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ORIGINAL ARTICLE

Short social interactions in male Japanese Quail fail to influence temporal dynamics of testicular and adrenocortical activities irrespective of photoperiodic reproductive status

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Abstract This study evaluates whether short daily male-conspecific visits influence the dynamics of endocrine testicular and adrenal photo-responsiveness. A group of male Japanese Quail was exposed to short photoperiods and another group to long photoperiods. The quail exposed to short photoperiods were classified as responsive (SD-R) or non-responsive (SD-NR), depending on whether or not they lost their reproductive condition after 5 weeks of short days (SD). Individuals kept on a long photoperiod were used as controls (LD). After photoperiodic classification and during four consecutive days, individually-caged SD-R, SD-NR and LD resident male quail received 5-min visits from photo-stimulated (and therefore sexually active) male quail (social treatment). Cloacal gland volume (CGV), and androgen and corticosterone metabolites (AM and CM, respectively) in droppings were measured bi-weekly and evaluated by repeated measures ANOVA over a 6-week period. Immediately after photoperiodic classification and before social treatment, CGV, AM and CM values showed a SD-R < SD-NR < LD pattern ($P < 0.001$). Along SD exposure, SD-NR quail showed spontaneous cloacal gland recovery and AM and CM concentrations were found to be similar to those of the LD quail. At the end of the study, the SD-R group had still not recovered LD control values. Although the social treatment induced an overall main effect on AM ($P < 0.02$) and CM concentrations ($P < 0.009$), these changes were not related to a particular photoperiodic treatment. These findings provide a novel insight into the social modulation of reproductive and adrenocortical responses, particularly during transition from the photosensitive state to photorefractoriness.

Keywords Photoperiod · Social challenge · Noninvasive methods · Androgens · Glucocorticoids · Birds

Zusammenfassung

Kurze soziale Interaktionen können bei der Japanwachtel unabhängig vom photoperiodischen Fortpflanzungsstatus den zeitlichen Verlauf der Testikel- und Nebennierenrinden-Aktivität nicht beeinflussen

In dieser Studie wurde untersucht, ob ein kurzes, tägliches Zusammensetzen männlicher Tiere die photoperiodische Reaktionsfähigkeit der Fortpflanzungsdrüsen und des Nebennieren-Systems beeinflussen kann. Eine Gruppe männlicher Japanwachteln wurde kurzen, eine andere Gruppe langen Photoperioden ausgesetzt. Die Vögel aus den kurzen Photoperioden wurden in reaktive (SD-R) und nicht-reaktive (SD-NR) Tiere eingeteilt, abhängig davon, ob sie nach 5 Kurztag-Wochen (SD) ihren

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Reproduktionsstatus verloren hatten oder nicht. Die Tiere aus den Langtagen (LD) fungierten als Kontrollen. Nach ihrer photoperiodischen Einteilung wurden die einzeln gehaltenen SD-R-, SD-NR- und LD-Männchen an vier aufeinanderfolgenden Tagen für jeweils fünf Minuten mit photo-stimulierten und somit fortpflanzungsaktiven anderen Männchen zusammengesetzt („social treatment"). Zweimal wöchentlich wurde die Größe der Kloakendrüse (CGV) gemessen und androgene und kortikosterone Stoffwechselprodukte (AM und CM) aus dem Kot bestimmt und nach wiederholten Messungen innerhalb eines Zeitraums von 6 Wochen mit einer Varianzanalyse (ANOVA) ausgewertet. Unmittelbar nach der photoperiodischen Einteilung und noch vor dem „social treatment"zeigten die Werte für CGV, AM und CM ein klares Muster: SD-RA < SD-NR < LD ($p < 0{,}0001$). Während ihrer Haltung in Kurztagen (SD) zeigten die SD-NR-Wachteln ein spontanes neues Wachstum ihrer Kloakendrüse und ihre AM- und CM-Konzentrationen waren vergleichbar denen der Langtag-Wachteln (LD). Am Ende der Studie hatte die SD-R-Gruppe die Werte der LD-Gruppe noch nicht wieder erreicht. Obwohl das „social treatment"einen Einfluss auf die AM- und CM-Konzentrationen hatte ($P < 0{,}009$), konnten diese Änderungen der Werte aber nicht zu einem bestimmten photoperiodischen Regime in Verbindung gebracht werden. Diese Ergebnisse bieten einen neuen Einblick in die soziale Modulation der Fortpflanzungs- und adrenokortikalen Reaktion vor allem während des Übergangs von der photosensitiven zur photostablien Phase.

