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Glucocorticoids enhance and suppress heart rate and behaviour in time dependent manner in greylag geese (*Anser anser*)

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ABSTRACT

Stress responses involve autonomic, endocrine and behavioural changes. Each of these responses has been studied thoroughly in avian species, but hardly in an integrative way, in free-living birds. This is necessary to reveal the temporal dynamics of the stress response. Towards that goal, we recorded heart rate (HR) and behaviour in free-ranging male greylag geese (*Anser anser*) simultaneously over 2 h. The geese were subjected to (a) unmanipulated control condition, (b) capture, handling and injection of ACTH, and (c) capture, handling and injection of a saline solution (SHAM). Fecal samples for the non-invasive determination of immuno-reactive glucocorticoid metabolite (BM) concentrations were collected for 7 h thereafter. The SHAM control caused a significant BM increase, a transient increase in HR, an initial increase of preening behaviour and a delay in feeding. ACTH treatment, relative to SHAM, produced significantly higher BM concentrations, and activation of "displacement behaviours" such as wing flapping, body shaking and preening. Also, feeding activity as well as resting was postponed and/or lower for a longer period of time after ACTH than after SHAM. ACTH injection had a greater effect than SHAM injection on HR increase in the first hour, but particularly on HR decline in the second hour following the injection. Hence, glucocorticoids had time- and dose-dependent stimulatory and suppressive effects on cardiovascular activity and behaviour. HR dynamics after ACTH actually matched with behavioural dynamics: both were first enhanced and later suppressed, which is in alignment with adaptive stress management involving the fight–flight response and recovery from stress, respectively.

1. Introduction

The ability to cope with the environmental and social challenges of life affects energy budgets and social performance and may be a major determinant of an individual's ability to survive and reproduce [10]. To generate the appropriate allostatic response to challenging stimuli [22], the body must coordinate and effectively modulate autonomic, endocrine and behavioural responses (Table 1).

Generally, stress responses consist of two phases. The first phase involves catecholamine release via activation of the sympathetic nervous system (SNS) and the sympatico-adrenergic system (SAS), a hypothalamic release of corticotrophin-releasing hormone into the pituitary portal circulation, and some seconds later, release of adrenocorticotrophic hormone (ACTH; [41]). Then, over the course of several minutes, in the second phase, ACTH stimulates glucocorticoids synthesis and secretion [41]. An enhanced activity of the SNS and SAS has stimulatory effects on the respiratory and cardiovascular systems and is responsible for the initial fight or flight response [6]. Glucocorticoids (GCs), such as corticosterone, act early via non-genomic mechanisms, producing behavioural effects via membrane-bound receptors [34]; slow genomic actions are exerted about an hour after the onset of corticosterone release [41]. GCs affect virtually all metabolic processes and act upon cardiovascular activity and behaviour. Traditionally, it was thought that GCs prevent the initial cardiovascular stress response from overshooting [27], whereas some evidence suggests that GCs facilitate, rather than suppress, the cardiovascular response to stress (for a review see [41]). The effects of GCs on behaviour appear to be complex, depending on timing, celerity, magnitude of release, duration (acute vs. chronically elevated corticosteroids), context, and coordination of physiological and behavioural responses [45].

Studies pertaining to stress responses usually focus on the first hour following a challenge. Thereby, slow GC effects on behaviour and the cardiovascular system remain undetected [41] (but see [24]). Recordings lasting over 1 h may reveal the dynamic patterns of adaptive changes occurring at certain intervals from the onset of the acute stressful stimulus to the recovery from stress. Furthermore, the

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Table 1
Statistical results of the generalized linear mixed models (GLMMs). In the full model only fixed terms with $P \leq 0.1$ are given.

