Animal Cognition
https://doi.org/10.1007/s10071-021-01476-6

ORIGINAL PAPER

A songbird strategically modifies its blinking behavior when viewing human faces

Jessica L. Yorzinski[1] · **Mary K. Walker**[1] · **Rebecca Cavalier**[1,2]

Received: 15 March 2020 / Revised: 4 January 2021 / Accepted: 8 January 2021

Abstract

Even though blinking is necessary to maintain clear vision in many species, blinking is likely costly because it temporarily impairs vision. Given this cost, individuals can strategically modify their blinking behavior to minimize information loss. We tested whether a songbird species modifies its blinking behavior when viewing potential threats (human faces). We recorded the blinking behavior of captive great-tailed grackles (*Quiscalus mexicanus*) before, during, and after they viewed human face stimuli or control stimuli (tree bark as well as scrambled versions of human faces and tree bark). We found that the birds inhibited their blinking behavior the most when viewing human faces versus controls. In addition, they inhibited their blinking behavior more when viewing human faces that were directed rather than averted. Furthermore, when viewing the human faces, their blinking behavior was modified based on reactivity. These results suggest that a songbird can strategically modify its blinking behavior based on its perceived level of risk.

Keywords Antipredator behavior · Attention · Eye blink · Gaze · Head movement · Threat

Introduction

Animals constantly gather sensory information from their environments. In particular, many species rely on visual information to inform their behavioral decisions. They acquire this visual information using specialized visual systems that are often tuned to their behavioral needs (Martin 2007). Due to constraints on their visual systems, many vertebrates are unable to keep their eyes open continuously: they must blink by sweeping their eyelids across their eyes. Blinking behavior is necessary to maintain clear vision because it cleans the surface of the eye, provides a stable tear film, and prevents optical aberrations (Koh et al. 2006; Sweeney et al. 2013). Despite these benefits, blinking behavior likely incurs a substantial cost because animals have limited visual information during blinks (Volkmann et al. 1980; Bristow et al. 2005). Given that blinking behavior is likely costly, we would expect animals to strategically adjust their blinking behavior (Hoppe et al. 2018).

Our growing understanding of strategic blinking and its impact on visual perception primarily comes from studies on humans. Blink rates are often modulated by cognitive demands. In particular, blinking decreases during cognitively demanding tasks and this, therefore, reduces the chances of missing information (Hoppe et al. 2018). For example, blinking decreases during reading (Bentivoglio et al. 1997), when viewing salient scenes (Shin et al. 2015), and when solving difficult mathematical computations (Tanaka and Yamaoka 1993). Conversely, blinks are more frequent when attention is shifting, such as at the end of a sentence (Hall 1945) or during a scene change (Nakano et al. 2009), and may facilitate attentional disengagement (Nakano et al. 2013).

We are aware of only a limited number of studies that have examined strategic blinking in nonhuman animals. These studies found evidence to suggest that nonhuman primates and birds decrease their blinking behavior when exposed to risky situations (Tada et al. 2013; Cross et al. 2013; Yorzinski 2016, 2020b; Beauchamp 2017; Matsumoto-Oda

---

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10071-021-01476-6.

✉ Jessica L. Yorzinski
jyorzinski@tamu.edu

[1] Department of Ecology and Conservation Biology, Texas A&M University, 534 John Kimbrough Boulevard, College Station, TX 77843-2258, USA

[2] Department of Biology, Texas State University, San Marcos, TX 78666-4684, USA

et al. 2018), presumably to minimize the chance of missing critical information. Across and within nonhuman primates, blink rates decrease as social group size decreases (Tada et al. 2013; Matsumoto-Oda et al. 2018); similarly, in an avian species, individuals decrease their blink rates when they are in smaller versus larger groups (Beauchamp 2017). Blink rates may decrease in smaller groups because individuals experience higher predation risk in smaller groups and lower blink rates can maximize their ability to detect threats (Tada et al. 2013; Beauchamp 2017; Matsumoto-Oda et al. 2018). Two studies have also found that birds inhibit their blinks the most when they are threatened (Cross et al. 2013; Yorzinski 2016) but these studies did not control for head movements even though head movements and blinks are often positively related (Evinger et al. 1994; Gandhi 2012; Yorzinski 2016).

The aim of this study was, therefore, to test the hypothesis that birds inhibit their blinks when encountering potential threats. This hypothesis was tested using captive great-tailed grackles (*Quiscalus mexicanus*), a songbird species that has many predators (such as raptors, owls, snakes, and squirrels; Johnson and Peer 2020). Grackles blink by sweeping their semi-transparent nictitating membranes across their eyes but their eyelids generally remain open when they are alert (Yorzinski and Argubright 2019; Yorzinski 2020a, b). To test our hypothesis, we performed two experiments in which we recorded the blinking behavior of the birds before, during, and after they viewed digital stimuli. In "Experiment 1", we tested whether grackles inhibit their blinking behavior in response to potentially threatening compared to non-threatening stimuli; the threatening stimuli consisted of human faces with directed gaze and the control stimuli consisted of tree bark and scrambled versions of the stimuli. In "Experiment 2", we tested whether grackles inhibit their blinking behavior relative to the degree of threat. Previous studies have shown that birds perceive threats that are facing toward them as more dangerous than threats that are facing away from them (Hampton 1994; Carter 2008; Freeberg et al. 2014, 2016). We, therefore, used threatening stimuli that consisted of human faces that were either directed (face oriented toward the bird) or averted (face oriented away from the bird). We used human faces as the threatening stimuli in both experiments because our anecdotal observations suggested that great-tailed grackles inhibit their blinking behavior when humans are holding them and directly gazing at them. In addition, many previous studies in birds have also used humans as the threatening stimuli (e.g., Hampton 1994; Carter 2008; Bateman and Fleming 2011).

We predicted that the grackles would inhibit their blinking behavior the most when viewing human faces versus control stimuli. We also expected that the birds would inhibit their blinking more when viewing human faces that are directed rather than averted. Lastly, when the birds were viewing the human faces, we predicted that their blinking behavior would be inhibited the most when they were most reactive.

Methods

Animals and housing

We examined the impact of human faces on the blinking behavior of captive great-tailed grackles (*Q. mexicanus*) in College Station, Texas (30.56° N, 96.41° W). We tested 32 birds between June and October 2018 in "Experiment 1" and another 32 birds between October 2018 and March 2019 in "Experiment 2". Adult birds were captured from the wild in College Station, Texas using mist nets and bownets. They were housed in outdoor aviaries (2.1 m × 2.1 m × 1.9 m) with up to ten other conspecifics. They were given food (Purina cat chow, Dumor poultry layer feed, and dried mealworms) and water ad libitum. Due to logistical difficulties in capturing male birds from the wild, we restricted our study to females only. Over half of the birds ($n = 18$) used in "Experiment 1" had been tested in an unrelated study (Yorzinski and Argubright 2019; Yorzinski 2020a) but at least 5 days had passed since they were previously tested; none of the birds used in "Experiment 2" had been tested in a previous study.

Stimuli

The visual stimuli consisted of photographs of human faces and controls (tree bark, scrambled versions of the human faces, and scrambled versions of the tree bark; Fig. 1). Human faces were used as the threatening stimuli because passerines perceive humans as threats (Carter et al. 2008) and grackles are known to mob humans (Johnson and Peer 2020). Even though human caretakers provided the captive grackles in this study with daily feed, the grackles still perceived the human caretakers as threatening (they emitted alarm calls and avoided the human caretakers during feedings); in addition, most grackles were tested soon after they were captured from the wild (median 28 days; range 8–135 days). Furthermore, many of the captive grackles exhibited pupil dilation and piloerection when viewing the human face stimuli (see "Results"). Tree bark was used as the control because the birds regularly see tree bark but it is not dangerous to them. The scrambled versions of the faces and bark were used as additional controls that preserved some of the low-level features of the stimuli (e.g., color) but did not resemble the original stimuli.

