviours. WL females pecked the environment more often than both blind lines but differences in males were small and not significant. Standing was higher in beg females compared with rdd and WL whereas standing in male WL was higher than in rdd and beg. Conversely, walking was relatively low in male WL compared with beg and rdd. Nesting was observed in WL but not in rdd or beg chickens which laid their eggs on the pen floor (data not shown).

3.1.2. Behavioural synchrony and group aggregation

Means and test results for the analyses of variance for behavioural synchrony (Bs) and group aggregation (NND) are presented in Tables 2 and 3 respectively for chicks (experiment 1) and adults (experiment 2). Bs in chicks was greater in WL than in beg and rdd which were similar and the behaviour of all three lines was more synchronous in week 1 than in weeks 5 and 9 (Table 2). In adult birds, sighted WL birds showed a significantly higher level of Bs compared with both blind rdd and beg (Table 3). There was a significant line x week interaction in the young chickens for NND since in weeks 5 and 9, rdd and WL had similar NND that were significantly less than that of beg (Table 2). In adult chickens, sighted WL males birds showed reduced NND compared with
males of the other two lines whereas no differences were found between the females leading
to a line x gender interaction (Fig. 3). Main effect means and levels of significance for Bs
and NND in the adult birds are presented in Table 3.

3.2. Social Isolation in young chicks

Statistically significant interactions of week, line, carryover effects and day were ignored for
clarity of presentation as these were nuisance factors correctly accounted for in the analysis.
Means and tests of significance on the transformed scale for the main effects and interactions
are given in Supplementary Table 3.

Highly significant (P<0.001) line x isolation interactions were detected for each trait
and back transformed means for line x isolation subclasses are plotted in Fig. 4. The latency
to move of beg chicks was greater in both isolation treatments compared with WL or rdd
chicks (Fig. 4A). The latency to move of WL and rdd chicks was not significantly different
from each other except under physical isolation where the latency to move by rdd was higher
than in WL. Similarly, for both beg and rdd, the number of lines crossed did not differ
between the two isolation treatments whereas for WL it was higher under physical compared
with visual isolation (Fig. 4B). Under physical isolation, the WL chicks crossed more lines
(i.e. were more active) than beg or rdd chickens, but under visual isolation, all three lines
were similarly inactive.

The number of pecks was greater under physical than visual isolation for all three
lines (Fig. 4C) and the number of pecks was greater in WL compared with rdd, and in rdd
compared with beg. The number of peeps made by WL and rdd was greater in visual than
physical isolation whereas for beg, the number of peeps was similar in both treatments. All
three lines made a similar number of peeps under physical isolation (Fig. 4D).
For both WL and rdd, time spent near pen mates was significantly greater under physical isolation than visual isolation (Fig. 4E). Under physical isolation, both WL and rdd chicks spent a similar amount of time near pen mates, although the percentage for WL was statistically higher (P<0.05), and was greater than that for beg. Under visual isolation, time spent close to pen mates was also higher for WL compared with both rdd and beg.

A significant interaction (P<0.05) of genotype and age was detected for the number of pecks and proportion of scans near pen mates (Fig. 5). The number of pecks was similar in rdd and beg at all 3 ages whereas the number of pecks in WL was lower at 1 week and higher at 5 and 9 weeks compared with both beg and rdd (Fig. 5A). The percentage of time that beg chicks spent near pen mates was lower in beg than in rdd and in rdd compared with WL at 1 week only (Fig. 5B).

3.3. Tests of sight

Results for the number of birds responding to different tests of sight at 3, 6 and 9 weeks of age are presented in Table 4. As expected, beg showed no response to any test and WL responded maximally to all of them. The responses of rdd declined with age and a degree of sight remained at 9 weeks of age in at least 25% of these birds. However, the adult rdd birds that were parents of these chicks did not respond to the red laser light and were functionally blind.