Outcome/fixed term	Full fixed model				Final model			
	Treatment		Interval		Treatment		Interval	
	Wald statistics	*P*	Wald statistics	*P*	Wald statistics	*P*	Wald statistics	*P*
Mean heart rate	2.04	0.1	51.81	<0.001	**2.04**	**0.1**	**51.81**	**<0.001**
Median BM	38.31	<0.001	11	0.001	**38.31**	**<0.001**	**11**	**0.001**
Aggression freq.	0.09	0.77	0.56	0.46				
Body shaking freq.	17.16	<0.001	14.71	<0.001	**17.16**	**<0.001**	**14.71**	**<0.001**
Wing flapping freq.	2.7	0.1	32.63	<0.001	**2.7**	**0.1**	**32.63**	**<0.001**
Vigilance freq.	0	0.96	0.33	0.57				
Feeding duration	1.97	0.1	3.19	0.08	**1.98**	**0.1**	**3.18**	**0.08**
Locomotory activity	0.01	0.92	0.23	0.63				
Preening duration	0.22	0.64	17.87	<0.001			**17.72**	**<0.001**
Resting duration	0.01	0.93	3.6	0.06			**3.77**	**0.05**

individual components of the stress response have been studied thoroughly in avian systems, but only few studies have investigated modulation of cardiovascular, endocrine and behavioural responses to a challenging stimulus simultaneously [29,30]. To our knowledge, such integrative studies were never done in free-living birds, where outcome may differ from studies in laboratory animals [7], because environmental conditions may modulate corticosterone effects on physiology and behaviour [50]. To reveal the temporal dynamics of fast and slow changes after activation of the stress response, we compared the modulation of cardiovascular activity and behaviour over a period of 2 h in five male greylag geese (ganders), which were subjected to (a) an unmanipulated control condition, (b) capture, handling and injection of ACTH (high glucocorticoids expected), and (c) capture, handling and injection of a physiological solution (i.e. SHAM injection expected to result in an immediate stress response due to handling). We conducted (a) and (c) to estimate the effect of handling alone on the stress response. This is because a goose always has to be handled when ACTH injection is applied. This way, the effect of handling could be distinguished from the effect of the ACTH injection. Heart rate (HR) was recorded in parallel to behavioural observations. Fecal samples for the non-invasive determination of excreted immuno-reactive glucocorticoid metabolites (BM), which reflect a proportional record of plasma GCs [17,18], were collected continuously for 7 h to determine effective GC levels.

We predicted biphasic physiological and behavioural responses to acute elevation of GC concentrations, i.e. increased cardiovascular activity, increased general activity and decreased feeding activity shortly after ACTH injection. Also, we expected an increase in preening activity and body shaking, because these "displacement behaviours" are related to corticosterone levels [46] and good indicators of an elevated stress level [21]. Following this, we expected HR to decrease below baseline and also, reduced behavioural activity due to "fatigue" after a full activation of the stress response. We further predicted that physiological and behavioural responses would differ quantitatively between conditions (b) and (c), because responses to elevated glucocorticoids are dose-dependent [4], and may be expected to be higher after a combination of handling and ACTH injection than after handling stress (SHAM) alone.

2. Materials and methods

2.1. Study animals

The free-living flock of greylag geese in Grünau (Austria) is a well suited system for studying behavioural and physiological changes after challenges, because these geese are approachable and habituated to human presence [42] but are living in the full complexity of their social system. In 1973, a non-migratory flock of greylag geese was established in the valley of the river Alm in Upper Austria by the late Konrad Lorenz [20]. Life-history data of all flock members have been recorded ever since [14]. The free-ranging geese roam the valley between the Konrad Lorenz Research Station (KLF) and a lake 10 km to the south, where they roost at night. Geese breed in the valley every year, either in natural nest sites or in breeding boxes provided by the KLF. About 30% of the geese are hand-raised and fully integrated into the flock. Up to 10% of the adult flock members are lost to natural predators every year [16]. Geese are provided with supplemental food on the meadows in front of the research station, year round twice daily. At the time of data collection the flock consisted of approximately 150 individually marked animals. We tested five greylag ganders fitted with internal HR transmitters. The subjects aged 6.2 ± 2.9 (mean $\pm$ SD) years, were paired to females and were not accompanied by offspring.

