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„Hormonal responses to temporary changes in group size and composition in ravens (*Corvus corax*)“

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Master Thesis

**Hormonal responses to temporary changes in group  
size and composition in ravens (*Corvus corax*)**

written by  
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*Key words:*

Common Raven (*Corvus corax*) • Social relationships  
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## ABSTRACT

Ravens (*Corvus corax*) exhibit a complex social organisation that in early life, when they form non-breeder groups, includes a high degree of fission-fusion dynamics. Within these groups, individuals have social relationships of varying quality and valence – “friends” provide benefits, such as social support during or after conflicts with others, presumably reducing corticosterone levels and alleviating stress. So far, little is known about the neuroendocrine activity in the context of fission-fusion dynamics, where long-lasting separations of affiliated birds may occur. The present study aims to elucidate these endogenous effects by separating sixteen ravens individually from their group for four days and subsequently reintroducing them. To determine stress response patterns, we measured amounts of excreted immunoreactive corticosterone metabolites (CM) in droppings using an enzyme immunoassay against 3 $\alpha$ ,11oxo-CMs, previously validated for ravens. Our findings suggest that most individuals seem to be stressed when they are isolated from their group, though this only applies to socially well integrated birds. For individuals that lack social bonds, or only have very few, group living appears to be even more stressful than being alone. The birds’ stress responses, thus, seem to depend on their social integration. In contrast to our expectations, we found that ravens that are separated from a certain affiliate, but still within a group, did not show larger stress responses than birds that are not bonded with the isolated individual. The former might either exhibit cognitive and behavioural stress coping mechanisms rather than neuroendocrine stress responses, or, in accordance with the social buffering model, their stress could be alleviated by other group members.





## INTRODUCTION

Social life is not always easy to handle, entailing many challenges since conflicts may arise due to different reasons; e.g. competition for food and mating partners. Therefore, social interactions are known to be one of the strongest potential stressors, for both humans and non-human animals (DeVries et al., 2003). However, living in a group can also be very beneficial, as individuals might for example, defend against predators (e.g. Pulliam, 1973) and access food more easily with the help of others (e.g. Creel and Creel, 1995), they may share information (e.g. Wright et al., 2003), facilitate thermoregulation (e.g. penguins huddling together for warmth; Zitterbart et al., 2011) and may even profit from cooperative breeding, including allolactation (e.g. Clutton-Brock, 2002; Creel et al., 1991).

However, social relationships, which form the basis of social life, may vary between individuals. Social relationships, defined as a series of interactions in time between two individuals known to each other (Hinde, 1976), require repeated dyadic encounters, and include parent-offspring (in species with parental care) and sibling relationships as well as dominance and affiliative relationships (Adkins-Regan, 2009). Relationship's quality (see Cords and Aureli, 2000; Fraser and Bugnyar, 2010; Fraser et al., 2008), determines whether the conspecifics provide social support or not. According to the buffering model social support leads to less intense (physiological) stress responses to stressful situations (e.g. in great tits, *Parus major*, Stöwe et al., 2009; reviewed by Cohen and Wills, 1985). Allies may, on the one hand, support others by their mere presence, which is also referred to as "passive" social support, on the other hand individuals may actively intervene in agonistic encounters between "friends" and others and therefore provide "active" social support (see Scheiber et al., 2009; Fraser and Bugnyar, 2012). Both kinds of intervention appear to buffer the effects of stress and attenuate adrenocortical activity (e.g. in zebra finches, *Taeniopygia guttata*, Ramage-Healey et al., 2003; ravens, *Corvus Corax*, Stöwe et al., 2008).

In a vertebrate's body stress responses are regulated by two systems, a) the sympathetic adrenomedullary system, which controls the quick adrenaline release from the medulla of the adrenals via electric signals from the hypothalamus, and b) the hypothalamic-pituitary-adrenal (HPA) axis, which works as follows (reviewed by Manteuffel, 2002): When a stressor (e.g. conflicts with conspecifics, predator attacks or

certain physical influences such as cold) acts on an individual, the paraventricular nucleus of its hypothalamus excretes corticotropin releasing factor (CRF). CRF then causes the anterior pituitary gland to release adrenocorticotrophic hormone (ACTH), which in turn causes the zona fasciculata of the adrenal cortex to release cortisol and corticosterone. While most mammal species excrete more cortisol than corticosterone (e.g. humans 95% vs. 5%), birds and also other animal groups exhibit higher amounts of corticosterone (Holmes and Phillips, 1976). Both hormones, however, belong to the glucocorticoid class of steroid hormones that play an important role in metabolism (e.g. stimulation of gluconeogenesis) and immunology (Råberg et al., 1998), but also affect cognitive processes, such as memory consolidation (Belanoff et al., 2001; Cahill and McGaugh, 1998). Given the range of its functions, the HPA axis has to be active at all times, but the more stressful a situation the more glucocorticoids will be excreted. The quantity of glucocorticoids measured in blood serum, saliva and milk or of the hormones' metabolites in urine and faeces, can therefore be used as a reliable measure of stress (reviewed by Möstl and Palme, 2002). Due to welfare concerns, but also because directly handling an animal and drawing blood might cause stress by itself and, thus, would be counterproductive, many researchers refuse applying phlebotomy techniques on animals and prefer non-invasive methods.

Considering the previously mentioned positive effects of affiliates, one could assume that separation from these social allies acts primarily as a psychological stressor and results in increased glucocorticoid levels. Ramage-Healey and colleagues (2003), for example, showed that pair mate separation (of 48 hours) and reunion, effects the adrenocortical activity in zebra finches (*Taeniopygia guttata*); corticosterone was elevated due to separation but returned to a baseline level upon reunion. The study also discovered that in species with strong social bonds, the presence of other conspecifics during separation from the bonded partner did not significantly attenuate HPA activation. So far, however, little is known about animals' adrenocortical activity in societies with a high degree of fission-fusion dynamics, where long-lasting separations of affiliates may occur frequently. Due to their extraordinary social characteristics and abilities, common ravens (*Corvus corax*) are a well-suited species for investigating this further (Fig. 1).

As a species of the corvid family, ravens are highly gregarious and are considered, besides apes, a few other mammalian species and psittacines (parrots), one of the most intelligent animals. According to the social brain hypothesis (Dunbar,

1998), which in the last decade has been shown to not only apply to mammals, but also corvids (Bond et al., 2007, 2003), the evolutionary development of the ravens' enhanced cognitive abilities might have been facilitated by their complex social system, that changes with the birds' life-history stages. When ravens become sexually mature at the age of about four years (Boarman and Heinrich, 1999), they form long-term monogamous breeding pairs and establish large territories (at least 10 km<sup>2</sup>; Rösner and Selva, 2005), which they defend all year round for the rest of their lives (Haffer, 1993; Heinrich, 1989). Until then, however, when fledglings become independent from their parents, they join non-breeder groups (Glutz von Blotzheim and Bauer, 1993) that exhibit high degrees of fission-fusion dynamics.



**Figure 1.** Study species: Raven, *Corvus corax*

Unlike previous uses of the term “fission-fusion” in the context of a special type of social system (i.e. “fission-fusion society”), it is now referred to in more dynamic terms defined by the degree of spatial and temporal cohesion of individuals in a group (Aureli et al., 2008). This means that groups can change in size and composition to varying extents, often involving the formation of subgroups. A recent study on a wild raven population revealed that during the day ravens could either be found alone or they formed small subgroups of a mean of 3.5 individuals, seemingly in order to engage in affiliative behaviours (Braun et al., 2012). Since ravens are scavengers, however, they often join bigger non-breeder groups to be able to compete with territorial breeding pairs (Marzluff and Heinrich, 1991) or potentially dangerous predators, like grey wolves (*Canis lupus*) (Stahler et al., 2002). Moreover, ravens gather at night roosts of up to 2000 birds (Wright et al. 2003). These communal roost appear to function as information centres where, for instance, in the morning naïve roost members sometimes follow knowledgeable ones to feeding sites and therefore gain benefits (Marzluff et al., 1996).

