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Yolk testosterone affects growth and promotes individual-level consistency in behavioral lateralization of yellow-legged gull chicks

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ABSTRACT

Behavioral lateralization is common in animals and may be expressed at the individual- and at the population-level. The ontogenetic processes that control lateralization, however, are largely unknown. Well-established sex-dependence in androgen physiology and sex-dependent variation in lateralization have led to the hypothesis that testosterone (T) has organizational effects on lateralization. The effects of T exposure in early life on lateralization can be efficiently investigated by manipulating T levels in the cleidoic eggs of birds, because the embryo is isolated from maternal and sibling physiological interference, but this approach has been adopted very rarely. In the yellow-legged gull (*Larus michahellis*) we increased yolk T concentration within the physiological limits and tested the effects on the direction of lateralization in two functionally fundamental behaviors (begging for parental care and escape to cover) of molecularly sexed hatchlings. We also speculated that T may intervene in regulating consistency, rather than direction of lateralization, and therefore tested if T affected the ‘repeatability’ of lateral preference in consecutive behavioral trials. T treatment had no effect on the direction of lateralization, but enhanced the consistency of lateral preference in escape responses. Sex did not predict lateralization. Neither behavior was lateralized at the population-level. We therefore showed for the first time in any species an effect of egg T on consistency in lateralization. The implications of the effect of T for the evolution of trade-offs in maternal allocation of egg hormones, and the evolutionary interpretations of findings from our studies on lateralization among unmanipulated birds are discussed.

Introduction

Behavioral lateralization, whereby behavioral functions are consistently biased towards either the right or the left side generally as a result of asymmetric control by either brain hemisphere, is common among vertebrates (Adret and Rogers, 1989; Franklin and Lima, 2001; Reddon and Hurd, 2008; Rogers, 2002, 2008; Rutledge and Hunt, 2004; Ströckens et al., 2013; Vallortigara, 2000; Vallortigara and Rogers, 2005; see Pfannkuche et al., 2009), and has also been documented in invertebrates (Frasnelli, 2013). Behavioral lateralization in animal populations may be expressed at different levels (Rogers et al., 2013): its occurrence at the individual-level, meaning that individuals perform a behavior preferentially on either side, may or may not translate into population-level lateralization (i.e. alignment of the direction of the lateralization in the majority of individuals) depending on the relative frequency of individuals showing a specific lateral preference. In humans, for example, handedness is apparent at the individual-level and also at the population-level, because the frequency of right-handed people largely exceeds that of left-handers (Schaafsma et al., 2009). Conversely, in fiddler crabs where antisymmetric distribution in the size of the chelae is associated with strongly lateralized socio-sexual behavior at the individual-level, no or low lateralization occurs at the population-level because right- and left-‘handed’ individuals occur at approximately the same frequency (Jennions and Backwell, 1996; Pratt et al., 2003). Thus, where no lateralization occurs at the population-level, individual-level lateralization may still exist. It has also been shown that lateralization may vary in ‘strength’, with lateralized individuals differing in the intensity of the preference for a specific side. Whether we see population-level lateralization or not will thus ultimately depend on genetic and epigenetic control of individual-level lateralization in combination with the frequency of individuals that inherit or develop any specific lateral preference. An additional dimension of individual-level variation in lateralization, which has seldom been investigated, is the consistency of individuals in their lateralization, i.e. the extent to which any particular individual systematically prefers either side or shows no specific lateral preference. Any particular lateralized individual may show highly or, conversely, poorly consistent lateral preference (i.e. the variance in lateral preference may vary) or

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may even show no lateral preference in a highly consistent way. We speculate that this component of individual-level lateralization may be partly influenced by the same developmental and physiological mechanisms that influence direction and strength, and may also be important in an evolutionary perspective, because of its consequences in e.g. social as well as predator–prey interactions.

Mechanistic studies of individual-level lateralization and, at a different level, functional interpretations of lateralization and of the selection processes that lead to the evolution of lateralized populations have flourished over the decades (Csermely and Regolin, 2013; Ocklenburg and Güntürkün, 2012; Rogers et al., 2013; Rogers and Vallortigara, 2015). However, much of this matter is still vividly debated.

The common, though not uncontroversial, observation of sex-dependent lateralization in diverse functions has directed attention to the potential role that 'sex hormones', and testosterone ('T' hereafter) in particular, may have in the development of lateralized behavior (Rajendra and Rogers, 1993; Schwarz and Rogers, 1992; Vallortigara et al., 1999; see Pfannkuche et al., 2009 and references therein). Stemming from the organizational-activational theory (Phoenix et al., 1959; see also Arnold, 2009; Wallen, 2009) of sexual differentiation of the brain, extensive experimental work has demonstrated that androgens have pervasive organizational but also activational effects on brain structures and functions (see Adkins-Regan, 2007; Groothuis et al., 2005; Partecke and Schwabl, 2008; Rogers, 2008).

Because many of the anatomical and physiological differences between the sexes are in fact rooted in differences in steroid hormone profiles since early life (Arnold and Breedlove, 1985; Balthazart and Ball, 1995; Ketterson and Nolan, 1999; Schlinger, 1997; Wingfield et al., 1994; see Adkins-Regan, 2005, 2007), it has been hypothesized that androgens affect lateralization, causing sex-dependent but also individual-level variation in lateralization. At least three hypotheses have been presented for the mechanisms that may mediate the effects of early exposure to T, mainly in the context of sex-dependent variation in lateralization in humans and other mammals (see Pfannkuche et al., 2009 for a review). Here, we will not go into the details of the putative mechanisms and specific predictions that these hypotheses generate because they may not be readily generalized across taxa, owing to major differences in brain structure and in the control pathways of steroid hormones on sex-dependent development and expression of behavior.