The human faces were life-size (mean length between top of head and chin: 22 cm), exhibited neutral expressions, and depicted women (Radboud Faces Database; Langner et al. 2010). The tree bark was the same size as the human

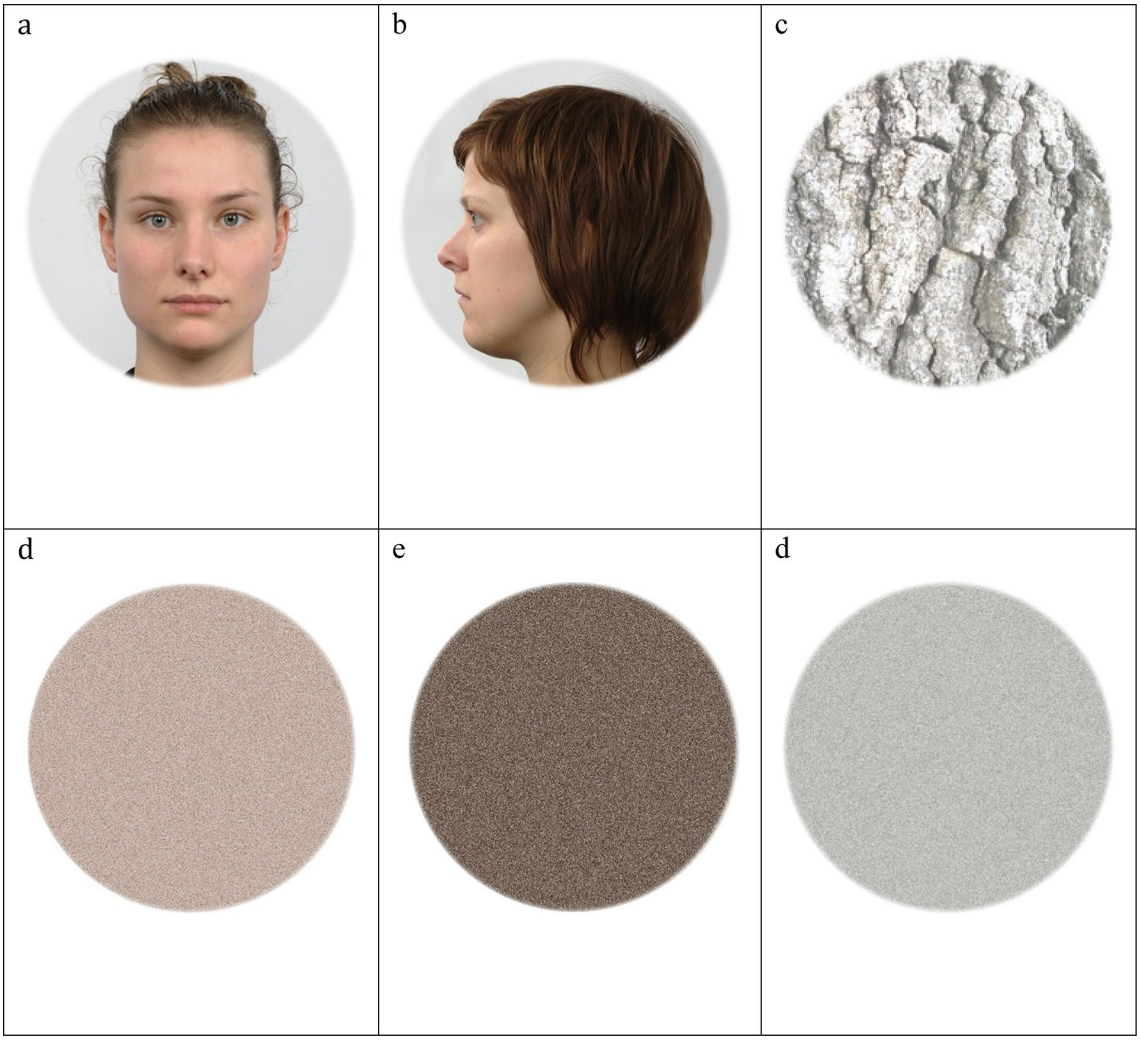

Fig. 1 Example experimental stimuli: **a** directed face, **b** averted face, **c** tree bark, **d** scrambled version of the directed face, **e** scrambled version of the averted face, and **f** scrambled version of the tree bark

faces and photographed (EOS Rebel T6, Canon, Inc.) from local trees. The scrambled version of the faces and tree bark were created by randomly repositioning every pixel within each stimuli. The stimuli were positioned atop a white background (2560 × 1440 px) that filled the display monitor. They were positioned close to one edge of the monitor (1.5 cm between the monitor's vertical edge to the closest edge of the stimuli) such that they were perpendicular to the birds' heads (Fig. S1). All images were matched for luminance.

In "Experiment 1", each bird viewed four stimuli: directed human face (eyes and face were oriented straight ahead), tree bark, scrambled version of the directed human face, and scrambled version of the tree bark. The order of the stimuli was pseudorandomized across birds such that each stimuli type appeared an equal number of times in each order (i.e., one-quarter of the birds viewed the human face first, one-quarter of the birds viewed the tree bark first, one-quarter of the birds viewed the scrambled version of the human face first, and one-quarter of the birds viewed the scrambled version of the tree bark first). To avoid pseudoreplication, five different faces and five different tree bark (along with their scrambled versions) were used across birds.

In "Experiment 2", the procedures were the same but the stimuli differed. Each bird viewed four stimuli: directed human face (eyes and face were oriented straight ahead), averted human face (eyes and face were oriented to the left or right), scrambled version of the directed human face, and scrambled version of the averted human face. Within a given bird, the directed face and averted face depicted different people. The faces of five different people were used across birds (these five people were different from those depicted in "Experiment 1"). Half of the averted faces were oriented to the left and the other half were oriented to the right.

Experimental design

For each trial, a bird was individually transported from its outdoor aviary to an indoor testing area (approximately 160 m apart). The testing arena consisted of a section (0.83 m × 0.63 m × 0.66 m) within a large plastic box (Wolverine model cooler; IRP Inc.) that contained two monitors (Dell 27″ S2716DG; 144 Hz; 2560 × 1440 pixels) positioned on each side of the bird (Movie S1; Fig. S1). An LED light strip on the roof of the box provided lighting (2.2 kLux; Extech Easyview 31 light meter positioned directly upwards at the location of the foam cradle). The bird was secured in a foam cradle using velcro straps and placed inside the testing arena atop a wooden block (0.18 × 0.25 × 0.08 m). The bill of the bird was fastened to a wooden dowel (0.14 m high) that was secured to the testing arena floor to keep the bird's head positioned at the same angle relative to the monitors. When the heads and bodies of grackles are restrained, the percentage of time they spend blinking is roughly similar to when only their bodies are restrained (Yorzinski and Argubright 2019; Yorzinski 2020a) or when they are unrestrained (Yorzinski 2020b). Two video cameras (Canon VIXIA HF R70; 60 frames/s) were located on opposite sides of the bird to record each eye. The bird was also monitored in real time using camcorders (SRPRO-T855CAM, Swann Communications, Inc.) multiplexed to a DVR (model 2600, Swann Communications, Inc.). The stimuli were displayed for 1 min each and 5 min elapsed between stimuli (the first stimulus did not appear until 5 min after the bird was inside the testing arena). The stimuli appeared on one monitor for half of the trials and the other monitor for the other half of the trials; the stimuli always appeared on the same monitor for a given bird while the other monitor displayed a white background. Custom Matlab scripts (Mathworks, Inc., Natick, MA) were used to present the digital stimuli. The temperature (Experiment 1, mean ± SE 23.9 ± 0.09 °C; range 20.1–26.7 °C; Experiment 2, mean ± SE 23.3 ± 0.07 °C; range 19.9–26.0 °C) and relative humidity (Experiment 1, mean ± SE 59.1 ± 0.2%; range 50.7–66.7%; Experiment 2, mean ± SE 41.8 ± 0.5%; range 29.3–69.6%) inside the testing arena were continuously recorded (HOBO MX2301; 1-s intervals).

