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Age-dependent and age-independent effects of testosterone in male quail

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ABSTRACT

Various studies in rodents recently concluded that puberty should be considered as a second period of organization of brain and behavior and that action of sex steroids at that time is long lasting and possibly permanent. We tested this notion in male Japanese quail that had been castrated before 3 weeks post-hatch by analyzing whether a similar treatment with exogenous testosterone initiated at 3, 5 or 7 weeks post-hatch has a differential influence on the development of testosterone-dependent morphological, behavioral and neural characteristics that are known to be sexually differentiated. The growth of the androgen-dependent cloacal gland was significantly faster when testosterone treatment was initiated later in life indicating that the target tissue is not ready to fully respond to androgens at 3 weeks post-hatch. The three groups of birds nevertheless developed a gland of the same size typical of intact sexually mature birds. When adults, all birds expressed copulatory behavior with the same frequencies and latencies and they displayed the same level of aromatase activity and of vasotocinergic innervation in the preoptic area as gonadally intact males despite the fact that they had been treated with testosterone for different durations starting at different ages. Surprisingly, the frequency of cloacal sphincter contractions, a measure of appetitive sexual behavior, was significantly higher when testosterone treatment had been initiated later. Together these data provide no clear evidence for an organizational action of testosterone during sexual maturation of male quail but additional experiments should investigate whether estrogens have such an action in females.

1. Introduction

Sex steroids actions have two distinct types of consequences on brain and behavior. During early ontogeny they organize brain and behavior during a more or less strictly delimited time window and these effects are considered irreversible (Phoenix et al., 1959). Later in life, they activate reproductive and other behaviors in parallel with the induction of related brain plasticity but these effects are transient and dissipate when the steroid is removed (McEwen, 1981). These two types of effects are classically considered completely independent (organization vs. activation) but a host of relatively recent studies have suggested that the activation of reproduction behaviors that takes place at the time of sexual maturation (puberty) could have long-lasting effects, a feature typical of organizing effects of steroids (Patlak, 2012; Schulz et al., 2009a; Sisk and Zehr, 2005).

In rodents, pre- or peri-natal testosterone (T), acting as such (an androgen) or via aromatization into an estrogen, masculinizes and defeminizes reproductive behaviors (mounts and intromissions, lordosis) and sexually differentiates multiple related brain structures (Arnold and Gorski, 1984; McCarthy, 2012; McCarthy et al., 2009). These behaviors will then be differentially activated by exposure to androgens or estrogens in adult males and females (Arnold and Gorski, 1984). It was however shown that deprivation of male Syrian hamsters (*Mesocricetus auratus*) of sex steroids during adolescence compromises activation by steroids of adult social behavior (Schulz et al., 2004). Furthermore in male hamsters gonadectomized on postnatal day 10, T treatment before or during, but not after, puberty facilitates mating behavior in adulthood (Schulz et al., 2009b). In parallel, the pre-pubertal treatments with T, although they did not induce sexual behaviors in the short-term, increased the volume of bed nucleus of the stria terminalis and of parts of the amygdala, two sexually dimorphic brain nuclei, to adult size (Schulz et al., 2009b). Studies in rats also showed that sex differences in sexually dimorphic nuclei of the brain such as the sexually dimorphic nucleus of the preoptic area, the anteroven-

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tral periventral nucleus of the hypothalamus and the medial amygdala are partly the result of a differential addition of new cells to these nuclei during puberty and that pre-pubertal gonadectomy eliminates these sex differences in cell addition (neurons but also other cell types) during puberty (Ahmed et al., 2008).

Puberty should in this context be considered as a second period of organization of brain and behavior since effects of steroids at that time are long lasting and possibly permanent (Schulz et al., 2009a; Sisk and Zehr, 2005). Although direct experimental data are lacking for obvious reasons, circumstantial evidence suggest that in humans, puberty could also be a period when sex steroids exert permanent organizational-like effects on sexually differentiated behaviors with behavioral traits potentially concerned ranging from gender identity to cognitive capacities through sexually differentiated forms of psychopathology (Beltz and Berenbaum, 2013; Berenbaum and Beltz, 2011).

Japanese quail (*Coturnix japonica*), a well-established model in neuroendocrine studies of sexual differentiation (Ball and Balthazart, 2011a; Balthazart and Ball, 1998), offers an interesting model to test the generality of these conclusions. Sexual behaviors and several neuroanatomical or neurochemical traits are sexually differentiated in this species with some of the differences depending on embryonic organizational effects of steroids while others simply result from a differential activation by T or estradiol in adulthood (Balthazart et al., 2009). Specifically in quail, male-typical copulatory behavior is demasculinized in females, mostly before day 12 of incubation ((Adkins, 1979; Adkins-Regan, 1983; Balthazart et al., 1992), but see (Balthazart and Schumacher, 1984b; Hutchison, 1978; Schumacher and Balthazart, 1984)) by estrogens secreted by the embryonic ovary, while in contrast, female receptivity does not appear to be differentiated by organizational effects of steroids and can be activated in adults of both sexes by a proper treatment with estrogens (Adkins, 1975). The cloacal gland, an androgen-dependent structure located at the back of the cloaca, is larger in males than in females and this difference in size results in part from the differential activation by T in adults (Balthazart et al., 1983) but also from a demasculinizing action of embryonic estrogens (Balthazart et al., 1992; Schumacher and Balthazart, 1983).