Furthermore, raven non-breeder groups are characterised by selective cooperation and competition and social bonding (Braun and Bugnyar, 2012; Heinrich, 2011). The latter not only presents an important social benefit, but could conceivably also enable individuals to assess the quality of a potential long-term partner (Braun and

Bugnyar, 2012). Recent findings revealed that ravens cannot just distinguish between familiar and unfamiliar individuals merely by their calls, but are even able to remember the relationship valence for years (Boeckle and Bugnyar, 2012). Affiliated birds reciprocate active agonistic support (Fraser and Bugnyar, 2012), console one another after conflicts with other individuals (Fraser and Bugnyar, 2010) and also reconcile conflicts between themselves (Fraser and Bugnyar, 2011). Stöwe and colleagues (2008) could even show, that the more time raven nestlings spent preening others the less corticosterone was released by them. All these findings suggest that in ravens affiliates are very valuable and do affect the birds' hormonal status. But how does this high degree of fission-fusion dynamics in non-breeder groups affect the ravens' endocrine status? What happens physiologically to group members when one bird (with social bonds to some, but not all members) is not present anymore and what happens to the individual that is separated from its group?

The present study aims to illuminate ravens' physiological stress responses to an experimentally induced fission-fusion situation. By individually separating group members for four days and subsequently reintroducing them into their group and measuring corticosterone metabolites in the birds' droppings we intend to investigate changes in the ravens' stress levels throughout the experiment.

Considering the positive impact of social bonds, we hypothesize that the corticosterone excretion in ravens is increasing while they are individually separated and declining when they are released back into the group and that the amplitude of the stress-induced corticosterone response is dependant on the social integration of the bird. Concerning the individuals remaining in the group, we expect that affiliates of the separated raven, in this study also referred to as "friends", experience stress, while "non-friends" could be influenced by the change of group structure in different ways, probably also leading to differences in stress levels.

## MATERIAL AND METHODS

### Subjects and housing

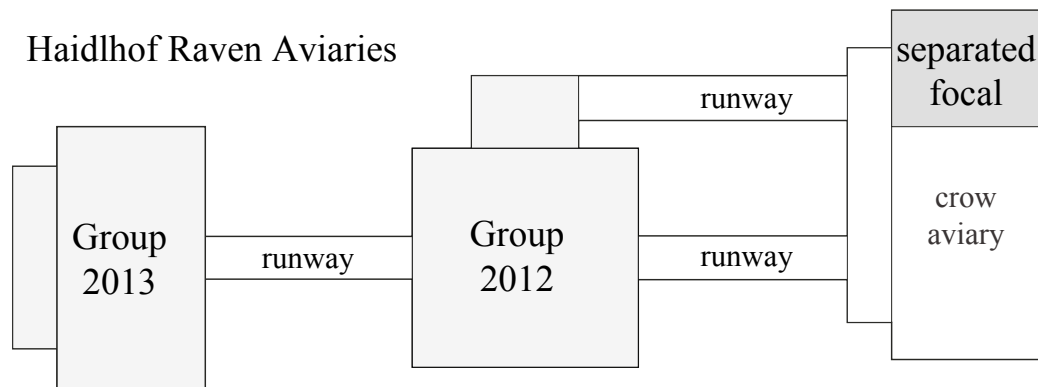
The study was conducted on sixteen ravens (seven males, nine females, Tab. 1) that were kept at the Haidlhof Research Station (joint research station of University of Veterinary Medicine Vienna and University of Vienna), Bad Vöslau, Austria. Individuals derived from eleven breeding pairs of different facilities (zoo, research station, private owner) and were grouped into two mixed sex non-breeder groups, ensuring a species-specific social situation that resembled natural conditions at that early stage of a raven's life. At the time of grouping they were less than a year old, while in the study period the birds were 1½ to 2 years old (two females were one year older), hence still sub-adult and sexually immature.

Initially each of the two groups consisted of 8 individuals, however, groups were merged after the first group was tested (in 2012), resulting in a group of 12 individuals (five males, seven females, 2013). The four remaining individuals (two males and females, respectively) from the already tested group were given to another research facility due to reasons not related with this project. To facilitate individual identification all birds were marked with coloured leg-rings.

**Table 1.** Overview of study subjects.

<b>Name</b>	<b>Group</b>	<b>Sex</b>	<b>Year hatched</b>
Heidi	2012 (2013)	Female	2010
Klara	2012 (2013)	Female	2010
Elen	2012	Female	2010
Sophie	2012 (2013)	Female	2010
Lena	2012	Female	2010
Anton	2012	Male	2010
Jonas	2012 (2013)	Male	2010
Jakob	2012	Male	2010
Astrid	2013	Female	2010
Joey	2013	Female	2010
Skadi	2013	Female	2011
Lellan	2013	Female	2011
Thor	2013	Male	2011
Ray	2013	Male	2011
Matte	2013	Male	2011
Orm	2013	Male	2011

The ravens' aviaries (approx. 180 m<sup>2</sup>) were constituted of freely accessible indoor as well as outdoor compartments (Fig. 2) and furnished with branches, trees, tree stubs, hollow bricks and also shallow water basins to allow the birds to bathe in. The ground was covered with wood chips, stones and partly with sand, offering plenty of opportunities to cache food and other items, which ravens do frequently. Their diet consisted of meat, chicken eggs, vegetables, fruits, yoghurt and kitchen leftovers and was provided on a daily basis, while water was available *ad libitum*.



**Figure 2.** Aviaries are connected by runways that can be opened or closed selectively. The merged group of 2013 also had access to the left half of the aviary that in 2012 was inhabited by the group tested first.

### Social integration

Since in ravens the dominance rank appears to be dependent on social bonds (Braun and Bugnyar, 2012; Gwinner, 1964), we included the birds' social integration instead of their dominance rank. Therefore we calculated a score for each bird using data from the behavioural parameter "contact sit" (birds sitting within one body length to each other). In the first group data gained from 5-minutes focal observations of 12 randomly selected days distributed over the six months study period was included in the analysis, while for the second group observations were only available from three months (Dez. 2012 - Feb. 2013). From these data we constructed weighted, undirected social networks for both groups and calculated normalized Freeman degree values for each individual, using UCINET 6 for Windows (Borgatti et al., 2002). Weighted degree values include the number of ties, i.e. number of interaction partners of an individual as well as tie weight, i.e. number of interactions with each partner. Contact sit as the used behavioural parameter reflects high spatial (social) tolerance between individuals and therefore seems a good indicator of the bird's social integration. Individuals were

arranged into two groups according to their normalized degree values. Those with values lower than 50 % of the maximal value of the group were assigned to the class of poorly integrated individuals, the others to the well integrated (Tab. 2).

