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Does a short-term increase in testosterone affect the intensity or persistence of territorial aggression? — An approach using an individual's hormonal reactive scope to study hormonal effects on behavior

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HIGHLIGHTS

- We use hormonal reactive scopes to see how surges of testosterone affect behavior
- Surges of testosterone did not increase territorial aggression in black redstarts
- Surges of testosterone did not convey a competitive advantage in black redstarts

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ABSTRACT

In this study, we describe an approach based on an individual's hormonal reactive scope to study short-term effects of hormones on behavior. The control of territorial aggression has been traditionally linked to testosterone. Males of some vertebrate species show an increase in testosterone during territorial interactions and implantation studies suggest that such an increase in testosterone enhances the intensity and persistence of aggression. Here, we tested whether a short-term maximum release of testosterone – based on an individual's hormonal reactive scope – affects the intensity or persistence of territorial aggression in male black redstarts, a bird species in which testosterone does not increase during territorial encounters. An injection with gonadotropin-releasing-hormone (GnRH) induced a physiological peak in plasma testosterone that was specific for each individual (= individual reactive scope). However, such short-term surges in an individual's testosterone concentration did not affect the intensity or persistence of aggression. In conclusion, this study demonstrated (1) that a species that naturally does not increase testosterone during male–male encounters would not benefit from such an increase in terms of being more aggressive, (2) that behavioral studies using GnRH-injections represent a promising approach to study species differences in androgen responsiveness, and (3) that injections of releasing or tropic hormones in general may be a suitable approach to study short-term influences of hormones on behavior. These injections effectively mimic the potential short-term changes in hormones that can occur in the real life of individuals and enable us to study the effects of hormonal changes on behavior or other traits within an ecological and evolutionary framework.

1. Introduction

A classical approach to study the effect of hormones in eco-physiological studies of animals is to insert implants containing a hormone and observe the effect of the elevated hormone concentrations on the trait in question (see e.g. [1–4] for pioneering examples of early studies in free-living birds). Typically, such implants elevate hormone concentrations for longer periods than normal (e.g. [5–7]) and thus may be of limited value to study the effects of short-term elevations of hormone concentrations on behavior.

Here, we exemplify an alternative approach to study short-term effects of changes in hormone levels on behavior, that is based on an individual's hormonal reactive scope, i.e. the range of concentrations of a hormone (from 'baseline' to the potential maximum) that an individual can express during a particular life-history stage in response to external or internal cues. Following Williams [8] this could also be considered a hormonal reaction norm. We use the hypothalamic–pituitary–gonadal axis (HPG) with testosterone (hormone) and territorial aggression (behavior) as an example to illustrate the practicability of

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this approach, but it could be easily adapted to other behaviors or hormonal systems.

The control of territorial aggression in birds and other vertebrates has been linked to testosterone (e.g. [9–11]). In a classic study on the song sparrow (*Melospiza melodia*) Wingfield [12] observed that implants containing testosterone (subsequently termed *testosterone implants*) increased the intensity and persistence of territorial aggression towards a simulated territorial intruder (a caged song sparrow and playback of song). This is particularly interesting in combination with the observation that song sparrows belong to the minority of bird species studied to date that show a short-term increase in testosterone concentrations during territorial interactions [13]. Together these studies by Wingfield suggest that short-term increases in testosterone observed during territorial encounters may affect a song sparrow's motivation to defend a territory during the reproductive period.

Unlike the song sparrow and a few other species, the majority of birds tested so far do not show a testosterone surge after simulated territorial intrusions (see [14–23] for recent studies and a review [24] for studies conducted before 2007). This includes the black redstart (*Phoenicurus ochruros*), a socially monogamous and bi-parental songbird of the western Palaearctic, and our main study species regarding territorial aggression (e.g. [15–17]). Although black redstarts do not increase testosterone in response to simulated territorial intrusions, they have the hormonal reactive scope to do so (and may increase testosterone following other cues, i.e. when stimulated by receptive females). We ask whether a short-term rise in testosterone could potentially mediate a higher intensity and persistence of aggression also in males of species – such as the black redstart – that do not elevate testosterone during male–male challenges. This androgen response may just not have (yet) evolved in such species, even though an increase in testosterone could be beneficial, i.e. increase the likelihood to deter a potential rival during territorial challenges (alternatively, the ancestors of black redstarts may have lost the androgen response to territorial challenges, because it did not have a selective advantage).

