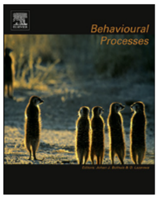
Behavioural Processes 157 (2018) 470–473

Contents lists available at ScienceDirect

Behavioural Processes

journal homepage: www.elsevier.com/locate/behavproc



# Are there facial indicators of positive emotions in birds? A first exploration in Japanese quail

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## ARTICLE INFO

*Keywords:*
Animal welfare
*Coturnix coturnix japonica*
Emotion
Facial expression
Positive affect

## ABSTRACT

The positive aspect of emotions, like pleasure, remains overlooked in birds. Our aim was to contribute to the exploration of facial indicators of positive emotions. To observe contrasting emotional expressions, we used two lines of Japanese quail divergently selected on their inherent fearfulness: a fearful line (long tonic immobility duration: LTI) and a less fearful line (short tonic immobility duration: STI). To induce positive emotions, we gave individual quail the opportunity to perform a rewarding behaviour, dustbathing, in an unfamiliar cage. More STI than LTI quail expressed dustbathing and latencies to dustbathe were significantly shorter in STI than LTI quail. This result indicated that the lines of quail differed in their fearfulness of the situation. We observed crown feather height, throat feather angle and pupil surface before (control) and during dustbathing. We found significant increases in crown feather height, pupil area and angle of throat feathers between the control and the dustbathing phases in STI quail, and pupil area correlated positively with crown feather height. In LTI quail, the angle of throat feathers increased during dustbathing, but the other parameters did not differ. We argue that variation in crown feather height and pupil area may provide indications of positive emotions in Japanese quail.

## 1. Introduction

From an evolutionary perspective, both positive and negative emotions have adaptive functions such as the regulation of avoidance and approach behaviours (Panksepp, 2004; Barrett et al., 2007). However, in birds, research remains largely focused on the capacity to experience and express emotions with negative valences, such as fear-related behaviors. The positive aspect of emotions, such as the capacity to feel pleasure, remains an unpaved avenue of research (Emery and Clayton, 2015). Emotions are composed of behavioural, neurophysiological and cognitive components (Boissy et al., 2007). One of the difficulties that may constrain the scientific investigation of pleasure in birds is the current lack of reliable indicators. In mammals, changes in facial expressions have become one of the most studied behavioural indicators of emotions (Waller and Micheletta, 2013). Although birds can move the feathers on their head without facial musculature (Homberger and de Silva, 2003), facial indicators of emotions remain overlooked.

Positive emotions are associated with appetitive motivational states (anticipation) and reward-seeking behaviours (Mendl et al., 2010). In poultry, the opportunity to dustbathe engenders anticipatory behaviours, and birds are willing to work (e.g., pushing doors) to perform this behaviour (McGrath et al., 2016). According to some authors, the motivation to dustbathe is driven not only by the need to clean the plumage but also by the possible pleasure engendered by performing this behaviour (Widowski and Duncan, 2000). To provide a first descriptive study of possible facial indicators of positive emotions in birds, we gave Japanese quail maintained in wire mesh cages the opportunity to dustbathe by placing them in an unfamiliar cage with friable material. We measured crown feather height, the angle of throat feathers and pupil area on quail before (control) and during dust bathing. We observed two lines of quail diverging in the way they appraise their environment. Selected for long or short duration of tonic immobility for generations (LTI or STI line), the quail strongly diverge in their inherent fearfulness (i.e., propensity to express fear related behaviours) (Mills and Faure, 1991). We argued that the contrast in behavioural responses between the selected lines would help reveal subtle behavioural variation in facial movements. Indeed, we expected STI quail (the less fearful line) to appraise the balance between fear of the novel environment (the test cage) and the rewarding properties of dustbathing

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https://doi.org/10.1016/j.beproc.2018.06.015
Received 14 May 2018; Received in revised form 28 June 2018; Accepted 29 June 2018

more positively than LTI quail (the more fearful line) would. We hypothesized that, if facial expressions reflect inner subjective emotions, then larger variations in the recorded parameters between the control phase and the dustbathing phase should be observed in STI quail than in LTI quail.