**Table 2.** Social integration class of the subjects.

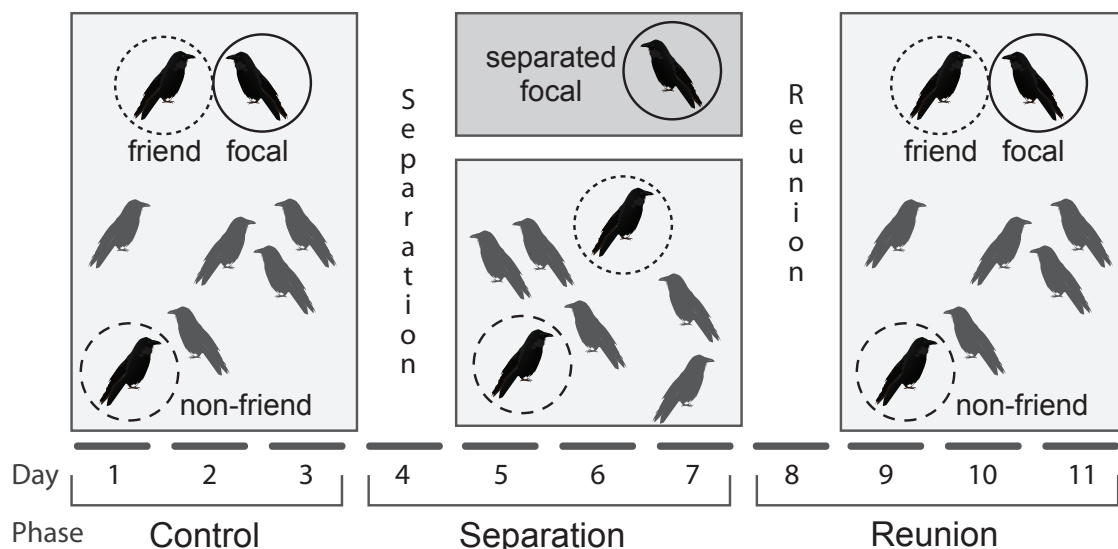
<b>Soc. integration class</b>	<b>Individual</b>	<b>Degree</b>	<b>Normalized degree</b>	<b>% of max. norm. degree</b>
<b>2012</b>				
Well integrated	Anton	49	23.33	100.00
	Jakob	47	22.38	95.92
	Sophie	38	18.10	77.55
	Lena	36	17.14	73.47
	Klara	36	17.14	73.47
	Heidi	32	15.24	65.31
Poorly integrated	Elen	18	8.57	36.73
	Jonas	14	6.67	28.57
<b>2013</b>				
Well integrated	Ray	24	10.39	100.00
	Astrid	21	9.09	87.50
	Heidi	21	9.09	87.50
	Jonas	21	9.09	87.50
	Thor	20	8.66	83.33
	Lellan	16	6.93	66.66
Poorly integrated	Matte	11	4.76	45.83
	Orm	10	4.33	41.67
	Skadi	0	0	0
	Joey	0	0	0
	Klara	0	0	0
	Sophie	0	0	0

## Data collection

Each raven was separated individually from his or her conspecifics for four days. On the first day the respective focal individual was captured with a net at around 9:30 am and transferred to another, familiar aviary that was situated approximately 20 m away from the group aviaries. Hence, the bird was isolated visually, but not acoustically. In separation the raven encountered nearly the same housing and dietary conditions as in the group in order to reduce environmental influences other than social aspects. After the separation phase the focal individual was allowed to move back to the group through a lattice fence runway to avoid any further handling stress and ensure that changes in HPA activity are due to the reunion.

For each separation we collected droppings of the focal individual and one or two “friends” and rather neutral “non-friends” (see supplementary material: *Friends and non-friends*). Samples were collected during the separation phase as well as three days before and after the separation to determine not only the stress-induced corticosterone response to the isolation itself, but also the metabolite level prior to separation and the adrenocortical response to the reunion. For the sake of brevity, the corticosterone level prior to the separation is referred to as “control”, even though it is hard to rule out that unknown or uncontrolled stress factors are acting on the animals. In the present study this could, for example, be a conflict with conspecifics (Fig. 3).

All droppings were collected in Eppendorf micro tubes (1.5 ml, Fig. 4.A) from 10:00 to 11:30 am to avoid measuring the corticosterone peak many bird species excrete in the early morning (Carere et al., 2003; Meier and Fivizzani, 1975). Moreover, to ensure that the researcher’s presence does not stress the ravens, only people that used to collect droppings already before the experiment started and therefore were well acquainted to the birds (Alexandru Munteanu, Martina Schiestl, Tanja Hampel, Martina Stocker), were allowed to take data. Immediately after the collection period samples were frozen at -20 °C (Möstl and Palme, 2002).



**Figure 3.** Experimental design: Social conditions (focal, friend and non-friend) during the three phases (control, separation, reunion). Droppings from day 4 and 8 were not included in the analysis because the birds might have been stressed due to moving from one aviary to another.

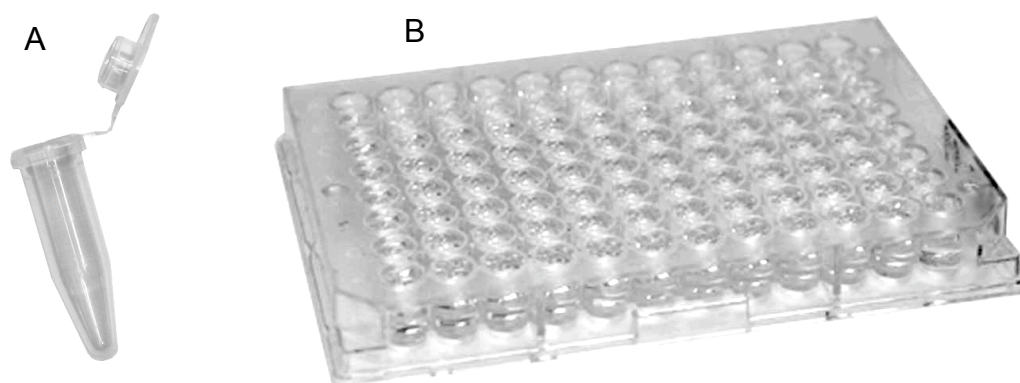


After each separation at least two weeks passed until the next one started to give the birds time to recover and re-stabilise relationships in the group that were potentially disturbed due to the separation.

Since hormone levels vary with age of the individual and season (Breuner, 2002; Stöwe et al., 2008), we tested the first group, of which all ravens hatched in 2010, between December 2011 and May 2012 and the second one, where most of the individuals hatched in 2011, between December 2012 and May 2013.

### **Extraction and analysis of immunoreactive corticosterone metabolites**

Droppings were frozen at - 20 °C until analysis. For the CM extraction 0.1 g of wet faeces and 1 ml 60 % methanol (0.6 ml 99.9 % methanol + 0.4 ml distilled water) were combined, shaken for 30 min and centrifuged for 15 min at 3000 rpm (Palme et al., 2013). If droppings weighed less than 0.1 g the amount of methanol was adjusted appropriately. The resulting eluate was diluted with assay buffer by a factor of 1:5 and analysed with an enzyme immunoassay (EIA) against 3 $\alpha$ ,11oxo-CM (using 11-oxoetiocholanolone as standard; the antibody was raised in rabbits against 5 $\beta$ -androstane-3 $\alpha$ -ol,11,17-dione,17-CMO: bovine serum albumin; detailed assay description see Möstl et al., 2002; Fig. 4.B). This assay was biologically validated for ravens (Stöwe et al., 2008). All samples were analysed in duplicates of which the resulting average forms the final value of each sample. The inter-assay coefficient of variance (CV) of the separations in 2012 and 2013 were 10.1 % and 7.5 %, respectively, while the intra-assay CV was 8 %.



**Figure 4.** A. Eppendorf micro tube (1.5 ml); B. Enzyme immunoassay plate.

## Statistical data analysis

To determine whether the amount of CMs in the birds' droppings is dependent on the condition (focal, friend, non-friend) or phase (control, separation, reunion) of the experiment or on social integration or sex of the individual Generalized Linear Mixed Models (GLMMs) were calculated, using IBM SPSS Statistics 20 (IBM Corp., 2011). This statistical method was chosen because it allows using clustered data (ravens in the same group) and repeated measures (multiple droppings of each raven) and is moreover, even able to deal with an unbalanced study design (number of droppings for each individual and times individuals were assigned friends or non-friends differed).

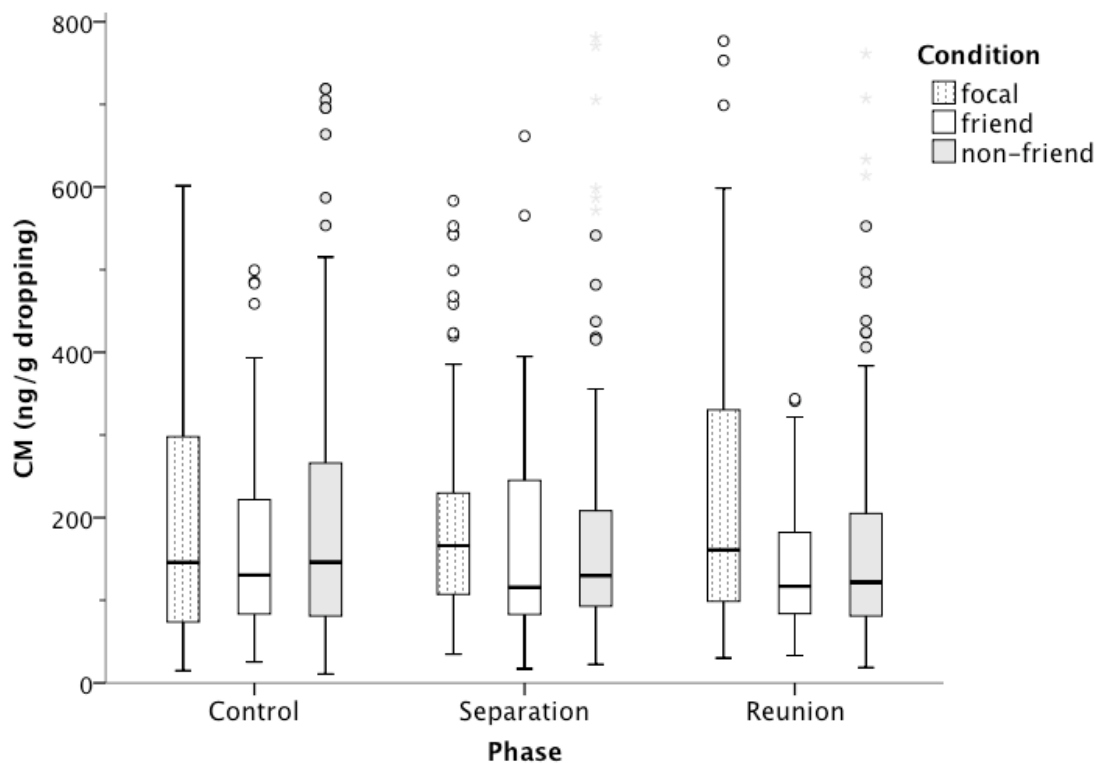
CM values above  $x + 2$  S.D of the whole data set were excluded from the statistical analysis ( $x = 250.69$ ; S.D. = 404.38;  $n = 18$ ) and further 17 samples were not included due to different reasons (see supplementary material: *Excluded data points*). The target variable "CM" was Ln transformed to reach normal distribution of the Pearson residuals obtained from the GLMMs. However, whether a distribution was normal or not was determined visually from Q-Q plots (see supplementary material: *Q-Q plot example*).

Instead of applying a backward step-wise selection procedure, only the global model, which consisted of all four predictor variables (condition, phase, social integration, sex) including their two-way interactions, was taken into account. Nevertheless, the data set was split into subsets in case of significant predictor interactions to allow the calculation of significance values within this subset (after Preininger et al., 2013). Significance levels were set to  $p < 0.05$ . In order to correct for possible influences of the respective separation experiment and the number of samples that were collected from different individuals on different days of the phases the nested term "Separation (Phase (Day of Phase (Individual (Sample of the Day))))" and to correct for differences between individuals "Individual" was included as random effect.

All models are presented in the supplementary material: *GLMMs (Generalized linear mixed models)*, page 26.

## RESULTS

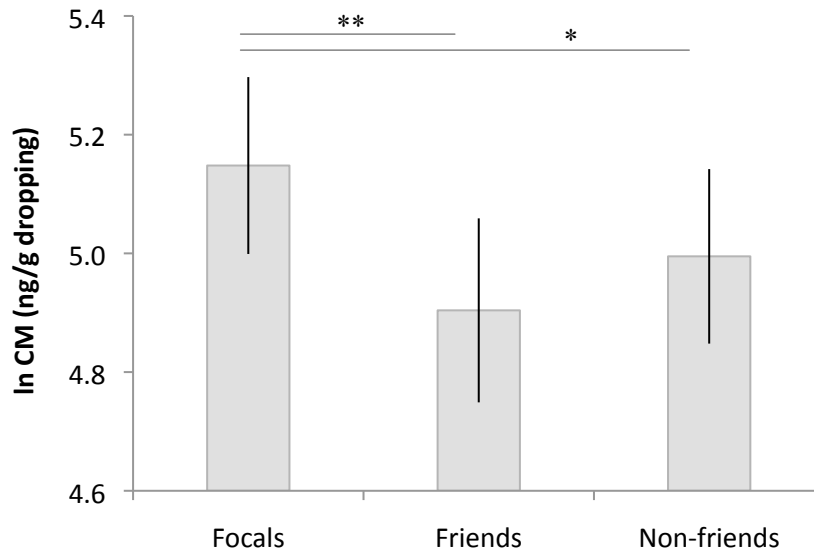
In the course of all 16 separation experiments 684 droppings were collected and their CM values included in the statistical data analysis. Despite huge individual variation, **visualisation of the data** showed that medians slightly differ between focal individuals and the birds that remain in the group throughout the whole experiment (friends and non-friends) during the separation and reunion phase (Fig. 5). To determine whether this signal is statistical significant, we calculated GLMMs.



**Figure 5.** Amounts of corticosterone metabolites (ng/g dropping) in focal individuals, friends and non-friends during the three experimental phases: control, separation and reunion. 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. Circles present outliers.

As the **GLMM**, including all four predictors (condition, phase, social integration, sex), revealed significant differences between the conditions, with focal individuals excreting more CMs than friends and non-friends, respectively (GLMM: pair-wise comparison: friends:  $\beta = -0.244$ ,  $SE = 0.083$ ;  $t = -2.956$ ,  $p = 0.003$ ; non-friends:  $\beta = -0.154$ ,  $SE = 0.070$ ;  $t = -2.179$ ,  $p = 0.030$ ; Fig. 6), and the model also revealed significant predictor interactions (GLMM: fixed factor: condition x social integration:  $F = 4.644$ ,  $P = 0.010$ ; phase x social integration:  $F = 3.418$ ,  $p = 0.033$ ) we calculated separate models for 1) focal individuals and 2) the birds that remain in the

group throughout the whole experiment – friends and non-friends. The predictor sex and its interactions with other factors were not included in these models as neither showed significant effects in this first model and reduction of predictors enhances the models' statistical power.



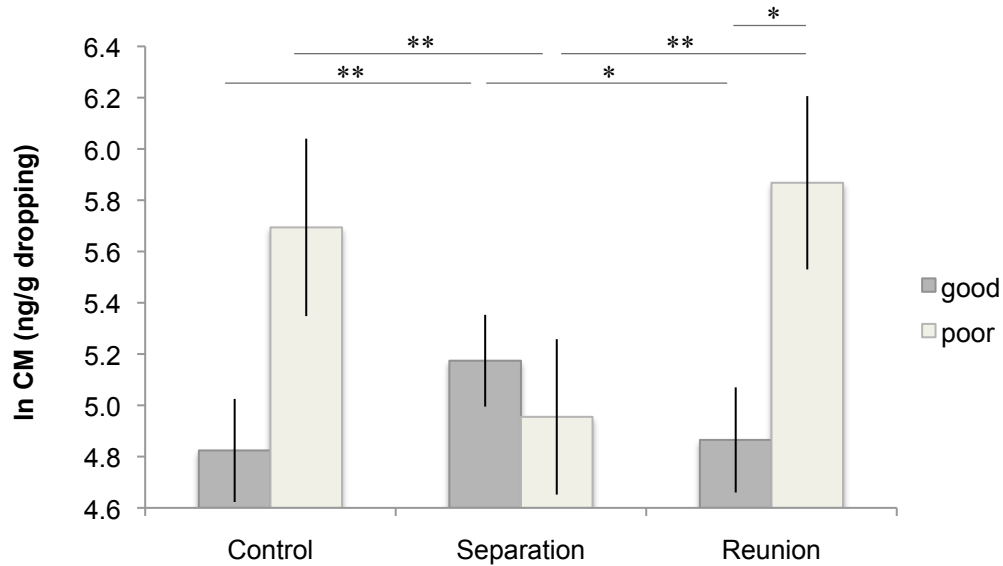
**Figure 6.** Estimated mean corticosterone metabolite values  $\pm$  SE (ln transformed, GLMM) of all three conditions: focal individuals, friends, non-friends (all phases together). Asterisks mark sign. between-condition differences as determined by pairwise comparisons of GLMM (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ).

### Focal individuals

The focal model was based on 212 samples of 16 individuals and incorporated the predictors phase and social integration. Since we found a significant interaction of these predictors (GLMM: fixed effects:  $F = 10.651$ ,  $p < 0.000$ ), we split the data into a) the two social integration classes and b) the experimental phases.

a) **Social integration** (see Material and Methods, Tab. 2): Well integrated focal subjects excreted more CMs during the separation as during the control and also reunion phase (GLMM: pair-wise comparison: control:  $\beta = -0.350$ ,  $SE = 0.131$ ;  $t = -2.672$ ,  $p = 0.009$ ; reunion:  $\beta = -0.310$ ,  $SE = 0.135$ ;  $t = -2.286$ ,  $p = 0.024$ ). In contrast, poorly integrated birds showed lower levels of CMs while they were separated, compared to higher levels before and after the separation (GLMM: pair-wise comparison: control:  $\beta = 0.739$ ,  $SE = 0.248$ ;  $t = 2.976$ ,  $p = 0.005$ ; reunion:  $\beta = 0.912$ ,  $SE = 0.240$ ;  $t = 3.806$ ,  $p = 0.001$ ). In both groups the CM levels during the control did not differ significantly from those of the reunion phase (Fig. 7).

b) **Experimental phase:** Even though the two social integration classes seemed to differ hugely from each other in the control and reunion phase, this effect was only significant in the latter, where poorly integrated focal individuals excreted much more CMs than well integrated ones (GLMM: pair-wise comparison:  $\beta = 0.728$ ,  $SE = 0.319$ ;  $t = 2.283$ ,  $p = 0.028$ ; Fig. 7).

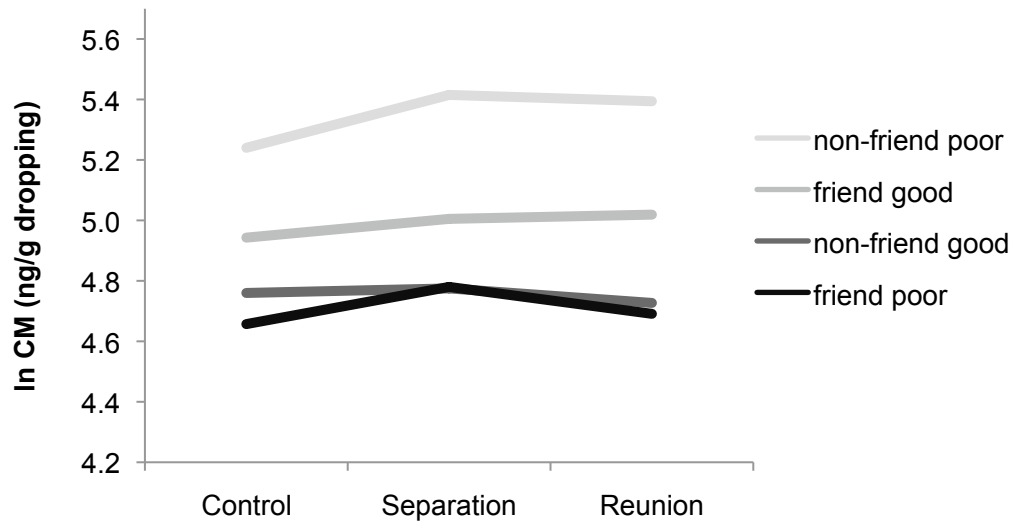


**Figure 7.** Estimated mean corticosterone metabolite values  $\pm$  SE (ln transformed, GLMM) of well and poorly integrated focal individuals during the three experimental phases: control, separation and reunion. Asterisks mark sign. differences as determined by pairwise comparisons of GLMMs (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ).

### Friends and non-friends in the group

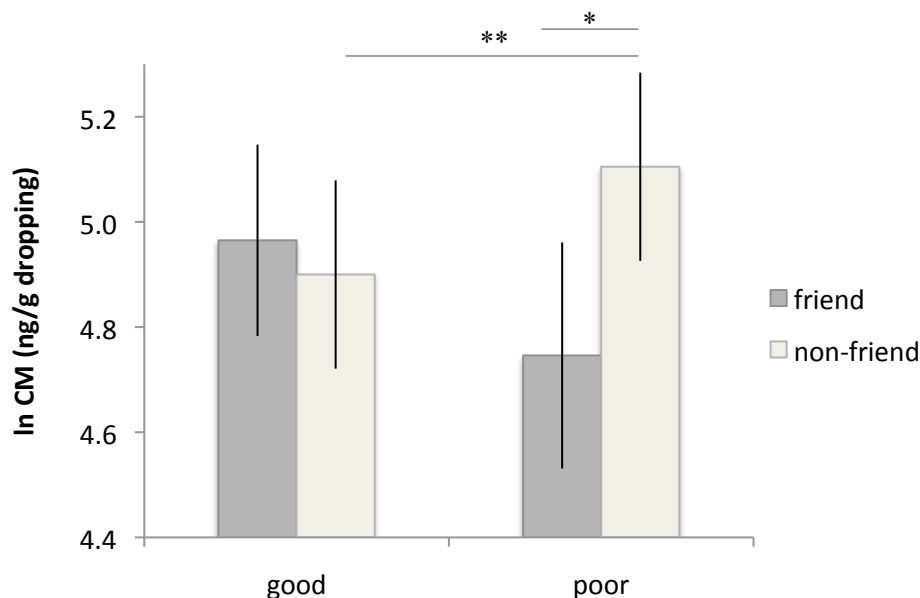
Since the model for individuals that remained in the group (based on 472 samples) revealed a significant interaction (GLMM: fixed effects: condition x social integration:  $F = 6.519$ ,  $p = 0.011$ ), the dataset was split according to a) the condition and b) the social integration.

a) **Condition:** Neither friends nor non-friends varied a lot in the excretion of CMs throughout the three experimental phases (Fig. 8). Poorly integrated non-friends, however, exhibited significantly higher CM levels than well integrated non-friends (GLMM: pair-wise comparison:  $\beta = -0.596$ ,  $SE = 0.178$ ;  $t = -3.347$ ,  $p = 0.001$ , Fig 9). There were no significant differences between friends of good and poor social integration.



