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Nestling testosterone controls begging behaviour in the pied flycatcher, *Ficedula hypoleuca*

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Abstract

Begging signals and endogenous testosterone (T) levels of young birds have been shown to be positively correlated. If T is causally involved in controlling the level of begging effort, an endocrine control mechanism could explain the evolution of begging as a costly signal reflecting need. We tested experimentally whether elevated circulating T levels enhanced begging behaviour in nestling pied flycatchers, *Ficedula hypoleuca*. A pilot study confirmed that nestling T levels could be elevated within a natural physiological range using an oral dose of T. After T-dosing, nestling begging behaviour was measured as: i) the duration of begging displays and ii) the maximum height of begging stretches. Our results show that nestling T levels were elevated at 90 min post dosing and that at this time point both measures of begging behaviour were performed more intensely by T-dosed nestlings than controls. Nestling begging displays in response to dosing varied between individuals, which in part was explained either by the date in the breeding season or nestling mass. The results of this study confirm the causal nature of T in controlling nestling begging signals and suggest that it may be part of the mechanism that controls begging behaviour in nestling birds.

Keywords: Begging; Offspring solicitation; Hormone; Androgen; Signal evolution; Testosterone; Dosing; Corticosterone

Introduction

Both altricial and precocial birds exhibit complex solicitation or begging behaviours in order to procure resources from their parents (e.g. Kilner, 2002). Such complex solicitation behaviours have evolved as a result of competition for resources both within the brood and between siblings and parents. The success of individual nestlings in receiving a share of the available resources determines their growth rate and ultimately their fledging success and survival (Mock and Parker, 1997). Theoretically, the evolution of such displays are of interest in terms of understanding conflict resolution (Godfray, 1995a; Parker and Macnair, 1979; Parker et al., 2002a; Royle et al., 2002). In contrast, mechanistically, such behaviours are poorly understood. Previous studies investigating the effect of hormones on animal behaviour have suggested that an endocrine mechanism may potentially control begging behaviour (e.g. Crook et al., in press; Eising and Groothuis, 2003; Engelhardt et al., 2006; Goodship and Buchanan, 2006; Kitaysky et al., 2001; Quillfeldt et al., 2006; Schwabl, 1996). A recent study on an insect model, the burying beetle, *Nicrophorus vespilloides* has shown that experimentally elevated juvenile hormone levels stimulate larval begging behaviour (Crook et al., in press). In birds, where studies on hormonal regulation of begging have mainly been focussed, correlative studies in a range of species have shown that circulating levels of testosterone (T) and corticosterone are positively related to nestling begging behaviour, suggesting that these steroid hormones may play a role in regulating begging responses (Goodship and Buchanan, 2006; Quillfeldt et al., 2006; Sasvári et al., 1999).

In a recent study, we have shown that circulating T levels correlate with begging behaviour and are positively related to fledging success in nestling pied flycatchers, *Ficedula hypoleuca* (Goodship and Buchanan, 2006). In our study, nestling T was positively correlated with two measures of begging behaviour: i) the duration of nestling begging displays and ii) the maximum height of begging stretches. Another study on white storks, *Ciconia ciconia* has also shown that first hatched

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nestlings have higher T levels, respond faster to the feeding parent and receive more food than their younger siblings (Sasvári et al., 1999). Furthermore, the number of younger siblings that died was higher when the difference in T levels between first and later hatched nestlings was greater (Sasvári et al., 1999). In thin-billed prion nestlings, *Pachyptila belcherie* both T and corticosterone levels have been found to correlate with begging behaviour (Quillfeldt et al., 2006). In addition to begging behaviour, nestling circulating T has also been positively correlated with aggressive behaviour in nazca boobies, *Sula granti* (Ferree et al., 2004), although studies in blue-footed boobies, *Sula nebouxii*, dominant nestlings did not have significantly higher T levels than subdominant nestlings (DelaMora et al., 1996; Ramos-Fernandez et al., 2000). It should be noted however that a number of studies have not shown such patterns. Two previous manipulative studies investigating begging behaviour have increased circulating T levels in black-headed gull nestlings, *Larus ridibundus* by implanting crystalline T pellets subcutaneously (Groothuis and Meeuwissen, 1992; Groothuis and Ros, 2005). However, it was found from these studies that although T-treated nestlings were more aggressive towards other siblings, there was a significant decrease in begging behaviour compared with control birds.

Circulating corticosterone levels of nestlings have also been experimentally increased in black-legged kittiwakes, *Rissa tridactyla* using silastic tubing filled with corticosterone (Kitaysky et al., 2003, 2001). In black-legged kittiwakes, corticosterone implanted nestlings begged more frequently and were provisioned more often with food from parents (Kitaysky et al., 2003, 2001), but chronic elevation of corticosterone was found to have a detrimental effect on cognitive ability (Kitaysky et al., 2003). However, in blue-footed booby nestlings, corticosterone implants have not been found to affect begging rates, but implanted nestlings were found to feed more frequently (Vallarino et al., 2006).

