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Loner or socializer? Ravens' adrenocortical response to individual separation depends on social integration

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ABSTRACT

Non-breeding common ravens (*Corvus corax*) live in complex social groups with a high degree of fission–fusion dynamics. They form valuable relationships and alliances with some conspecifics, while taking coordinated action against others. In ravens, affiliates reconcile their conflicts, console each other after conflicts with a third party, and provide each other with social support — all behaviors that presumably reduce corticosterone levels and alleviate stress. However, how well an individual is socially integrated in a (sub)group might vary substantially. This raises the question whether the social integration of a raven affects its stress responses to fission–fusion dynamics. The present study aims to investigate this effect experimentally by separating single ravens ($n = 16$) individually from their group for four days and subsequently reintroducing them. To determine stress response patterns in the separated individuals we measured the amounts of immunoreactive corticosterone metabolites (CM) in droppings. We compared two enzyme immunoassays, which we validated by conducting an ACTH challenge, and finally decided to apply an 11-oxoetiocholanolone enzyme immunoassay. Additionally, we determined levels of social integration using focal observations. Our findings suggest that a strong social integration is related to low CM levels when the individuals are within the group and high levels during separations, implying that separation leads to stress in these birds. In contrast, poorly socially integrated ravens seem to exhibit the opposite pattern, indicating that to them group living is more stressful than being temporarily separated. We, therefore, conclude that the birds' adrenocortical activity is modulated by their social integration.

Introduction

Social life does not only bring benefits, but also entails many challenges. Interactions between individuals can, therefore, depending on the circumstances, either alleviate or cause stress (DeVries et al., 2003). One of the mechanisms serving to diminish the effects of stressors is social support, which leads to less intense (physiological) responses to stressful situations ("buffering model" reviewed by Cohen and Wills, 1985). It is important to note, however, that social support is not directed towards random individuals, but rather specifically towards close affiliates or bonding partners. Hence, the quality of the social relationship (see Cords and Aureli, 2000; Fraser et al., 2008) determines the amount of social support given and received. Close social bonds are therefore very valuable and accordingly, one could assume that being separated from a social ally acts primarily as a psychological stressor and results in increased glucocorticoid levels. Indeed, Remage-Healey et al. (2003) showed that in zebra finches (*Taeniopygia guttata*) corticosterone, the major glucocorticoid in birds (Holmes and Phillips, 1976), is elevated due to pair mate separation (48 h) and returns to baseline levels upon reunion. The study also discovered that the presence of other conspecifics during separation from the bonded partner did not significantly attenuate the hypothalamic-pituitary-adrenal axis activation.

So far, however, little is known about animals' adrenocortical activity in groups of affiliated, but not pair-bonded individuals with a high degree of fission–fusion dynamics, where long-lasting separations of affiliates may occur frequently. Unlike previous uses of the term "fission–fusion" in the context of a specific 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 over time groups can change in size and composition to varying extents (e.g. in Guiana dolphins, *Sotalia*

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guianensis, Lunardi and Ferreira, 2014; spider monkey, *Ateles geoffroyi*, Ramos-Fernández and Morales, 2014).

Non-breeding common ravens (*Corvus corax*) are highly gregarious and live in groups that exhibit high degrees of fission–fusion dynamics (Braun et al., 2012). Individuals usually remain in these groups until they gain sexual maturity and form long-term monogamous pairs that establish large breeding territories (size depends on the availability of food and breeding sites, Drack and Kotrschal, 1995), which they defend all year round (Heinrich, 1989). Non-breeding ravens often form small subgroups of individuals for socializing during the day (Braun et al., 2012), and join bigger non-breeder groups to be able to compete for food with territorial breeding pairs (Marzluff and Heinrich, 1991) or potentially dangerous predators, like gray wolves (*Canis lupus*, Stahler et al., 2002).

Additionally, raven non-breeder groups are structured by close social bonds, characterized by affiliative interactions and selective cooperation (Braun and Bugnyar, 2012; Heinrich, 2011). 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 with each other (Fraser and Bugnyar, 2011). Recent findings have further revealed that ravens are able to remember the relationship valence even for years (Boeckle and Bugnyar, 2012) and understand third-party relations without having to interact physically with the respective conspecifics (Massen et al., 2014a). All these findings suggest that in ravens social bonds are pivotal in respect to solving problems in daily social life. Whether or not individuals can rely on a bonding partner affects their status in the group and ability to secure resources (Braun and Bugnyar, 2012). It might also affect their response to challenges like being separated from and reunited with conspecifics, as it is typical in fission–fusion dynamics.