3.4. Body weights, HL ratios and mortality in chicks

The average body weights of beg, rdd and WL chicks, sex-average body weight gain from 0-7 and 8-14 days and body weight at 10 weeks of age are given in Table 5. Differences between genders were significant from week 2 onwards and sex x line interactions were
significant from week 5 (P<0.05): These were not significant when the data were logged, indicating that the interaction was a scale effect. Line means for the HL ratio and mortality are also presented in Table 5. Week and week x line had no detectable effect on the HL ratio whereas rdd had a very low ratio compared with both WL and beg (P<0.01). Mortality in beg chicks was higher than in rdd and WL chicks during the first 2 weeks (Table 5) but there were no subsequent deaths during the course of experiment 1. In experiment 2, two adult rdd females, one male and one female beg died before the experiment started. Cannibalism of a WL female resulted in this bird being culled before the observations began and an additional WL female from the same pen was culled for cannibalism of the vent during the study.

4. Discussion

The main aim of this study was to study the behaviour of rdd birds and how this changed as their loss of sight progressed in comparison to the blind beg and sighted WL. It was hypothesised that for the first 3 weeks of life, while sighted, rdd would behave similarly to WL and continue to do so, at least to some degree, once they began to lose sight. The lack of abnormal behaviours in rdd in experiment 1 (Fig. 1) suggests that they were experiencing greater visual stimulation than beg even if their sight was compromised. However, as adults there were generally few differences between rdd and beg suggesting that the differences in the behaviour of blind birds is a consequence of their lack of sight and that there are in adulthood no significant long term effects of vision at an early age, or of behaviour that was learnt when the birds were sighted. The results emphasise the critical importance of vision for the display of normal behaviour. Whereas the beg and WL birds in the experiment of Collins et al. (2011) were genetically similar except for the genetic mutation (they were from a backcross), both beg and rdd chickens in this experiment were from parents which differed both from each other and from the parents of the WL. The similarities of the results for beg
and controls from both experiments suggest that comparisons in the present experiments are
valid tests of the effect of developing blindness in rdd. Furthermore the comparisons between
lines (state of vision) are made at the same age in identically treated birds.

The results from experiment 1 confirm previous observations that beg birds exposed
to social isolation showed reduced social reinstatement behaviour (Collins et al., 2011).
However, the behaviour of rdd was intermediate or more like one of the other two lines
suggesting that they were visually aware of the location of their conspecifics while being as
inactive as beg (Fig. 4 A, B, C and E). Vocalisation is strongly dependent on social contacts
in the chick (Marx et al., 2001) and the greater mean number of peeps for rdd compared with
WL and beg suggests that they were under greater stress from limited ability to see their
conspecifics compared with the blind or sighted line.

Social aggregation was greatest in week 1 for all three lines and reflects the fact that
the birds huddled together under the heat lamp. In contrast to behavioural synchrony, in
weeks 5 and 9, rdd and WL had significantly higher group aggregation (i.e. lower NND) than
beg. Behavioural synchrony and group aggregation are visually dependent and beg performed
poorly in this respect which is in line with previous results (Collins et al., 2011). Chickens
naturally carry out behaviours in synchrony and there are possibly strong social facilitation
and physiological effects motivating the birds to act in this way (Mench et al., 1986) and,
conversely, separation and isolation from social companions has been shown to be stressful to
chickens (Jones and Williams, 1992; Marx et al., 2001).

Both beg and rdd spent proportionally less time feeding at week one than the sighted
controls and differences in body weight and mortality in beg confirm previous suggestions
that chicks that are blind at hatch have difficulty learning to feed, have lower subsequent
growth rates and higher mortality during the first 2 weeks of life (Collins et al., 2011).
However the low rate of feeding and body weight of rdd was unexpected and may indicate
that their vision was compromised even though they were able to see sufficiently well to
locate feed and water and conduct normal behavioural activity, at least to 3 weeks of age. Furthermore, the relative lack of interaction of line and age in the isolation experiment suggests that the vision of rdd was compromised from an early age. In support of this suggestion, degenerative changes associated with abnormal gene expression patterns have been observed in the retina of rdd chicks at hatch (Finnegan et al., 2010). Taken together the results emphasise the importance of vision to chicks and growing birds for locating and ingesting food (Goodale, 1983; Bermejo and Zeigler, 1998).