## 2. Material and methods

### 2.1. Animals and housing

We used 12 adult male Japanese quail from the STI line and 12 adult male Japanese quail from the LTI line. As fully described in several papers, the LTI line presents a higher level of inherent fearfulness compared to the STI line (e.g., Mills and Faure, 1991). We used quail available at the end of another experiment where they were trained in spatial learning tasks between 4 and 6 weeks of age. All the quail were thus habituated to human handling and transport. All animals were maintained in the same room (20 ± 2 °C), in individual wire mesh cages (41 cm × 51 cm × 25 cm) under a 12:12 h light:dark schedule. Each cage was enriched with a piece of synthetic turf on the floor. Food and water were provided *ad libitum*.

### 2.2. Protocol and video recording

All quail were observed on the same day and tested only once. Each quail was gently transported in a familiar transport box and placed in the unfamiliar test cage, in an adjacent room (20 ± 2 °C). The test cage was composed of a glass window and two compartments of equal size (50 cm x 45 cm x 45 cm) separated by a removable opaque wall and kept under similar luminosity (587 ± 4 lx). Each quail was placed first in the control compartment with a wire mesh grid on the ground for a 5-min duration (control phase). Then, the wall was removed and the quail could freely access the compartment with wood shavings on the floor for a 10-min duration (dustbathing phase). We used a Sony HDRP PJ410 video camera capturing up to 24 images per seconds to record each quail. During recordings, the experimenter sat in dimmed light (30 lx) behind two light bulbs facing the glass window in order to remain unseen by the birds. The quail were returned to their cage after the session, and the cage was cleaned between birds. The testing order of the lines was counterbalanced between trials.

### 2.3. Analysis of images

We used the software MPCSTAR to extract images from the movies with a scan sampling method every 5 s. Only images of clear profiles were kept for the analysis (Fig. 1). During the dustbathing phase, for both lines, images were extracted only from sequences where quail expressed dustbathing: a recumbent posture combined with vertical wing shaking, head rubbing, bill raking and scratching (Olsson and Keeling, 2005). We used the software ImageJ to estimate crown feather height, angle of throat feathers and area of the pupil. To correct for variation in the distance of the bird from the camera, we determined an invariant distance on each bird. For each bird, the day before the test, we measured the real distance between the eyelid corners with a digital calliper ( ± 0.01 mm). Then, for each bird, we used the function “straight” to draw this distance on the image and then the function “set scale” to convert the distance in pixels to distance in real millimetres. To measure crown feather height, we drew an angle with a vertical plan going from the top of the beak (boundary between the nostril and the beak) and the external corner of the eye (Fig. 1A) and a 90° angle from the external corner of the eye going to the top of the head. The length of the line was estimated with the function “analyse” and “measure”. With the function “angle”, we measured the angle between the vertical plan and throat feathers (Fig. 1A). The pupil area was assessed with the function “oval”, with which we circled the black pupil and then used the function “measure” to obtain the area of the circle.

For the STI line, 11 out of the 12 quail expressed dustbathing. One quail with some feathers missing from the top of the head was removed from the analysis thereafter. We analysed a mean of 14.20 ± 1.35 clear profiles per bird for the control phase and 20.30 ± 3.22 clear profiles per bird for the dust bathing phase. For the LTI line, 6 out of the 12 quail expressed dustbathing behaviour. We were able to obtain a mean of 16.66 ± 1.56 clear profiles per bird for the control phase and a mean of 15.33 ± 3.37 clear profiles per bird for the dustbathing pahse. The same well-trained experimenter, blind to the line, scored all the images.