**Figure 8.** Estimated mean corticosterone metabolite values  $\pm$  SE (ln transformed, GLMM) of well and poorly integrated friends and non-friends during the three experimental phases: control, separation and reunion.

b) **Social integration:** Non-friends of poor social integration also excreted more CMs than poorly integrated friends (GLMM: pair-wise comparison:  $\beta = -0.362$ , SE = 0.173;  $t = -2.098$ ,  $p = 0.038$ ; Fig. 9). In well integrated birds no significant differences in CMs levels could be found.



**Figure 9.** Estimated mean corticosterone metabolite values  $\pm$  SE (ln transformed, GLMM) of well and poorly integrated friends and non-friends (all phases together). Asterisks mark sign. differences as determined by pairwise comparisons of GLMMs (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ).

## DISCUSSION

The study demonstrates that ravens do show changes in CM levels due to being separated from their group as well as due to being reunited. Interestingly, the expected pattern of CM levels in the course of the experiment – an elevation of CMs following the separation and a decrease upon the reunion – could only be found in socially well integrated focal individuals. Poorly integrated ravens did not just exhibit a weaker stress response during separation, but even seemed to be more relaxed than during the control and reunion phase, excreting less CMs while being socially isolated and more when they are within the group (Fig. 7). Thus, as hypothesized, the amplitude of the stress-induced corticosterone response indeed seems to be dependant on the social integration of the bird. While well integrated birds were less stressed when in the group and more when isolated, poorly integrated ones showed the opposite pattern. The social integration classes even differed significantly in the reunion phase.

These results indicate that the adrenocortical activity of well integrated subjects is more likely to be attenuated when they are in the group, because they have more socio-positive interaction partners with which they spend more time, while ravens that are poorly integrated are more stressed because they might lack social support and are therefore also prone to experience conflicts (see Fraser and Bugnyar, 2012). In separation, however, these individuals do not have to compete for food, nor can they fall victim to agonistic interactions. The reunion seems to be even more stressful for them as they suddenly might have to cope with competition and socio-negative behaviours again. Being alone, thus, appears to be stressful for well integrated individuals, but more tolerable, or maybe even comfortable for ravens of poor social integration.

Interestingly, ravens remaining in the group did not vary a lot in their stress levels throughout the three experimental phases (Fig. 8), even though changes of group size and structure during the separation phase could have resulted in modifications of hormonal states. The structural change could have caused instability of the group, and not just non-friends, but also other birds in the group could have used the window of opportunity and taken advantage of the friend missing one of his or her allies. They could either have taken action against this friend and maybe even dominated it, or formed new or strengthened already existing bonds with this individual. Both possibilities could have result in changing CM levels.

Concerning friends, our findings suggest that the separation of an affiliated individual is, in contrast to our expectations, barely affecting its stress level. It is likely, therefore, that due to the presence of other individuals the stress that friends might experience while one affiliated bird is separated, is alleviated. This would indicate that social integration has a stronger impact on the friend than its affiliate being separated. However, it is important to note, that the focal individual was only isolated visually, but not acoustically. Given this setting, birds remaining in the group could probably have communicated with the separated one and, hence, have known that this individual is still in proximity (Boeckle and Bugnyar, 2012).

Unlike our findings, a study on Australian zebra finches (*Taeniopygia guttata*) demonstrated, that the presence of other familiar conspecifics did not reduce the stress reaction that resulted from the separation from an affiliate individual (Ramage-Healey et al., 2003). However, this concerned pair mates of a monogamous species, whereas in our study birds were not pair mated yet, but rather formed bonds that resemble friendships.

Throughout all phases poorly integrated non-friends appeared to be more stressed than well integrated non-friends and also poorly integrated friends (Fig. 8, 9). This effect of constantly elevated CMs in non-friends of poor social integration might have resulted from the selection of subjects for this condition. Looking at the social integration scores (see supplementary material) it appears that within poorly integrated individuals some did not have any social contacts, while others had at least a few. Birds that are at the bottom of this range, and maybe therefore very high on the stress axis, were more likely to be assigned to the category non-friends because we wanted to ensure that they are not affiliated with the focal individual. In comparison, poorly integrated friends derived from the upper range of this social integration class, indicating that they have at least one, but maximum two affiliates, which could probably lead to a higher acceptance in the group and, hence, lower stress levels. This could presumably explain why friend and non-friends of poor social integration vary a lot from each other, whereas well integrated do not.

Corvids are known to be very flexible in their behaviour. For instance, they are able to adjust their behaviour to the social context and to the identity of familiar conspecifics (e.g., ravens, *Corvus corax*, Bugnyar and Heinrich, 2006) and they are able to use tools and even modify them according to their needs (e.g., New Caledonian crows, *Corvus moneduloides*, Hunt, 1996; Weir and Kacelnik, 2006). In recent years it



was hypothesized that enhanced cognition, which includes behavioural flexibility, might represent an alternative stress coping strategy to neuroendocrine stress responses. Lendvai and colleagues (2013), for example, conducted a metaanalysis to examine the association of relative brain size and corticosterone levels in 119 bird species, including one corvid, the Florida scrub jay (*Aphelocoma coerulescens*). Their findings suggest that only within long-lived bird species, enlarged brains may act as a compensatory mechanism, allowing the animals to avoid or anticipate stressors and reduce corticosterone levels (Lendvai, 2013). Ravens were not taken into account in this analysis, however, as a long-lived and large-brained species these findings might also apply to them. Accordingly, our finding that friends do not show elevated CM levels due to the separation of their affiliate could result from the ability to cope with this situation in terms of cognition. As in the wild raven non-breeder groups exhibit high degrees of fission-fusion dynamics, which also entail the separation of affiliated birds, this stress coping mechanism is highly beneficial because it evades health burdens caused by chronically elevated corticosteroid levels (Sapolsky, 2000). However, this does not explain the different neuroendocrine responses of the separated focal individuals.

Despite the rather small sample size of only sixteen ravens, our findings seem to be similar to patterns found in other bird species. CM levels in greylag geese (*Anser anser*), for instance, are during mating season high in single ganders, which are socially poorly integrated, and relatively low in paired males (Kotrschal et al., 1998). Moreover, adult as well as subadult female geese profit from passive social support when they are part of a secondary family and therefore exhibit reduced adrenocortical responses during socially stressful situations (Scheiber et al., 2009). Hence, like in our study the birds' social position appears to play a very important role.

Altogether, our study showed a large variation in corticosterone levels between individuals, indicating that personality (also called behavioural syndrome) might have had an impact on our results. In a review on stress and avian personality, Cockrem (2007) reported a link between corticosterone and behaviour, both being dependent on each bird's personality. Individuals with proactive personalities exhibit relatively active, fast and bold behavioural reactions and low adrenocortical stress responses, whereas birds of reactive personalities show relatively passive, slow and shy behavioural and strong adrenocortical responses (e.g. in great tits, *Parus major*, Stöwe et al., 2010; Cockrem, 2007). In the course of our study we did collect behavioural data during all

three experimental phases and also conducted personality tests. However, this data was not analysed yet. Hence, whether and how these factors are connected with the birds' neuroendocrine responses remains to be elucidated in a further step.