In birds pre-natal exposure to different T levels affects diverse morphological, physiological and behavioral traits that are expressed in early post-hatching stages but also in adulthood, suggesting the existence of organizational besides activational effects (Bonisoli-Alquati et al., 2007; Groothuis et al., 2005; Strasser and Schwabl, 2004; see Williams and Groothuis, 2015). These effects, however, can be independent of the process of sexual differentiation (Groothuis and Schwabl, 2008; Schlinger, 1998; Williams and Groothuis, 2015). Hence, exposure to prenatal T in birds may have different consequences as compared to other vertebrates, possibly because of differences in the processes of sexual differentiation (Carere and Balthazart, 2007). In addition, sexual differentiation in birds is considered to be induced mainly by the action of estrogens in females, rather than by the masculinizing effects of T in males (Carere and Balthazart, 2007). Hence, variation in pre-natal T levels in birds may be expected to have consequences on post-natal phenotype, which are independent of sex or, alternatively, that depend on sex partly because of aromatization of T to estrogens and the feminizing effects of estrogens on females.

Experimental studies of epigenetic effects of pre-natal T in eutherian mammals are hampered by the intimate physiological connection between the offspring and their mother, as well as, in multiparous species, among siblings, which prevents controlled manipulation of the pre-natal milieu, besides of course ethical constraints to experimentation on humans. Pathological conditions such as CAH (Congenital Adrenal Hyperplasia), which entails altered prenatal exposure to androgens (Mathews et al., 2004, 2009; see also Hines, 2006), may not be fully representative of the consequences of variation in the pre-natal hormone milieu in the general, healthy population. Conversely, birds represent excellent models to investigate the influence of pre-natal environment as determined by maternal allocation of materials to the eggs (Gil, 2008; Mousseau and Fox, 1998; Riedstra et al., 2013) because their eggs are cleidoic, meaning that no exchange of materials occurs between the internal egg milieu and the outer environment, except for oxygen, carbon dioxide and water vapor (Groothuis et al., 2005). Being isolated from the maternal body, bird eggs afford the opportunity of testing how experimentally altered concentrations of egg substances influence pre-natal offspring development, without incurring any interference by maternal or sibling physiology.

Several studies of birds have been used for insightful experimental approaches to investigate the role of epigenetic effects mediated by pre-natal exposure to T on a wide variety of traits, ranging from pre- and early post-natal development, growth and immunity, social or other forms of behavior, and survival (Rubolini et al., 2006; Rutkowska and Cichoń, 2006; Siefferman et al., 2013; Tobler et al., 2010; von Engelhardt et al., 2006; see also Muriel et al., 2013 and references therein; see Schaafsma and Groothuis, 2012 for an experiment on fish). Some experiments have even extended to the investigation of organizational T effects, like those on socio-sexual behavior or susceptibility to activational effects of T that are expressed in adulthood (e.g. Bonisoli-Alquati et al., 2011a, 2011b; Cooke et al., 1998; Groothuis et al., 2005; Rhen and Crews, 2002; Rubolini et al., 2007; Strasser and Schwabl, 2004). Given these advantages of cleidoic bird eggs as experimental models, it is surprising that the *in ovo* manipulation approach to the mechanistic study of behavioral lateralization has been previously adopted, to the best of our knowledge, only once (Riedstra et al., 2013). In that study, no evidence for an effect of egg experimental T manipulation on the direction or strength of lateralization in three behavioral tasks could be observed in chicken (*Gallus gallus domesticus*) chicks.

In the present study we manipulated the concentration of T in unincubated eggs of the yellow-legged gull (*Larus michahellis*) and tested for the effect on lateral preference exhibited by newly hatched chicks, relative to chicks from sham-injected, control eggs, in two tasks: solicitation of food provisioning by pecking at the bill of dummy parental heads (hereafter 'begging') (Romano et al., 2015) and escape to a safe, dark place in a test apparatus, reflecting response to fear (hereafter 'escape response'). We tested if T treatment influenced the direction but also the consistency of individual-level lateralization (see above for a definition). An effect of T on consistency in lateralization was expected based on the evidence that in poultry chicks T increases persistence of attention in searching for a particular type of food or in a particular place (Andrew, 1972, 1975; Andrew and Jones, 1992; Andrew and Rogers, 1972; Klein and Andrew, 1986), suggesting that T treatment may also induce greater consistency in lateral behavior by boosting attention to the stimulus. Indeed, the consequences of exposure to T on lateralization may be seen not only in terms of direction but also in terms of 'strength' of lateralization, but this component of lateralization has been relatively neglected in favor of a prominent focus on the direction of lateralization.

Moreover, we tested if lateralization existed at the population-level. Finally, we analyzed variation in the direction and consistency of lateralization according to sex and position of the original egg in the laying sequence. The effect of laying order was tested because in the same population that we studied here (Rubolini et al., 2011), as well as in many other bird species (e.g. Badyaev et al., 2006; Groothuis et al., 2006), variation in T concentration according to egg laying order has been observed, suggesting strategic maternal effects mediated by T according to laying order and/or that chicks differ in susceptibility to T effects depending on laying order.

In designing the experiment we paid special attention to administer a dose of T into the egg yolk that resulted in post-manipulation yolk T levels within the natural range of variation of the yolk T concentration