2.2. Heart rate telemetry

Focal ganders were fitted with fully implanted sensor-transmitter packages with internal antennas and a battery lifetime of 18 months. At the time of data collection geese were implanted for more than six months. The implanted equipment, weighing approximately 60 g, was in the range of 2.5% of body weight even in the lightest individuals. The electronic packages measured $60 \times 30 \times 11$ mm, were embedded in epoxy resin, and were implanted into the abdominal cavity using non-absorbable polyester mesh for intra-peritoneal fixation [47]. The implantation was approved under animal experimental license (6268.210/41-BRGT/2003) by the Austrian government. The electronic package was implanted into the abdominal cavity by an experienced team of veterinarians in a properly equipped surgery room at the veterinary clinic Cumberland in Gmunden, the closest clinic to the study site. For anaesthesia the geese were randomly assigned to three groups. Group 1 received medetomidine (0.1 mg/kg), ketamine (10 mg/kg) and butorphanol (1 mg/kg), group 2 received midazolam (3 mg/kg) and ketamine (30 mg/kg) and group 3 received midazolam (3 mg/kg), ketamine (30 mg/kg) and butorphanol (0.5 mg/kg), all given intramuscularly. Endotracheal intubation was done if necessary following mask induction and anaesthesia was maintained with isoflurane in 100% oxygen delivered with a semiopen anaesthesia system. For surgery, the geese were placed in dorsal recumbency. A standard ventral midline celiotomy approach 1 cm caudal of the sternum was used with an incision length of 4 cm. The transmitter and one of the two electrodes were fixed in the coelum and the incision for the second electrode was placed subcutaneously near the axilla. The electrode was then subcutaneously pulled from the coelum through a subcutaneous tunnel prepared with a sterile gynaecological catheter (4 mm in diameter) and fixed to the subcutis. After the proper function of the transmitter was ensured, the abdominal wall and skin incisions were closed with sutures of absorbable material (U. Auer, I. Wiederstein & W. Zenker, unpublished data; W. Zenker, U. Auer, G. Fluch, F. Schober & I. Wiederstein, unpublished data). Twenty-four hours after implantation the geese

were released and returned to the flock. After full recovery, 2–7 days after the surgery, the implanted geese could not be distinguished from non-implanted ones neither in their appearance nor behaviour. For details on HR telemetry see Wascher et al. [48].

2.3. Data collection

Fecal samples, HR recordings and behavioural protocols were collected during August and September 2006, i.e. the non-breeding season. Weather was warm and fairly stable throughout the data collection. For three subsequent days, each focal was observed (a) in a non-manipulated control condition, (b) after capture, handling and injection with ACTH (1 ml per goose of Synacthen®, Novartis containing 0.25 mg tetracosactid (ACTH-analogue) into the pectoral muscle) and (c) after capture, handling and injection with 1 ml of saline solution per goose into the pectoral muscle (SHAM). The order of experimental conditions could not be randomized (a→b→c), because catching becomes increasingly difficult with every catching event. The non-random order ensured that geese were at least injected with ACTH if a second trapping was not possible. In (b) and (c) the focal gander was hand-captured around the morning feeding and held embraced for the time of injection. The gander was released into the flock immediately after the injection. Approval of the Austrian government for this experiment was granted for five geese only (license BMBWK-66.006/0039-BrGT/2005).

Data collection started just prior to the morning feeding (0800) and lasted from 2.5 to 3.5 h for behavioural observations and HR recordings (depending on the laptop's battery time and the time when a focal was caught), whereas fecal samples were collected continuously until 1500 h. Fecal samples were frozen at −20 °C within 1 h after collection. The observer recorded behaviour and HR along the same time axis and kept a distance of approximately 10 m to the focal goose. We recorded frequency of aggressive behaviours (threat, peck, and chase), frequency of vigilance behaviours (head up and extreme head up), frequency of body shaking (whole body, leg, neck, and tail) and wing flapping as well as duration of feeding, preening and resting. The time when geese did not rest was considered as activity time. Behaviours were scored according to the greylag goose ethogram by Lorenz [20].

Catching could only be done opportunistically; therefore, geese were caught at different times after beginning of the morning feedings, i.e. between 0815 and 0850, and for one SHAM the individual was caught at 0920. During this time the everyday behavioural dynamics of the flock change, e.g. geese are first fed with grains, then pluck grass, move to the local stream or ponds to drink afterwards, and then they settle down, preen and rest. The behavioural dynamics of the flock over time around feeding remain similar. To match control and ACTH data, and control and SHAM data, we started collecting data for control and experimental days at the same time, for each individual. This was done to adjust to the change in behavioural dynamics of the flock over the course of the morning.

2.4. Determination of immuno-reactive glucocorticoid metabolite (BM) levels from fecal samples

We extracted 0.5 g of feces per sample in methanol [17]. BM values were determined by enzyme immuno-assay (EIA, [18,26,36]) using group-specific antibodies [11]. The assay methods were evaluated appropriately for greylag geese [35]. The sensitivity of the assay was less than 2 pg/well, and concentration limits for reliable measurement ranged from 7.25 ng/g to 501.4 ng/g feces. The intra- and inter-assay coefficients of variation for BM were 12.5% and 17%, respectively.