Video analysis and interobserver reliability

The blinking behavior of the birds was measured from the videos using Quicktime (version 7; Apple Inc.). All the videos from a given trial were synchronized and a 3-min clip was extracted from each trial that included three time periods for each of the four stimuli: a 1-min period before each stimulus appeared, a 1-min period while each stimulus was present, and a 1-min period after each stimulus was no longer present; 12 min of videos was, therefore, analyzed for each bird (3 min per trial × 4 trials). For each trial, the frame at which each blink began and ended during the 3-min clip was recorded. A blink start was defined as the first frame when the nictitating membrane was visible and the blink end was defined as the first frame when the nictitating membrane was no longer visible. The blinks in the left and right eye were recorded separately because the birds did not always synchronize their blinks between the eyes. In addition to scoring the blinking behavior of the birds, we also recorded when the birds exhibited piloerection (feathers atop their heads becoming erect), an indication of reactivity (Hilton 1982; Caine and Weldon 1989; Coss 1991; Benedek and Kaernbach 2011; Yorzinski and Platt 2012). Even though the birds were restrained, they sometimes moved their heads and bodies within the cradle, likely trying to escape from the restraint; we assessed this movement behavior by quantifying their latency to first move their heads or bodies after each stimulus appeared. Lastly, we measured pupil size (in pixels of the eye viewing the stimuli; ImagJ) four times for each bird for each stimulus: immediately before the stimulus appeared, 1 s after the stimulus appeared, 30 s after the stimulus appeared, and immediately before the stimulus disappeared. We converted the pupil size to millimeters by approximating the diameter of the birds' irises to be 5.3 mm.

To ensure reliability in coding the blinking behavior, the coders practiced their scoring methods on a video from one of the trials. After an initial training period in which they scored at least 20 blinks and received feedback on their scoring from a trainer (JLY), they scored another 20 blinks and these blinks were compared to those scored by the trainer. The blinks from the coders (three coders in "Experiment 1" and two coders in "Experiment 2") and the trainer were scored similarly (100% of the blinks of each coder were scored within one frame of how they were scored by the trainer). Piloerection and movement behavior were scored by a single coder (JLY).

Using customized Matlab scripts, the blink rate (mean number of blinks in the left and right eye divided by the time period; blinks per min), blink duration (mean duration of each blink in the left and right eye; s), and time the birds spent blinking (mean amount of time the left and right eye were blinking divided by the time period and multiplied by 100; percentage) were calculated for each time period. Individual blinks lasted at least two frames (33.3 ms).

Statistical analysis

The data were analyzed using linear mixed-effects models with repeated measures in SAS (PROC MIXED; unstructured covariance structure; Version 9.4; SAS Institute Inc., Cary, NC). The denominator degrees of freedom were computed with the DDFM = CONTAIN option in SAS, the default method that uses the containment method. Since the blinking variables were highly correlated, a factor analysis (using principal components as the method of extraction) was performed on the blinking variables (blink rate, blink duration, and time spent blinking) to extract a single factor ("blinking behavior;" Minitab version 18.1; Minitab Inc., State College, PA). This factor score was used as the dependent variable. The independent variables were the stimuli (Experiment 1: directed face, bark, scrambled version of the directed face, and scrambled version of the bark; Experiment 2: directed face, averted face, scrambled version of the directed face, and scrambled version of the averted face), time period (before, during, or after the stimuli appeared), the interaction between stimuli and time period, and eye viewing (whether the left or right eye was viewing the stimuli) as well as the ambient temperature (mean across each minute time period) and ambient relative humidity (mean across each minute time period). Stimuli were nested within bird identity and they were included as random effects. A priori contrasts were performed to compare the blinking behavior between stimuli and time periods; 12 comparisons were performed and the false discovery rate correction was used to evaluate statistical significance (the false discovery rate was controlled at $q^* = 0.05$; Benjamini and Hochberg 1995). This model was also rerun using the individual blinking variables—blink rate, blink duration (natural log transformed to meet underlying assumptions of normality), and time spent blinking—as the dependent variables. We used the mean values of the left and right eyes because the blinking behavior in the left and right eyes were highly correlated (Experiment 1, blink rate: $F_{1,30} = 7965.99$, $p < 0.0001$; blink duration: $F_{1,30} = 144.98$, $p < 0.0001$; time the birds spent blinking: $F_{1,30} = 5191.95$, $p < 0.0001$; Experiment 2, blink rate: $F_{1,31} = 5192.55$, $p < 0.0001$; blink duration: $F_{1,31} = 323.20$, $p < 0.0001$; time the birds spent blinking: $F_{1,31} = 3570.75$, $p < 0.0001$).

We performed a follow-up analysis because some birds exhibited piloerection when the face stimuli were present (they did not exhibit piloerection in any of the before time periods or in response to the control stimuli). We examined whether the blinking behavior (factor score of blink rate, blink duration, and time spent blinking; see above) was related to piloerection using linear mixed-effects models (Experiment 1 independent variables: piloerection, time period, eye viewing, temperature, and relative humidity; Experiment 2 independent variables: piloerection, time period, stimuli, eye viewing, temperature, and relative humidity). Stimuli were nested within bird identity and they were included as random effects. This model was also rerun using the individual blinking variables—blink rate, blink duration (natural log transformed), and time spent blinking—as the dependent variables.

We assessed the grackles' movement behavior by performing a survival analysis based on the Cox proportional hazards model (PROC PHREG in SAS). If the birds moved within the during or after time period, the dependent variable was their latency to first move after the stimuli appeared; if the birds did not move within the during or after time period, the dependent variable was the experimental time limit (2 min). The independent variables were the stimuli, eye viewing, temperature and relative humidity. Bird identity was included as a repeated measure.

Lastly, we performed linear mixed-effects models to assess whether pupil size varied relative to the stimuli. The dependent variable was the pupil size while the stimuli were visible (an average of the pupil size 1 s after the stimuli appeared, 30 s after the stimuli appeared, and immediately before the stimuli disappeared). The independent variables were the stimuli, pupil size immediately before the stimuli appeared, eye viewing, temperature and relative humidity. Bird identity was included as a random effect.

Results

Experiment 1

A single factor derived from varimax rotation explained 61.0% of the variance in the blinking variables. The blinking variables loaded positively on a single factor. The factor score coefficients was highest for the time spent blinking (blink rate 0.34; blink duration 0.37; time spent blinking 0.54); similarly, the proportion of variability explained by the factor (communality) was highest for the time spent blinking (blink rate: 0.39; blink duration: 0.45; time spent blinking: 0.99).

The birds modified their blinking behavior in response to the stimuli ($F_{3,123} = 17.13$, $p < 0.0001$; Table 1; Fig. 2). During the directed face stimuli, the blinking behavior decreased when the face appeared ($t_{1,246} = 15.72$, $p < 0.0001$; movie S1) and increased after the face disappeared ($t_{1,246} = 8.93$, $p < 0.0001$). Even after the face disappeared, the blinking behavior was lower compared to before the face appeared ($t_{1,246} = 6.77$, $p < 0.0001$). The blinking behavior was similar before, during, and after the bark, scrambled version of the directed face, and scrambled version of the bark were presented ($q^* > 0.05$). The eye viewing the stimuli did not impact blinking behavior ($F_{1,246} = 1.10$, $p = 0.30$). The results were similar when the analysis was performed on the individual blinking variables (blink rate, blink duration, and time spent blinking; Table S1; Fig. S2-S4).

