

# Effects of experimental increase of corticosterone levels on begging behavior, immunity and parental provisioning rate in house sparrows

Claire Loiseau <sup>a,b,\*</sup>, Gabriele Sorci <sup>a,1</sup>, Stéphanie Dano <sup>b</sup>, Olivier Chastel <sup>b</sup>

<sup>a</sup> *Laboratoire de Parasitologie Evolutive, CNRS UMR 7103, Université Pierre et Marie Curie, Bât. A, 7ème étage, 7, quai St Bernard, Case 237, F-75252 Paris Cedex 05, France*

<sup>b</sup> *Centre d'Etudes Biologique de Chizé, CNRS UPR 1934, F-79360 Beauvoir-sur-Niort, France*

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## Abstract

Begging is a complex display that is supposed to honestly indicate the need for food of nestlings, and, usually, parents use this information to adjust their investment in food provisioning. However, the mechanisms that ensure the honesty of begging as an indicator of need are still poorly known. It has been shown that levels of corticosterone (Cort), the hormone released during the stress response, raises during food shortage and is associated with increased begging rate. However, Cort also entails costs and these costs might prevent nestlings cheating. We tested this hypothesis in nestlings of the house sparrow. We experimentally increased levels of circulating Cort and investigated (1) the behavioral responses of nestlings, (2) the parental allocation of food and (3) the consequences on nestling growth and immune response. We found that Cort significantly increased begging rate but did not affect posture and position in the nest. Surprisingly, when begging effort was controlled statistically, control nestlings received more food from parents than Cort-treated nestlings. We also found that nestlings injected with Cort showed a weaker immune response and had lower body mass than controls. We suggest that Cort might have affected multiple aspects of nestling signaling, such as mouth color, and that parents use these multiple signals to adjust their feeding effort.

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## 1. Introduction

In altricial birds, nestlings are entirely dependent on their parents to feed them from hatching to fledging, and the arrival of an adult with food at the nest provokes a frenzy of nestling begging activity (Kilner, 2002). Nestling begging is a conspicuous signal that is supposed to serve

the parents to assess the need of the offspring (Godfray, 1991, 1995; Johnstone, 1999). Accordingly, parents are expected to adjust their provisioning rate to the intensity of begging behavior (Kilner, 1995; Kölliker et al., 1998; Leonard and Horn, 2001). This idea is based on the implicit assumption that parents can reliably use the information on the offspring need because begging is costly and honestly reflects nestling need (Godfray and Johnstone, 2000; Johnstone and Godfray, 2002). Costs of begging have been investigated in several studies. An increase in the intensity of begging has been reported to enhance the risk of predation (Leech and Leonard, 1997; Briskie et al., 1999), to slow down growth (Rodríguez-Gironés et al., 2001a; Kilner, 2001) or to increase energy expenditure (Harper, 1986; Weathers et al., 1997; Verhulst and Wiersma, 1997;

\* Corresponding author. Address: Laboratoire de Parasitologie Evolutive, CNRS UMR 7103, Université Pierre et Marie Curie, Bât. A, 7ème étage, 7, quai St Bernard, Case 237, F-75252 Paris Cedex 05, France. Fax: +33 1 44273516.

E-mail address: [cloiseau@snv.jussieu.fr](mailto:cloiseau@snv.jussieu.fr) (C. Loiseau).

<sup>1</sup> Present address: BioGéoSciences, Université de Bourgogne, CNRS UMR 5561, 6 Boulevard Gabriel, 21000 Dijon, France.

Rodríguez-Gironés et al., 2001a, but see Bachman and Chappell, 1998). Yet, little is known on the physiological mechanisms underlying the costs of begging.

In vertebrates, fasting is associated with a series of physiological responses, most of which are mediated by the stress response hormone, corticosterone (Cort). Fasting increases the production of Cort, which in turn, stimulates foraging and locomotor activities (Wingfield et al., 1990; Breuner et al., 1998), increased food intake (Astheimer et al., 1992; Wingfield and Silverin, 1986; Koch et al., 2002), enhanced plasma glucose levels via gluconeogenesis (Norris, 1997; Remage-Healey and Romero, 2001), and decreased triglyceride concentration in the blood (Remage-Healey and Romero, 2001). All of these responses allow the organism to cope with the period of food restriction. However, it should be noted that the effect of exogenous corticosterone may be dependent to a degree upon the physiological state of the individual as there were no effects in a number of species, including dark-eyed juncos (*Junco hyemalis*), mountain chickadees (*Parus gambelii*), white-crowned sparrows (*Zonotrichia leucophrys*) and domestic fowl (*Gallus domesticus*) (for review see Landys et al., 2006).

