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Steroids in the Avian Brain: Heterogeneity across Space and Time

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Abstract

Sex steroids influence a diversity of neural and behavioral endpoints in birds, including some that have little to do with reproduction per se. Recent advances in neurochemistry and molecular biology further indicate that the avian brain is comprised of a network of unique sex steroid microenvironments. Factors involved in steroid synthesis and metabolism are present in the avian brain with expression levels that vary from region to region and with activities that are, in some cases, subject to regulation over relatively slow or rapid time intervals. Advances in our ability to a) isolate steroids from brain tissue and b) precisely measure their concentrations reveal how steroid levels vary spatially and temporally. A full appreciation of sex steroid effects on the avian brain require not only measures of hormones in blood but also an understanding of the numerous and varied mechanisms whereby the brain creates such a heterogeneous steroidal environment.

Introduction

Studies in avian biology have a long history linking testicular androgen synthesis and secretion with the activation of masculine reproductive behavior (Wingfield 2005). Measures of circulating sex steroid hormones remain popular and are especially instructive for clarifying endocrine control of natural avian behavior. Nevertheless, while peripheral hormone stimulation of neural function is of undisputed importance, a thorough understanding of hormonal control of brain, behavior and physiology in birds requires a deeper inspection of specific neural circuits on which hormones act. Neural expression of receptors for steroidal signaling molecules renders specific circuits “sensitive” to steroidal action. However, as new receptor forms and receptor-mediated mechanisms of action are discovered, we must regularly update our notions about spatial and temporal forms of neural steroid action. Additional refinement of our concepts of steroid signaling in brain is required when we consider that steroids can be synthesized de novo in brain (neurosteroidogenesis) and there exists circuit-specific enzymatic activation and inactivation of traditional circulating steroids, such as testosterone (T), or non-traditional, non-gonadal steroids, like dehydroepiandrosterone (DHEA).

Birds seem to show appreciable complexity in the synthesis, metabolism and actions of steroids in brain. By describing some relatively new concepts regarding steroid neurochemistry in this brief report, I hope to provide investigators of avian behavior a better sense of the challenges we face in understanding the connection between the hormones measured in blood and the control of steroid-dependent behavior.

Steroids Impact Diverse Neural Functions

It is crucial to first recognize the diversity of neural endpoints impacted by sex steroids in the avian brain. Of course, steroids play an unquestioned role in controlling reproductive physiology and in activating copulatory and aggressive behaviors by actions primarily, but not exclusively, on diencephalic (hypothalamic) neural circuits (Ball and Balthazart 2009). In addition, steroids exert effects on neural functions secondary to reproduction per se, such as on courtship and vocal signaling behaviors, and these often involve diverse non-hypothalamic neural circuits (Schlinger and Brenowitz 2009). One such example is the neural song system of oscine songbirds comprised of several telencephalic (forebrain) and mesencephalic (midbrain) nuclei that express receptors for androgens and, in some cases, estrogens (Gahr et al. 1987; Metzdorf et al. 1999). Most of these aforementioned circuits express steroid receptors in abundance that are readily visualized by steroid-binding assays, by autoradiography, and by molecular approaches such as in situ hybridization. Examination of diverse species can also reveal novel sites of sex steroid action (Fusani et al. 2014).

Importantly, steroids also influence avian neural circuits that express non-traditional steroid receptors or traditional nuclear receptors at low abundance, and some of these circuits may not typically be associated with reproduction. Two examples help illustrate these points. First, there is evidence that estrogens act on the hippocampus (HP) to influence a bird's ability to find food and remember where available food exists in a maze or in an open arena (Bailey et al. 2013; Oberlander et al. 2004; Rensel et al. 2013). The estrogen synthetic enzyme aromatase is expressed in relatively high amounts in the songbird HP where it remains elevated, or even increases, outside of the breeding season (Saldanha et al. 1999; Saldanha et al. 1998; Soma 1999.; Soma et al. 2003), implicating a role for estrogens in HP-dependent learning and memory in non-breeding birds. Second, there is both behavioral and physiological evidence for estrogen action in an auditory processing region of the oscine songbird brain, the caudomedial neopallium (NCM; (Remage-Healey et al. 2010; Remage-Healey and Joshi 2012; Tremere et al. 2009). The NCM also expresses aromatase in significant amounts (Saldanha et al. 1998; Saldanha et al. 2000), suggesting that it is a site of estrogen action, though estrogen receptors themselves are not prominent. Indeed, little is known about the receptors through which estrogens act in both of the HP and NCM. In NCM, estrogens rapidly modulate both neural firing as well as a behavior that depends on accurate auditory processing (Remage-Healey et al. 2010; Tremere et al. 2009), suggesting that estrogens act via membrane-bound receptors in the NCM, such as the recently identified G-protein coupled receptor GPR-30 (Acharya and Veney 2012). Clearly, numerous sex steroid neural circuits exist in the avian brain. Some of these circuits likely rely solely (or largely) on sex steroids derived from the periphery when the H-P-G is active during periods of reproduction. Given the disparate functions of some of the other circuits it is likely that the provision of sex steroids to these regions is spatially and temporally disassociated and accomplished by local steroid synthetic and metabolic mechanisms.

