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Testosterone and BDNF interactions in the avian song control system

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Abstract

Interaction between steroid sex hormones and brain-derived neurotrophic factor (BDNF) is a common feature of vertebrate brain organization. The avian song control system provides an excellent model for studying such interactions in neural circuits that regulate song, a learned sensorimotor behavior that is often sexually dimorphic and restricted to reproductive contexts. Testosterone (T) and its steroid metabolites interact with BDNF during development of the song system and in adult plasticity, including the addition of newborn neurons to the pallial nucleus HVC and seasonal changes in structure and function of these circuits. T and BDNF interact locally within HVC to influence cell proliferation and survival. This interaction may also occur transsynaptically; T increases the synthesis of BDNF in HVC, and BDNF protein is then released on to postsynaptic cells in nucleus RA where it has trophic effects. The interaction between sex steroids and BDNF is an example of molecular exploitation, with the evolutionarily ancient steroid-receptor complex having been captured by the more recently evolved BDNF. The functional linkage of sex steroids to BDNF may be of adaptive value in regulating the trophic effects of the neurotrophin in sexually dimorphic and reproductively relevant contexts.

Introduction

Brain-derived neurotrophic factor (BDNF) regulates numerous aspects of development, growth, and plasticity of the nervous system. This neurotrophin influences a range of cellular processes including proliferation, differentiation, survival, growth of processes, electrical activity, neurotransmission, and synaptic plasticity (Bothwell, 1995, Murer et al., 2001). Steroid sex hormones, including androgens and estrogens, may have similar trophic effects on the brain as does BDNF, and act by common cellular and molecular mechanisms (Scharfman and MacLusky, 2006) (Scharfman and MacLusky, 2005). These commonalities suggest that steroids can play an important role in regulating the expression of the genes for BDNF and its cognate tropomyosin receptor kinase B (trkB), and/or that steroids and BDNF may act by convergent pathways (Scharfman and MacLusky, 2006).

The avian neuroendocrine song control system provides an excellent model for examining interactions between steroids and BDNF. Sex steroids and BDNF interact to influence development of the song control regions, the addition of new neurons to a cortical nucleus in

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adults, and seasonal plasticity of the song control circuits in adults. NT-3 is also present in song control regions and appears to play a role in development and adult plasticity (Johnson et al., 1997, Wissman and Brenowitz, 2009). NT-4 does not occur in avian brains (Sehgal and Lovette, 2003). Given the focus of this special issue, I will discuss steroid interactions with BDNF in the song control system.

The avian song control system

Song is a learned behavior that is widely produced among the 4000 species of oscine songbirds and many other avian taxa (Catchpole and Slater, 2008). Songs have well-defined acoustic structures that are characteristic of each species. In species that breed in temperate and high latitude regions, song is produced largely or only by males in most species. In many tropical species, however, females also sing and may join with males in producing complex vocal duets. There is extensive taxonomic diversity in the age when song is learned (Beecher and Brenowitz, 2005, Brenowitz and Beecher, 2005). In many species song learning is completed by the onset of sexual maturity late in the first year of life. Other species may continue to learn new songs as adults. Song plays important roles in avian reproduction (Catchpole and Slater, 2008). In many species, song is used to declare a territory from which other birds are aggressively excluded. Both males and females may use song in this context. In most songbird species males also use song to attract females. Females may select among potential mates on the basis of individual song characteristics. The male's song may directly stimulate reproductive behavior in females. In addition to these two main functions, song may be used in other behavioral contexts. For example, song may be important in mediating dominance behavior among members of a social group.

Song behavior is regulated by a discrete network of interconnected nuclei that arises in the pallium, projects to brainstem nuclei that control vocalization and respiration, and receives input from pallial auditory regions. (Some authors regard the avian pallium as homologous to mammalian cortex (Jarvis, 2009) (Wang et al., 2010). The song control system is organized into two functional pathways (for review see Zeigler and Marler, 2008, Fig 1). The main pathway for the motor production of song in adult birds includes projections from HVC to RA in the telencephalon, and from RA to nXIIts in the medulla, which innervates the muscles of the sound-producing organ, the syrinx, and brainstem respiratory premotor nuclei. The anterior forebrain pathway (AFP) includes HVC, area X in the striatum, DLM in the thalamus, LMAN in the pallium, and RA; it is analogous to the mammalian basal ganglia-thalamocortical circuit (Luo et al., 2001) The AFP is essential for song learning, for the motor production of song in juveniles (Ölveczky et al., 2005), and for adult song variability (reviewed in Brainard, 2008).