Interestingly, Cort has also been reported to affect begging behavior in chicks of the black-legged kittiwake (*Rissa tridactyla*), in which dietary restriction caused a chronic elevation of Cort (Kitaysky et al., 2001a), and an experimental elevation of baseline Cort increased begging (Kitaysky et al., 2001b). As expected, parents adjusted their food provisioning to the level of begging and Cort-treated chicks received more food (Kitaysky et al., 2001b).

Although the stress response (production of Cort in response to an environmental stressor) is probably adaptive, since it allows the organism to mobilize needed energy reserves (Wingfield, 2005), chronic stress might entail severe costs. These costs range from the alteration of cognitive functions, such as learning or memory (De Kloet et al., 1999; Sapolsky et al., 2000; Kitaysky et al., 2003) to immunosuppression (Apanius, 1998).

In the light of these previous results, one might suggest that Cort is a physiological link between hunger and begging (Kitaysky et al., 2001a) and that increased Cort in nestlings induces more intense begging behavior. The association between hunger, high hormone level (with its potential costs) and begging intensity would then ensure the honesty of the signal because intense begging would only occur if the nestling was truly hungry.

The aim of the present study was to test the hypothesis that Cort affects begging vigor, but also entails costs. We experimentally increased levels of circulating Cort (repeated acute stress), in house sparrow nestlings (*Passer domesticus*) to mimic a food shortage. We then assessed the effect of the treatment on (i) the behavioral response of nestlings, (ii) the parental allocation of food and (iii) the nestling growth rate and immune response. We predicted that if begging is a signal of need then (i) Cort-treated nestlings should beg more than controls, (ii) parents

should adjust their rate of provisioning according to the nestling begging rate (providing more food to nestlings that beg more), (iii) Cort-treated nestlings should have a slower growth rate and a weaker immune response than controls, unless parents fully compensate for increased begging rate of Cort-nestlings.

## 2. Materials and methods

The study was carried out in spring 2004 in a house sparrow population breeding in nest boxes at the Centre d'Etude Biologique de Chizé, France (46°09'N, 0°24'W). Fifty-seven broods were studied from April 14th–June 30th 2004.

All nests were checked regularly before and during egg laying to determine date of clutch initiation, clutch size and hatching date. Five days after hatching, all nestlings were marked with numbered metal leg rings. Nestling body mass ( $\pm 0.1$  g) was measured daily from day 5 to day 10, and tarsus length was measured on day 5 and 10.

Nestling Cort levels were experimentally increased by daily subcutaneous injections in the internal side of the leg from day 5 to day 9 of age ( $n = 237$  nestlings and 57 broods). Within each brood, half of the nestlings were injected with Cort and the other half were used as control. Nestlings were alternatively assigned to one of the two treatments (the first nestling taken out of the nest injected with corticosterone, the second with oil and so on). The order of injection (cort first, oil second) was also alternated between nests (cort first, oil second; oil first, cort second). In broods with an odd number of chicks, one nestling was randomly allocated one of the two treatments. Cort-treated nestlings received 40  $\mu$ g of Cort, dissolved in 20  $\mu$ l of peanut oil, at day 5 and 6 and 49  $\mu$ g of Cort, at day 7, 8 and 9 [to adjust the doses (2.67 mg Cort/kg) to the body mass of older nestlings]. These doses were based on published information on the dose response of Cort injections in Starlings *Sturnus vulgaris* (Remage-Healey and Romero, 2001) and adjusted to the body mass of house sparrow nestlings, because we did not find any previous work done on house sparrows. Control nestlings received a daily injection of 20  $\mu$ l of peanut oil only. Nestlings were always injected in the morning (between 8:00 and 11:00 am). When chicks were 10 days old, blood samples were collected ( $\sim 100$   $\mu$ l), within 3 min, to measure the baseline Cort level.

Nestling and parent behavior was recorded using an infrared video camera (28  $\times$  28  $\times$  30 mm) fixed on the roof of the nest box. Nest boxes were recorded, in the afternoon (starting 1:00 pm), during 4 h, when chicks were 8 days old (i.e., after the 4th Cort injection). Thirty-three broods ( $n = 136$  nestlings) were videotaped between May 7th and June 12th. All nestlings were marked individually on their head with small dye spots. Parents usually resumed normal provisioning about 15 min after the installation of the camera.