Introduction

Seasonal reproductive cycles in birds result from interactions between endogenous cycles and exogenous factors, particularly photoperiod. Photosensitivity and photorefractoriness occur as a result of internal changes in photoperiodic response (Deviche and Silverin 2003; Bradshaw and Holzapfel 2007; Dawson 2008). If birds relied entirely on photoperiod to control the time of gonadal maturation and regression, then these events would occur at the same time every year. However, the exact timing of breeding can clearly differ between years, within the same year, and between different habitats at the same latitude. Apparently, non-photoperiodic environmental cues (such as food, temperature, rainfall and social interaction) modulate the timing of breeding at the individual level, which could explain the flexibility of the reproductive schedule (Dawson 2008). For example, Dawson (2008) postulated that in male Common Starling, spring-summer increments in testicular volume may begin early due to the effects of non-photoperiodic cues such as social factors. Several hypotheses, not necessarily mutually exclusive, have been proposed to explain why the testosterone levels in male birds of some populations are socially modulated whereas in others they are not (Wingfield et al. 2007).

Glucocorticoids are steroid hormones that play an important role in energy mobilization during energetically costly times of the year, such as the breeding season (Romero 2002) or during stressful social interactions (Blas 2015). They can be considered mediators allowing birds to adjust their physiological responses across both predictable and unpredictable environmental challenges (Blas 2015). Further studies are therefore required to fully understand the nature of the HPG axis response to social factors and also to elucidate long-term changes in glucocorticoids as signaling molecules or final messengers of the HPG axis, all of which affect reproductive performances in birds. We were particularly interested in monitoring the activity of the hypothalamic-pituitary-adrenocortical (HPA) axis associated with reproductive flexibility since this may also play an essential role in supporting the mechanisms through which birds adjust their physiological stages (Carsia and Harvey 2000).

Japanese Quail (*Coturnix japonica*) breed during spring and summer. In the laboratory, under long-day conditions (long photoperiod), they can be indefinitely maintained in a breeding state (Ubuka et al. 2013). One of the most important physiological effects of long photoperiods is the stimulation of gonadal growth and the subsequent increase in plasma sex steroids by triggering gonadotropin production and release. Under short-day conditions, reproduction is inhibited. However, not all quail fall out of the reproductive condition on short days (Mills et al. 1997). This phenomenon was first assessed by Oishi and Konishi (1983), who described a procedure to discriminate photoresponsiveness in birds. Using this procedure with slight modifications, combining cloacal gland volume values with foam production, we observed the same phenomenon in our bird population (Busso et al. 2013). This modified procedure is an effective tool to predict the fertilizing ability of the male Japanese Quail (Ottinger and Brinkley 1979; Mohan et al. 2002; Biswas et al. 2007; Henare et al. 2011).

In a previous study, we assessed reproductive functioning in male Japanese Quail exposed to different photoperiods and demonstrated that the length of the photoperiod also affected adrenocortical activity (Busso et al. 2013). In addition, adrenocortical response was associated with photoresponsiveness on short and long days. In particular, male quail non-responsive to short days had high androgen and corticosterone metabolite concentrations, probably with similar general and metabolic activity to that of quail exposed to long photoperiods. It was then confirmed that quail that were non-responsive to short days exhibited long-term changes in both HPG and HPA axes, suggesting that some quail exposed to this condition (8L:16D) are able to reach similarly high levels of reproductive performance as photo-stimulated quail (Dominchin et al. 2014).

The aim of the current study was to determine whether daily short (5-min) male conspecific visits influence the dynamics of endocrine testicular and adrenal photo-responsiveness. A group of male Japanese Quail was exposed to short photoperiod and another group was maintained on long photoperiods. In quail, as in other bird species, when male encounters occur in a restricted area, they usually lead to vigorous sexual and/or aggressive interactions (Adkins-Regan 2014; Berger et al. 2014). The male of a sparrow species (*Melospiza melodia*) showed different neuroendocrine responses to simulated territorial intrusions (Wingfield 2012). We, therefore, proposed that opportunistic encounters between resident male quail exhibiting varied levels of reduction in gonadal development and fully developed male counterparts would be a relevant social challenge. We hypothesized that males under short days would consider social interaction with a fully developed male as a stimulatory environmental signal for breeding and that male encounters would therefore have long-term effects on the development of their reproductive physiology even though they were being maintained under a non-stimulatory photoperiod (short days).