Some birds (34.4%) exhibited piloerection while the directed face was present. The birds that exhibited piloerection when the face was present exhibited less blinking behavior compared to the birds that did not exhibit piloerection when the face was present ($t_{1,58} = 3.13$, $p = 0.0027$; Table 2; Fig. 3). Even after the face disappeared, the birds

Table 1 The effect of stimuli, time period, eye viewing, temperature and relative humidity on blinking behavior during Experiment 1

Overall model	Numerator *df*, denominator *df*	*F* value (*p* value)	
Stimuli	3, 123	17.13 (<0.0001)*	
Time period	2, 246	34.33 (<0.0001)*	
Stimuli × time period	6, 246	30.94 (<0.0001)*	
Eye viewing	1, 246	1.10 (0.30)	
Temperature	1, 246	8.61 (0.004)*	
Relative humidity	1, 246	0.14 (0.71)	
Comparisons	**Numerator *df*, denominator *df***	**Difference of least-squares means (standard error)**	***t* value (*p* value)**
Directed face			
Before vs. during	1, 246	1.57 (0.10)	15.72 (<0.0001)*
During vs. after	1, 246	0.89 (0.10)	8.93 (<0.0001)*
Before vs. after	1, 246	0.68 (0.10)	6.77 (<0.0001)*
Bark			
Before vs. during	1, 246	0.16 (0.10)	1.57 (0.12)
During vs. after	1, 246	0.04 (0.10)	0.36 (0.72)
Before vs. after	1, 246	0.19 (0.10)	1.92 (0.06)
Directed face scramble			
Before vs. during	1, 246	0.03 (0.10)	0.25 (0.80)
During vs. after	1, 246	0.06 (0.10)	0.55 (0.58)
Before vs. after	1, 246	0.08 (0.10)	0.80 (0.43)
Bark scramble			
Before vs. during	1, 246	0.09 (0.10)	0.94 (0.35)
During vs. after	1, 246	0.03 (0.10)	0.28 (0.78)
Before vs. after	1, 246	0.07 (0.10)	0.66 (0.51)
During			
Directed face vs. bark	1, 246	1.43 (0.12)	11.46 (<0.0001)*
Directed face vs. directed face scramble	1, 246	1.48 (0.14)	10.54 (<0.0001)*
Directed face vs. bark scramble	1, 246	1.48 (0.14)	10.31 (<0.0001)*
Bark vs. directed face scramble	1, 246	0.05 (0.13)	0.41 (0.68)
Bark vs. bark scramble	1, 246	0.05 (0.13)	0.36 (0.72)
Directed face scramble vs. bark scramble	1, 246	0.005 (0.10)	0.05 (0.96)

Statistically significant variables or comparisons are indicated with an asterisk

that exhibited piloerection exhibited less blinking behavior compared to the birds that did not exhibit piloerection ($t_{1,58} = 2.13$, $p = 0.037$). Regardless of whether the birds exhibited piloerection, their blinking behavior decreased after the face appeared (no piloerection: $t_{1,58} = 7.75$, $p < 0.0001$; piloerection: $t_{1,58} = 10.91$, $p < 0.0001$). The results were similar when the analysis was performed on the individual blinking variables (blink rate, blink duration, and time spent blinking; Table S2; Fig. S5-S7).

After the stimuli appeared, the birds remained still for more time when viewing the directed faces compared to the controls ($q^* < 0.05$; Table 3; Fig. S8). The pupil size of the birds was larger when viewing the directed faces (mean ± SE 2.99 ± 0.050 mm) compared to the controls (bark: 2.83 ± 0.047 mm; directed face scramble: 2.82 ± 0.045 mm; bark scramble: 2.79 ± 0.044 mm; Table 4).

Experiment 2

A single factor derived from varimax rotation explained 60.8% of the variance in the blinking variables. Blink rate and time spent blinking loaded positively while blink duration loaded negatively on the factor. The factor score coefficients were highest for the blink rate and time spent blinking (blink rate: 0.53; blink duration: − 0.03; time spent blinking: 0.52); similarly, the proportion of variability explained by the factor (communality) were highest for the blink rate and time spent blinking (blink rate: 0.96; blink duration: − 0.061; time spent blinking: 0.94).

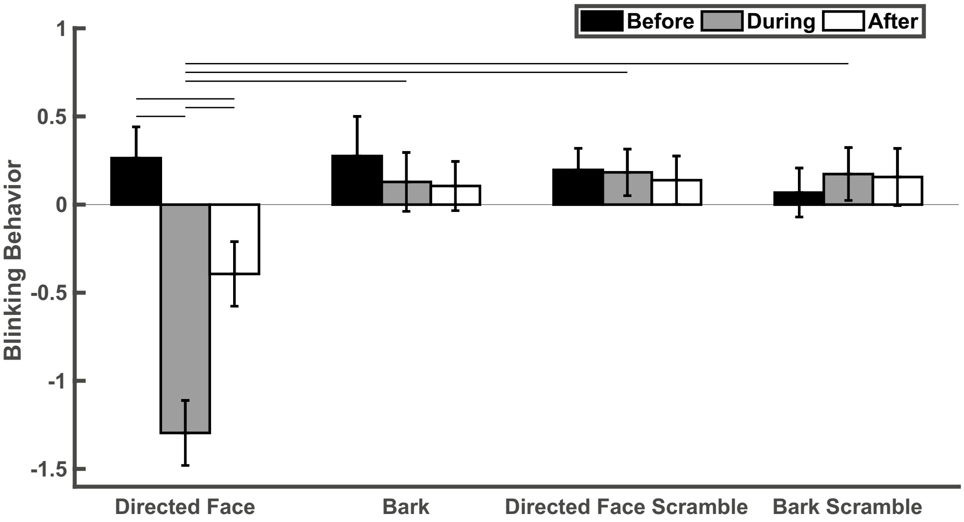

Fig. 2 Blinking behavior (composite factor including blink rate, blink duration, and time spent blinking) before, during, and after the stimuli in "Experiment 1". Means and standard error bars are shown; horizontal lines indicate planned comparisons that were statistically significant

Table 2 The effect of piloerection, time period, eye viewing, temperature and relative humidity on blinking behavior during Experiment 1

Overall model	Numerator *df*, denominator *df*	*F* value (*p* value)	
Piloerection	1, 58	3.77 (0.06)	
Time period	2, 58	89.74 (< 0.0001)*	
Piloerection × time period	2, 58	9.60 (0.0003)*	
Eye viewing	1, 58	1.21 (0.28)	
Temperature	1, 58	0.48 (0.49)	
Relative humidity	1, 58	0.36 (0.55)	
Comparisons	Numerator *df*, denominator *df*	Difference of least-squares means (standard error)	*t* value (*p* value)
No piloerection			
Before vs. during	1, 58	1.18 (0.15)	7.75 (< 0.0001)*
During vs. after	1, 58	0.77 (0.15)	5.07 (< 0.0001)*
Before vs. after	1, 58	0.41 (0.15)	2.67 (0.0098)*
Piloerection			
Before vs. during	1, 58	2.30 (0.21)	10.91 (< 0.0001)*
During vs. after	1, 58	1.13 (0.21)	5.38 (< 0.0001)*
Before vs. after	1, 58	1.16 (0.21)	5.52 (< 0.0001)*
Piloerection vs. no piloerection			
Before	1, 58	0.01 (0.36)	0.03 (0.98)
During	1, 58	1.13 (0.36)	3.13 (0.0027)*
After	1, 58	0.77 (0.36)	2.13 (0.037)*

Statistically significant variables or comparisons are indicated with an asterisk