2.5. Data analyses

Glucocorticoid metabolite (BM) levels were assessed using the medians of BM concentrations over 30 min intervals. We also determined minimum and maximum BM concentration per treatment. Duration or frequency of behaviours was calculated for each 30 min interval for each treatment. 30 min long time intervals were chosen on the basis of other studies [3,13,15,23,25,33,39,41].

Similarly, we determined minimum and maximum heart rate (HR) per treatment as well as calculated means of HR frequencies over 30 min intervals for each test condition. ACTH and SHAM treatments were expected to cause pronounced HR changes, because geese were caught and handled. Since we were interested in HR time dynamics over an intermediate time, i.e. sustained HR changes rather than those caused by transitory stimuli and locomotion, we filtered short-term changes in HR by calculating running averages of 10 min intervals (e.g. average of HR from 0 to 600 s, 1 to 601 s, 2 to 602 s, etc.). Using running averages as new HR values we considered a) stabilization time: time elapsed from the injection to the first HR value ≤ mean HR in control; and (b) duration of HR decline: time from (a) to the first HR value, which is ≥ mean HR in control.

In one ACTH treatment the first 30 min of HR recordings were disrupted due to technical problems.

To see the effect of treatments on BM, HR and behaviours (median BM, mean HR, and frequencies or durations of behaviours over 30 min intervals), we conducted generalized linear mixed models (GLMMs) using the GenStat 10.1 statistical package [12]. We sequentially deleted fixed terms in order of decreasing significance; only terms, with $P \leq 0.1$ remained in the final model. Excluded terms were re-entered one by one into the final model to confirm that they did not explain a significant part of the variation [38]. Due to our small sample size, which renders statistical power to be low [28], we were unable to compare all three treatments (control, ACTH, and SHAM) simultaneously. We, therefore, constructed the GLMMs to compare control versus SHAM, and control versus ACTH treatments and SHAM versus ACTH separately. Besides differences in BM concentrations, parameters did not differ between SHAM treatment and control condition (see Results for differences between intervals). Therefore, to be conservative, we compared behaviours and mean HR during ACTH treatment with behaviours and HR during SHAM treatment in the further analyses.

All GLMMs were constructed with BM, HR and behaviours as response variables, individual identity as a random factor; and treatments and time intervals as fixed terms. We present Wald statistics for final models including fixed terms with $P \leq 0.1$ only.

3. Results

Relative to the non-manipulated control condition, SHAM treatment caused a significant BM increase (Wald = 5.12, $df = 1$, $P = 0.026$), changed HR dynamics (Wald = 5.41, $df = 1$, $P = 0.024$) and tended to modulate behavioural dynamics: It caused short-term HR increase, the initial increase of preening durations (Wald = 3.75, $df = 1$, $P = 0.059$) and postponed feeding (Wald = 3.51, $df = 1$, $P = 0.068$).

The increase of BM concentration, however, was significantly more pronounced after ACTH than SHAM (Wald = 38.31, $df = 1$, $P < 0.001$, Fig. 1). After ACTH treatment, BM concentrations initially increased (30–60 min after injection), peaked at 180–248 min (quartile 1–quartile 2), and then decreased towards initial baseline (control condition; Wald = 11, $df = 1$, $P = 0.001$, Fig. 1). We found no specific pattern of median BM fluctuations after SHAM treatment. Maximum BM was found 139–194 min (Q1–Q2) after SHAM injection.

Mean HR and its dynamic over time were affected significantly more by ACTH than by SHAM treatment (treatment: Wald = 2.04, $df = 1$, $P = 0.1$; interval: Wald = 51.81, $df = 1$, $P < 0.001$). After ACTH treatment, mean HR first increased, then decreased below baseline and later increased toward baseline again (Fig. 2). Similar to HR dynamics after ACTH injection, HR increased initially also after SHAM injection (see above, Fig. 2), then mean HR dropped to values similar to HR in the control condition. HR stabilization and decline tended to last longer and