Overall, we conclude that most individuals seem to be stressed when they are isolated from their group, however, for individuals that lack social bonds, or only have very few, group living appears to be even more stressful than being alone. Birds that are separated from a certain affiliate, but still within a group, might either exhibit cognitive and behavioural stress coping mechanisms, rather than neuroendocrine stress responses or, according to the social buffering model, their stress could be alleviated by other group members. Unexpectedly, however, it seems that social integration, measured by time individuals spent in close proximity, is even more important than having one good friend. It is important to keep in mind, however, that this study was conducted on captive ravens. Hence, firstly, future research including separations of whole sub-groups is needed to better understand the endocrine influences of fission-fusion dynamics on the remaining group and secondly, further work on wild raven populations in a more natural setting is required to test whether our findings also apply to them.

## **ACKNOWLEDGEMENTS**

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## APPENDIX

### Supplementary material

#### *Friends and non-friends*

Sep.	Date	Focal	Friend		Non-friend	
			Male	Female	Male	Female
1	16. - 20. Dec. 2011	<b>Heidi</b>	Anton		Jakob	Lena
2	05. - 09. Jan. 2012	<b>Lena</b>		Sophie	Anton	Elen, Klara
3	26. - 30. Jan. 2012	<b>Anton</b>		Heidi	Jakob	Lena
4	16. - 20. Feb. 2012	<b>Elen</b>	Jakob	Klara	Jonas	Sophie
5	05. - 09. March 2012	<b>Jonas</b>	Anton	Klara	Jakob	Heidi
6	23. - 27. March 2012	<b>Klara</b>	Jakob	Elen	Anton	Sophie, Lena
7	10. - 14. April 2012	<b>Jakob</b>		Elen, Klara	Anton	Heidi
8	04. - 08. May 2012	<b>Sophie</b>		Lena	Jakob	Klara
9	18. - 22. Dec. 2012	<b>Thor</b>		Astrid	Orm	Lellan
10	08. - 12. Jan. 2013	<b>Orm</b>	Ray		Thor	Astrid
11	26. - 30. Jan. 2013	<b>Lellan</b>	Matte		Thor	Sophie
12	26. Feb. - 02. Mar. 2013	<b>Ray</b>	Orm	Lellan	Thor	Astrid
13	19. - 23. March 2013	<b>Matte</b>		Lellan	Thor	Sophie
14	09. - 13. April 2013	<b>Astrid</b>	Thor		Ray	Lellan
15	26. - 30. April 2013	<b>Skadi</b>	Thor		Orm	Astrid
16	12. - 16. May 2013	<b>Joey</b>			Matte	Skadi, Sophie

#### *Excluded data points*

Number of values	Reason
18	CM value over $x + 2 \text{ S.D} = 1059.45 \text{ ng/g}$ dropping.
3	Methodological issues during the collection or extraction
2	2 values of other samples from the same individual on the same day lie within a range that differs hugely from the excluded value.
5	Outliers due to statistical test; 2 of these values derive from controls and lie above $1000 \text{ ng/g}$ dropping, while other values from this bird in the same phase are below $356 \text{ ng/g}$ . Thus, an unexpected stressor could have affected the bird on this day.
2	Outliers due to statistical test

*GLMMs (Generalized linear mixed models)*

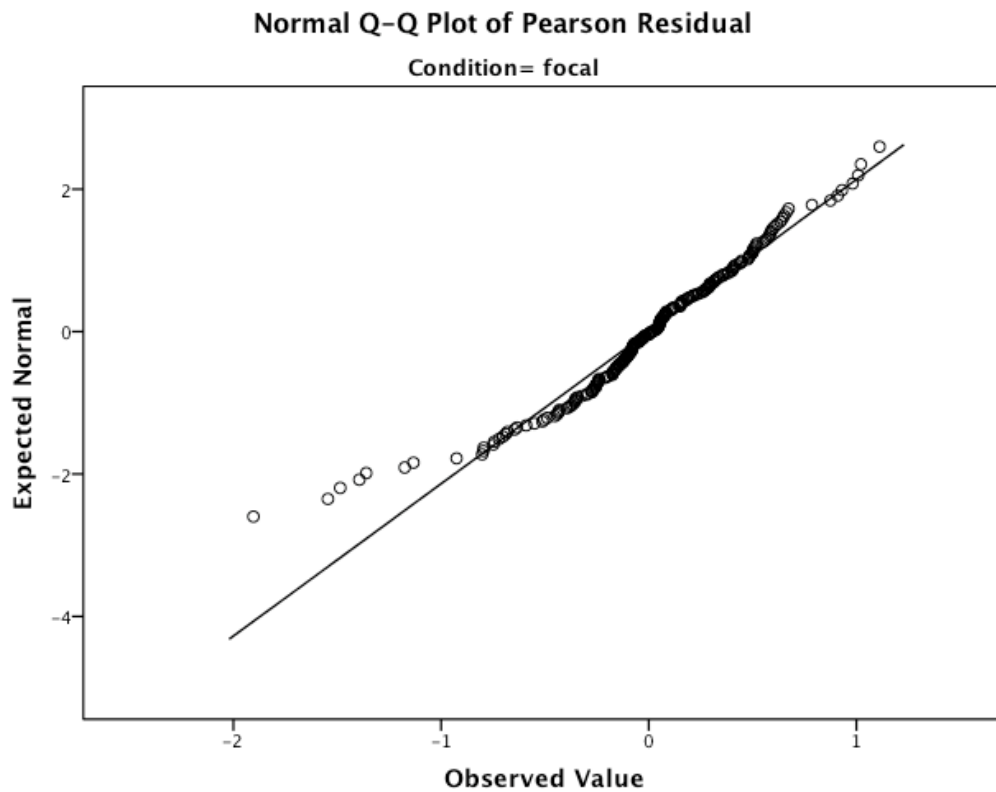
List of all models, including number of individuals (n) and samples, and the fixed factors, also called predictors, with their significance values.

<b>Model</b>	<b>n</b>	<b>Samples</b>	<b>Fixed factors (Predictors)</b>	<b>Sig.</b>
<b>Global</b>	16	684	<b>Condition</b>	<b>0.009</b>
			Social integration	0.277
			Sex	0.879
			Phase	0.201
			<b>Condition:Social integration</b>	<b>0.010</b>
			Condition:Sex	0.722
			Condition:Phase	0.774
			Social integration:Sex	0.377
			<b>Social integration:Phase</b>	<b>0.033</b>
			Sex:Phase	0.402
<b>1. Focals</b>	16	212	Social integration	0.200
			Phase	0.574
			<b>Social integration:Phase</b>	<b>0.000</b>
<b>1 a. Soc. integration good</b>	10	133	<b>Phase</b>	<b>0.009</b>
<b>1 a. Soc. integration poor</b>	6	39	<b>Phase</b>	<b>0.001</b>
<b>1 b. Phase control</b>	16	44	Social integration	0.335
<b>1 b. Phase separation</b>	16	124	Social integration	0.408
<b>1 b. Phase reunion</b>	16	44	<b>Social integration</b>	<b>0.028</b>
<b>2. In group (friends, non-fr.)</b>	15	472	Condition	0.077
			<b>Social integration</b>	<b>0.037</b>
			Phase	0.397
			<b>Condition:Social integration</b>	<b>0.011</b>
			Condition:Phase	0.908
			Social integration:Phase	0.681
<b>2 a. Condition friends</b>	13	173	Social integration	0.467
			Phase	0.751
			Social integration:Phase	0.923
<b>2 a. Condition non-friends</b>	15	299	<b>Social integration</b>	<b>0.001</b>
			Phase	0.687
			Social integration:Phase	0.652
<b>2 b. Soc. integration good</b>	10	362	Condition	0.335
			Phase	0.839
			Condition:Phase	0.824
<b>2 b. Soc. integration poor</b>	6	110	<b>Condition</b>	<b>0.038</b>
			Phase	0.577
			Condition:Phase	0.972



### *Q-Q plot example*

Most of the data points are aligned on the line, indicating that the data are normally distributed. This method of determining the distribution is very useful when other tests do not show normality due to outliers.