Materials and methods

Animals and husbandry

The study animals were male domestic Japanese Quail. Egg incubation, chick brooding and lighting procedures were similar to those described elsewhere (Nazar and Marin 2010), with the exception that chicks were brooded in wood cages (85 cm long × 45 cm wide × 50 cm tall) in mixed-sex groups from 1 day up to 4 weeks of age. Briefly, birds were fed a starter ration (28% protein content; 2800 kcal of metabolic energy/kg) and water ad libitum. They were kept under long photoperiod conditions (14L:10D; lights on at 6:00 a.m.) and controlled temperature (brooding temperature was 37.5 °C during the first week of life, with a weekly decrease of 3 °C until room temperature, 24–27 °C, was achieved). At 4 weeks the birds were sexed by plumage coloration and 107 male quail were randomly housed in cages (length 45 cm; width 20 cm; height 25 cm) in order to individually collect droppings over the course of the experiment; birds were not visually or acoustically isolated. From this time onwards, birds were switched to a breeder ration (21% protein content; 2750 kcal of metabolic energy/kg). All environmental conditions (temperature, photoperiod and feeding) were constantly controlled throughout the study period.

From 10 weeks of age onwards, by which time all birds had reached full reproductive development, cloacal gland measurements and foam production were recorded bi-weekly for the duration of the study (20 weeks of age; see further details below), with an extra measurement at week 15 in order to classify quail exposed to short photoperiod. Cloacal gland size, foam production and body weight were also measured weekly throughout the study (see details below). All procedures complied with applicable Argentine laws and with the guidelines of the local Argentine Association for Science and Technology Laboratory Animals–(AACyTAL Bulletins number 15 and 16, 2001) and were approved by the Institutional Committee for Care and Use of Laboratory Animals (IIByT/CONICET-UNC: local reference: CICUAL, 1-2015).

At 10 weeks of age, once all measurements were taken, male Japanese Quail ($n = 54$) exposed to long photoperiods were switched to short photoperiods (8L:16D; lights on at 10:00 a.m.). Other male Japanese Quail were maintained under long photoperiods and used as controls (LD; $n = 17$) or intruder (visitors) males ($n = 36$), all reproductively active. Following the procedure of Busso et al. (2013), after 5 weeks of exposure to short photoperiod (Oishi and Konishi 1983; Busso et al. 2013), 15-week-old quail were classified into those showing a reduction in cloacal gland volume below 1000 mm^3 and not exhibiting any cloacal foam in response to short days (SD-R; $n = 33$) and those that were unresponsive to short photoperiod (cloacal gland volume >1000 mm^3; still exhibiting cloacal foam production (values ≥ 2); SD-NR; $n = 21$). The expression of cloacal gland foam production was evaluated after its ejection upon manual squeezing of the gland, using a scale of 0 (no foam expressed) to 5 (maximum foam expression) as previously performed in the laboratory.

Male-male social interactions (social treatment)

Social treatment was applied in each group (SD-NR, SD-R and LD): about half the residents of each photoperiodic group received visits (treatment) and the remaining birds did not (control). Thus, the number of animals (treatment/control) in the three experimental groups (SD-NR/SD-R/LD) was 9/11/16 (treatment) and 8/10/16 (control), respectively.

One day after photoperiodic classification, male-male social interactions were performed for 5 min; these consisted of individually caged LD, SD-NR and SD-R residents being visited by an unfamiliar photo-stimulated male quail intruder ($n = 36$; Dominchin et al. 2017; Guzmán et al. 2013). After completing the 5-min visits, the intruder male was allowed to return to its home cage. Male-male encounters were repeated over the following three consecutive days in a random order. During these 4 days, the intruders were always unfamiliar to the resident male. We chose 5-min visits because we considered that more than 5-min could lead to physical damage as a consequence of aggression. Aggressive behavior: latency of first attack, the number of aggressive pecks of residents to the intruder and vice versa were recorded (for further details see Dominchin et al. 2017).