Environmental pecking, as well as feeding and drinking, is also primarily visually mediated (Green, 1998; Prescott et al., 2003). As previously demonstrated (Collins et al., 2011), beg chicks showed less environmental pecking at week 5, and both beg and rdd showed less at week 9 (Fig. 1) and in adult females (Fig. 2), compared with WL. Environmental pecking was the most common behaviour performed by WL adults, emphasising the importance of visual stimuli for pecking and feeding behaviour (Rogers, 1995; Appleby et al., 2004). However, differences in the pattern of responses for rdd, which were similar to WL at 1 and 5 weeks, suggest that a high degree of visual ability is not as critical for exploratory behaviour as for ingestion. It is, however, possible that the differences in environmental pecking between the two lines of blind chickens may be linked to the behaviour that was learnt while rdd birds had some vision during early life.

A higher frequency of sitting in beg and rdd at 1 and 5 weeks and in beg at 9 weeks compared with WL may be a consequence of a relative lack of visual stimulation. The significantly higher frequency of sitting behaviour in the beg adults than both rdd and sighted birds may be due to the continuation of behaviour learnt as chicks in response to a lower level of environmental stimulation. It is possible that the relatively high rate of walking and drinking in rdd at 9 weeks is a sign of frustration as the visual ability of these birds was clearly compromised at this age.
Abnormal behaviours occurred in rdd and beg and were not observed in WL.

Abnormal behaviour such as repetitive circling is the most obvious characteristic of the blind birds and is presumed to be a response to the lack of awareness of the environment (e.g. conspecifics, potential predators and obstacles). Similarly star-gazing may be an attempt to better detect, locate and distinguish auditory stimuli that are present in the surrounding environment (Schnyder et al., 2014). The blind birds frequently collide with other birds, pen walls and equipment and collisions were usually followed by circle walking. The absence of abnormal behaviour in rdd to 9 weeks of age suggests that their putative limited vision was sufficient to allow them to fulfil normal orienting behaviours whereas when the birds became blind, as adults, they started to perform abnormal behaviours using their aural sense as a compensation for the inability to fulfil essential visually based orienting behaviour (Fig. 1 and 2).

The observation that rdd retained some vision at 9 weeks (Table 4) was unexpected and is consistent with behavioural differences and the results for Behavioural Synchrony (Bs) and Nearest Neighbour Distance (NND; Table 2). Bs declined in rdd to a similar level as beg at 9 weeks whereas NND was similar to WL until 9 weeks ($P<0.05$). It is likely that rdd have sufficient sight to be aware of conspecifics but are increasingly unable to differentiate different behaviours at 9 weeks. This suggests that they have extremely poor eyesight by 9 weeks of age because chickens are normally able to detect social signals even at low light intensities (Kristensen et al., 2009). In the adult birds, Bs was similar in rdd and beg and was significantly lower than in WL whereas NND did not differ among the 3 lines. The latter observation may be a consequence of the relatively small size of the pen compared with the number and size of the birds.