### **Picture and graph sources**

Fig. 1. Raven	<a href="http://www.firstnations.de/fisheries/kwakwakawakw-kwakiutl.html">http://www.firstnations.de/fisheries/kwakwakawakw-kwakiutl.html</a> ; 9.11.2013; © Guy L. Monte, BC, Canada.
Fig. 2. Haidhof aviaries	created by author
Fig. 3. Experimental design	created by author
Fig. 4. A. Micro tube	created by author
Fig. 4. B. ELISA plate	created by author
Fig. 5.-9. Results	created by author



## Deutsche Zusammenfassung

Raben (*Corvus corax*) weisen ein komplexes Sozialsystem auf, das im frühen Lebensstadium, wenn sie nicht-brütende Junggesellengruppen bilden, einen hohen Grad an fission-fusion Dynamik beinhaltet. Innerhalb dieser Gruppen haben Individuen sozialen Beziehungen von unterschiedlicher Qualität und Wertigkeit - "Freunde" bieten Vorteile, wie soziale Unterstützung während oder nach Konflikten mit anderen, was zu reduzierten Corticosteronlevels und damit zur Stresslinderung führen kann. Bisher ist nur wenig über die neuroendokrine Aktivität im Zusammenhang mit fission-fusion Dynamiken, bei denen langanhaltende Trennungen von affilierten Vögeln auftreten können, bekannt. Ziel der vorliegenden Studie ist diese endogenen Effekte aufzuklären, indem sechzehn Raben erst einzeln für vier Tage von ihrer Gruppe getrennt und anschließend wieder in die Gruppe eingeliebert werden. Um die resultierenden Stressreaktionen zu untersuchen, wurden immunreaktive Corticosteron-Metaboliten (CM) aus dem Kot der Raben mit Hilfe eines Enzym-Immunoassay gegen  $3\alpha$ ,  $11\text{-oxo}$  - CMs gemessen, der zuvor für Raben validiert wurde. Unsere Ergebnisse deuten darauf hin, dass die meisten Individuen, gestresst sind, wenn sie von ihrer Gruppe getrennt werden, obwohl dies nur auf sozial gut integriert Vögel zuzutreffen scheint. Für Individuen, die keine oder nur sehr wenige soziale Bindungen aufweisen, dürfte das Gruppenleben stressiger sein als die Isolation. Die Stressantwort der Vögel scheint daher von ihrer individuellen sozialen Integration abhängig zu sein. Im Gegensatz zu unseren Erwartungen, fanden wir, dass jene Raben die von einem Affilierten getrennt werden, sich jedoch immer noch innerhalb der Gruppe befinden, keine stärkeren Stressreaktionen zeigen als Vögel, die keine Bindung zu dem isolierten Individuum haben. Erstere könnten entweder Stressbewältigungsmechanismen anwenden, die auf Kognition und Verhalten basieren, anstatt neuroendokrinen Stressreaktionen zu zeigen, oder Stress könnte durch andere Gruppenmitglieder gelindert werden.

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## EDUCATION

- 1997-2005 Bundesgymnasium auf der Schmelz (BRG 15), Vienna**  
Final examination (Matura): June 2005
- 2005-2007 College for Fashion and Clothing Design “Die Herbststraße”, Vienna**  
Focus: Project management and marketing  
Thesis: “Corporate identity of a newly established company”
- 2007-2010 Bachelor program Biology, University of Vienna**  
Focus: Zoology  
Thesis: “Alpine bird monitoring – A comparison of point counts method and line assessment”
- 2010-2013 Master program Behaviour, Neurobiology and Cognition, University of Vienna**  
Thesis: „Hormonal responses to temporary changes in group size and composition in ravens (*Corvus corax*)“  
Supervisor: Prof. Thomas Bugnyar

## PRACTICAL COURSES AND PROJECTS AT UNIVERSITY

### Master program

- **PhD course on “Non-invasive Monitoring of Steroid Hormones”,** University of Veterinary Medicine, Vienna, 17<sup>th</sup> – 21<sup>st</sup> September 2012
- **Methods of fieldwork** – Focus: small mammals (Europ. hamster (*Cricetus cricetus*), Europ. ground squirrel (*Spermophilus citellus*)) and birds; use of Tomahawk live traps, mist nets and telemetry; taking measurements; summer term 2012
- **Konrad Lorenz Research Station, Grünau, Austria;** Effect of the hand rearer’s presence on the behaviour of juvenile greylag geese (*Anser anser*), July 2011
- **Cross-modal transfer of predator recognition:** Do pigeons (*Columba livia*) have an abstract concept of the buzzard? summer term 2011
- **Effect of different nutritional components on the HPA axis,** social and agonistic behaviour in guinea pigs (*Cavia aperea f. porcellus*), winter term 2010

### Bachelor program

- **Field research on a common hamster population** (*Cricetus cricetus*) in Vienna; volunteered for one week in summer 2010
- **Alpine bird monitoring** (Bachelor thesis), field trip to the Zillertaler Alps, Austria, summer term 2010
- **Bioacoustic analysis** of the vocalisations of two merging elephant groups (*Loxodonta africana*) at the zoo Schönbrunn, winter term 2009
- **Zoo animal observation:** Behavioural repertoire of lions (*Panthera leo*) and their distance to conspecifics, summer term 2009

## STAYS ABROAD FOR SCIENTIFIC PURPOSES

**Tropical Biology Association: field course in tropical ecology and conservation,**  
Kibale Forest, Uganda, 30<sup>th</sup> June - 30<sup>th</sup> July 2012,  
Funding: **British Ecological Society Scholarship**

**Internship at “Living Links to Human Evolution Research Centre” at Edinburgh Zoo**  
Edinburgh, Scotland, 1<sup>st</sup> August - 5<sup>th</sup> October 2013,  
Topic: Emotional contagion in capuchin monkeys (*Sapajus apella*)  
Funding: **KWA** (Kurzfristiges Auslandsstipendium)

## WORK EXPERIENCE

**Department of Behavioural Biology**, University of Vienna, March - June 2013  
Working in a hormone lab

**Department of Cognitive Biology**, University of Vienna, November 2012 - April 2013  
Recording and coding videos of ravens' behaviour (focal protocols)

**Department of Neurobiology**, University of Vienna, October 2012 - February 2013  
Tutor at a practical course in physiology

## ADDITIONAL QUALIFICATIONS

**Computing Skills:** ECDL qualifications in MS Word, PowerPoint, Excel and Access, and knowledge of the following software: Solomon Coder, The Observer, Sigma Plot, SPSS, STx (Acoustics Research Institute, Vienna), Adobe: Illustrator, Photoshop, Premiere Pro

**Languages:** Fluent German (mother tongue) and English, basic Spanish and French

**Monitoring of parasite infestation in birds** (droppings)

**Driving licence**

## PUBLICATIONS

### Poster presentation:

Stocker M, Munteanu A, Stöwe M, Bugnyar T: See you soon? Adrenocortical responses to experimentally induced fission-fusion dynamics in ravens (*Corvus corax*).

Presented at Behaviour 2013: Joint meeting of the 33<sup>rd</sup> International Ethological Conference (IEC) & the Association for the Study of Animal Behaviour (ASAB);  
Newcastle Gateshead, UK, 4-8 August 2013

### Papers:

Stocker M, Munteanu A, Stöwe M, Bugnyar T: Hormonal responses to temporary changes in group size and composition in ravens (*Corvus corax*). (in prep)

Munteanu A, Stocker M, Stöwe M, Bugnyar T: Adaptive responses to social distress in ravens (*Corvus corax*). (in prep)