Cloacal gland volume (CGV)

When quail were 10 weeks of age, CGV was measured bi-weekly in all males up to 20 weeks of age. Cloacal gland volume was also measured at 15 weeks of age in order to classify birds according to photoperiodic response in SD. Cloacal gland size length (mm) and width (mm) were measured using a digital caliper and CGV was calculated from these measurements according to Chaturvedi et al. (1993). Cloacal gland volume $= (4/3 \times 3.5414 \times a \times b2)$, where $a = 0.5 \times$ long axis and $b = 0.5 \times$ short axis of the cloacal gland. After 5 weeks of SD exposure, quail exhibiting cloacal gland volume increments were considered to be at a photorefractory stage.

Sampling and steroid measurements

Collection of droppings

Droppings were individually collected bi-weekly (four samples per bird) during the entire experimental period (from week 14 to week 20) prior to CGV measurement in order to avoid stress disturbance due to manipulation. Briefly, cage trays were cleaned immediately after lights were automatically turned on at 6:00 a.m. or 10 a.m. (quail maintained under long or short photoperiods, respectively) and fecal samples were collected 1 h later. All samples were stored immediately at −20 °C until analysis.

Steroid extraction and immunoassays

A total of 0.5 g of each homogenized sample was extracted with 5 mL of 60% aqueous methanol by shaking for 30 min (Palme et al. 2013). After centrifugation (2500*g*, 15 min), aliquots of the supernatant were evaporated and transported to the Vetmeduni Vienna. Fecal steroid extracts were then re-suspended in assay buffer (1:10 dilution) and measured with a cortisone EIA and an epiandrosterone EIA. Standards or samples (50 µL) were incubated with antibody and label (each 100 µL) overnight at 4 °C in duplicate. After incubation, the plates were washed and blotted dry prior to the addition of 250 µL streptavidin horseradish peroxidase conjugate (=4.2 mU, Art. No. 1089153, Boehringer, Mannheim, Germany) to each well. Plates were left in the dark on stirring tables for 45 min at 4 °C after which each well was subjected to a further washing step. After another washing step 250 µL tetramethylbenzidine (=69.4 nmol/well; Art. No. 87748, Fluka Chemika, Vienna, Austria) was added, and the plates were incubated for an additional 45 min at 4 °C before the enzymatic reaction was stopped by the addition of 50 µL/well of 1 mol/L sulphuric acid. Absorbances were measured at a wavelength of 450 nm (reference filter 620 nm) with an automatic plate reader. Further details of the EIAs (including immunogens, biotinylated steroid labels and cross-reactions of the antibodies) are given elsewhere (Palme and Möstl 1994; Rettenbacher et al. 2004); both are group-specific assays, the cortisone EIA measuring immunoreactive corticosterone metabolites (CM) with a 3,11-dione structure and the epiandrosterone EIA measuring immunoreactive androgen metabolites (AM) with a 17-oxo structure. Sensitivity was 10 and 25 ng/g, respectively. Inter-assay CVs of high and low controls were 6.5% and 6.2% for the cortisone EIA, and 3.7% and 5.0% for the epiandrosterone EIA, respectively. The EIA for androgen metabolites in male Japanese Quail was previously validated by Hirschenhauser et al. (2008). The cortisone EIA was successfully validated for Japanese Quail droppings and used to monitor adrenocortical activity in a previous study (Busso et al. 2013).

Statistical analyses

All variables were subjected to repeated measures ANOVA. A mixed linear model was performed to evaluate the effects of photoperiodic responses (SD-R, SD-NR and LD), exposure to male social interaction (with or without visits) and time (14, 16, 18 and 20 weeks of age) as well as their interaction on CM and AM concentrations and CGV measurements. Photoperiodic responses and social interaction were included as fixed variables. The time range encompasses CGV recordings at the time of classification (basal measurement) and after male interactions. “Resident male quail” was incorporated as a random effect across all analyses. Since a repetition factor model was applied, in those cases in which a bird did not naturally defecate during a particular time interval, the concentrations between the previous and following collection time intervals were interpolated to obtain a value and avoid losing the whole hormonal profile for that bird. Hormonal data were rank-transformed to meet the ANOVA assumptions. Whenever repeated measures ANOVA reached significance ($P < 0.05$), Di Rienzo, Guzman, Casanove (DGC) post hoc tests were performed (Di Rienzo et al. 2002).

Values are expressed as mean ± SEM. All statistical analyses were performed using Infostat (2000).