The welfare and ethical consequences of keeping blind chickens for commercial egg laying have been discussed elsewhere (Collins et al., 2011; Sandøe et al., 2014). Additional data from this experiment confirm the lower body weights of beg and rdd compared with WL
and contrast with those of Ali and Cheng (1985) who reported no difference between adult
blind *rd* hens and sighted White Leghorn controls. Higher mortality and lower body weight in
blind birds is evidence of lower welfare compared with sighted birds whether blind from
hatch or becoming blind during rearing. A higher HL ratio is indicative of a greater level of
stress (Gross and Siegel, 1983) and whereas the HL ratio was significantly different between
the lines, the means were all relatively low and well within the normal physiological range.
These data are also not consistent with Ali and Cheng (1985) who concluded that the blind-
from-hatch birds were less stressed than the sighted ones partly on the basis of a lower HL
ratio. Furthermore the reduced rates of behavioural synchrony and group aggregation seen in
both the *rdd* and *beg* adults are indicative of reduced welfare of blind hens independently of
whether they are blind from hatch or become blind during early growth. The inability to
perform behaviours in synchrony may reduce a positive state of welfare linked to social
activities and indirectly affect the willingness to engage in other normal behaviour such as
feeding.

5. **Conclusions**

It is concluded that abnormal behaviours are a response to a complete loss of vision,
regardless of initial sight. Thus in general the behaviour of *beg* and *rdd* as adults is similar.
Greater drinking, walking and preening in *rdd* compared with WL at 9 weeks or as adults
may be a consequence of frustration at the loss of visual acuity. Behavioural synchrony was
comparable in birds that are blind from hatch or have limited vision and develop blindness at
a later age whereas group aggregation may be different only in birds that have totally lost
their vision. Taken together, the difficulty that blind birds have in feeding and drinking and
their effects on body weight, mortality and behaviour (fewer environmental pecks, lower BS
and greater NND), decreased social reinstatement behaviour and lower activity in *beg*
chickens, and the increase of such effects in $rdd$ as they become blind, is consistent with the view that the wellbeing of blind birds is severely compromised. The conclusion that blind birds are less stressed than sighted counterparts (Ali and Cheng, 1985) is therefore refuted.

Acknowledgements

We are grateful Graeme Robertson for carrying performing the HL counts and to Kim Bernard and the staff of Poultry Unit for helping to care for the birds. We are also grateful to the Department of Food and Resource Economics, University of Copenhagen, for financial support. Parts of the research were submitted in partial fulfilment of the MSc degree (K-AH) and an Honours BSc project (EMD). The Roslin Institute is supported by an Institute Strategic Programme Grant from the BBSRC.

References


**Figure legends**

**Fig. 1.** Back transformed means for the proportion of observations of different behaviours for *beg* (black bar), *rdd* (light grey bar) and WL (dark grey bar) chickens at 1, 5 and 9 weeks of age. Note the different scales within rows one and two and the large difference in the scale for sitting compared with the other behaviours in the third row. Tests of significance are provided for *beg* vs. *rdd* above the first column and *rdd* vs. WL over the third column for each age: *P<0.05, ** P<0.01, *** P<0.001.

**Fig. 2.** Back transformed means for the proportion of observations of different behaviours for female (left) and male (right) from three lines of adult chickens: *beg* (blind at hatch, black bar), *rdd* (blind as adults, light grey bar) and WL (sighted, dark grey bar). Note the different scales and the large differences in the scales for standing and pecking the environment. Tests of significance are provided for *beg* vs. *rdd* above the first column and *rdd* vs. WL over the third column for each gender: ** P<0.01, *** P<0.001, § test of *rdd* vs. zero P<0.001.

**Fig. 3.** Back transformed means for Nearest Neighbour Difference for female (left) and male (right) chickens from three genetic lines that were blind (*beg* and *rdd*) or sighted (WL) as adults. Interaction of line x gender, F(2,5)=8.25, P<0.05.

**Fig. 4.** Back transformed means of different behaviours at 1, 5 and 9 weeks of age in three lines of chicks subjected to physical isolation (black bars) or visual isolation (grey bars) showing significant (P<0.001) line x isolation interactions. A, latency to move from the start box; B, number of lines crossed (a measure of activity); C, number of pecks and B, number of peeps (potential measures of frustration); and E, percentage of scans near pen mates (a proxy
measure of sight). Tests of significance are provided for beg vs. rdd above the first pair and
ddd vs. WL over the second pair of columns. * P<0.05, ** P<0.01, *** P<0.001.