Videotapes were screened to extract several variables: nestling position, begging intensity, begging rate and feeding rate. Nestling position was assessed as the mean distance to the entrance hole during the 4 h of the recording. Nestling begging behavior was scored as: 0, no begging; 1, gaping; 2, gaping with neck fully stretched; 3, gaping with neck fully stretched and wing flapping (Lotem, 1998). Begging intensity was defined as the time spent expressing each of these behaviors. This was done using the software Observer 2.01 (Observer, 1991). Begging rate was expressed as the number of begs (mouth opening) per chick per hour. We distinguished primary begging (occurring when parents arrived at the nest) and secondary begging (occurring between feeding visits in the absence of the parents). We defined the first nestling to beg as the first nestling to open its mouth when a parent entered the nest. Finally, we defined the feeding rate as the number of feeds per chick per hour.

When nestlings were 10 days old, we tested their cell-mediated immune response. Nestlings were subcutaneously injected with 0.025 mg of phytohemagglutinin (PHA) dissolved in 0.04 ml of phosphate buffered saline (PBS), in the right wing patagium (Bonneaud et al., 2003). The other wing web was injected with the same amount of PBS to serve as a control. We quantified the immune response by subtracting the thickness of the right

wing patagium prior to injection from the thickness of the same wing 24 h post-injection (with a thickness gauge  $\pm 0.01$  mm); a strong immune response was indicated by a large swelling (Goto et al., 1978). Indeed, injection with PHA results in local activation and proliferation of T-cells, followed by local recruitment of inflammatory cells (Cheng and Lamont, 1998). The PHA assay is a reliable indicator of *in vivo* cellular immunity (Goto et al., 1978; McCorkle et al., 1980) and it is used commonly to assess cell-mediated immune response in immunoeology studies (Lee et al., 2005; Martin et al., 2004).

### 2.1. Justifications of the Cort doses

To measure changes in Cort levels induced by the exogenous administration, blood samples were taken from an independent group of 5 days old nestlings ( $n = 25$  from a population at a distance of 1 km (Zoodyse Center, France) from the experimental site), (i) before injection (basal level), (ii) 1 h, and (iii) 2 h after the Cort injection.

We also checked whether food restriction would produce a raise in Cort. To do this, we collected 5 days old nestlings ( $n = 24$  from the same population as above) and brought them to the lab. When we took a chick out of the nest, we immediately took a blood sample, and then placed the nestlings (two individuals together) in a heated box (ca 25 °C) in the lab. We fed them with a mealworm to reduce the potential interindividual variation in hunger. Another blood sample was taken after 3 h of fasting to assess the effect of food restriction on baseline plasma Cort. We did not induce begging before taking the second blood sample. At the end of the experiment, we fed the chicks and returned them to their nest.

### 2.2. Corticosterone assays

Blood samples were centrifuged, the plasma separated and frozen at  $-20$  °C. Total corticosterone levels were determined at the Centre d'Etudes Biologiques de Chizé following Lormée et al. (2003). Plasma corticosterone was measured in samples (30  $\mu$ l) after ethyl ether extraction by radioimmuno-assays using a commercial antiserum, raised in rabbits against corticosterone-3-(ocarboxy-methyl) oxime bovine serum albumin conjugate (Biogenesis, UK). Cross-reaction was 9% with 1-desoxycorticosterone and less than 0.1% with other plasma steroids. Duplicate aliquots (100  $\mu$ l) of the extracts were incubated overnight at 4 °C with 8000 cpm of  $^3$ H-Corticosterone (Amersham Pharmacia Biotech-France) and antiserum. The bound and free corticosterone were separated by adding dextran-coated charcoal. After centrifugation, the bound fraction was counted in a liquid scintillation counter. Minimal detectable corticosterone levels were 0.1 ng/ml. Recovery values of all samples were greater than 95%. Two assays were performed and samples have been analysed during five consecutive days (using the same antibody and the same assay conditions). All samples were run in duplicate. The intra-assay coefficient of variation was 8%. In addition to the standard concentrations, two plasma samples of known corticosterone concentration (samples for which the corticosterone had been previously assessed) were also added in duplicate (the two samples came from domestic rabbit and wild boar). The

inter-assay coefficient of variation (between days variation) was 11% (domestic rabbit) and 13% (wildboar). Moreover, the samples were analysed in a random sequence.