Results

Repeated measures ANOVA detected an interaction ($P < 0.001$; $F_{6,260} = 3.86$) between photoperiod and time (weeks of age), but no effect of social treatment on CGV ($P = 0.949$; $F_{1,260} = 0.004$). For example, at 14 weeks of age the analysis showed statistical differences in mean CGV values (mm^3) due to photoperiodic response: LD > SD-NR > SD-R quail (2544 ± 158; 1821 ± 196 and 302 ± 31 mm^3, respectively). Under short photoperiod, CGV increased between 16 and 18 weeks of age in both SD-NR and SD-R males. However, compared to LD and SD-NR quail, CGV in the SD-R group remained reduced until the end of the study (LD = SD-NR > SD-R quail).

Mean AM concentrations showed differences due to photoperiodic response ($P < 0.001$; $F_{2,196} = 124.21$) and time ($P < 0.001$; $F_{3,196} = 15.91$). An interaction between these two variables was also detected ($P < 0.001$; $F_{6,196} = 8.06$; Fig. 1). The post hoc test revealed that the AM pattern of SD-NR and SD-R quail was similar, with biweekly increments up to the end of the study. At 20 weeks of age, SD-NR quail showed similar mean AM concentrations than the LD quail. Overall social effects were also evident ($P = 0.02$; $F_{1,196} = 5.83$). The AM concentration in Japanese Quail without visits (504 ± 32 ng/g; control) remained higher than in their counterparts (422 ± 27 ng/g; social treatment) receiving a daily male visit during 4 days ($P < 0.05$; $F_{1,196} = 6.03$).

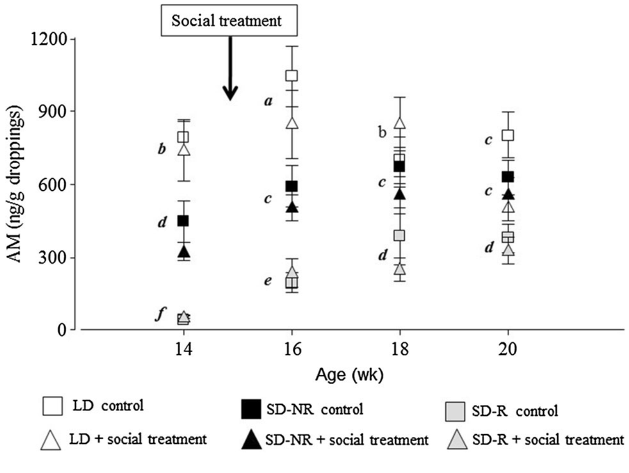

Fig. 1 Androgen metabolites (AM) in droppings individually collected from male Japanese quail during 1 h. At 10 weeks of age, birds were assigned to a photoperiodic treatment (long or short photoperiod). At 15 weeks of age, quail under short photoperiods were classified according to their cloacal gland photoresponsiveness; 1 day later half the groups of SD-NR, SD-R and LD quail individually received social treatment (visited quail, indicated by an *arrow*). The other half served as controls (unvisited quail). All values are expressed as mean ± SEM. Treatment failed to influence the temporal dynamics of AM, thus *different letters* differ significantly between groups SD-NR, SD-R and LD quail ($P < 0.05$)

Differences in mean CM concentrations according to photoperiodic response ($P < 0.001$; $F_{2,196} = 54.37$) and time ($P < 0.001$; $F_{3,196} = 15.60$) were highly significant, and an interaction between photoperiod and time was also detected ($P < 0.001$; $F_{6,196} = 10.29$; Fig. 2). In particular, social treatment had no evident effect on the CM concentrations ($P = 0.8013$; $F_{2,196} = 0.22$). Post-hoc tests revealed that CM concentrations increased during the first 2 weeks of the study in both groups of Japanese Quail exposed to short photoperiods and that SD-NR quail showed similar mean CM values to LD quail, after which no further changes were observed in either group. Increments in CM values in SD-R continued, but were not higher than in SD-NR or LD counterparts. Furthermore, social effects on CM concentrations were also evident, irrespective of the photoperiodic reproductive status ($P = 0.009$; $F_{1,196} = 6.91$). The post hoc test showed ($P < 0.05$) that CM concentrations were lower in male Japanese Quail (182 ± 9 ng/g) receiving photo-stimulated male visits than in those without visits (219 ± 11 ng/g).