Fig. 5. Back transformed means for of different behaviours at 1 (black bar), 5 (light grey bar)
and 9 (dark grey bar) weeks of age in three lines of chicks subjected to physical or visual
isolation showing significant (P<0.05) line x week interactions. A, number of pecks; B,
percentage of scans near pen mates. Tests of significance are provided for beg vs. rdd above
the first set and rdd vs. WL over the second set of columns. *** P<0.001.
Supplementary Table 1.

Mean and SED on the transformed scale (logit) of the proportion of different behaviors of beg, rdd and White Leghorn (WL) chicks at 1, 5 and 9 weeks of age. The WL is a normal sighted line, beg and rdd have a similar genetic background as WL but inherits a recessive gene causing blindness throughout life (beg), or are sighted at hatch and progressively loses sight with age (rdd).

Degrees of freedom were 2, 2 and 4 for line, week and line x week interaction with denominator degrees of freedom of 6-10 for line and 87-97 for week and line x week. Tests of significance for line and week are from an analysis with no interactions. Significance levels for time were similar to those in the analysis with time and line x week. **, P<0.01; ***, P<0.001.

<table>
<thead>
<tr>
<th>Week</th>
<th>Line</th>
<th>Average SED</th>
<th>Line</th>
<th>Week</th>
<th>Line x week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drinking</td>
<td>beg</td>
<td>-4.875</td>
<td>0.4022</td>
<td>6.87***</td>
<td>3.20</td>
</tr>
<tr>
<td></td>
<td>rdd</td>
<td>-2.723</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>-2.428</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal</td>
<td>beg</td>
<td>-5.560</td>
<td>0.7668</td>
<td>31.93***</td>
<td>17.97***</td>
</tr>
<tr>
<td></td>
<td>rdd</td>
<td>0.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>0.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>beg</td>
<td>-5.354</td>
<td>0.7550</td>
<td>3.07</td>
<td>6.03**</td>
</tr>
<tr>
<td></td>
<td>rdd</td>
<td>0.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>-6.949</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preen</td>
<td>beg</td>
<td>-4.648</td>
<td>0.4214</td>
<td>2.66</td>
<td>38.00***</td>
</tr>
<tr>
<td></td>
<td>rdd</td>
<td>-4.289</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>-4.747</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stand</td>
<td>beg</td>
<td>-2.086</td>
<td>0.3231</td>
<td>2.14</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>rdd</td>
<td>-2.274</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>-1.857</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walk/run</td>
<td>beg</td>
<td>-1.700</td>
<td>0.2549</td>
<td>8.59</td>
<td>7.46</td>
</tr>
<tr>
<td></td>
<td>rdd</td>
<td>-2.053</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>-1.858</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feeding</td>
<td>beg</td>
<td>-3.250</td>
<td>0.3143</td>
<td>0.91</td>
<td>10.69***</td>
</tr>
<tr>
<td></td>
<td>rdd</td>
<td>-1.925</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>-1.069</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peck environment</td>
<td>beg</td>
<td>-2.914</td>
<td>0.3666</td>
<td>16.92**</td>
<td>5.04**</td>
</tr>
<tr>
<td></td>
<td>rdd</td>
<td>-2.107</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>-1.843</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Siting</td>
<td>beg</td>
<td>0.482</td>
<td>0.2513</td>
<td>25.77***</td>
<td>7.95***</td>
</tr>
<tr>
<td></td>
<td>rdd</td>
<td>-0.079</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>-1.158</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Supplementary Table 2.