The birds modified their blinking behavior in response to the stimuli ($F_{3,123} = 18.52$, $p < 0.0001$; Table 5; Fig. 4). The birds decreased their blinking behavior when viewing the directed ($t_{1,245} = 16.61$, $p < 0.0001$) and averted ($t_{1,245} = 9.53$, $p < 0.0001$) faces. Interestingly, the birds decreased their blinking behavior even more when viewing the directed faces compared to the averted faces ($t_{1,245} = 5.34$, $p < 0.0001$). After the faces disappeared, their blinking behavior increased (directed faces: $t_{1,245} = 10.99$, $p < 0.0001$; averted faces: $t_{1,245} = 7.05$, $p < 0.0001$). Their blinking behavior was lower after the faces disappeared compared to before they appeared (directed faces: $t_{1,245} = 5.67$, $p < 0.0001$; averted faces: $t_{1,245} = 2.48$, $p = 0.014$). The blinking behavior was similar before, during, and after the scrambled versions of the faces were presented ($q^* > 0.05$). The eye viewing the stimuli did not impact blinking behavior ($F_{1,245} = 0.03$, $p = 0.86$). The results were similar when the analysis was performed on the individual blinking variables (blink rate,

Fig. 3 Blinking behavior during the face stimuli relative to whether the birds exhibited piloerection in "Experiment 1". Means and standard error bars are shown; horizontal lines indicate planned comparisons that were statistically significant

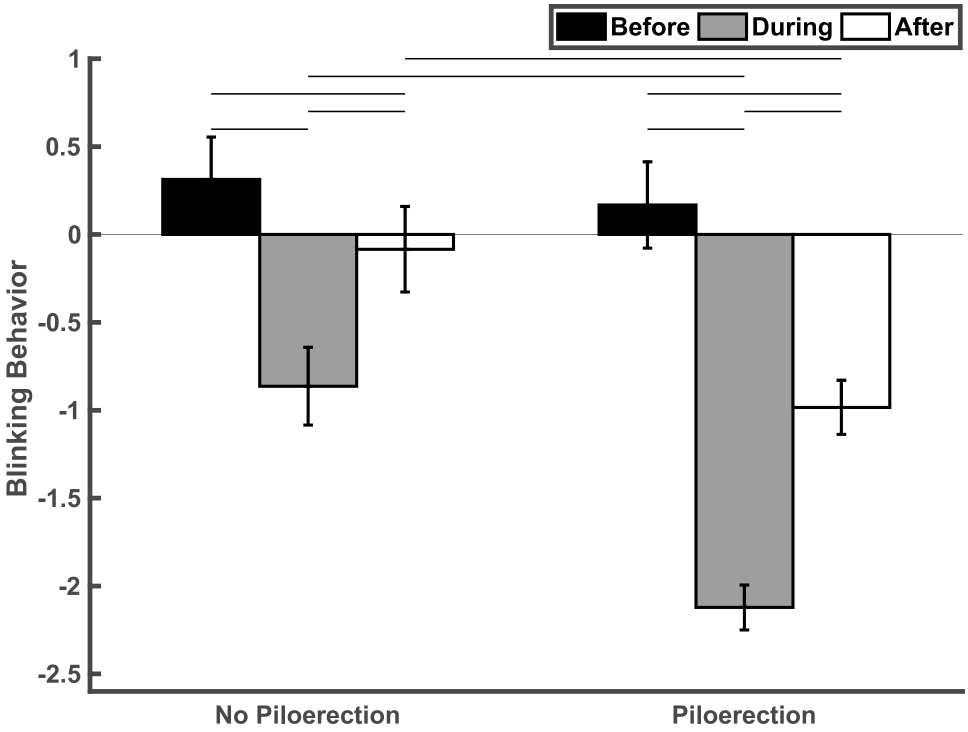

Table 3 The effect of stimuli, eye viewing, temperature and relative humidity on movement behavior during Experiment 1

	χ^2	p value
Overall model		
Stimuli	38.13	< 0.0001*
Eye viewing	0.03	0.86
Temperature	0.63	0.43
Relative humidity	1.41	0.24
Comparisons		
Directed face vs.		
Bark	28.94	< 0.000*
Directed face scramble	31.30	< 0.0001*
Bark scramble	27.51	< 0.0001*
Bark vs.		
Directed face scramble	0.73	0.39
Bark scramble	0.002	0.97
Directed face scramble vs.		
Bark scramble	0.50	0.48

Statistically significant variables or comparisons are indicated with an asterisk

blink duration, and time spent blinking; Table S3; Fig. S9–S11).

When the faces were present, some birds exhibited piloerection (directed faces: 34.4%; averted faces: 18.8%). The birds that exhibited piloerection when the faces were present exhibited less blinking behavior compared to the birds that did not exhibit piloerection when the faces were present (directed faces: $t_{1,116} = 3.68$, $p = 0.0004$; averted faces: $t_{1,116} = 2.42$, $p = 0.017$; Table 6; Fig. 5). Regardless of whether the birds exhibited piloerection, their blinking behavior decreased after the directed faces (no piloerection: $t_{1,116} = 9.01$, $p < 0.0001$; piloerection: $t_{1,116} = 11.29$, $p < 0.0001$) or the averted faces (no piloerection: $t_{1,116} = 5.66$, $p < 0.0001$; piloerection: $t_{1,116} = 6.66$, $p < 0.0001$) appeared. The results were similar when the analysis was performed on the individual blinking variables (blink rate, blink duration, and time spent blinking; Table S4; Fig. S12–14).

After the stimuli appeared, the birds remained still for more time when viewing the directed faces compared to the averted faces. Furthermore, they remained still for more time following both the directed and averted faces relative to the controls (directed face scramble and averted face scramble; $q^* < 0.05$; Table 7; Fig. S15). Pupil size was larger when the birds were viewing the face stimuli (directed face: 2.92 ± 0.040 mm; averted face: 2.91 ± 0.035 mm) compared to the controls (directed face scramble: 2.78 ± 0.037 mm; averted face scramble: 2.80 ± 0.038 mm; Table 8).

Discussion

Great-tailed grackles inhibited their blinks the most when they viewed human faces, especially when the human faces exhibited directed gaze. The grackles likely perceived the human faces as potentially threatening, as evidenced by the

Table 4 The effect of stimuli, pupil size before the stimuli appeared, eye viewing, temperature and relative humidity on pupil size during Experiment 1

Overall model	Numerator *df*, denominator *df*	*F* value (*p* value)
Stimuli	3, 90	17.10 (0.0001)*
Pupil size before stimuli appeared	1, 90	4.46 (0.04)*
Eye viewing	1, 90	0.38 (0.54)
Temperature	1, 90	6.01 (0.02)*
Relative humidity	1, 90	0.06 (0.80)

Comparisons	Numerator *df*, denominator *df*	Difference of least-squares means (standard error)	*t* value (*p* value)
Directed face vs.			
Bark	1, 90	0.17 (0.03)	5.37 (< 0.0001)*
Directed face scramble	1, 90	0.18 (0.03)	5.62 (< 0.0001)*
Bark scramble	1, 90	0.20 (0.03)	6.39 (< 0.0001)*
Bark vs.			
Directed face scramble	1, 90	0.009 (0.03)	0.27 (0.78)
Bark scramble	1, 90	0.03 (0.03)	1.03 (0.31)
Directed face scramble vs.			
Bark scramble	1, 90	0.02 (0.03)	0.76 (0.45)

Statistically significant variables or comparisons are indicated with an asterisk

birds' piloerection, remaining relatively still, and pupil dilation when viewing the faces. The grackles' blinking behavior did not change when they viewed the non-threatening stimuli (tree bark, scrambled versions of the faces, or scrambled versions of tree bark).