### 2.4. Statistics

We compared the number of quail expressing dustbathing by using a Chi-square test. For each bird and each parameter, we pooled the measures obtained from all images to obtain a total mean per phase. The data were not normally distributed (Shapiro-Wilk test) and did not have the homogeneity of variances (Levene tests) required to apply parametric statistics. A non-parametric Mann-Whitney *U* test was conducted to compare latencies to express dustbathing between lines. Wilcoxon tests were applied within each line to compare the parameters between the control phase and the phase with dustbathing. We report effect size correlational coefficients (*r*) with coefficients between 0.1 to 0.5 indicating small to intermediate effects and coefficients above 0.5, strong effects (Cohen, 1988; Fritz et al., 2012). We used Spearman’s correlation coefficients to assess associations between the parameters. All analyses were performed using the software Statview (SAS), with significance accepted at $P < 0.05$.

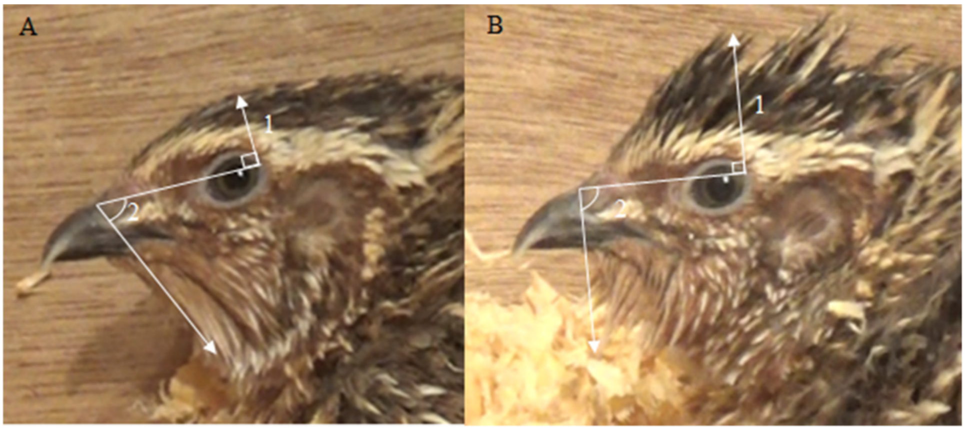

**Fig. 1.** Photographs of the same quail during dustbathing with schematic representations of the measures of 1) crown feather height and 2) throat feathers. A) Crown feathers and throat feathers were sleeked. B) Crown feathers and throat feathers were ruffled (higher crown feather height and wider angle of throat feathers).

**Table 1**
Means ± SE crown feather height, throat feather angle and pupil area in quail from the LTI (N = 6) and STI lines (N = 10). Different letters indicate significant differences between the control phase and the dustbathing phase within each line (Wilcoxon tests, $P < 0.05$).

| | Line LTI<br>Control phase | Dustbathing phase | Line STI<br>Control phase | Dustbathing phase |
|---|---|---|---|---|
| Crown feathers (mm) | $11.58 \pm 0.43^{a}$ | $11.89 \pm 0.31^{a}$ | $11.11 \pm 0.34^{a}$ | $12.03 \pm 0.49^{b}$ |
| Throat feathers (°) | $103.61 \pm 3.72^{a}$ | $113.96 \pm 5.63^{b}$ | $119.18 \pm 3.27^{a}$ | $134.79 \pm 1.86^{b}$ |
| Pupil area (mm$^2$) | $8.74 \pm 0.30^{a}$ | $9.07 \pm 0.46^{a}$ | $8.23 \pm 0.40^{a}$ | $9.60 \pm 1.54^{b}$ |

### 2.5. *Ethical note*

Animal care complied with the guidelines of the French Ministry of Agriculture for animal experimentation and European regulations on animal experimentation (86/609/EEC). The experiment was performed in accordance with the local animal regulation (authorized N°006352 of the French ministry of Agriculture in accordance with EEC directive) and with the Val de Loire ethical committee (agreement N°01833.01).

## 3. Results

A significant higher number of STI than LTI quail expressed dustbathing (Chi$^2$ = 5.04, $P = 0.02$). The latency to express dustbathing was shorter in STI than in LTI quail (68.00 ± 13.38 s vs. 141.16 ± 37.92 s, Mann-Whitney $U$ test, $U = 12.5$, $P = 0.05$, $r = 0.75$).