Discussion

In the present study we evaluated the temporal endocrine testicular and adrenocortical activity in adult Japanese Quail subjected to short (inhibitory) or long (stimulatory; control group) photoperiods and its potential modulation

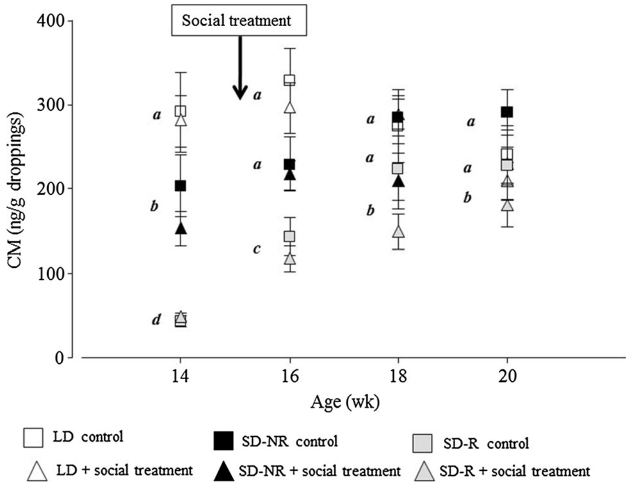

Fig. 2 Corticosterone metabolites (CM) in droppings individually collected from male Japanese quail during 1 h. An *arrow* indicates application of social treatment, which failed to influence temporal dynamics of CM. For further details see legend to Fig. 1

by social interactions. We were particularly interested in birds under short photoperiods, during their transition from the photosensitive to the photorefractory state. Our results reveal that cloacal gland development and temporal dynamics of endocrine testicular and adrenocortical glands in Japanese Quail are not modulated by short, repetitive male social encounters. This pattern did not differ between birds exposed to short or long days. Particularly, under short days in the laboratory, the influence of social treatment was not related to the photoresponsiveness characteristics of the quail. Our findings, therefore, do not support the hypothesis that social factors (non-photoperiodic cues) affect the timing of testicular and adrenocortical activity. However, regardless of time and/or photoperiodic effects, we found that overall endocrine testicular and adrenocortical activities were lower in male Japanese Quail receiving photo-stimulated male intruders than in birds that were not visited.

Previous data on the endocrine testicular response to short photoperiods by androgen metabolite measurements indicated that short photoperiods can induce long-term effects on HPG activity in quail (Dominchin et al. 2014). In the present study, after 6 weeks of exposure to short photoperiods, SD-NR quail of 18 weeks of age exhibited spontaneous cloacal gland recovery and increments in androgen metabolite concentrations. Since gonadal regression induced by short photoperiod in quail is associated with a decrease in the secretion but not in the synthesis of hypothalamic GnRH (Dawson 2015), it may be surmised that the secretion of this neuropeptide underwent modification in SD-NR quail, most likely in terms of an increase in the rate of release. However, further studies are required to test this hypothesis. The same phenomenon probably occurred in SD-R quail, although they showed increments in androgen metabolites simultaneously with SD-NR quail, spontaneous cloacal gland recovery was delayed by 14 days. This delay is to be expected since in castrated Japanese Quail between 7 and 14 days of treatment with exogenous testosterone were required to significantly modify cloacal gland development. Internal changes (such as in testosterone) were detected earlier in birds than external changes (such as cloacal gland volume; Charlier et al. 2013), from which we infer that the androgen values reached in SD-R at 18 weeks of age, after 6 weeks of exposure to SD seem to represent a threshold for inducing cloacal gland recovery under short day treatment.

At 14 weeks of age AM levels exhibited an LD > SD-NR > SD-R pattern, with LD quail showing almost 10 fold higher AM concentrations than SD-R birds; this pattern was maintained during the following five weeks, until at 20 weeks of age SD-NR quail reached similar mean AM levels to LD quail. Similar differences were previously detected in the circulating concentrations of testosterone for other quail exposed to long or short days (Ball and Balthazart 2010), but it could also be due to the AM oscillations observed in the LD birds. Since AM metabolites in SD-NR quail were similar to those observed in photo-stimulated, sexually active males, our endocrine data suggest that SD-NR males may also be sexually active and able to fertilize females. A reproductive performance assessment has recently been tested in Dominchin et al. (2017). Charlier et al. (2013) reported that not only testosterone but also steroid metabolizing enzymes, steroid receptors and their coactivators are associated with neuroplasticity in quail, calling for further studies to deepen our understanding of the SD-NR phenotype. New insights along these lines could enhance the usefulness of non-responsive phenotypes as abiotic-resistant birds.