While blinking behavior is necessary to maintaining clear vision (Koh et al. 2006; Sweeney et al. 2013), blinking likely limits information gathering. When birds blink, their nictitating membranes move across their eyes. Studies in humans have shown that blinks suppress neural activity in areas of the brain associated with perceiving environmental change (Volkemann et al. 1980; Bristow et al. 2005) but it is not known whether birds have suppressed neural processing as well. Regardless, the semi-transparent nature of grackles' nictitating membrane likely causes some visual impairment during blinks. By strategically modifying their blinks during potentially dangerous contexts (i.e., inhibiting their blinks during human face stimuli), it is possible that the grackles can maximize information gathering. During encounters with potential threats, birds may maximize visual input to learn about the threat or look for escape opportunities. It is also possible that the grackles inhibited their blinking behavior when under threat to avoid detection or feign death. The grackles remained relatively still (except for eye movements, piloerection, and breathing) after the threatening stimuli appeared. Additional studies that explore how blinks are perceived by predators would provide insight into this possibility.

Previous work in humans has demonstrated that individuals strategically modify their blinking behavior: people suppress their blinks before predictable events that require their attention (Hoppe et al. 2018). Studies in nonhuman primates and birds have also suggested that blinking behavior is strategic. Anubis baboons (*Papio anubis*) and chickens (*Gallus gallus*) exhibit lower blink rates when they are in smaller compared to larger groups, presumably because individuals in small groups experience high predation and need to be more vigilant (Beauchamp 2017; Matsumoto-Oda et al. 2018). Peacocks (*Pavo cristatus*) also alter their blinking behavior when under threat by inhibiting their blinks the most when they are highly vigilant (Yorzinski 2016) and American crows (*Corvus brachyrhynchos*) blink less in response to possible danger (Cross et al. 2013). Similarly, great-tailed grackles inhibit their blinks when engaging in potentially risky locomotion (Yorzinski 2020b). In addition, rhesus macaques (*Macaca mulatta*) blink less when viewing conspecifics engaging in social behavior (Ballesta et al. 2016).

The grackles in this study were not simply inhibiting their blinks in response to faces. When viewing the faces, their blinking behavior was modified based on their perceived level of risk. The grackles inhibited their blinks the most when viewing the human faces that were directed toward them rather than away from them. Since birds often perceive human faces that are directed toward them as more dangerous than human faces that are directed away from them (Hampton 1994; Carter 2008; Freeberg et al. 2014, 2016), the grackles also likely perceived the human faces that were directed toward them as more threatening than the human faces that were directed away from them. This suggests that

Table 5 The effect of stimuli, time period, eye viewing, temperature and relative humidity on blinking behavior during Experiment 2

	Numerator *df*, denominator *df*	*F* value (*p* value)	
Overall model			
Stimuli	3, 123	18.52 (< 0.0001)*	
Time period	2, 245	86.76 (< 0.0001)*	
Stimuli × time period	6, 245	35.47 (< 0.0001)*	
Eye viewing	1, 245	0.03 (0.86)	
Temperature	1, 245	10.30 (0.0015)*	
Relative humidity	1, 245	23.60 (< 0.0001)*	
Comparisons	Numerator *df*, denominator *df*	Difference of least-squares means (standard error)	*t* value (*p* value)
Directed face			
Before vs. during	1, 245	1.62 (0.10)	16.61 (< 0.0001)*
During vs. after	1, 245	1.07 (0.10)	10.99 (< 0.0001)*
Before vs. after	1, 245	0.55 (0.10)	5.67 (< 0.0001)*
Averted face			
Before vs. during	1, 245	0.92 (0.10)	9.53 (< 0.0001)*
During vs. after	1, 245	0.68 (0.10)	7.05 (< 0.0001)*
Before vs. after	1, 245	0.24 (0.10)	2.48 (0.014)*
Directed face scramble			
Before vs. during	1, 245	0.000005 (0.10)	0.00 (1.00)
During vs. after	1, 245	0.05 (0.10)	0.51 (0.61)
Before vs. after	1, 245	0.05 (0.10)	0.51 (0.61)
Averted face scramble			
Before vs. during	1, 245	0.04 (0.10)	0.43 (0.66)
During vs. after	1, 245	0.007 (0.10)	0.07 (0.95)
Before vs. after	1, 245	0.04 (0.10)	0.37 (0.72)
During			
Directed face vs. averted face	1, 245	0.75 (0.14)	5.34 (< 0.0001)*
Directed face vs. directed face scramble	1, 245	1.76 (0.16)	11.31 (< 0.0001)*
Directed face vs. averted face scramble	1, 245	1.76 (0.15)	11.92 (< 0.0001)*
Averted face vs. directed face scramble	1, 245	1.013 (0.12)	8.52 (< 0.0001)*
Averted face vs. averted face scramble	1, 245	1.008 (0.11)	8.86 (< 0.0001)*
Directed face scramble vs. averted face scramble	1, 245	0.005 (0.09)	0.05 (0.96)

Statistically significant variables or comparisons are indicated with an asterisk

grackles can strategically adjust their blinking behavior relative to their perceived level of risk. It is also possible that the grackles inhibit their blinking behavior in response to any animal stimuli (threatening or non-threatening), especially those with directed gaze. In fact, other avian species are most fearful of faces that are directed toward them and display two eyes (Hampton 1994; Freeberg et al. 2014). Future experiments that examine the specificity of blinking behavior would be valuable.

Reactivity also influenced blinking behavior in the grackles. Some of the birds exhibited piloerection when viewing the faces, indicating a high level of reactivity, whereas other birds did not exhibit piloerection, indicating a lower level of reactivity (Hilton 1982; Caine and Weldon 1989; Coss 1991; Benedek and Kaernbach 2011; Yorzinski and Platt 2012). The grackles that exhibited piloerection suppressed their blinks the most when viewing the human face stimuli. When the birds did not exhibit piloerection, the percentage

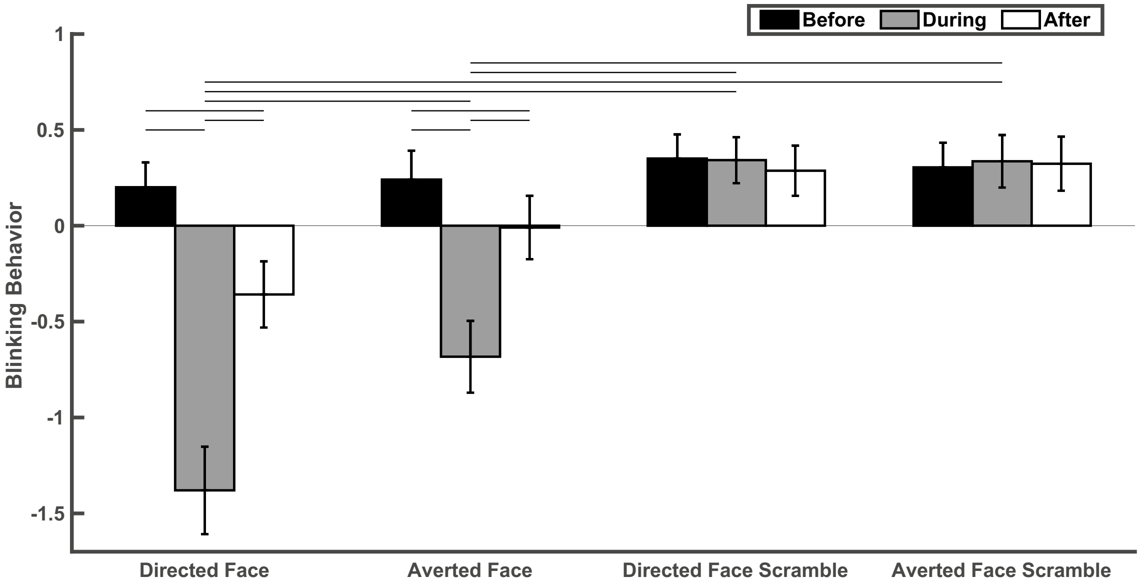

Fig. 4 Blinking behavior (composite factor including blink rate, blink duration, and time spent blinking) before, during, and after the stimuli in "Experiment 2". Means and standard error bars are shown; horizontal lines indicate planned comparisons that were statistically significant

of time they spent blinking decreased by 41% after the human face stimuli appeared; in contrast, when the birds exhibited piloerection, the percentage of time they spent blinking decreased by 81% after the human face stimuli appeared ("Experiment 1"). Mechanistically, piloerection can occur when individuals have high levels of epinephrine (DeCatanzaro and Graham 1992), a hormone and neurotransmitter released from the adrenal glands during stress. As such, epinephrine may also influence blink inhibition in birds during threatening contexts.