To answer this question we elicited a short-term increase in plasma testosterone within an individual's hormonal reactive scope using injections of gonadotropin releasing hormone (GnRH). GnRH is the hypothalamic releasing hormone that causes the pituitary to secrete luteinizing hormone into the circulation, which then induces the testes to produce testosterone. Injections of GnRH are a well-established method to study the functioning of the hypothalamic–pituitary–gonadal axis (HPG) and have been used in mammals and domesticated birds for many years (reviewed by [25]). The use of GnRH injections as an indicator of gonadal activity in wild birds has been pioneered by John Wingfield [26] and since then this approach has been extensively used by many researchers to study the gonadal potential to produce testosterone in free-living wild birds (e.g.[15,17,19,20,27–46]). However, such GnRH injections have been rarely used as a means to test the effect of brief elevations in testosterone on the behavior of free-living animals (but see [47]).

Birds and other vertebrates show large individual differences in baseline breeding concentrations of testosterone, and also the capacity to maximize testosterone output differs largely between individuals (and life-history stages). For example, baseline concentrations of testosterone during the mating stage range from less than 0.2 ng/ml up to about 12 ng/ml between male black redstarts that hold a territory and are paired with a female [48]. Upon injection of a sufficient amount of GnRH the testes of male black redstarts presumably maximize testosterone production. This results in a wide range of short-term maxima in plasma testosterone concentrations that reflect each individual's capacity to produce this hormone. Thus, by injecting GnRH one can induce a short-term rise in testosterone from an individual's post-capture baseline to the respective individual's maximum concentration. This increase represents the individual's hormonal reactive scope of testosterone during a particular life-history stage and is repeatable within individuals [33,49]. Unlike testosterone implants GnRH injections cannot produce a peak in testosterone that would go beyond an individual's physiological maximum. Instead, GnRH injections lead to short-term increases of testosterone within the individual physiological range. This physiological increase of testosterone allows us to link the change in hormone concentration with changes in territorial aggression. We consider GnRH-injections (and more generally releasing hormone or tropic hormone injections) a useful approach to study short-term behavioral effects of transient increases in testosterone within the physiological reactive scope of an individual. This may be particularly useful in studies within an eco-physiological framework.

In this study, we first present reanalyzed data from our previous studies on male black redstarts to show that GnRH-injections lead to a short-term surge in testosterone during the mating and parental phases of the first brood and to demonstrate that there is large variation in the potential maxima between individuals. These data show that in both periods natural and GnRH-induced testosterone concentrations of black redstarts were high indicating that the HPG axis is active [48]. Second, we present a new experiment, in which we passively caught territorial male black redstarts during the parental phase of their first brood. According to the challenge hypothesis testosterone could interfere with parental care. Therefore, in a socially monogamous and bi-parental species, this should be the period with the largest benefit of maintaining lower testosterone levels but a high potential to increase testosterone (androgen responsiveness) in case of social instability [50]. Also, during this period black redstarts are easiest to catch without a dummy or playback lure. After capture, we quickly injected an experimental group with GnRH and a control group with saline. After measurement and banding we immediately released them back onto their territory to conduct a simulated territorial intrusion 35 min after the injection of GnRH (when testosterone concentrations were presumably maximal) or saline. If short-term surges of testosterone increase the intensity and persistence of territorial aggression also in a bird species that does not elevate testosterone during territorial encounters, we predicted that GnRH-injected birds would respond more intensely towards a simulated territorial intrusion and also would be more persistent in their territorial response in the period after the simulated territorial intrusion in comparison to control birds that were injected with saline. GnRH injections have been used to study changes in behavior before (e.g. [51–55]), but to the best of our knowledge this is only the second time the approach is used in free-living animals within an ecologically relevant setting. The first time it was used in free-living European ground squirrels (*Spermophilus citellus*), in which GnRH injections led to an increase in testosterone and agonistic behavior of males during the premating phase [47].

2. Methods

The study was performed in small villages in the vicinity of the Max-Planck-Institut für Ornithologie, Germany (N 47°, E 11°, 500–600 m above sea level). Using data from our previous studies [15,17,33] we demonstrate that GnRH induced an increase in testosterone within 30 min after injection during the mating and the first parental phase in male black redstarts. 67 male black redstarts that were in the mating phase before their first clutch (April–May) and 31 male black redstarts that were feeding their first nestlings or fledglings (May–June) were caught and an initial blood sample of about 100 μl was taken within 5 min after capture. Then, birds were injected intramuscularly with 1.25 μg chicken GnRH-I (Bachem H 3106) in 50 μl isotonic saline (see [15–17,44]), a dose that has been demonstrated to elicit a maximal testosterone response in the dark-eyed junco (*Junco hyemalis*), with about 20–22 g body mass an at least 25% larger bird than the black redstart [33]. Thirty minutes after the injection, a second blood sample of 50–100 μl was taken to measure the GnRH-induced increase in testosterone.

Plasma was immediately separated by centrifugation with a Compur minicentrifuge (Bayer Diagnostics). The amount of plasma was