At the beginning of the study (between 14 and 16 weeks of age), adrenocortical activity was associated with photoresponsiveness in short and long photoperiods. From 16 weeks of age onwards, SD-NR quail reached a similar adrenocortical activity to LD quail. Considering that we had previously detected a high level of adrenocortical activity in both phenotypes from another population (Dominchin et al. 2014), we expected high values of corticosterone metabolites, with our results contributing to the energy mobilization hypothesis (Romero 2002). Under this hypothesis, high glucocorticoid concentrations during breeding result from high energetic demands during the reproductive season. Accordingly, we observed increments in CMs and AMs throughout the study in SD-R quail; however, neither value reached the level of those observed in the other two groups of birds. This pattern is expected for birds during the non-breeding period.

Little information is available on the long-term effects of non-photoperiodic control such as that of social modulation on male reproductive physiology development when males are maintained under a non-stimulatory photoperiod (short days; Dawson and Sharp 2007). Previous studies have reported on the reproductive responses of Japanese Quail exposed to different temperature cycles and short photoperiods (Tsuyoshi and Wada 1992) or to auditory and short-photoperiod social cues (Kerlan et al. 1988, 1991), but to our knowledge no data is available on reproductive physiology during short photoperiods of other relevant social cues such as the sudden appearance of a fully mature male in the territory of another male. In the current study we rejected the hypothesis that short social interactions with a fully mature male exert an effect on the timing of testicular and adrenocortical activity. Regardless of photoperiodic responses, no changes in androgen and corticosterone profiles were observed after male quail interactions during 4 consecutive days. Under our experimental conditions, shortly after Japanese Quail have completed gonadal regression (possibly analogous to the physiological stage of early fall in nature), quail exposed to short days did not appear to show sensitivity to social modulation by the presence of other reproductively active birds. The Japanese Quail exposed to short days possibly become increasingly sensitive to non-photic cues only later, as reported by Deviche and Small (2001). Furthermore, a recent review points out that in male song sparrows, exposure to simulated territorial intrusions leads to increases in plasma levels of testosterone in spring, but not in autumn (Wingfield 2012). Moreover, in our experiment LD quail did not respond to the applied social treatment. Similarly, Lambrechts and Perret (2000) reported that a Mediterranean Blue Tit (*Parus caeruleus*) population subjected to long-day treatment showed no response to non-photoperiodic factors and that the importance of non-photoperiodic cues decreases with the progress of the breeding season. Further studies going beyond 20 weeks age are therefore required in order to assess whether the effect of social cues on the testosterone profiles of our Japanese Quail population becomes significant later on in the breeding season.

Finally, in terms of overall endocrine activity, in our experiment male quail without visits showed higher endocrine testicular and adrencortical activities than their counterparts visited by another male quail. Since the social environment can have a strong impact on testosterone secretion (Hirschenhauser et al. 2013), it is possible that in our experiment, in which male quail were not visually isolated, the mere observance of a fight has a stronger consequence than actually taking part in it. It should also be taken into account that the studied Japanese Quail without visits may have been under some degree of stress due to physical isolation. Acoustic and visual signals of neighbouring birds were apparently not sufficient to avoid adrenocortical activity overload. Other authors also pointed out that Japanese Quail showed normal corticosterone response following acute stress when birds were placed for 30 min in a restraint cage (Dickens et al. 2011). However, differences in the experimental conditions may explain the lack of consistency between studies. Furthermore, we cannot discard a "social defeat effect" in resident loser males, probably acting as a negative stimulus on AM excretion. This hypothesis is consistent with the lower levels of endocrine testicular activity found in those males exposed to male visits than in their unvisited counterparts. Further studies are necessary to test both hypotheses.

Conclusions

Short social interactions of male Japanese Quail did not affect temporal variations in testicular endocrine and adrenocortical activities, irrespective of the photoperiodic reproductive status, but did affect overall endocrine activity. The social treatment neither affected cloacal gland development nor spontaneous recovery of reproductive activity.

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