Since blinking likely interferes with visual perception, animals can strategically time their blinks to avoid missing critical information. While evidence indicates that humans strategically time their blinks (Hoppe et al. 2018), very little is known about nonhuman animals. Additional studies that measure the costs of blinking in nonhuman animals would be informative. Nonhuman animals vary widely in their blinking behavior across species (Blount 1927; Kirsten and Kirsten 1983; Tada et al. 2013) and within species (Cross et al. 2013; Yorzinski 2016; Beauchamp 2017; Matsumoto-Oda et al. 2018); this variation could influence their strategic blinking behavior. For example, individuals that blink at high rates may be slow to detect predators, and therefore, suffer high predation risk. Future studies that experimentally determine the costs associated with blinking in nonhuman animals would be informative.

Table 6 The effect of piloerection, time period, eye viewing, temperature and relative humidity on blinking behavior during Experiment 2

Overall model	Numerator *df*, denominator *df*	*F* value (*p* value)
Piloerection	1, 116	1.95 (0.16)
Time period	2, 116	126.29 (< 0.0001)*
Stimuli	1, 60	8.16 (0.0059)*
Piloerection × time period × stimuli	7, 116	7.13 (< 0.0001)*
Eye viewing	1, 116	0.49 (0.49)
Temperature	1, 116	1.01 (0.32)
Relative humidity	1, 116	25.36 (< 0.0001)*

Comparisons	Numerator *df*, denominator *df*	Difference of least-squares means (standard error)	*t* value (*p* value)
No piloerection: directed face			
Before vs. during	1, 116	1.30 (0.14)	9.01 (< 0.0001)*
During vs. after	1, 116	0.80 (0.14)	5.56 (< 0.0001)*
Before vs. after	1, 116	0.50 (0.14)	3.50 (0.0007)*
Piloerection: directed face			
Before vs. during	1, 116	2.21 (0.20)	11.29 (< 0.0001)*
During vs. after	1, 116	1.54 (0.20)	7.90 (< 0.0001)*
Before vs. after	1, 116	0.66 (0.20)	3.39 (0.0009)*
No piloerection: averted face			
Before vs. during	1, 116	0.72 (0.13)	5.66 (< 0.0001)*
During vs. after	1, 116	0.54 (0.13)	4.29 (< 0.0001)*
Before vs. after	1, 116	0.17 (0.13)	1.38 (0.17)
Piloerection: averted face			
Before vs. during	1, 116	1.88 (0.28)	6.66 (< 0.0001)*
During vs. after	1, 116	1.24 (0.28)	4.38 (< 0.0001)*
Before vs. after	1, 116	0.64 (0.29)	2.22 (0.029)*
Piloerection vs. no piloerection: directed face			
Before	1, 116	0.09 (0.27)	0.32 (0.75)
During	1, 116	1.00 (0.27)	3.68 (0.0004)*
After	1, 116	0.25 (0.27)	0.94 (0.35)
Piloerection vs. no piloerection: averted face			
Before	1, 116	0.41 (0.34)	1.22 (0.23)
During	1, 116	0.75 (0.31)	2.42 (0.017)*
After	1, 116	0.06 (0.34)	0.17 (0.87)

Statistically significant variables or comparisons are indicated with an asterisk

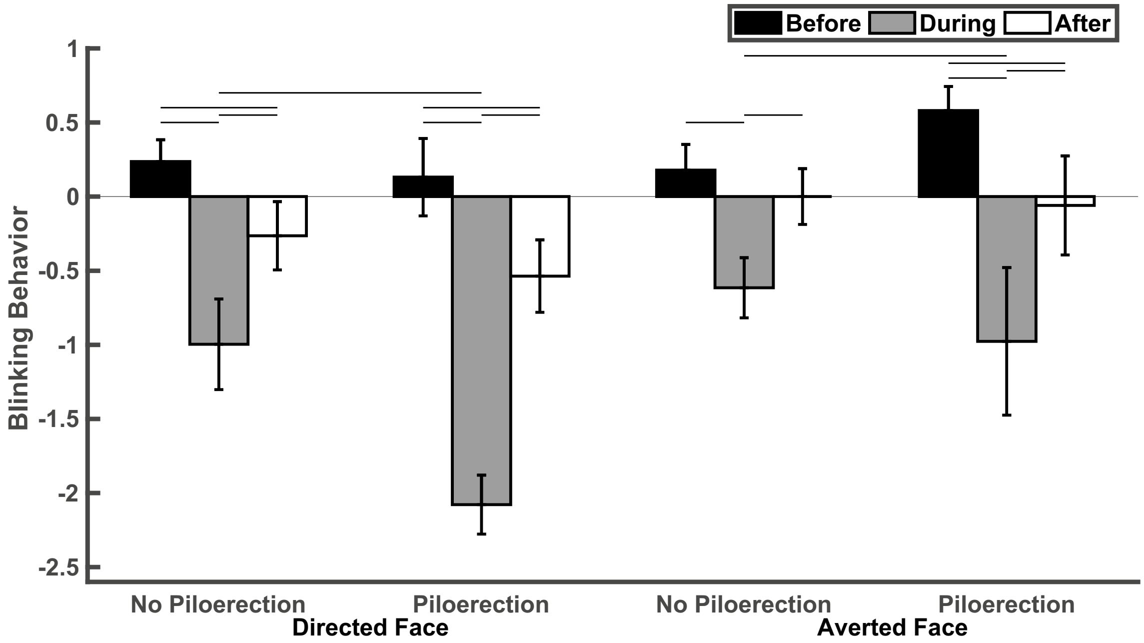

Fig. 5 Blinking behavior during the face stimuli relative to whether the birds exhibited piloerection in "Experiment 2". Means and standard error bars are shown; horizontal lines indicate planned comparisons that were statistically significant

Table 7 The effect of stimuli, eye viewing, temperature and relative humidity on movement behavior during Experiment 2

	χ^2	*p* value
Overall model		
Stimuli	44.91	< 0.0001*
Eye viewing	4.50	0.034*
Temperature	0.006	0.94
Relative humidity	4.95	0.026*
Comparisons		
Directed face vs.		
Averted face	9.28	0.0023*
Directed face scramble	37.54	< 0.0001*
Averted face scramble	33.81	< 0.0001*
Averted face vs.		
Directed face scramble	41.03	< 0.0001*
Averted face scramble	30.09	< 0.0001*
Directed face scramble vs.		
Averted face scramble	0.01	0.92

Statistically significant variables or comparisons are indicated with an asterisk

Table 8 The effect of stimuli, pupil size before the stimuli appeared, eye viewing, temperature and relative humidity on pupil size during Experiment 2

	Numerator *df*, denominator *df*	*F* value (*p* value)
Overall model		
Stimuli	3, 90	16.41 (< 0.0001)*
Pupil size before stimuli appeared	1, 90	3.62 (0.060)
Eye viewing	1, 90	0.79 (0.38)
Temperature	1, 90	6.10 (0.015)*
Relative humidity	1, 90	5.43 (0.022)*

Comparisons	Numerator *df*, denominator *df*	Difference of least-squares means (standard error)	*t* value (*p* value)
Directed face vs.			
Averted face	1, 90	0.02 (0.03)	0.59 (0.55)
Directed face scramble	1, 90	0.14 (0.03)	5.66 (< 0.0001)*
Averted face scramble	1, 90	0.12 (0.03)	4.71 (< 0.0001) *
Averted face vs.			
Directed face scramble	1, 90	0.13 (0.03)	5.07 (< 0.0001) *
Averted face scramble	1, 90	0.11 (0.03)	4.11 (< 0.0001) *
Directed face scramble vs.			
Averted face scramble	1, 90	0.02 (0.03)	0.93 (0.36)

Statistically significant variables or comparisons are indicated with an asterisk

Acknowledgements We thank Sarah Gheida, Jon McLeod, and Cyann Taylor for scoring some of the videos.