Mean and SED on the transformed scale (logit) of the proportion of different behaviors of \textit{beg}, \textit{rdd} and White Leghorn (WL) male and female adult chickens. The WL is a normal sighted line, \textit{beg} and \textit{rdd} have a similar genetic background but the \textit{beg} line inherits a recessive gene causing blindness throughout life, whereas \textit{rdd} is sighted at hatch and is blind as an adult. * P<0.05; **P<0.01; ***P<0.001.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Breed</th>
<th>Gender</th>
<th>Avg SED</th>
<th>Line</th>
<th>Week</th>
<th>Line x Week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drinking</td>
<td>\textit{beg}</td>
<td>-2.822</td>
<td>-3.417</td>
<td>0.6029</td>
<td>3.79</td>
<td>3.67</td>
</tr>
<tr>
<td></td>
<td>\textit{rdd}</td>
<td>-2.048</td>
<td>-3.334</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>-3.627</td>
<td>-4.096</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal</td>
<td>\textit{beg}</td>
<td>-1.870</td>
<td>-1.749</td>
<td>0.4009</td>
<td>1.08</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>\textit{rdd}</td>
<td>-2.003</td>
<td>-2.463</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>0.000</td>
<td>0.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>\textit{beg}</td>
<td>-5.555</td>
<td>-4.475</td>
<td>0.9158</td>
<td>1.69</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>\textit{rdd}</td>
<td>-5.080</td>
<td>-4.501</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>-3.264</td>
<td>-4.539</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preening</td>
<td>\textit{beg}</td>
<td>-1.642</td>
<td>-2.396</td>
<td>0.2958</td>
<td>4.44</td>
<td>6.92*</td>
</tr>
<tr>
<td></td>
<td>\textit{rdd}</td>
<td>-1.308</td>
<td>-1.401</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>-1.421</td>
<td>-2.127</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standing</td>
<td>\textit{beg}</td>
<td>-1.864</td>
<td>-0.909</td>
<td>0.2641</td>
<td>1.77</td>
<td>44.29***</td>
</tr>
<tr>
<td></td>
<td>\textit{rdd}</td>
<td>-2.992</td>
<td>-1.031</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>-2.901</td>
<td>-0.197</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feeding</td>
<td>\textit{beg}</td>
<td>-2.224</td>
<td>-2.911</td>
<td>0.3032</td>
<td>3.04</td>
<td>4.85</td>
</tr>
<tr>
<td></td>
<td>\textit{rdd}</td>
<td>-2.125</td>
<td>-2.784</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>-2.003</td>
<td>-2.026</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walk/run</td>
<td>\textit{beg}</td>
<td>-2.597</td>
<td>-1.799</td>
<td>0.4785</td>
<td>0.09</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>\textit{rdd}</td>
<td>-2.209</td>
<td>-2.171</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>-1.605</td>
<td>-3.287</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peck environment</td>
<td>\textit{beg}</td>
<td>-1.868</td>
<td>-2.954</td>
<td>0.3577</td>
<td>9.29**</td>
<td>30.30***</td>
</tr>
<tr>
<td></td>
<td>\textit{rdd}</td>
<td>-1.424</td>
<td>-2.258</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>-0.661</td>
<td>-1.962</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Siting</td>
<td>\textit{beg}</td>
<td>-1.539</td>
<td>-1.682</td>
<td>0.3893</td>
<td>4.63*</td>
<td>2.73</td>
</tr>
<tr>
<td></td>
<td>\textit{rdd}</td>
<td>-2.508</td>
<td>-1.999</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>-3.211</td>
<td>-2.059</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Degrees of freedom were 2, 1 and 2 for line, gender and line x gender interaction with denominator degrees of freedom of 5-9 for line, gender and line x gender. Tests of significance for time, line and week are from an analysis with no interactions. * P<0.05; **, P<0.01; *** P<0.001.
### Supplementary Table 3

Transformed (back transformed) mean latency to move, numbers of lines crossed, proportion of scans near pen mates, number of peeps and environmental pecks for *beg, rdd* and WL in physical or visual isolation from their pen mates at 1, 5, and 9 weeks of age.