With the present study we aimed at investigating changes of adrenocortical activity in ravens during experimentally induced fission–fusion situations. By individually separating group members for four days and subsequently reintroducing them into their group and simultaneously measuring immunoreactive corticosterone metabolites (CM) in the birds' droppings, we intend to investigate changes in the ravens' stress levels throughout the experiment. Considering the positive effect of social bonds, we predicted that corticosterone secreted in ravens is increasing while they are individually separated and declining once they are released back into the group, and we hypothesize that the stress-induced changes in the adrenocortical activity depend on how well an individual is socially integrated in the group.

Material and methods

Subjects and housing

The study was conducted on 16 ravens (7 males, 9 females) at the Haidlhof Research Station in Bad Vöslau, Austria. With the exception of one female who hatched in the wild, all individuals were born and raised in captivity. In the study period the birds were 1½ to 2 years old, hence still sub-adult and sexually immature. Individuals were grouped into two mixed-sex non-breeder groups of 8 birds each, ensuring a species-specific social situation that resembles natural conditions at that early stage of a raven's life. To facilitate individual identification all birds were marked with colored leg-rings.

The ravens were housed in an aviary complex, consisting of three main units (approx. 230 m^2 each), which were connected by lattice fence runways. While the birds had permanent access to one main unit, all the others were at least temporary accessible, hence, the ravens were acquainted with all areas. Like all units, the ravens' main aviary consisted of freely accessible indoor and outdoor compartments and was furnished with branches, trees, and shallow water basins. The ground was covered in equal manner with wood chips, stones and sand, offering plenty of opportunities to cache food and other items, which ravens do frequently. Their diet consisted of meat, chicken eggs, vegetables, fruits and yoghurt and was provided on a daily basis, while water was available ad libitum.

Social integration

We calculated a social integration score for each bird using data from the behavioral parameter "*contact sit*" (birds sitting within one body length to each other), which proved to be an appropriate indicator for close socio-positive relationships in a previous study (Schwab et al., 2008). For each bird the data was extracted from 5-minute focal observations of 12 randomly selected days (except for experiment days) distributed over the study period (group 1: Dec. 2011–May 2012; group 2: Dec. 2012–May 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 (Borgatti et al., 2002). Weighted degree values consider the number of interaction partners of an individual as well as the number of interactions with these partners. To get comparable individual scores from both groups, weighted degree values were transformed into relative values (in %) measured against the individual with the highest value of the respective group, which then represented an individual's relative social integration in its respective group (for details see Supplementary material Tab. S1).

Experimental procedure

Each tested individual (hereafter "focal" individual) was subjected to an 11-day experiment, which started with a 3-day control phase during which the focal individual was housed in its everyday social group. On day 4 the focal raven was separated individually from his or her conspecifics for four days. On this day, around 9:30 am, the respective focal individual was isolated in a side compartment of the main aviary and caught with a net within only a few minutes. Afterwards the raven was transferred to a familiar compartment (80 m^2) of another aviary unit that was situated approximately 20 m away from the group's aviary. Hence, the bird was isolated visually, but not acoustically. During the separation phase the focal raven was presented with the same dietary conditions as the group to minimize environmental influences other than social aspects. On day 8, after the separation phase, the bird was allowed to move back to the group through a runway to avoid any further handling stress. This reunion event was followed by a 3-day reunion phase (Fig. 1).

Droppings of each focal individual were collected each day during the entire experiment with the exception of day 4 when the focal bird was separated and day 8 when it was reunited, because the birds might have been stressed due to moving from one aviary to another (Fig. 1). This allowed us to determine not only the changes in adrenocortical activity in response to the separation itself, but also the metabolite level prior to separation and the adrenocortical response to the reunion (Fig. 1). 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 were acting on the animals.

To be sure about the origin of a dropping the focal individual was visually tracked until it produced a sample, which was then immediately collected. The collection happened between 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). Immediately after the collection period samples were frozen at −20 °C (Möstl and Palme, 2002).

Between experiments there was a minimum of a two-week period in order to give the birds time to re-stabilize 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 all but two individuals hatched in 2011, between December 2012 and May 2013. All procedures were approved by the Austrian