Multiple brain features are also differentiated between males and females including the volume of the medial preoptic nucleus (POM; (Panzica et al., 1996b; Viglietti-Panzica et al., 1986)), the aromatase activity (AA) in the preoptic area (Schumacher and Balthazart, 1986) and more specifically the POM (Balthazart et al., 1990b; Cornil et al., 2011), and the vasotocinergic innervation of the POM (Panzica et al., 1997, 1998). All these traits are T-dependent in adulthood (Balthazart and Ball, 2007; Panzica et al., 1996a, 1996b) but clear evidence for an organizational action of embryonic estrogens on these sex differences is available only for vasotocinergic innervation of the POM (Panzica et al., 1998). Indeed, no effect of embryonic exposure to estrogens on POM volume could be detected (Aste et al., 1991; Panzica et al., 1987, 1991). Likewise, variable results were obtained for the preoptic AA. Some experiments detected a significantly higher AA in males than in females when all subjects were gonadectomized and treated with a same dose of T (Schumacher and Balthazart, 1986) but several other studies failed to find such a difference or found a quantitatively much smaller difference that was not statistically significant (Balthazart, 1989; Balthazart et al., 1990b; Cornil et al., 2011).

Based on the information on long term effects of steroids at the time of mammalian puberty described just before, we wondered whether these variations from one experiment to another in the magnitude of the sex difference affecting brain AA could not be related to the age at which birds had been gonadectomized and when their treatment with T had been initiated. The age at gonadectomy was reasonably standardized (±3 weeks post-hatch) between experiments for practical reasons (gonads must be removed before they start growing due to extensive vascularization). But since we had shown that T restores full copulatory behavior in birds gonadectomized before sexual maturity (Balthazart et al., 1990a, 1983), we had not controlled to the nearest week the specific age when T treatment was initiated. We had also serendipitously discovered that there is around 40–60 days post-hatch an increase in cell divisions as identified by BrdU labeling in the preoptic area of male and female quail (Mouriec and Balthazart, 2013). This suggested that, in quail also, the rise in sex steroid concentrations occurring at the time of sexual maturation (Ottinger and Brinkley, 1979a) could be associated with a remodeling of the preoptic area, a brain region critically implicated in the control of reproduction where multiple sex differences have been identified (Balthazart and Ball, 2007; Panzica et al., 1996b).

We therefore decided to test whether T could have long-lasting organizing effects in quail around the time of sexual maturation. More specifically we analyzed whether the age at which administration of T is initiated in male quail that have been castrated around 3 weeks post-hatch has an influence on the development of T-dependent morphological, behavioral and neural characteristics known to be affected by sex differences as a result of either a different organization or activation by steroids. We show here that the growth rate of the cloacal gland was markedly affected by the age when T was first administered (faster in older birds) but in contrast, no effect of the age of T treatment initiation was detected on the adult level of behavioral and neural T-dependent features that were considered with the possible exception of the measure of sexual motivation that was higher when T treatment had been initiated at a later age. This apparent discrepancy with the results of previous studies in rodents might relate to the mode of sexual differentiation in these two groups of vertebrates (masculinization by T of male rodents vs. demasculinization by estrogens of female quail) and future studies should test whether long-lasting peripubertal effects of estrogens would be present in female quail.

2. Methods

2.1. Subjects

This experiment was performed with a total of 49 male Japanese quail (*C. japonica*) derived from our breeding colony at the University of Liège. Sexually mature females obtained from the same colony served as stimuli during the behavioral tests. From hatching, chicks were initially raised in groups in heated brooding cages. Temperature in these cages was progressively dropped from 40 °C to room temperature (20–25 °C) during the first three weeks of life. At 9 weeks of age, 2 days before the beginning of behavioral tests, all males were isolated in individual cages where they stayed until the end of the experiment. In these cages birds were in physical isolation but they could see and hear each other. Throughout the experiment all birds were maintained under a 16L:8D photoperiod simulating long summer days and they were provided with food and water *ad libitum*. All experimental procedures were in agreement with the Belgian laws on the protection of experimental animals and were approved by the local animal care committee.

2.2. Hormonal treatments

At the age of 17–18 days, 40 males were bilaterally castrated (CX) through a unilateral incision behind the last rib on the left side as described previously (Balthazart et al., 1998; Schumacher and Balthazart, 1984). The remaining 9 males were sham-operated: