In: Handbook of Social Interactions in the 21st Century ISBN 978-1-60692-860-8
Editor: A. T. Heatherton and V. A. Walcott

Chapter 9

SOCIAL CONTEXT AND WITH-IN PAIR BEHAVIOUR MAY MODULATE HORMONAL STRESS RESPONSE IN GREAT TITS (PARUS MAJOR)

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ABSTRACT

The presence of social allies may buffer adverse consequences of social stress. This has mainly been demonstrated in mammals and recently also in birds. The behaviour of social allies might crucially influence to which extent social context may buffer the behavioural and hormonal response to stress. We here examined the influence of social context on the hormonal response to handling stress in great tits (Parus major) selected for fast and slow exploration. We tested 16 male-female pairs (8 fast-fast pairs, 8 slow-slow pairs) after the breeding season. We subjected females to handling stress and thereafter observed their behaviour and collected droppings for the following 2½h with their mate being either absent or present when the females came back into her home cage. As control the same females were not handled prior to observation and their mate was present. In addition, we tested 7 fast and 7 slow unpaired females in the conditions control and mate absent. We measured immunoreactive corticosterone metabolites (CM) in droppings using an enzyme immunoassay. Fast females excreted significantly higher CM mean values when they were alone after handling stress (mate absent) than in the control condition and in condition mate present. Slow females tended to show a similar pattern. While fast females increased their locomotory activity, slow females sat close to their mates longer after handling stress compared to control days. Pair mates resting and feeding synchronously excreted lower CM than asynchronous pairs, irrespective of their

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behavioural phenotype. Paired and unpaired females did neither differ in behavioural nor in hormonal stress response, indicating that observed differences between condition mate absent and mate present in paired females were not due to an accumulation of stressors (mate absent plus handling) in condition mate absent. We here show for the first time, that depending on behavioural phenotype birds increased social proximity after a stressful event and that pair synchrony may modulate corticosterone excretion.

Keywords: *corticosterone, stress, social support, selection lines, Parus major*

INTRODUCTION

Benefits of social support have been described in the buffering model (Cohen and Wills 1985), which proposes that due to social context a stressful event might be perceived less intense and/or the magnitude of the physiological stress response might be less pronounced.

Investigations supporting the buffering model have mainly been conducted in mammals with a major focus on mother-infant (squirrel monkeys, *Saimiri*: Levine *et al.* 1993, rats (diverse strains): Levine 2001, reviewed in Hennessy 1997) or peer separations (cattle (diverse strains): Boissy and Le Neindre 1997, guinea pigs, *Cavia aperea*: Hennessy *et al.* 2006, prairie voles, *Microtus ochrogaster*: Ruscio *et al.* 2007). In addition, the presence of a social ally may also reduce behavioural and hormonal stress response to novelty (marmosets, *Callithrix kuhli*: Smith *et al.* 1998, rats: Terranova *et al.* 1999, prairie and meadow, *M. pennsylvanicus*, voles: Stowe *et al.* 2005), leading to increased exploration (e.g. novel food: zebra finches, *Taeniopygia guttata*: Coleman and Mellgren 1994, rats, *Rattus norvegicus*: Galef and Whiskin 2000, capuchin monkeys, *Cebus apella*: Visalberghi and Addessi 2003, novel objects: ravens, *Corvus corax*: Stöwe *et al.* 2006a,b, Stöwe and Kotrschal 2007).

Several factors determine to which extent social context may buffer stress response: firstly the social system of the species: whether, for how long and to how many different individuals affiliative bonds are established. In addition, the familiarity and identity of the social partner may modulate effects of social context. Another crucial factor seems to be the social experience (guinea pigs: Kaiser *et al.* 2007) and the developmental state (guinea pigs: Hennessy *et al.* 2006) of the focus individual determining to which extent social context may buffer stress response and which social partner may efficiently provide social support. Finally, sex-specific differences in hormonal response to social support have been observed (humans, *homo sapiens*: Kirschbaum *et al.* 1995, guinea pigs: Kaiser *et al.* 2003) as well as individual differences in response to social context (van Oers *et al.* 2005, Stöwe and Kotrschal 2007).

After decades of social support mainly being studied in mammals (e.g. reviewed in de Vries *et al.* 2003), more recently evidence for the buffering model and social support in birds is accumulating. In greylag geese, *Anser anser,* the presence of social allies (human foster parents: Frigerio *et al.* 2003, conspecifics: Scheiber *et al.* 2005a) affected the outcome of agonistic interactions and glucocorticosteroid excretion (assessed via immunoreactive corticosterone metabolites in droppings, CM). After agonistic interactions post-conflict affiliation between former opponents (reconciliation) or between former opponents and a bystander (third-party affiliation) may have calming effects (de Waal and van Roosmalen

1979). Not only in primates (Schino 2000), but also in corvids (rooks, *Corvus frugilegus*: Seed *et al.* 2007, ravens: own observation) and in greylag geese (Kotrschal pers. com.) both, initiators and targets of aggression were observed to engage in third-party affiliation with a social partner, exchanging socio-positive behaviours during the post-conflict period.

Generally, calming effects of social context are more pronounced if socio-positive behaviours are exchanged (e.g. pigtail macaques, *Macaca nemestrina*: Boccia *et al.* 1989, baboons, *Papio hamadryas*: Wittig *et al.*2008). Thus the behaviour of the social partner seem to be a factor modulating hormonal stress response. In ravens socio-positive behaviour (allopreening) was related to CM excretion already in nestlings (Stöwe *et al.* 2008). In cockatiels, *Nymphicus hollandicus*, pairs showing high frequencies of affiliative behaviour and a high degree of behavioural compatibility were more stable than behaviourally less compatible pairs (Spoon *et al.* 2004) and they produced larger clutches and raised more chicks (Spoon *et al.* 2006). Especially for females facing energetic bottle-neck situations such as egg-laying and breeding, stress management and buffered corticosterone excretion may crucially affect fitness and reproductive success. Beside other detrimental consequences on immune defence and body condition (e.g. Sapolsky 2002, Korte *et al.* 2004), chronically elevated levels of corticosteroids lead to increased mobilization of energy reserves (e.g. Holberton *et al.* 1999, Jenni *et al.* 2000, Cockrem *et al.* 2006), which consequently cannot be allocated in reproduction.

Individuals differ in suites of correlated behavioural and physiological characteristics ("personality", "behavioural syndrome", "coping style") leading to a cross-context consistency in how they deal with challenges (Gosling and John 1999, Sih *et al.* 2004, Kralj-Fiŝer *et al.* 2007). Behavioural phenotypes are genetically and epigenetically heritable (Dingemanse *et al.* 2002, Drent *et al.* 2003, Daisley *et al.* 2004) and they are one factor determining how an individual will respond to stress (reviewed in Cockrem 2007). In the social domain, studies on behavioural phenotypes mainly focussed on aggressive behaviour (Verbeek *et al.* 1996, D'Eath and Burn 2002, lines selected on the base of attack latency: Benus *et al.* 1990, Benus 2001) and coping with defeat (e.g. Carere *et al.* 2001, 2003, Ebner *et al.* 2005). Individual differences in response to social context have been observed with respect to exploratory behaviour (Stöwe and Kotrschal 2007). But despite the wide ranging effects of socio-positive behaviour on physiology (body condition, hormone excretion) and well being, none of the studies so far considered effects of behavioural phenotypes on between pair mate behaviour and stress response modulation due to social context.

We here focus on social support in great tit pairs of lines selected for fast and slow exploration (Drent *et al.* 2003, van Oers *et al.* 2004). Great tits are territorial, non-migratory passerines, which establish monogamous pair bonds. To investigate potential buffering effects of social context on hormonal stress response, we subjected the females to handling stress and once back in their home cage we observed the females with their mate being either absent (neither in visual nor in acoustical contact, condition mate absent) or present (condition mate present). In the control condition we observed the pairs without handling the females prior to observations. Since the mere absence of a pair mate may be a stressor itself, as has been shown for example in zebra finches (Remage-Healey *et al.* 2003), we also tested unpaired females in the control condition and after handling, to compare their stress response to the one of paired females when being without their mate.

In the majority of experiments examining buffering effects of social context, individuals were temporarily transferred into a novel cage either alone or together with a significant

social partner. In this testing paradigm it is sometimes difficult to distinguish which stressor (social separation and novel environment) triggered the increased excretion of corticosteroids (see Hennessy 1997 for detailed discussion). Moreover, none of these studies considered the mutual influence of the social partners affecting each other behaviourally and in the perception of the novel environment, leading to a potential increase in neophobia. To avoid this mutual influence of behaviour towards a novel environment, we chose to stress the females with handling, a commonly used effective stressor (also applied in great tits e.g. Cockrem and Silverin 2002, Carere and van Oers 2004) and compare the females' hormonal and behavioural response after this stressful event in her home-cage in presence or absence of her (when present) unhandled mate.

Since the stressor we used was the same in conditions mate absent and mate present and only *after* the handling stress females were either alone or with their mate, we expected no difference between pairs of the same selection line concerning in maximum CM excretion after the handling stress, but we predicted a modulation of the CM excretion curve due to calming effects of the presence of the mate. We predicted between line differences in hormonal and behavioural stress response patterns. We assumed males to increase socio-positive behaviour towards their mates after handling stress. Since differences in personality have wide-ranging effects on behaviour in different contexts (Koolhaas *et al.* 1999), birds selected for fast and slow exploration may also differ in within-pair behaviour (e.g. differ in amounts of socio-positive behaviours exchanged) both during control trials and after a stressful event.

METHODS

Animals and Housing

We tested adult great tits of lines selected for fast and slow exploration (Drent *et al.* 2003). Breeding pairs were housed in aviaries (2 x 4 x 2.5m). One wall consisting of wire-mesh was facing surrounding garden, the other three walls were opaque. Birds had visual and acoustical contact to other breeding pairs and to same-sex groups of unpaired birds (n_{max}=8 per aviary). Birds had *ad libitum* access to seeds, fruit, water and a mixture of minced meat, seeds and herbs. Once a day they were additionally fed mealworms. Round dishes filled with water served as bathing pools. After birds had completed the clutches, eggs were removed for other studies (cross-fostering, hand-raising).

Not earlier than at least two weeks after the end of the egg-laying pairs were subjects in the present experiment. Mid of May (2007) the first set of subjects (consisting of three fast-fast, three slow-slow male-female pairs and two unpaired females of each line) was transferred from the aviaries to the experimental room (artificial light, day: night rhythm: 14h:10h). Three rows of five cages (0.95 x 0.45 x 0.5m) each were fixed on opposite walls. Cages had solid top, rear and side walls, and wire-mesh at the front side. The bottom of each cage consisted of a drawer filled with wood-chips. Cages were equipped with three perches, diverse feeding trays and bathing tub. Feeding regime was as in the aviaries.

Each pair was kept in two connected adjacent cages, unpaired females alone in one cage each. All birds were familiar with these cages and housing conditions due to earlier

experiments. Still, we allowed three days of habituation to the housing conditions, the social environment in the experimental room and to the experimenter (M.S.) being present and collecting droppings before the first pair was tested. Only after *all* birds housed in the experimental room at a time, have been subjects in the experiments, they were returned into the aviaries, in same-sex groups of maximal eight individuals. We did not move subjects from the experimental room back into the aviaries immediately after they have been tested in all three conditions to keep the social environment constant for all birds. Mid of June the second set of birds was transferred into the experimental room (5 fast-fast pairs, 5 slow-slow pairs, 5 unpaired fast females, 5 unpaired slow females), which remained there until the end of the experiment (end of July 2007).

To facilitate dropping collection we covered the floor of the cages with brown paper, which M.S. put into the cage 24 hours before the onset of the control condition, to habituate the birds to the paper. Before the observations started, M.S. replaced the paper sheets with clean ones in each trial (control condition, condition mate absent and mate present). One day prior to the control condition M.S. repeatedly moved the drawers and simulated sample collection to habituate the birds to the procedure.

Experimental Set-up

In all conditions tests lasted from 10.00 to 12.30 am. On control days M.S. initiated the video-recording and dropping collection after having renewed the paper on the bottom of the cages.

On test days one of the caretakers would enter the room before the onset of the observation, divide the cages of the pairs with an opaque sliding wall, separating the male and the female. After catching of the female (catching time: $\overline{X}\pm SD=15.3s \pm 14.5$, min: 3s, max: 61s) the caretaker left the experimental room, keeping the female in the hand for 1 min. Thereafter the female was kept for 5 min in a cotton bag, then handled another min and put back into her home-cage. The caretaker removed the separating sliding wall and left the room. M.S. entered and started the video-recording as well as the collection of droppings. We asked the caretakers to handle the birds to avoid the tits associating M.S. to the handling stress and consequently becoming nervous each time she approaches the cage to collect droppings.

In the mate absent condition, the caretaker caught the male during the 5min the females was in the cotton bag. We kept the male in a cage (dimensions like the cages in the experimental room, food and water provided) in another room out of visual or acoustical contact to the female. The male remained separated for the 2½hours of observation. Thereafter he was brought back to the female. During the mate separation, males were not completely isolated. They were kept singly in a cage, but tits taking part in other experiments were housed in the same room.

In test condition mate present, males remained in the cage, thus the pair was united with the caretaker removing the separation wall after having returned the female.

We alternated observations between fast and slow pairs and conducted either condition mate absent or condition mate present first. We observed one female/pair at a time. This way we avoided that a stressed females could potentially affect the behaviour of other females under observation (i.e. a female in a control condition being nervous because of the presence

of a stressed female). Only in the control condition of unpaired females, we collected data of two females parallel. We conducted the stress test with half of the fast unpaired females the day following the control day and the other half after one day of interruption. We did the same for the slow females. Every third week of the experiment we tested unpaired females, the two weeks in between pairs, to balance observations of paired and unpaired females over time (mid May- end of July, detailed testing scheme in Table 1). For logistic reasons we had to test two pairs and four unpaired females in the afternoon (14.00-16.30 pm). Since CM values were in the range of those of birds tested in the morning we included the birds tested in the afternoon in the further data analyses.

Table 1. Scheme of testing sequence. During two weeks we tested four pairs (one fast and one slow pair per week). Every third week we conducted the tests with unpaired females (2 fast and two slow females). In the test conditions we handled the females and thereafter observed their behaviour and collected droppings with their mate being either present (a) or absent (b) when the female came back to her home cage. During the control condition females were not handled prior to observation and their mate was present. We alternated between weeks which test condition (a or b) females experienced first

day 1	day 2	day 3	day 4	day 5	day 6	day 7
pair A control	pair A condition a			pair A condition b	- -	
		pair B control	pair B condition a		- -	pair B condition b
day 8	day 9	day 10	day 11	day 12	day 13	day 14
pair C control	pair C condition b			pair C condition a	- -	
		pair D control	pair D condition b		- -	pair C condition a
day 15	day 16	day 17	day 18	day 19	day 20	day 21
female I control	female I condition a		female III control	female III condition a		
female II control		female II condition a	female IV control		female IV condition a	

BEHAVIOURAL OBSERVATIONS

We videotaped (JVC, digital video camera, GR-DVX7) the behaviour of the subjects during the first hour of observation in each experimental condition and observed the behaviour of males and females or the unpaired females from minutes 1-10, 25-35, 50-60. Each parameter was measured separately for each individual. We assessed the number/duration of a behaviour per minute of observation We noted locomotory activity (number of: hops and flights between perches, between a perch and the wire or the ground),

the time birds spent resting (seconds, sitting with one leg lifted up, sitting longer than 3s without moving, the feathers slightly ruffled, the body lowered) and the feeding duration (manipulation/ingestion of food items, seconds). Sitting within a distance of less than 20cm was recorded as socio-positive behaviour (duration in seconds). We never observed birds preening or feeding each other.

FAECAL SAMPLE COLLECTION AND ANALYSIS OF CORTICOSTEROID HORMONE METABOLITES

During the 2½hours data collection M.S. sat if front of the cages to track which dropping belonged to which bird (the male or the female of the pair). In 15min intervals droppings were collected in plastic tubes and stored on ice. Immediately after the end of the observation samples were frozen at -20°C until analysis. Before pooling the samples per individual and 15min interval, M.S. noted the number of droppings the male and female / the unpaired female excreted (see Table 2). In case a dropping could not be attributed to the male or the female (i.e. M.S. did not see which bird it came from), it was not collected. This was the case for mean±SD = 4±2 droppings out of 18-37 droppings per pair in the control and mate present condition. However, it is not likely that this loss of data in the control and mate present condition remarkably affected the results. Scheiber *et al.* (2005b) determined in greylag geese that three samples were sufficient to consistently assess differences in CM between a control condition and after a social density stress, when CM maxima were used for analysis. Four or more samples were required when working with the mean. In the present study we pooled samples of two 15min intervals, in case the amount of faeces per 15min interval was too low for analysis, resulting in mean±SD = 6±2 pooled samples per bird per condition. For the hormone analysis we followed the protocol described in Stöwe *et al.* (2008), only quantities of faeces and proportionally also of chemicals differed. In brief, 0.025g of wet faeces were shaken in a mixture of methanol (0.15ml, 96%) and distilled water (0.1ml). 0.05ml of this extract were evaporated and afterwards dissolved in 0.5ml Na-acetate buffer and 0.2µl β-glucuonidase-arylsulfatase (Merck 4114) and hydrolyzed for 18h. The enzyme immunoassay assay used in Stöwe (*et al.* 2008) has been validated for great tit droppings previously (Carere *et al.* 2003). It shows crossreactions not only with $C_{19}O_3$ steroids but also with $C_{21}O_4$ metabolites that have a 3α-ol, 11-oxo structure, therefore measuring 3α,11oxo-CM (detailed description in Möstl *et al.* 2002). Samples were assayed in duplicate. Intra-assay variation was 10%, inter-assay variations were 10.37% for the low level pool (we pooled droppings of females on control days) and 6.13% for the high level pool (here we used samples after we had handled the females).

CM peaked after $\overline{X}$±SD=1h 46min ± 30min, which indicates that the main part of immunoreactive corticosterone metabolites detected, were excreted in the faeces, because in the urine CM peaks in response to a stressor are excreted earlier (Palme *et al.* 1996, Touma *et al.* 2003)

The number of droppings (see Table 2) excreted during the 2½hours of observation did not relate to mean CM values. In non of the test conditions, the number of droppings excreted related to mean CM values neither in males, nor in females (Spearman rank order correlation coefficients ranging from r_s=-0.01 to r_s=-0.49, $p>0.05$).

Table 2. The number of droppings birds excreted in the different test conditions

subjects	n droppings, mean±SD		
	control condition	mate present	mate absent
fast paired females, n=6	13±3	13±5	16±6
fast paired males, n=6	14±4	13±4	
slow paired females, n=8	12±4	12±2	15±5
slow paired males, n=8	13±4	11±3	
fast unpaired females, n=6	20±7		16±2
slow unpaired females, n=6	22±2		20±4

DATA PROCESSING

Females of two fast pairs and two unpaired females (one of the fast and one of the slow selection line) excreted higher amounts of CM in the control condition than after handling stress. In addition, their CM means on control days were five to nine times higher than those of the other same selection line females (on control days), and the CM excretion pattern looked like a stress response curve. After handling the CM values were in the range of the those of the other same line females. Therefore, we assume unnoticed disturbances or stressful events on (on four out of 76 days of observation) before the onset of the control trials with these four females. Consequently we are missing valuable control data of these individuals and we excluded these two pairs and two females from the data analysis.

Data of the two sets of birds tested (transferred into the experimental room mid of Mai and mid of June respectively) did not differ significantly, therefore we do not distinguish between the two sets of birds in the data analysis. Between line differences did not reach significance level.

For data analysis we calculated the sum (number or duration depending on the parameter) per behavioural parameter measured during the 30min of behavioural observation and the mean of the CM values measured per individual during the 2½hours of observation.

To asses within pair synchrony we calculated Kendall-Tau-b correlation coefficients the time the female of a pair spent feeding /resting per minute of observation, with the time the male spent feeding /resting per observation minute (n=30 per pair). If the time birds spent resting/feeding correlated between pair-mates, we defined the pair as behaving synchronously. We did not include locomotory behaviour in this calculation, because if one bird is active in the relatively small experimental cages, the pair mate might be active too, in order to retreat, avoid contact or search contact. Thus, locomotory behaviour expressed synchronously could have been a by effect of keeping condition rather than a parameter of pair bond quality or affiliative relationships. Three pairs did not feed during the observation period, therefore n=11 pairs in the analysis of feeding synchrony.

We run a stratified data analysis with the selection line as stratification variable. When n=8 or lower, data were analysed by hand according to Siegel and Castellan (1988). Kendall-Tau-b correlation coefficients were calculated using the software package SPSS (2001). Only

non-parametric tests were used. Test results are given two-tailed. 0.1>alpha>0.05 were considered as trends.

RESULTS

Pairs

Fast paired females excreted significantly higher CM mean values when they were alone after handling stress (condition mate absent) compared to the control and the mate present condition (Friedman test: df=2, n=6, χ^2=7, p=0.03, post-hoc test for multiple comparison between conditions: control<mate absent, p<0.05, Figure 1a), slow females tended to show a similar pattern (Friedman test: df=2, n=8, χ^2=4.75, p=0.093, Figure 1b). CM maxima excreted after handling stress did not differ between conditions mate absent and mate present (Wilcoxon signed ranks test, hereafter referred to as Wilcoxon test: fast females: n=6, T^+=12, p=0.84, slow females: n=8, T^+=19, p=0.94). Fast females significantly increased their locomotory activity when they were without their mate (condition mate absent) compared to conditions mate present and control (Friedman test: df=2, n=6, χ^2=7.0, p=0.03, post-hoc test for multiple comparison between conditions: mate absent>mate present, p<0.05). Slow females, in contrast, did not show augmented locomotory activity when alone (Friedman test: df=2, n=8, χ^2=0.84, p=0.66). There was no between condition difference in the time birds spent resting (Friedman test: df=2, fast birds: n=6, χ^2=2.33, p=0.31 slow birds: n=8, χ^2=2.25, p=0.33). Slow females tended to feed longer in the control condition than after handling stress (conditions mate absent and mate present; Friedman test: df=2, n=8, χ^2=5.85, p=0.054, post-hoc test for multiple comparison between conditions: control>mate absent, control>mate present, p<0.05). We did not observe any between condition difference in feeding activity of fast females (Friedman test: df=2, n=6, χ^2=0.087, p=0.96). After handling slow birds tended to spend more time sitting close to their mates as compared to the control condition (Wilcoxon test: n=8, T^+=32, p=0.054, Figure 2), while fast females did not (Wilcoxon test: n=6, T^+=16, p=0.31, Figure 2). There was no difference between males and females in how often they initiated sitting close to their mate (Mann Whitney-U test: control condition: fast pairs: $n_{f=m}$==6, W_f=37,5, W_m=40,5, p=0.87, slow pairs: $n_{f=m}$=8, W_f=60, W_m=76, p=0.44; condition mate present: fast pairs: $n_{f=m}$==6, W_f=27, W_m=44, p=0.48, slow pairs: $n_{f=m}$=8, W_f=71.5, W_m=64.5, p=0.72).

Mean CM values did not differ between females that were tested in condition mate absent first and those that were tested this condition secondly (Mann Whitney-U test: resting: n_1=5, n_2=9, W_x=36, W_y=69, p=0.89).

In males mean CM values did not differ between test conditions (control and mate present, Wilcoxon test: fast males: n=6, T^+=16, p=0.31, slow males: n=8, T^+=20, p=0.84), nor did locomotory activity (Wilcoxon test: fast males: n=6, T^+=13, p=0.69, slow males: n=8, T^+=24, p=0.46) and feeding duration (Wilcoxon test: fast males: n=6, T^+=15, p=0.44, slow males: n=8, T^+=25, p=0.38). Slow males tended to spend more time resting in condition mate present than during the control trials (Wilcoxon test: fast males: n=6, T^+=14, p=0.56, slow males: n=8, T^+=31, p=0.078).

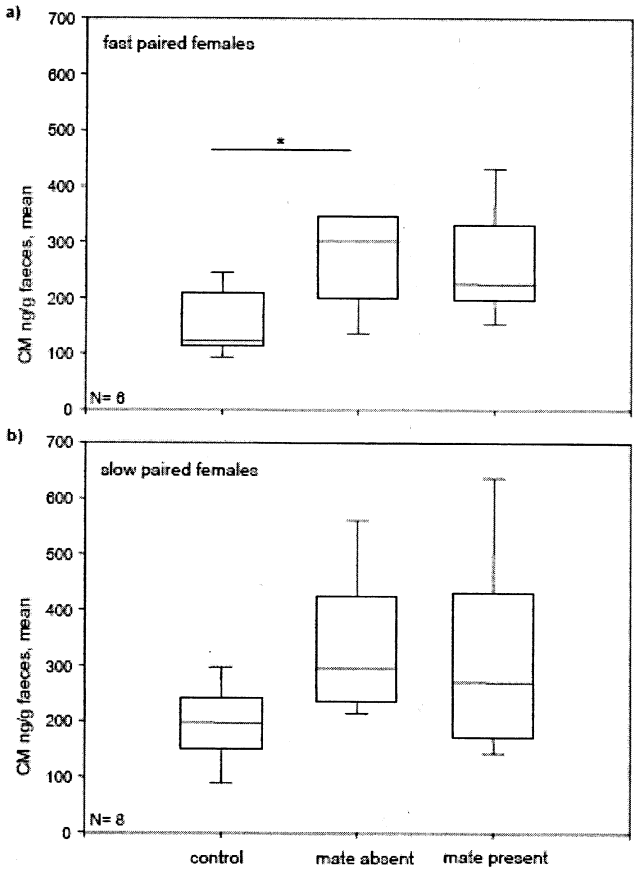

Figure 1. Amounts of immunoreactive corticosterone metabolites (CM, mean, ng/g faeces) fast (a) and slow (b) paired females excreted during the differed test conditions. N=number of birds, box plots show the median and the interquartile range from the 25th to the 75th percentile. Whiskers above and below the box indicate the 10th and the 90th percentiles. The asterisk marks significant between-condition difference as determined by post hoc tests for multiple comparisons for Friedman two-way analyses of variance by ranks (* $p<0.05$).

WITHIN PAIR SYNCHRONY

Irrespective of behavioural phenotype (fast or slow), in pairs resting synchronously, females excreted significantly less CM in the control condition than females in asynchronous pairs (Mann Whitney-U test: females: $n_1=6$, $n_2=8$, $W_x=28$, $W_y=77$, $p<0.029$, Figure 3, males: $n_1=6$, $n_2=8$, $W_x=35$, $W_y=70$, $p=0.22$). Males feeding synchronously with their mates had lower CM values compared to males feeding asynchronously during control trials (Mann Whitney-U test: females: $n_1=6$, $n_2=5$, $W_x=32$, $W_y=34$, $p=0.51$, males: $n_1=6$, $n_2=5$, $W_x=22$, $W_y=44$, $p<0.01$).

In the condition mate present, females tended to excrete lower mean CM when feeding synchronously with their mates compared to females that did not (Mann Whitney-U test: $n_1=4$, $n_2=7$, $W_x=15$, $W_y=51$, $p=0.089$).

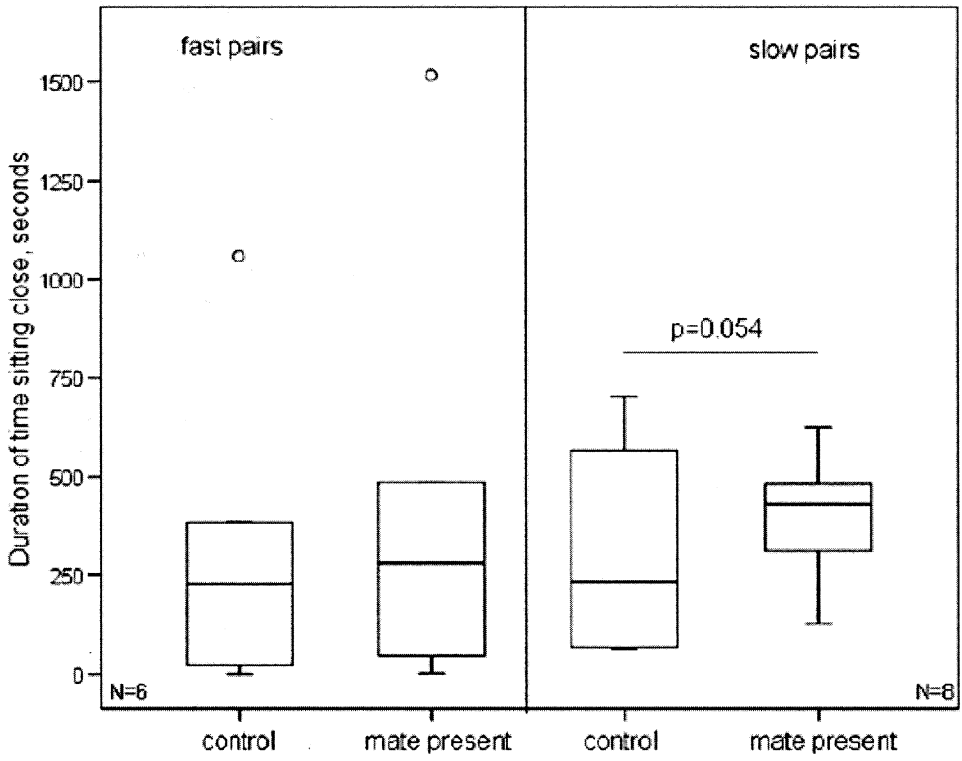

Figure 2. Time spent sitting close to the mate (distance between pair mates <20cm) in the control and mate present condition (between condition comparison using the Wilcoxon signed ranks test). N=number of pairs.

We did not observe any difference in CM concerning resting synchronously (Mann Whitney-U test: n_1=8, n_2=6, W_x=62, W_y=43, p=0.89). Males, in contrast, tended to excrete lower CM when resting synchronously with their mate, but not when feeding in synchrony (Mann Whitney-U test: feeding: n_1=4, n_2=7, W_x=19, W_y=47, p=0.41 resting: n_1=8, n_2=6, W_x=45, W_y=60, p=0.058).

UNPAIRED FEMALES

All unpaired females excreted significantly higher amounts of CM after having been handled compared to control days (Wilcoxon test: n=6, fast females: T^+=21, p=0,031, slow females: T^+=21, p=0,031). Females of both lines tended to feed longer during control trials than on test days (Wilcoxon test: n=6, fast females: T^+=19, p=0,092, slow females: T^+=20, p=0,063). Slow but not fast females tended to rest longer during test trials as compared to control trials (Wilcoxon test : n=6, fast females: T^+=12, p=0,84, slow females: T^+=19, p=0,092).

In none of the parameters measured paired fast females differed significantly from unpaired fast females, neither in the control condition nor in the mate absent condition (Table 3).

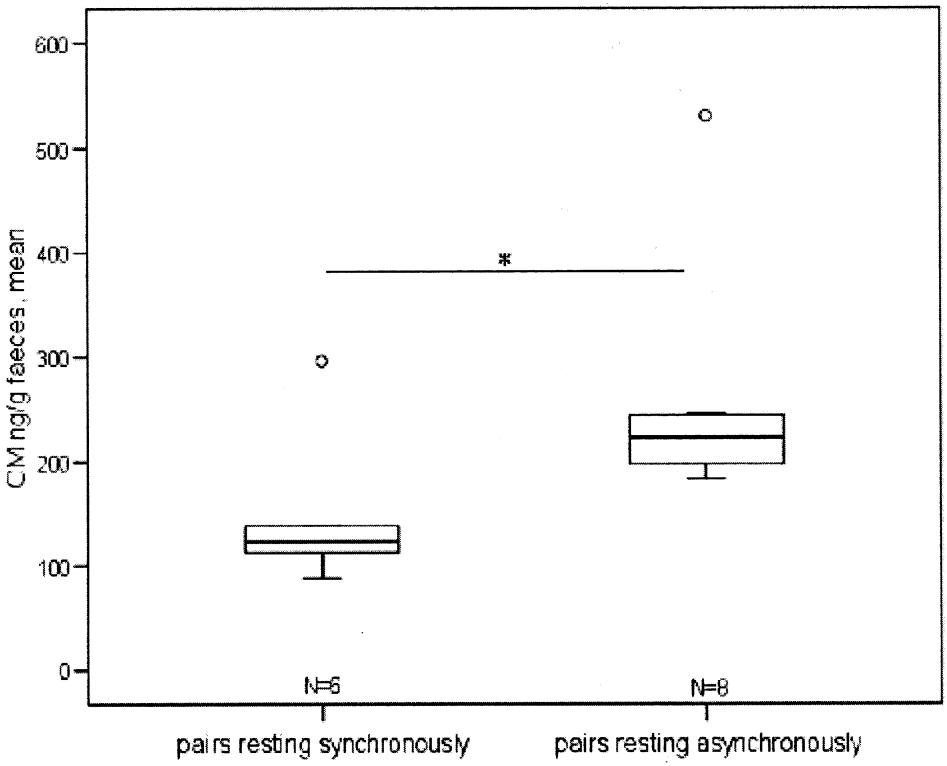

Figure 3. Comparison of females' CM (mean, ng/g faeces) levels during the control condition of pairs resting synchronously or asynchronously, (irrespective of selection line, Mann Whitney-U test, *p<0.05). N=number of pairs.

Table 3. Comparison of paired and unpaired females. We used the Mann Whitney-U test to compare behaviour and CM excretion of paired and unpaired females in the control condition and after handling stress (condition a). Wx: unpaired females, Wy: paired females

parameter	condition	fast females, n1=n2=6	slow females, n1=6, n2=8
CM	control	Wx=39, Wy=39, p=1.0	Wx=35, Wy=70, p=0.22
locomotory activity	control	Wx=49, Wy=29, p=0.13	Wx=56, Wy=49, p=0.18
resting, duration (s)	control	Wx=37, Wy=41, p=0.82	Wx=38, Wy=67, p=0.41
feeding, duration (s)	control	Wx=42, Wy=36, p=0.70	Wx=42, Wy=63, p=0.74
CM	test a	Wx=37, Wy=41, p=0.82	Wx=45, Wy=60, p=0.99
locomotory activity	test a	Wx=41, Wy=37, p=0.82	Wx=45, Wy=60, p=0.99
resting, duration (s)	test a	Wx=40, Wy=38, p=0.94	Wx=43, Wy=62, p=0.85
feeding, duration (s)	test a	Wx=36, Wy=42, p=0.70	Wx=40.5, Wy=60.5, p=0.57

DISCUSSION

The presence of the pair mate after a stressful event indeed affected the hormonal and behavioural stress response in great tit females. Fast females excreted significantly higher mean amounts of CM when alone after handling stress as compared to the control condition or when their mate was present. This indicates that the presence of the social partner had buffering effects on hormonal stress response. Slow females tended to show a similar pattern, however the differences between conditions were less pronounced. Alternatively to the buffering hypothesis (Cohen and Wills 1985), the absence of the pair mate in the mate absent condition could have been an additional stressor (handling *and* absence of the mate) leading to higher mean CM values. Mate separation commonly leads to increased corticosteroid secretion (e.g.: zebra finches: Remage-Healey *et al.* 2003). However, comparing unpaired great tit females in our experiment to paired females when without their mate, neither hormonal nor behavioural stress response differed significantly. On test days (with handling stress) unpaired females were not exposed to a potential accumulation of stressors (mate absent and handling stress) and they did not excrete lower levels of CM compared to paired females. Therefore, it seems that the presence of the mate had calming effects rather than an accumulation of stressors being responsible for the increased CM excretion of paired females in the mate absent condition compared.

In both conditions (mate absent, mate present) females remained with their mates until the handling stress, the stressor was the same and females were always alone during handling, thus the circumstances eliciting the hormonal stress response did not differ between conditions. Thus, we expected a modification in the CM excretion curve due to the presence of the mate rather than a difference in CM maxima. Indeed, we did neither observe a significant difference in the time until CM peaked nor did CM maxima differ between conditions mate absent and mate present, irrespective of the females' behavioural phenotypes.

A potential mechanism how calming effects of social context may influence CM could be a modulation of adrenocorticotrophic hormone (ACTH) secretion after a stressful event. Such that stressed individuals stop ACTH secretion in response to a stressor quicker when a social ally is present compared to when alone. If so, social support should lead to a steeper decrease in CM after the CM peak in response to a stressful event. Since the females excreted CM maxima only after $\overline{X}$±SD=1h 46min ± 30min and they did not return to CM control levels during the 2½hours observation period, we unfortunately can not compare CM decrease curves between conditions. However, CM mean values differed between conditions. These observed between condition differences in CM mean values could be due to CM excreted in the urine fraction (where CM are excreted with less delay compared to faeces, e.g. Palme *et al.* 1996, Touma *et al.* 2003). In addition to the urine contained in droppings, the metabolization in the liver and excretion via gut and kidneys, immunoreactive corticosterone metabolites may diffuse through the gut walls and thus be in part measured before the peak excretion. Nevertheless, it would be useful in future experiments to extend the test duration to be able to properly compare CM decline curves between the selection lines with and without social support after a stressful event.

In the present study between line differences surprisingly did not reach significance level. With an extended period of dropping collection and the comparison of CM decline curves after the handling stress, differences between the selection lines might have been more

pronounced. Besides, the relatively low sample size per line and individual variation within the lines may have rendered between line differences less pronounced.

Carere *(et al.* 2003) observed that CM excretion increased in slow male great tits 45min after having been defeated in an agonistic male-male interaction. The day after the conflict slow males had lower CM values compared to baseline levels. Fast males did not show these changes in CM excretion. In our experiment females of both lines significantly increased CM excretion in response to handling stress, with fast females being hormonally more responsive to the presence of the mate after a stressful event than slow females.

Females of the slow line tended to spend more time sitting close to their mate, after having been handled compared to control days. This could reflect an increased need of social support after a stressful event. When comparing how often males and females initiated sitting close to their mate (i.e. how often the male approached the female and vice versa), we did not observe differences between the sexes. This could be due to the fact that both, the males and the females increased socio-positive behaviour. The males might have increased the time sitting close to their stressed mate, as we expected. While stressed females might have actively searched proximity to their mate. Following agonistic interactions in rooks, both, individuals involved in the fight and uninvolved third parties initiated affiliative contacts (Seed *et al.* 2007). Thus, also in the rooks' case not only those individuals directly involved in a stressful event increased social proximity.

We here show for the first time, that depending on the behavioural phenotype birds show differences in social support expressed: only slow birds spent more time sitting close to their mate after a stressful event. This differences between females of the selection lines in need/use of passive social support could be an additional factor influencing mate choice. Indeed, assortative mated free living great tit pairs, assortative with respect to their behavioural phenotype (slow-slow pairs and fast-fast pairs), produced offspring in best condition (Both *et al.* 2005). Concerning the number of recruits (young being present in the following spring) the pattern is less clear: after a winter with abundant food (mass seeding of European beech, *Fagus sylvatica*) most recruits came from assortatively mated parents. After a winter with worse food conditions birds of an intermediate exploratory behaviour produced most recruits (Dingemanse *et al.* 2004).

Beside slow males resting more in the mate present condition compared to control trials, we neither observed differences in behaviour nor in CM in males. This indicates that the catching of the mate, her absence for 7min and her coming back stressed, did not elicit increased agitation (e.g. reflected in locomotory activity) or a hormonal stress response in the males. The increased time slow males spent resting in the test condition could be due to them being more familiar to the test procedure, including dropping collection compared to control trials, even though birds were habituated to the dropping collection procedure (i.e. to M.S. partly pulling out the drawers) before the onset of the experiment.

Irrespective of behavioural phenotype, pair mates feeding and resting synchronously excreted lower CM compared to those behaving asynchronously. To a certain extent, synchronous resting and feeding could be related to daily rhythms, time after feeding (all birds were fed in the morning before the onset of the experiment) and motivation transfer (*Stimmungsübertragung*). However, preferred food (mealworms and seeds mixed with meat) could be monopolised and some males did (males are dominant over females in great tits, Dingemanse and de Goede 2004). Thus, in pairs feeding synchronously males tolerated females feeding at the same time and did not monopolise food sources while they were

feeding themselves. In addition, feeding motivation transfer should not be related to CM excretion in the dominant males, because they would not be limited in behaving according to their needs (e.g. unlimited access to food). Therefore, synchronous behaviour observed seems to reflect partner compatibility or harmony rather than being just a by-product of daily rhythms. Mate compatibility is an important attribute of pair bond quality affecting long term success and fitness. Spoon (*et al.* 2004, 2006), for example, could demonstrate in cockatiels, *Nymphicus hollandicus*, that pairs showing higher frequencies of affiliative behaviour and a high degree of behavioural compatibility were more stable and raised more chicks than behaviourally less compatible pairs. Synchrony between pair partners is not limited to behaviour. Year round testosterone co-variation was positively correlated with the pair's long-term productivity (mean number of fledged young per year of the pair-bond) in greylag geese (Hirschenhauser *et al.* 1999). In addition, pairs successfully rearing young until fledging, were acting synchronously more often and for longer periods of time than mates in unsuccessful pairs did (Nedelcu pers. com.). Our results highlight, that partner compatibility also plays an important role in coping with stressful events and in buffered stress response, which is indirectly linked to reproductive success. Elevated corticosteroids lead to increased mobilisation of fat reserves, which are consequently not available to be invested in reproduction (e.g. number and weight of eggs). Thus, a good "stress management" and low corticosteroid values have long term fitness consequences on body condition, health, immune response and reproductive success. We here could observe calming effects due to the presence of the mate and increased socio-positive behaviours exchanged following a stressful event, even if pairs had not been allowed to freely choose their mates. Even if we did not observe between line differences in within-pair behaviour, it seems precipitate to conclude that fast and slow explorers generally do not differ in behaviour towards their mate. Before doing so, pairs formed on the basis of mate choice should be observed in different seasons. Between line differences may in stages (i.e. during pair bond formation) be more pronounced than in others.

Long term mate separation (several weeks) and social isolation are linked to an increase of HPA-axis function, anxiety and inactivity (e.g. siberian dwarf hamster, *Phodopus sungorus*: Castro and Matt 1997, prairie voles: Ruscio *et al.* 2007). In our experiment, unpaired females did neither differ from paired females in behavioural nor in hormonal parameters. Unlike individuals kept in social isolation (for example in the above mentioned studies), unpaired females in our experiments were housed in same-sex groups until we transferred them into the experimental room, where they remained in visual and auditory contact with conspecifics. These social housing conditions without mate separation before the onset of the experiment may explain, why we did not observe differences between paired and unpaired females in baseline CM.

CONCLUSION

In line with the buffering model of social context, the presence of the pair mate after a stressful event indeed reduced hormonal stress response in great tit females. We here show for the first time, that depending on the behavioural phenotype birds show differences in social support expressed: only slow birds spent more time sitting close to their mate after a

stressful event. In addition, pair mates resting and feeding synchronously excreted lower CM than pairs behaving asynchronously. These results highlight that partner compatibility and synchrony affect corticosterone excretion also in response to stressful events. Since individuals with different behavioural phenotypes seem to differ in the use/need of social support and social proximity, assortative mating could be advantageous with respect to stress management and the buffering effect due to the presence of the mate.

ACKNOWLEDGEMENTS

We would like to thank the animal caretakers at NIOO-KNAW for collaboration with handling the females. A. Kuchar's laboratory assistance/analysis was indispensable. We are grateful for statistical advice by Professor I. Sommerfeld-Stur. M.S.: I tightly embrace Angelo, my home. Financial support for the faecal samples analysis came from the NIOO-KNAW, Department of Animal Population Biology, Heteren, NL. We thank Professor J. F. Cockrem and Professor K. Kotrschal for reviewing the manuscript and their helpful comments.

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