Neural steroid synthesis and metabolism

Neurosteroidogenesis—We have long known that peripheral steroids, like testosterone, could undergo metabolism in brain into downstream potent or inactive products. This is not neurosteroidogenesis, which involves the synthesis of steroidal molecules in brain from non-

steroidal precursors (Baulieu 1998). The synthesis of bioactive steroids starts with cholesterol as a substrate and, via sets of cholesterol transporters and enzyme catalyzed reactions, a variety of steroids can be produced. There is substantial evidence from studies of both non-songbirds and songbird species, for expression of the key molecules that accomplish de novo steroid synthesis in the brain (London et al. 2009; Schlinger and Remage-Healey 2012; Tsutsui et al. 2006a). Cells in the periphery (testicular Leydig cells, ovarian granulosa and theca or adrenal cortical cells) that synthesize steroids in part to enrich the vasculature for signaling throughout the body, express many of these transporters and enzymes in abundance (Miller 1988; Stocco and Clark 1996). While also expressed in brain, many of these steroidogenic factors are expressed at relatively low levels, most likely because the actions of the steroidal products are local, likely restricted only to proximal neural circuits. In addition, examination of their neuroanatomic distributions show that neural circuits differ widely in the kinds steroidogenic factors expressed, suggesting regional heterogeneity in the steroidal products produced (London et al. 2006; Schlinger et al. 2014). Some brain areas stand out with relatively rich expression of these factors, including the cerebellum and areas of the neural song system and related auditory processing centers in oscine species (London et al. 2006; Tsutsui et al. 2006b). In some cases, only truly sensitive techniques can adequately measure the expression of genes encoding steroidogenic factors (e.g. qPCR or in situ hybridization). As has been the case with aromatase (e.g. (Saldanha et al. 2000)), isolation of specific antibodies to help define the location of neurosteroidogenic cells by immunocytochemistry will help refine concepts regarding steroidal signaling in the avian brain.

Neural Steroid Metabolism and Novel Peripheral Substrates—As pointed out above, there is substantial evidence that biologically important steroidal products are formed in the avian brain from substrates synthesized and secreted peripherally. Perhaps the best studied is the conversion of circulating testosterone into estradiol by action of the enzyme aromatase (Balthazart and Ball 2013). In addition we know that testosterone can undergo conversion in brain into the potent androgen 5 β -DHT by actions of the enzyme 5 β -reductase (Schlinger and Callard 1987; Schumacher and Balthazart 1987). In the avian brain, testosterone can also be inactivated by its conversion into 5 β -DHT by the enzyme 5 β -reductase (Hutchison and Steimer 1981; Schlinger and Callard 1987; Schumacher and Balthazart 1987). There is a substantial literature describing the importance of these metabolic pathways in the control of avian brain development and in the activation of adult behavior, especially masculine copulatory behaviors (Hutchison and Steimer 1984; Schlinger and Brenowitz 2009; Balthazart and Ball, 2013).

The significance of these enzyme catalyzed reactions has recently expanded as we see them also positioned as terminal reactions involving substrates from the periphery other than testosterone. The best example come from studies that show that the adrenal androgen DHEA circulates at relatively high levels in blood of many bird species (Landys et al. 2013; Shah et al. 2011; Soma et al. 2004; Soma and Wingfield 2001), and that DHEA can undergo conversion in brain by the enzyme 3 β -HSD (3 β -hydroxysteroid dehydrogenase/isomerase) into androstenedione, which can undergo further conversions into neurobiologically active products like estradiol (Soma et al. 2004; Tam and Schlinger 2007; Vanson 1996). Although

3 β -HSD has been identified in a few species (Pradhan et al. 2010; Ukena et al. 1999; Vanson 1996), more work is needed to confirm its widespread presence and importance in utilizing peripheral DHEA as a substrate. Consequently, whereas identifying DHEA in blood is a good first step, it is necessary to identify the brain's capacity to utilize and respond to this hormone before reaching conclusions as to its behavioral and neurobiological importance. Moreover, as the brain may have the capacity to synthesize DHEA on its own as part of a larger neurosteroidogenic capability (see above), DHEA secreted by the adrenals may be of limited importance.

Measuring Steroids in Brain—Whereas measures of sex steroids in blood and the identification of steroid synthetic and metabolic enzymes in brain provides some guidance into the presumptive steroid environment of the avian brain, direct measures of steroids are needed to accurately assess local neurosteroid concentrations. This can be quite challenging because steroids are lipoidal molecules and the brain is rich with lipids which makes isolation and purification of the steroids especially problematic. Dissected brain tissues can be subject to organic (biochemical) and/or solid-phase extraction and steroids within identified and/or measured by mass spectroscopy, RIA or ELISAs (Chao et al. 2011; Charlier et al. 2011; Shah et al. 2011; Taves et al. 2011; Taves et al. 2010). Application of such approaches reveals significant regional differences in the concentrations of hormones across the brain as well as significant differences between concentrations found in blood and what is seen in some brain regions (Chao et al. 2011; Charlier et al. 2011; Shah et al. 2011; Taves et al. 2011; Taves et al. 2010). Another approach to assessing brain steroids is with the use of in vivo microdialysis. This procedure has the advantage that local steroid levels can be assessed in live, behaving birds across time (Remage-Healey et al. 2012; Remage-Healey et al. 2009) and the procedure itself separates freely diffusing steroids from inert lipids in the brain reducing the need for complex downstream separation procedures. Microdialysis suffers from its own drawbacks including the need for complicated surgical procedures on captive held birds. Despite limitations, the application of microdialysis, newly adopted biochemical or solid-phase steroidal separation techniques and increasingly sensitive steroidal assays are providing a new window into the complex steroidal milieu of the avian brain.

Rapid synthesis and actions of estrogens

Gonadal steroid secretion fluctuates over rather lengthy time-scales, as periods of reproduction wax and wane across the year (Wingfield and Farner 1993) but also over more rapid time intervals, such as in response to changing social contexts (Wingfield 1990). The brain also has the capacity to modulate local steroid concentrations on a rapid time scale that is independent of steroid levels in blood. We have used in vivo microdialysis to measure estradiol levels in discrete brain regions of freely behaving zebra finches. Exposure of male and females to conspecific song or even to conspecific visual information induces a rapid rise (well within 30mins) in NCM estradiol levels, a change not seen in blood or in brain regions adjacent to the NCM. In some cases, T levels in NCM are inversely related to those of estradiol, suggesting that rapid changes in aromatase activity mediate the local flux in steroid levels. There is a substantial body of evidence showing the phosphorylation/dephosphorylation of the aromatase enzyme rapidly modulates its activity in the brain

(Cornil and Charlier 2010; Cornil et al. 2012) and we assume this is the basis for the changes seen in the NCM. Thus, sex steroid levels in specific brain regions can exhibit both spatial and temporal independence from one another and from levels seen in the periphery.

Steroid Concentrations Likely Fluctuate at a Subcellular Level of Resolution

Steroid levels may not only differ across regions of the avian brain but may do so at the level of individual cells or subcellular compartments. Studies of the avian brain indicate that the aromatase enzyme can be enriched in synaptic terminals and regulation of activity at the synapse can lead to changes in local levels of estradiol (Peterson et al. 2005; Remage-Healey et al. 2011; Saldanha et al. 2011). Although we cannot as yet measure estradiol concentrations at the synapse per se, we believe that estradiol functions as a neuromodulator capable of influencing the activity of specific neurons, their downstream neural targets and, ultimately, behavior (Balthazart and Ball 2006; Saldanha et al. 2011). Such fine scale signaling represents a conspicuous difference from the system wide signaling produced by steroids secreted gonadally emphasizing the diverse ways in which steroids impact brain and behavior.

While we have evidence to support such fine-scale modulation of estradiol levels and action in the avian brain, other steroidal molecules (such as allopregnanolone or 7 α -hydroxypregnenolone) have been shown to rapidly influence avian neurophysiology and behavior (Carlisle et al. 1998; Tsutsui et al. 2009). They likely do so by actions on post-synaptic membranes like traditional neuromodulators. More work is needed to understand the role of such molecules in controlling natural avian behavior.

Conclusion

In conclusion, when viewed through an endocrine-focused lens, the avian brain can be seen an intricate landscape of sex steroids that is regularly modulated across space and time. Some of the behavioral and physiological endpoints of these steroidal actions are well-understood, whereas other areas are just emerging in importance. I encourage investigators of avian behavior to consider this complexity when designing experiments or when interpreting data on plasma hormone levels. While we have focused here on the sex steroids, there is good evidence that similar considerations must also be applied to studies of adrenal steroid action on the avian brain (Newman et al. 2008; Newman and Soma 2009; Rensel et al. 2014). No doubt, our appreciation of the richness of this neuroendocrine landscape will expand even further as we investigate novel bird species with unique neural functions and behavioral capabilities. There is much to look forward to in avian behavioral neuroendocrinology.

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