Author contributions JLY conceived the study, designed the analyses, conducted the analyses, and wrote the paper. MKW and RC scored the videos.

Funding JLY was supported by the National Science Foundation (BCS #1926327), the College of Agriculture and Life Sciences at Texas A&M University and Texas A&M AgriLife Research.

Data availability Data are supplied as ESM.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study was approved by Texas A&M University's Institutional Animal Care and Use Committee (no. 2019-0219), Texas Parks and Wildlife Department (SPR-1116-279), United States Fish and Wildlife Service (MB160637-0 and MB47977D-0) and United States Geological Survey Bird Banding Laboratory (24067).

References

Ballesta S, Mosher CP, Szep J, Fischl KD, Gothard KM (2016) Social determinants of eyeblinks in adult male macaques. Sci Rep 6:38686

Bateman PW, Fleming PA (2011) Who are you looking at? Hadeda ibises use direction of gaze, head orientation and approach speed in their risk assessment of a potential predator. J Zool Lond 285:316–323

Beauchamp G (2017) Half-blind to the risk of predation. Front Ecol Evol 5:131

Benedek M, Kaernbach C (2011) Physiological correlates and emotional specificity of human piloerection. Biol Psychol 86:320–329

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc 57:289–300

Bentivoglio AR, Bressman SB, Cassetta E, Cerretta D, Tonali P, Albanese A (1997) Analysis of blink rate pattern in normal subjects. Mov Disord 12:1028–1034

Blount P (1927) Studies of the movements of the eyelids of animals: blinking. Q J Exp Physiol 18:111–125

Bristow D, Haynes JD, Sylvester R, Frith CD, Rees G (2005) Blinking suppresses the neural response to unchanging retinal stimulation. Curr Biol 15:1296–1300

Caine NG, Weldon PJ (1989) Responses by red-bellied tamarins (*Saguinus labiatus*) to fecal scents of predatory and non-predatory Neotropical mammals. Biotropica 21:186–189

Carter J, Lyons NJ, Cole HL, Goldsmith AR (2008) Subtle cues of predation risk: starlings respond to a predator's direction of eye-gaze. Proc R Soc Lond B Biol Sci 275:1709–1715

Coss RG (1991) Context and animal behavior III: the relationship between early development and evolutionary persistence of ground squirrel anti-snake behavior. Ecol Psychol 3:277–315

Cross DJ, Marzluff JM, Palmquist I, Minoshima S, Shimizu T, Miyaoka R (2013) Distinct neural circuits underlie assessment of a diversity of natural dangers by American crows. Proc R Soc B Biol Sci 280:1046

DeCantezaro D, Graham C (1992) Influences of exogenous epinephrine on two reproductive parameters in female mice: disruption of receptivity but not implantation. Horm Behav 26:330–338

Evinger C, Mannin KA, Pellegrini JJ, Basso MA, Powers AS, Sibony PA (1994) Not looking while leaping: the linkage of blinking and saccadic gaze shifts. Exp Brain Res 100:337–344

Freeberg TM, Krama T, Vrublevska J, Krams I, Kullberg C (2014) Tufted titmouse (*Baeolophus bicolor*) calling and risk-sensitive foraging in the face of threat. AnimCogn 17:1341–1352

Freeberg TM, Book DL, Weiner RL (2016) Foraging and calling behavior of Carolina chickadees (*Poecile carolinensis*) in response to the head orientation of potential predators. Ethol 122:10–19

Gandhi NJ (2012) Interactions between gaze-evoked blinks and gaze shifts in monkeys. Exp Brain Res 216:321–339

Hall A (1945) The origin and purposes of blinking. Br J Ophthalmol 29:445–467

Hampton RR (1994) Sensitivity to information specifying the line of gaze of humans in sparrows (*Passer domesticus*). Behaviour 130:41–51

Hoppe D, Helfmann S, Rothkopf CA (2018) Humans quickly learn to blink strategically in response to environmental task demands. Proc Natl Acad Sci 115:2246–2251

Johnson K, Peer BD (2020) Great-tailed grackle (Quiscalus mexicanus), version 10. In: Poole AF, Gill FB (eds) Birds of the world. Cornell Lab of Ornithology, Ithaca

Kirsten J, Kirsten EB (1983) Spontaneous blink rates of birds. Condor 85:92–93

Koh S, Maeda N, Hirohara Y, Mihashi T, Ninomiya S (2006) Serial measurements of higher-order aberrations after blinking in normal subjects. Invest Ophthalmol Vis Sci 47:3318–3324

Langner O, Dotsch R, Bijlstra G, Wigboldus DHJ, Hawk ST, van Knippenberg A (2010) Presentation and validation of the Radboud Faces Database. Cogn Emot 24:1377–1388

Martin GR (2007) Visual fields and their functions in birds. J Ornithol 148:547–562

Matsumoto-Oda A, Okamoto K, Takahashi K, Ohira H (2018) Group size effects on inter-blink interval as an indicator of antipredator vigilance in wild baboons. Sci Rep 8:10062

Nakano T, Yamamoto Y, Kitajo K, Takahashi T, Kitazawa S (2009) Synchronization of spontaneous eyeblinkswhile viewing video stories. Proc Biol Sci 276:3635–3644

Nakano T, Kato M, Morito Y, Itoi S, Kitazawa S (2013) Blink-related momentary activation of the default mode network while viewing videos. Proc NatlAca Sci USA 110:702–706

Shin YS, Chang W-d, Park J, Im C-H, Lee SI, Kim IY, Jang DP (2015) Correlation between inter-blink interval and episodic encoding during movie watching. PLoS ONE 10:e0141242

Sweeney DF, Millar TJ, Raju SR (2013) Tear film stability: A review. Exp Eye Res 117:28–38

Tada H, Omori Y, Hirokawa K, Ohira H, Tomonaga M (2013) Eyeblink behaviors in 71 species of primates. PLoS ONE 8:e66018

Tanaka Y, Yamaoka K (1993) Blink activity and task difficulty. Percept Mot Skills 77:55–66

Volkmann FC, Riggs LA, Moore RK (1980) Eyeblinks and visual suppression. Science 207:900–902

Yorzinski JL (2016) Eye blinking in an avian species is associated with gaze shifts. Sci Rep 6:32471

Yorzinski JL (2020a) Blinking behavior in great-tailed grackles (*Quiscalus mexicanus*) increases during simulated rainfall. Ethol 126:519–527

Yorzinski JL (2020b) A songbird inhibits blinking behaviour in flight. Biol Lett 16:20200786

Yorzinski JL, Argubright S (2019) Wind increases blinking behavior in great-tailed grackles (*Quiscalus mexicanus*). Front Ecol Evol 7:330

Yorzinski JL, Platt ML (2012) The difference between night and day: antipredator behavior in birds. J Ethol 30:211–218