### 2.3. Statistical analyses

We used mixed linear models with fixed and random effects (Proc Mixed, Sas, 1999). Given that nestlings share genes and the environment (the nest), they cannot be considered as independent observations, from a statistical point of view. To take into account this non-independence, the nest identity was always included in the models as a random factor. The Cort-treatment was included as a fixed factor and covariates (body mass, brood size, hatching date) were also added. Repeated measurements models were used when variables were measured several times for the same individual, such as body mass and tarsus length. The assumptions underlying the use of the linear model were systematically checked and the  $\log_{10}$ -transformation was applied when necessary. We always started with a model including all the two-way interactions, which were dropped when non significant.

## 3. Results

### 3.1. Effect of exogenous Cort on plasma Cort

First of all, we checked to what extent the administration of exogenous Cort produced a raise in the level of circulating hormone (Table 1a). Cort-nestlings had significantly more Cort than controls 1 h after the injection ( $F_{1,18} = 32.47$ ,  $P < 0.0001$ ), whereas this difference disappeared 2 h after the injection ( $F_{1,19} = 0.13$ ,  $P = 0.7274$ ). It is interesting to note that PBS injected nestlings also exhibited a significant increase in plasma Cort levels both at 1 h post injection ( $F_{1,10} = 9.42$ ,  $P = 0.0118$ ) and at 2 h post injection ( $F_{1,11} = 5.66$ ,  $P = 0.0366$ ), suggesting that they responded to the handling stress.

### 3.2. Effect of fasting on nestling corticosterone levels

Cort levels of nestlings that experienced a 3 h fasting period significantly increased by a factor of 4.5 (Table 1b). Baseline Cort levels of nestlings increased significantly after 3 h of fasting ( $F_{1,46} = 31.55$ ,  $P = 0.0001$ ).

### 3.3. Effect of administration of exogenous Cort on nestling begging behavior

Cort significantly affected both primary and secondary begging rate. Cort nestlings begged more frequently than

Table 1

(a) Corticosterone concentration (ng/ml) before, 1 and 2 h after a subcutaneous injection of corticosterone (Cort) or oil (Control)

Time	Cort			Control		
	Means $\pm$ SE	Median	Min–max	Means $\pm$ SE	Median	Min–max
Before injection	11.09 $\pm$ 1.22	11.56	3.35–17.32	12.75 $\pm$ 1.58	13.92	4.04–21.28
1 h after injection	127.05 $\pm$ 15.80	119.50	49.44–241.10	39.32 $\pm$ 9.26	23.30	14.54–98.11
2 h after injection	31.87 $\pm$ 6.62	18.95	7.17–84.90	26.44 $\pm$ 4.55	23.85	5.81–53.50

(b) Corticosterone concentration (ng/ml) before and after 3 h of fasting

Fasting	Means $\pm$ SE	Median	Min–max
Before fasting	2.94 $\pm$ 0.45	2.60	0.18–8.67
3 h after fasting	13.28 $\pm$ 1.78	12.31	2.91–45.24

Table 2

Results of generalized linear mixed models exploring the effect of the hormonal treatment (Cort vs. oil) on nestling behaviors, primary and secondary begging rate

Fixed effect	Primary begging rate			Secondary begging rate		
	$F_{1,98}$	Estimate (SE)	$P$	$F_{1,98}$	Estimate (SE)	$P$
Treatment	12.29	0.903 (0.257)	0.0007	5.19	0.0413 (0.018)	0.0249
Body mass	0.02	0.009 (0.071)	0.898	11.64	-0.0164 (0.005)	0.0009
Brood size	7.18	3.162 (1.180)	0.0086	0.27	0.028 (0.055)	0.6068
Feeding rate	56.66	7.434 (0.988)	<0.0001	31.32	0.4038 (0.072)	<0.0001
Hatching date	18.58	-0.391 (0.091)	<0.0001	13.41	-0.0164 (0.004)	0.0004
Random effect	$Z$	$P$		$Z$	$P$	
Nest	3.78	<0.0001		3.71	<0.0001	

Hormonal treatment was included in the models as a fixed factor, brood size, feeding rate, hatching date and body mass as covariates and the nest as a random factor. The positive estimate value for the hormonal treatment indicates that Cort-injected nestlings beg more than oil-injected chicks.

control nestlings both when parents were in the nest (Table 2; Fig. 1) and in their absence (Table 2). The effect of Cort on begging was also significant after the removal of feeding rate from the model ( $F_{1,98} = 5.57$ ,  $P = 0.0202$ ), showing that Cort-nestlings begged more both in absolute terms and when correcting for the amount of food they received. In addition, primary begging was significantly and positively correlated with brood size and feeding rate and negatively correlated with hatching date (Table 2). Secondary begging rate was negatively correlated with nestling body mass, smaller nestlings begging more in the absence of parents (Table 2). None of the other behavioral variables was affected by the treatment (Table 3).

### 3.4. Behavioral response of parents

The provisioning rate of Cort-injected nestlings did not differ from the feeding rate of control chicks ( $F_{1,99} = 2.96$ ,  $P = 0.0886$ ). However, when correcting for the begging effort produced by the chicks, Cort-nestlings received significantly less food than oil-injected nestlings (Table 4,

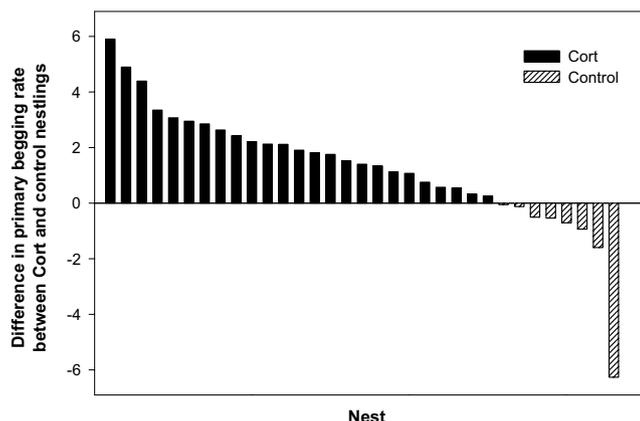


Fig. 1. Difference in primary begging rate (number of begs per nestling per hour) between Cort and control nestlings. Positive values correspond to higher begging rate in Cort nestlings (black bars) and negative values correspond to higher primary begging rate in control nestlings (hatched bars). Each bar represents a nest.

Fig. 2). Parental feeding rate was also positively correlated with nestling body mass and negatively correlated with brood size (Table 4).

### 3.5. Effects of exogenous Cort on nestling growth, immune response and apparent survival

We used a repeated measurements model to assess the effect of daily injections of Cort on body mass and tarsus length. The treatment had a significant effect on body mass gain (Table 5, Fig. 3), with Cort-nestlings growing slower than control chicks. Feeding rate was also significantly correlated with body mass gain both in the repeated measurements model (Table 5) and when looking at body mass of 8 days old (the day when feeding rate was actually measured;  $F_{1,99} = 11.61$ ,  $P = 0.0009$ ) and 10 days old nestlings ( $F_{1,99} = 24.51$ ,  $P < 0.0001$ ). Contrary to body mass, tarsus growth was not affected by the hormonal treatment ( $F_{1,229} = 0.90$ ,  $P = 0.344$ ).

Cort-nestlings exhibited a weaker response to the PHA challenge than control nestlings ( $F_{1,71} = 47.90$ ,  $P < 0.0001$ ; Fig. 4).

Finally, levels of baseline Cort at day 10 were not explained by the treatment ( $F_{1,153} = 1.85$ ,  $P = 0.176$ ), body mass gain ( $F_{1,153} = 0.92$ ,  $P = 0.338$ ) or brood size ( $F_{1,153} = 0.40$ ,  $P = 0.277$ ).

In spite of decreased body mass and weaker immune response, fledging success of Cort nestlings was not significantly different from fledging success of controls (GLIMMIX with nest as a random factor:  $F_{1,183} = 0.34$ ,  $P = 0.5585$ ). Similarly, recaptures performed from 12th March to 18th April 2005 with mist nets, showed that apparent over-winter survival did not differ between Cort and control nestlings (Cort 24/135, 17.8%; control 13/109, 11.9%; GENMOD:  $\chi^2_1 = 1.63$ ,  $P = 0.2014$ ). We also checked whether Cort nestlings were less able to acquire a breeding site (a nest-box in the following spring). As for apparent over-winter survival, the proportion of individuals that acquired a nest-box in 2005 did not differ between the two treatments (Cort 13/135, 9.6%; control 5/109, 4.6%; GENMOD:  $\chi^2_1 = 2.34$ ,  $P = 0.1259$ ).

Table 3

Results of generalized linear mixed models exploring the effect of the hormonal treatment (Cort vs. oil) on several nestling behaviors (first begging, position, begging intensity)

Fixed effect	First begging			Position			Begging intensity		
	$F_{1,102}$	Estimate (SE)	<i>P</i>	$F_{1,92}$	Estimate (SE)	<i>P</i>	$F_{1,92}$	Estimate (SE)	<i>P</i>
Treatment	0.75	0.037 (0.042)	0.3884	0.01	−0.003 (0.027)	0.9210	0.93	115.22 (119.25)	0.3366
Body mass	0.076	−0.008 (0.009)	0.3849	1.07	0.008 (0.008)	0.3037	4.43	−45.124 (21.432)	0.0380
Brood size	1.80	−0.057 (0.042)	0.1826	0.00	−0.005 (0.074)	0.9497	4.01	196.15 (97.930)	0.0481
Feeding rate	37.93	0.663 (0.108)	<0.0001	6.06	−0.241 (0.098)	0.0157	1.58	447.29 (355.39)	0.2115
Hatching date	0.02	−0.001 (0.004)	0.8938	1.42	−0.007 (0.006)	0.2367	0.05	−2.352 (10.768)	0.8276
Random effect	<i>Z</i>	<i>P</i>		<i>Z</i>	<i>P</i>		<i>Z</i>	<i>P</i>	
Nest	2.07	0.019		3.54	0.0002		1.78	0.0379	

Hormonal treatment was included in the models as a fixed factor, brood size, feeding rate, hatching date and body mass as covariates and the nest as a random factor.

Table 4

Results of a generalized linear mixed model exploring the effect of the hormonal treatment (Cort vs. oil) on the parental feeding rate

Fixed effect	Feeding rate		
	$F_{1,98}$	Estimate (SE)	<i>P</i>
Treatment	8.17	−0.059 (0.021)	0.0052
Begging rate	66.03	0.028 (0.003)	<0.0001
Body mass	12.10	0.016 (0.005)	0.0008
Brood size	11.40	0.016 (0.004)	0.0011
Hatching date	1.13	−0.004 (0.003)	0.2907
Random effect	<i>Z</i>	<i>P</i>	
Nest	3.24	0.0006	

Hormonal treatment was included in the models as a fixed factor, begging rate, brood size, hatching date and body mass as covariates and the nest as a random factor. The negative estimate value for the hormonal treatment indicates that Cort-injected nestlings were fed less than oil-injected chicks.

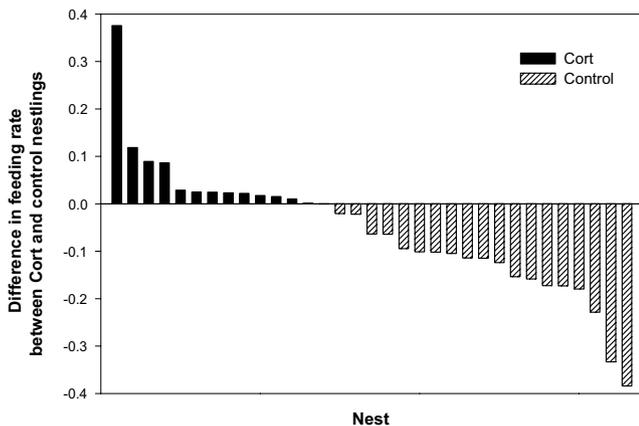


Fig. 2. Difference in parental feeding rate (number of feedings per nestling per hour) between Cort and control nestlings. Positive values correspond to higher feeding rate in Cort nestlings (black bars) and negative values correspond to higher feeding rate in control nestlings (hatched bars). Each bar represents a nest.

#### 4. Discussion

As predicted, exogenous administration of Cort had a substantial effect on nestling begging behavior. Cort-injected nestlings begged more than controls both when

Table 5

Results of a generalized linear mixed model (repeated measurements) exploring the effect of the hormonal treatment (Cort vs. oil) on the body mass

Fixed effect	Body mass	
	$F_{1,674}$	<i>P</i>
Age	3568.89	<0.0001
Treatment	1.83	0.1786
Feeding rate	7.55	0.0068
Age*Treatment	8.45	0.0038
Nestling (Nest)	7.96	<0.0001

Hormonal treatment was included in the models as a fixed factor, feeding rate as covariates and the nest as a random factor.

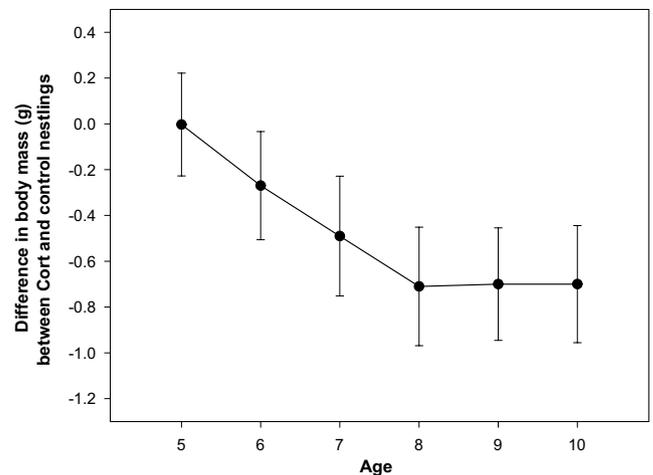


Fig. 3. Within nest difference (means  $\pm$  SE) in body mass (g) of Cort and control nestlings as a function of age.

parents entered the nest with food and in their absence. They also begged more after having removed statistically the effect of the amount of food received from the parents. Conversely, contrary to the prediction, Cort-injected nestlings did not receive more food from parents and actually they were fed significantly less than control chicks, when correcting for their begging effort. Moreover, Cort injections depressed both growth rate (in terms of mass gain)

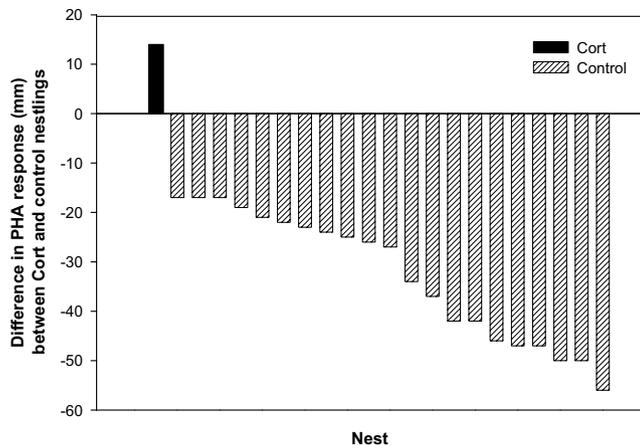


Fig. 4. Difference in immune response against a PHA challenge between Cort and control nestlings. Positive values correspond to stronger immune response in Cort nestlings (black bars) and negative values correspond to stronger immune response in control nestlings (hatched bars). Each bar represents a nest.

and the immune response. Because of the costs associated with the raise in Cort levels, the stress response hormone is a likely proximal candidate that could ensure the honesty of nestling begging as a signal of need.

There exists a considerable amount of theoretical work exploring the signaling function and the honesty of begging in the resolution of the parent-offspring conflict (Rodríguez-Gironés et al., 2001b; Johnstone and Roulin, 2003). Models based on the idea that begging reflects offspring need provided a number of testable predictions that have in several cases received empirical support (Saino et al., 2000a; Krebs, 2001; Neuenschwander et al., 2003). These predictions can be listed as follows: (i) an experimental manipulation of nestling condition should affect begging rate, (ii) parents should adjust their rate of provisioning according to the nestling begging rate, (iii) begging should be costly to avoid the spread of cheaters. We aimed to test these predictions in the current study by looking at one of the possible physiological determinants of begging in nestling house sparrows.

Much attention has been devoted in the last years by behavioral ecologists to the role played by hormones in the expression of behaviors and life history traits (Wingfield et al., 1998). The reason for this probably relies on the observation that hormones have multiple effects on the organism and this multi-regulatory role could explain the trade-offs involving life history traits. Hormonal constraints could also explain the maintenance of phenotypic variation of several traits and the honesty of signals. Two hormones have been recently reported to affect nestling begging behavior: corticosterone and testosterone. However, the direction of the effects is not always consistent across studies. For instance, Kitaysky et al. (1999, 2001a) showed that kittiwake chicks release corticosterone in response to short-term food deprivation and that experimental increase of corticosterone levels trigger chick begging rates. Conversely, injections of corticosterone in eggs

of yellow-legged gull resulted in the reduction of nestling begging intensity (Rubolini et al., 2005). Concerning testosterone Goodship and Buchanan (2006) found that the intensity of begging was positively correlated to plasma levels of testosterone in pied flycatcher chicks, *Ficedula hypoleuca*, whereas an experimental increase of testosterone in black-headed gull nestlings suppressed begging (Groothuis and Ros, 2005). The reasons for this apparent heterogeneity, at least for corticosterone, might reside in the organizational and activational effect of hormones in embryos vs. nestlings. In addition, developmental effects, such as the maturation of hypothalamic-pituitary-adrenal axis, the production of corticosteroid binding globulin (CBG), or the availability of receptors might also explain the discrepancy between studies conducted on embryos vs. juveniles (Levine, 2005; Vazquez et al., 2006).