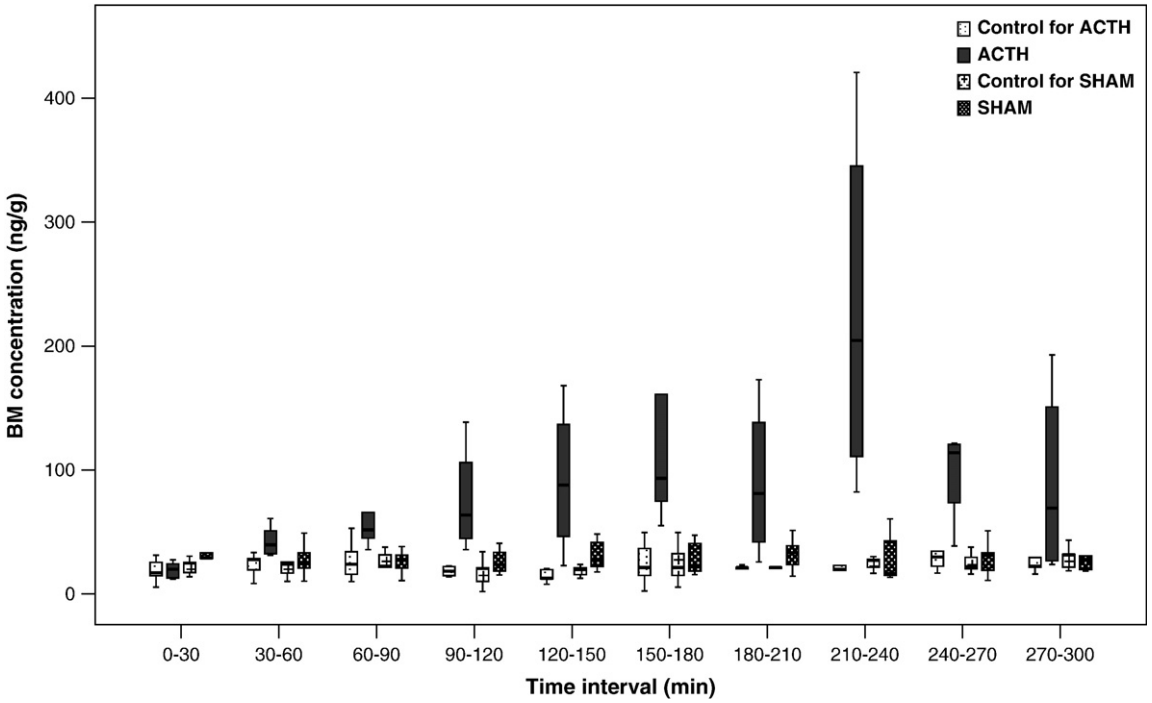

Fig. 1. Immuno-reactive corticosterone metabolite (BM) concentrations after ACTH treatment and in matched control conditions; and after SHAM treatment and in matched control conditions. Data are shown for 30 min intervals. Boxplots show medians and 1st and 3rd quartiles.

reached lower minimum HR after ACTH than SHAM injection (stabilization: $Z=-2.02$, $P=0.04$; decline: $Z=-1.83$, $P=0.06$; minimum HR: $Z=-2.02$, $P=0.04$). However, we observed higher frequencies of body shaking and wing flapping, but lower feeding durations after ACTH than after SHAM treatment (body shaking: Wald = 17.16, $df=1$, $P<0.001$; wing flapping: Wald = 2.7, $df=1$, $P=0.1$; feeding: Wald = 1.98, $df=1$, $P=0.1$; Fig. 3). These differences were particularly pronounced in the initial phase after injections (body shaking: Wald = 14.71, $df=1$, $P<0.001$; wing flapping: Wald = 32.63, $df=1$, $P<0.001$; feeding: Wald = 3.18, $df=1$, $P=0.08$; Fig. 3). Also, after ACTH injection geese preened longer and generally started feeding and resting (i.e. were behaviourally passive) later than after SHAM injection (preen: Wald = 17.72; $df=1$, $P<0.001$; feeding: Wald = 3.18, $df=1$, $P=0.08$; rest: Wald = 3.77, $df=1$, $P=0.05$; Fig. 3).