Several studies investigating the influence of maternal hormones on offspring behaviour and development, have manipulated steroid hormone levels of egg yolks and recorded the begging behaviour of nestlings hatching from treated eggs (Boncoraglio et al., 2006; Eising and Groothuis, 2003; Engelhardt et al., 2006; Freire et al., 2006; Rubolini et al., 2005; Schwabl, 1996). In an experimental study on chickens, *Gallus domesticus* chicks hatching from corticosterone treated eggs emitted more distress vocalisations and had an increased pecking rate compared with controls (Freire et al., 2006), although in a similar study on yellow-legged gulls, *Larus michahellis* corticosterone treatment reduced the loudness of late embryonic vocalisations and the intensity of nestling begging displays post hatching (Rubolini et al., 2005). In canaries, *Serinus canaria* (Schwabl, 1996), black headed gulls (Eising and Groothuis, 2003) and zebra finches, *Taeniopygia guttata* (Engelhardt et al., 2006), nestlings hatching from T-treated eggs have been shown to have more intense begging behaviour than controls. In addition, a recent study has also shown that yellow-legged gull embryos developing within T treated eggs can produce louder pre-hatch vocalisations than controls (Boncoraglio et al., 2006).

No study has yet tested the effect of T on begging behaviour by manipulating nestling levels in a passerine species. As passerines have well documented complex begging displays (Kilner, 2002), we sought to experimentally elevate circulating T levels in nestling pied flycatchers and to investigate the effect of increased T on nestling begging behaviour. This study was carried out in two phases: pilot study (2003) and experimental tests (2004). It was predicted that T-dosing would increase nestling begging behaviour in comparison to controls. To the best of our knowledge, this is the first study to investigate how manipulated nestling T levels affect begging behaviour in a passerine species.

Methods

Study subjects

A nestbox ($n=199$) population of pied flycatchers was studied at Llysdinam Field Centre in mid Wales, which consists of a 26.96 ha area of oak and coniferous woodland situated in steep sided valleys. Nestboxes were monitored to record: laying date; hatching date (hatching = day 0); number of eggs hatching and brood size.

Pilot study

In 2003, a pilot study was carried out to manipulate circulating T levels of pied flycatcher nestlings aged 13–14 days old ($n=64$) with an oral dose of T in peanut oil. The pilot study investigated a suitable concentration of T to be given in an oral dose that would elevate, but would also keep T levels within a normal physiological range. Due to restrictions in the number of nestlings available in 2003, nestlings used in the pilot study were older than those used in the main experimental study. Each nestling was temporarily (<10 min) removed from a nestbox ($n=17$) and dosed orally using a blunt tipped syringe containing either 0.5 or 1 μg of T (Sigma) in 10 or 20 μl of peanut oil respectively, before being placed back in the nestbox. Each nestling was blood sampled (approx. 100 μl) once at either: 0, 30, 60, 90 and 120 min after dosing, to assess circulating T concentration, weighed (accuracy 0.25 g) and placed back in the nestbox.

Experimental tests

In 2004, the population of pied flycatcher nestlings in the study site hatched between 20th May and 14th June. In the early to mid nesting season in 2004, experimental tests were conducted on 7 day old nestlings (consistent with our earlier study on pied flycatcher nestlings; Goodship and Buchanan, 2006) between 30th May and 14th June. Nestlings ($n=18$) were temporarily removed (<5 h) from individual nestboxes (containing 6–8 nestlings), fed to satiation with Nectarblend rearing mix (Haiths Seeds, Cleethorpes, UK) made up to a standard concentration (Kilner, 2001) and transported to the field laboratory. At 30 min post-satiation, each nestling received one oral dose of either 4 μg of T in 20 μl peanut oil (T dosed nestlings, $n=13$) or 20 μl of the peanut oil vehicle only (control nestlings, $n=5$). Nestling allocation to treatment was balanced through the season. After dosing, each nestling was placed alone on an artificial nest inside a laboratory test box and begging behaviour was recorded in begging trials at 20, 30, 70 and 90 min post dosing. In each begging trial begging behaviour was recorded as: (i) the duration (s) of the begging display and (ii) the maximum height (mm) of the begging stretch (Goodship and Buchanan, 2006). All data from video recordings were recorded blind to treatment. At 90 min post dosing, a blood sample (approx. 100 μl) was collected with a heparinized capillary tube from the brachial vein of each nestling to record nestling sex using molecular techniques and to measure circulating T concentration. Nestlings were then weighed (accuracy 0.25 g) and returned to their original nestbox in the field.

Nestlings were temporarily removed from the field and blood sampled under licence of the local administration authorities. The protocol for studying begging behaviour closely followed previously published methodology (Goodship and