Our results are in agreement with the hypothesis that corticosterone enhances begging rate as it reflects a deterioration of the body condition of the chick. Fasting usually induces a raise in the secretion of corticosterone and our study also provided evidence showing that a 3-h food restriction produced a 4.5-fold increase in the levels of circulating corticosterone. These results are therefore suggestive that by experimentally increasing the amount of circulating corticosterone we manipulated nestling need and, in response, nestlings begged more. This result is therefore in agreement with the predictions of the signaling of need models.

One potential problem with this study is that Cort-injections produced a supra-physiological raise in Cort levels. However, we did not find any statistically significant difference between Cort and control nestlings in a number of fitness linked traits such as fledging success, apparent overwinter survival and the likelihood to acquire a breeding site the following spring. This suggests that the injected dose did not produce pharmacological effects.

An important aspect of this work deals with the costs associated with Cort manipulation. Whereas costs of signals are at the heart of the signaling theory, few empirical studies have assessed them and even fewer have investigated the proximal basis of the costs. We found that exogenous Cort depressed both growth rate (body mass gain) and immune responsiveness. The effect on body mass gain could be due to the energetic cost of begging as already shown in Black-billed Magpie *Pica pica* (Rodríguez-Gironés et al., 2001a) and canary chicks (Kilner, 2001). They are also consistent with the known physiological effects of Cort on the metabolism (Munck et al., 1984). Cort-nestlings also exhibited a weaker T cell-mediated immune response than control nestlings. Again this result is in line with previous studies that have reported an immunosuppressing effect of corticosterone (Khansari et al., 1990; Apanius, 1998; El-Lethey et al., 2003; Rubolini et al., 2005). Obviously, if hunger means producing more Cort, and Cort modulates the intensity of the begging behavior, this would ensure the reliability of begging as a signal of need. Interestingly, the effect of Cort on body mass leveled

off at day 8 (the difference between treatments was constant between day 8 and 10, Fig. 3). Although we do not know the exact mechanism that might account for this developmental effect, it could be that repeated injections produced an increase in CBG.

The last prediction of the signaling of need models concerns the parental adjustment of their provisioning effort to the intensity of the signal. Although we found that parental feeding rate was strongly positively correlated with begging rate, Cort-nestlings were not fed more than controls. Actually, the opposite was true and control chicks got more food than Cort-treated nestlings. These results show that although begging was seen by the parents as a nestling signal and accordingly used to adjust their feeding rate, Cort-injected nestlings paid the cost of begging with no apparent rewards. There are several possible explanations for this result. First, persistently high begging levels may be seen by the parents as an indication of irreversibly poor condition and, accordingly, parents might have decided to sacrifice investment into these nestlings, with presumably low survival prospects, at the benefits of control chicks. Second, it could be that parents fed more cort-nestlings in the few hours that followed the injection and that in the face of persistent signaling they decided to down-modulate their effort. Finally, a third possible explanation could rely on both the multiple actions of corticosterone and the multiple signals used by parents to perceive nestling condition. Among the multiple traits reflecting body condition that parents use to adjust their feeding effort, it has been shown that mouth and flange color of nestlings can affect parental preference (Götmark and Ahlström, 1997). Carotenoids have been suggested to be involved in the intensity of mouth and flange coloration in certain passerine species (Hunt et al., 2003) and because of the physiological role of carotenoids as immunomodulators (Chew and Park, 2004), it has been suggested that mouth color could reflect nestling health (Saino et al., 2000b, 2003). It is possible that the administration of exogenous Cort has produced a reallocation of carotenoids towards the immune system at the expense of the signaling function and that parents responded to this re-allocation by investing more into healthy control chicks. Similar reallocations of carotenoids between the immune system and the production of signals have been documented in birds in the context of sexual selection (Faivre et al., 2003; Alonso-Alvarez et al., 2004). Of course, we should keep in mind that this is a hypothesis that relies on several assumptions that should be experimentally tested before we could draw any firm conclusion.

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