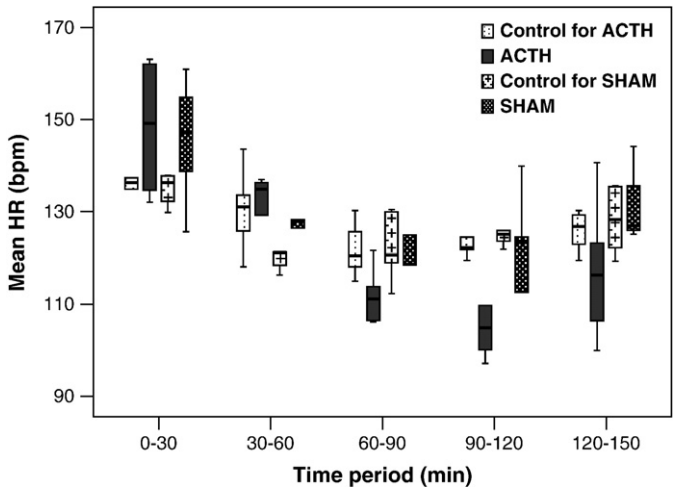

Fig. 2. Heart rate (HR, bpm) after ACTH treatment and in matched control conditions; and after SHAM treatment and in matched control conditions. Data are shown for 30 min intervals. Boxplots show medians and 1st and 3rd quartiles. Even though control was one per individual (from 0800 to cca. 1130), matched control condition for ACTH differs from matched control condition for SHAM treatment. This is because we matched the beginning of the control with the beginning of the ACTH/SHAM treatment. In ACTH and SHAM however, we caught and injected geese at different times (cca. between 0815 and 0920).

Similar to the above results were results of the comparison between the non-manipulated control condition and ACTH treatment. Relative to the non-manipulated control condition, ACTH treatment caused a significant BM and HR increase followed by BM and HR decline (BM: treatment: Wald = 44.92, $df=1$, $P<0.001$, interval: Wald = 12.43, $df=1$, $P<0.001$; HR: treatment: Wald = 5.34, $df=1$, $P=0.025$, interval: Wald = 17.02, $df=1$, $P<0.001$. Initially after ACTH treatment we observed higher frequencies of body shaking, wing flapping and more preening than in the control (body shaking: treatment: Wald = 9.51, $df=1$, $P=0.003$, interval: Wald = 10.23, $df=1$, $P=0.003$ wing flapping: treatment: Wald = 3.31, $df=1$, $P=0.075$, interval: Wald = 7.71, $df=1$, $P=0.008$; preening: Wald = 6.33, $df=1$, $P=0.015$). Besides, after ACTH injection geese were generally more locomotory active and fed less time than in the non-manipulated control condition (locomotory activity: Wald = 3.4, $df=1$, $P=0.072$; feeding: Wald = 3.31, $df=1$, $P=0.075$). Similarly, geese started to rest later after the ACTH treatment than in the control condition (Wald = 3.57, $df=1$, $P=0.065$).

4. Discussion

In our study we attempted to unravel the interplay between autonomic (cardiovascular), glucocorticoids and behavioural responses to a dual challenge, i.e. ACTH injection and handling, in five males from a free-living flock of greylag geese. This was compared to the geese' responses to handling and SHAM injection.

Glucocorticoid metabolite (BM) data indicate that ACTH treatment caused glucocorticoid synthesis and secretion in several minutes, whereas it remained high at least 5 h. BM concentrations after ACTH treatment increased up to cca. 500 ng/g feces, which is similar to values that we recorded after "separation test" (a goose was separated from the flock and put in the box for 20 min), when BM concentrations were between cca. 300 and 650 ng/g feces (Kralj-Fišer et al., unpublished). This implies that ACTH treatment was extremely stressful for our geese. Stress concentrations however are highly variable between individuals