<table>
<thead>
<tr>
<th>Trait and line</th>
<th>Week 1 Physical</th>
<th>Week 1 Visual</th>
<th>Week 5 Physical</th>
<th>Week 5 Visual</th>
<th>Week 9 Physical</th>
<th>Week 9 Visual</th>
<th>SED&lt;sup&gt;a&lt;/sup&gt;</th>
<th>F-ratios and significance&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latency to move (log s)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Line (L)</td>
</tr>
<tr>
<td><em>beg</em></td>
<td>2.50</td>
<td>3.26</td>
<td>3.22</td>
<td>3.33</td>
<td>2.88</td>
<td>2.84</td>
<td>0.848</td>
<td>17.99 **</td>
</tr>
<tr>
<td><em>rdd</em></td>
<td>1.11</td>
<td>2.39</td>
<td>-0.21</td>
<td>0.32</td>
<td>0.91</td>
<td>2.51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WL</td>
<td>-2.51</td>
<td>1.17</td>
<td>-1.32</td>
<td>1.54</td>
<td>0.09</td>
<td>1.86</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lines crossed (√n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>beg</em></td>
<td>3.00</td>
<td>2.69</td>
<td>2.77</td>
<td>2.56</td>
<td>2.38</td>
<td>3.06</td>
<td>0.499</td>
<td>34.44 **</td>
</tr>
<tr>
<td><em>rdd</em></td>
<td>2.57</td>
<td>3.57</td>
<td>3.32</td>
<td>3.92</td>
<td>3.43</td>
<td>3.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WL</td>
<td>6.40</td>
<td>3.48</td>
<td>6.81</td>
<td>3.02</td>
<td>4.63</td>
<td>3.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion near pen mate scans (logit %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>beg</em></td>
<td>0.64</td>
<td>0.22</td>
<td>1.49</td>
<td>0.66</td>
<td>0.64</td>
<td>0.44</td>
<td>0.420</td>
<td>175.59 ***</td>
</tr>
<tr>
<td><em>rdd</em></td>
<td>3.94</td>
<td>-0.01</td>
<td>2.86</td>
<td>0.38</td>
<td>1.64</td>
<td>0.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WL</td>
<td>4.61</td>
<td>1.85</td>
<td>3.32</td>
<td>0.48</td>
<td>1.75</td>
<td>0.86</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of peeps(√n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>beg</em></td>
<td>7.75</td>
<td>8.96</td>
<td>2.48</td>
<td>5.35</td>
<td>3.20</td>
<td>4.21</td>
<td>0.927</td>
<td>35.15 **</td>
</tr>
<tr>
<td><em>rdd</em></td>
<td>6.75</td>
<td>13.25</td>
<td>3.17</td>
<td>9.94</td>
<td>2.90</td>
<td>6.79</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WL</td>
<td>7.13</td>
<td>12.45</td>
<td>2.02</td>
<td>6.95</td>
<td>3.14</td>
<td>5.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of pecks(√n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>beg</em></td>
<td>3.41</td>
<td>2.11</td>
<td>2.97</td>
<td>2.01</td>
<td>2.08</td>
<td>2.38</td>
<td>0.401</td>
<td>0.99 ns</td>
</tr>
<tr>
<td><em>rdd</em></td>
<td>4.23</td>
<td>1.88</td>
<td>3.26</td>
<td>1.42</td>
<td>2.78</td>
<td>1.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WL</td>
<td>4.17</td>
<td>0.58</td>
<td>4.19</td>
<td>1.83</td>
<td>4.32</td>
<td>1.77</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Maximum standard error of a difference between two means.

<sup>b</sup> Variance ratios and significance levels are indicated for the main effects and interaction; degrees of freedom were 2,4 for L and A; 1,12 for I; 2,12 for L x I and 4,8 for L x A and L x A x I.

* P<0.05; ** P<0.01; *** P<0.001; ns = not significant.
Figure 1.
Figure 2.
Figure 4.
Figure 5.