Within the STI line, significantly higher crown feather height ($z = -1.88$, $P = 0.05$, $r = 0.6$), larger angle of throat feathers ($z = -2.59$, $P < 0.01$, $r = 0.82$) and larger pupil area ($z = -2.80$, $P < 0.01$, $r = 0.88$) were observed during dustbathing than during the control phase (Table 1). The area of the pupil correlated positively with crown feather height ($df = 8$, $N = 10$, $rs = 0.93$, $P < 0.01$) but not with the angle of throat feathers ($df = 8$, $N = 10$, $rs = 0.04$, $P = 0.89$).

Within the LTI line, no significant differences were observed between the control phase and the dustbathing phase in crown feather height ($z = -1.15$, $P = 0.24$, $r = 0.44$) or pupil area ($z = -1.36$, $P = 0.17$, $r = 0.5$) (Table 1). A wider angle of throat feathers was observed during dustbathing than during the control phase ($z = -2.20$, $P = 0.02$, $r = 0.89$). The area of the pupil did not correlate with crown feather height ($df = 4$, $N = 6$, $rs = -0.37$, $P = 0.40$) or throat feather angle ($df = 4$, $N = 6$, $rs = 0.6$, $P = 0.18$).

## 4. Discussion

As expected, greater variations in feather displays and pupil area were observed in STI than in LTI quail. STI quail expressed a significant increase in crown feather height, pupil area and the angle of throat feathers during dustbathing. In addition, the higher the crown feathers, the larger the pupil surface was. Ruffling of body feathers is part of the dustbathing display in birds (Olsson and Keeling, 2005). While ruffling of throat feathers was observed (increase in the angle during dustbathing) in both lines, this was not the case for the crown. In fact, the absence of an increase of crown feather height during dustbathing in LTI quail is of importance since it indicates that dustbathing is performed without crown ruffling. No significant variation in pupil area was found in the LTI line between the control and the dustbathing phases. In addition, LTI quail took longer and were slower to express dustbathing. As fearfulness is inversely correlated with locomotor activities and the expression of comfort behaviours (Jones, 1996), this higher latency indicates a stronger behavioural inhibition in LTI than in STI quail. These differences indicate that, as expected from the literature (e.g. Faure et al., 2006), LTI and STI quail differed in their fearfulness when exposed to unfamiliar environments. Less inhibited by fear, STI quail were more motivated to express dust bathing and the significant increase in crown feather height and pupil area in STI quail during dustbathing may indicate a stronger pleasurable state in this line. In the same direction, crown ruffling was recently described in blue-and-yellow macaws (*Ara ararauna*) during positive human-animal interactions or activities with positive valences (Bertin et al. in revision).

Not yet studied in birds, variation in pupil area may provide interesting facial indicators of emotions in addition to feather displays. Contrarily to mammals, pupillary size in birds is under the control of striated muscles and can thus vary voluntary (Schmidt-Morand, 1992). Unexplored by the scientific community, eye pinning describes the rapid dilation and contraction of the pupils of a bird's eye, and this behaviour is observed in contexts having positive or negative valences. In Psittacidae, eye pinning is reported when birds anticipate positive events like stroking, or during pleasant activities like eating a favourite food but also during negative events like defense of territory (Moustaki, 2011). The increase in the pupil size in the STI line during dustbathing was likely a consequence of eye pinning that may have been engendered by a positive subjective feeling during dustbathing.

Although preliminary, our data show that crown ruffling and variation in pupil area could be two potential indicators of positive emotions in Japanese quail. In the field of animal welfare of farm or captive animals, positive emotions are considered as primary components of subjective well-being. The presented patterns represent a first pioneer approach; however, this may open a new research line for studying positive emotions in birds based on facial indicators.

## Declarations of interest

None.

## Acknowledgements

We thank the experimental unit PEAT and the department PHASE INRA for the financial support. This funding source had no role in the study or the preparation of the article.

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