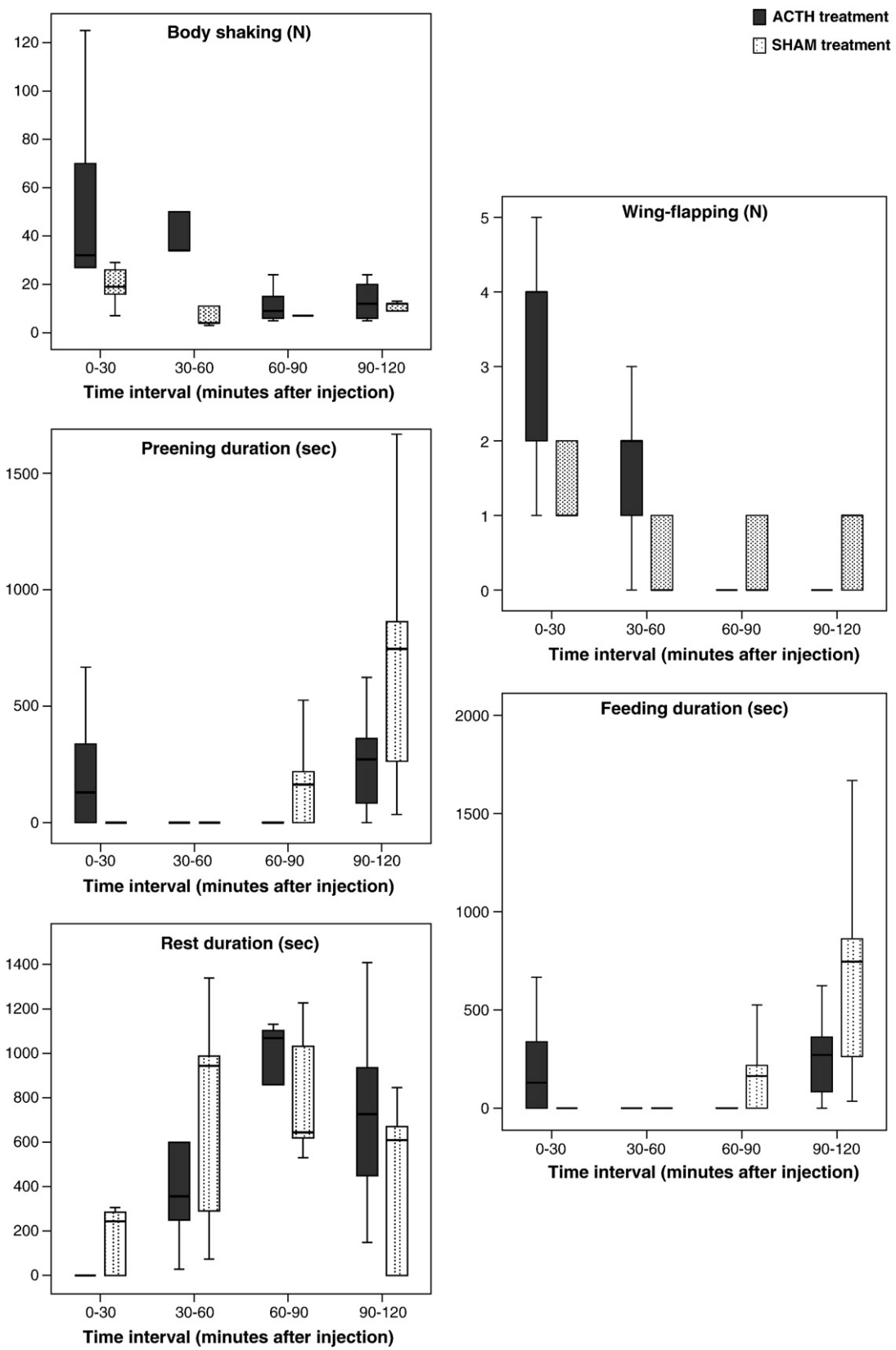

Fig. 3. Frequencies of body shaking, frequencies of wing flapping, feeding durations, preening durations and duration of rest. Graphs represent data after ACTH and SHAM treatments. Data are shown separately for the 30 min intervals. Boxplots show medians and 1st and 3rd quartiles.

[19,40]. As predicted, administering ACTH in combination with handling produced immediate peaks in heart rate (HR) and behavioural activation in the first hour after injection, but a decrease in HR and behavioural activity in the second hour after injection (e.g. [32]). HR increased up to a maximum of approximately 450 bpm, which is similar to HR response due to extensive physical activity as well as social interactions [48,49] and stabilized in roughly 1 h. In the second hour, HR decreased to as low as 80 bpm, which is similar to the expected resting

HR frequency in these geese [48]. Like HR dynamics, behavioural activation (comfort behaviours) was immediate and lasted about 1 h, followed by a high increase of feeding and rest durations. Similar increases of behavioural activity after glucocorticoid administration were also shown in other studies (e.g. [5]), whereas, hitherto, a delayed decrease in HR and behaviours was never shown in birds.

SHAM treatment caused significantly lower BM than ACTH, but still significantly higher BM relative to the non-manipulated control condition. SHAM treatment also had some initial effect on HR increase and behavioural dynamics. These results indicate that both ACTH and SHAM treatment affected the fast and slow stress axis, whereas behaviours (frequencies and durations) and "long-term" differences in mean HR were seen only after the ACTH treatment, when GC concentrations were particularly high. BM concentrations in response to SHAM treatment however might be only slightly elevated above baseline BM due to fixed experimental order, in which SHAM followed ACTH treatment and thus a goose could be already habituated to the handling. The fixed experimental order might change the GCs concentration; yet, the relationship between cardiovascular, glucocorticoids and behavioural responses was the main objective of this study, not the single parameter.

Within 2 h following a challenge, cardiovascular responses to ACTH and SHAM treatments were biphasic: an initial increase in HR was followed by a HR decline. ACTH injection, however, had a greater effect than SHAM injection on HR increase in the first hour and particularly on HR decline over the second hour following the injection. Thereafter HR frequency stabilized to control value. Results indicate that glucocorticoids had time- and dose-dependent stimulatory as well as suppressive effects on cardiovascular activity (e.g. [41]). The participation of GCs on HR increase, though longer and higher after ACTH compared to SHAM, is impossible to distinguish from the effect of fast stress axis in this study. However, the clear decrease of HR after the initial increase after ACTH injection suggests that glucocorticoids may indeed play an important role as a "buffering mechanism", preventing the stress response from overshooting [41]. The decreases of HR below baseline (control condition) over the second hour after ACTH injection match the increased resting and feeding found in this interval. This may imply that our focal males later compensated for the energy they lost in the initial stress response [8], when mean HR and behavioural activity was higher (e.g. [3]). The later results also suggest that behaviours and cardiovascular mechanisms were inter-related in geese similarly as was shown in starlings [31]; however, they were still (directly or indirectly) modulated by GCs.

The most robust behavioural changes were seen shortly after the geese were injected with ACTH. Compared to the SHAM treatment, we observed more body shaking and wing flapping. In birds, wing flapping, body shaking and preening are primarily parts of comfort behaviours [20]. They might have been caused by handling, because we thereby ruffled the ganders' feathers. However, this is unlikely because males were handled in both treatments, whereas frequencies in wing flapping and body shaking were elevated particularly after ACTH, but not after SHAM treatments. Also, the initial preening lasted longer after ACTH than SHAM injection. This may be therefore explained by differences in GCs level, which were much higher during ACTH than SHAM treatment. Increased preening, wing flapping and body shaking may express internal tension and thereby, represent "displacement behaviours", related to stress and anxiety due to increased GCs [21]. In birds these behaviours are also indicative of increased activity of the arginine–vasotocin (AVT) system and of motivational conflict [37]. Wing flapping is not only comfort behaviour, but it also reflects dominance and aggression. Interestingly, the more frequently the ganders performed body shaking, the less aggressive they tended to be ($r=-0.8$, $P=0.1$), supporting the motivational conflict hypothesis. However, as in male tree sparrows [2] a short-term glucocorticoids increase did not relate to any immediate modulation of aggression in geese.

In birds, glucocorticoids may have very diverse effects on behaviours, which are dependent upon species, breeding status, gender, metabolic state, environmental conditions, etc. [1,43,44,50,51]. For example, in our geese and in house sparrows [5] increased glucocorticoids caused more preening, whereas in starlings increased glucocorticoids decreased preening [29,30]. Notably, increased glucocorticoids affect feeding rate depending upon prior food availability, which was high in our geese. Also, low glucocorticoids stimulate food intake, whereas high increase inhibits food intake [9]. Therefore, it is not surprising that increased glucocorticoids resulted in reduced feeding in geese, whereas in some other studies glucocorticoids increased feeding rate [1,8]. It is important to emphasize that stress responses are usually extremely variable among individuals; therefore a study with higher sample sizes as permitted by the government in our case might reveal more behavioural changes after full activation of the stress response.

To conclude, ACTH injection and handling caused time- and dose-dependent effects on geese' HR and behaviours, probably mediated by glucocorticoids. ACTH injection in combination with handling had short-term enhancing, and long-term suppressing effects on HR and behaviour, indicating adaptive stress management from fight–flight response to recovery from stress.

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