The variation between species in sexual patterns of song behavior noted above is accompanied by concomitant variation in the neural song control system. In species in which only males sing, there are extreme sexual dimorphisms in the structure of the song nuclei. In the Zebra Finch (*Taeniopygia guttata*), the male song system is substantially more developed than that of the female (Nottebohm and Arnold, 1976). HVC and RA are larger in volume and have more and larger neurons in males than females. Area X of the basal ganglia is large and well-defined in males, but cannot be identified in females. The pallial nucleus LMAN also cannot be detected in female brains (Bottjer et al., 1985). Comparable patterns of sexual dimorphism are observed in the brains of other species in which only males sing (Ball et al., 1994, MacDougall-Shackleton and Ball, 1999). In those species in which females are able to sing, however, brains of males and females have the same network of song nuclei (Nottebohm and Arnold, 1976, Brenowitz et al., 1985, Brenowitz and Arnold, 1986, Brenowitz, 1997).

Steroid sex hormones affect song learning and production, and the juvenile development and adult plasticity of the song circuits (reviewed in Schlinger and Brenowitz, 2009). Nuclear androgen receptors are present in the song nuclei HVC, RA, LMAN, area X, ICo (intercollicular nucleus), and nXIIIts, as well as in the muscles of the syrinx. Classical nuclear estrogen receptors are found in HVC and ICo (Fig. 1; (reviewed in Schlinger and Brenowitz, 2009).

In juvenile male zebra finches, BDNF protein, as measured by immunocytochemistry, is present in neurons in HVC, RA, X, and LMAN (Johnson et al., 1997, Akutagawa and Konishi, 1998, Dittrich et al., 1999). Some of these HVC and LMAN neurons project to nucleus RA where the release of BDNF may influence the formation of connections between these nuclei (Akutagawa and Konishi, 1998; Dittrich et al., 1999), perhaps in part by regulating neuron survival in nucleus RA (Johnson et al., 1997). BDNF protein in RA and X is decreased in adult brains (Akutagawa and Konishi, 1998). BDNF mRNA is expressed in HVC and LMAN, but not X or RA, in juvenile or adult birds (Dittrich et al., 1999, Wissman and Brenowitz, 2009). Together these observations suggest that the BDNF gene is expressed in HVC and LMAN, and the protein is transported anterogradely to RA from HVC and LMAN, and to X from HVC (Dittrich et al., 1999). TrkB is a sex chromosome (Z)-linked gene and is expressed by day 6 posthatching at greater levels in male brains than in females, particularly in nucleus HVC (Chen et al., 2005). Consequently, males may be inherently more sensitive than females to the presence of BDNF.

TrkB receptor protein, measured by immunocytochemistry, is present in HVC, RA, and LMAN, and in auditory regions functionally related to the song system (Wade, 2000). TrkB mRNA is expressed in HVC, LMAN, RA, and X (Dittrich et al., 1999, Chen et al., 2005, Wissman and Brenowitz, 2009). Receptor expression in the song nuclei seems to be genetically rather than hormonally regulated (Chen et al., 2005, Wissman and Brenowitz, 2009).

Steroid and BDNF interactions during development of the song system

The role of BDNF in development of the song system has been studied, albeit incompletely, in the zebra finch, a species with extreme neural and behavioral sexual dimorphisms as discussed above.

Steroid sex hormones influence the development of multiple attributes of the song system, whereas other traits appear to be under direct genetic regulation (Schlinger and Brenowitz, 2002, Wade and Arnold, 2004, McCarthy and Arnold, 2011). Estrogens, and to a lesser extent androgens, stimulate masculine growth of the song system when they were injected into very young (1–3 week posthatching) females (Gurney and Konishi, 1980, 1981; Gurney, 1982). Since these early studies, the masculinizing effects of sex steroids have been confirmed repeatedly. Steroids induce growth of the overall volumes of HVC, RA, Area X and LMAN, stimulate increases in neuronal size and number, and alter biochemical properties and connectivity between neurons in these nuclei (Arnold, 1992). The axonal projection from HVC to RA seen in males but not females seems particularly sensitive to estrogen exposure (Holloway and Clayton, 2001). In an *in vitro* slice culture preparation, co-culture of male and female slices or exposure to exogenous E2 stimulated formation of this projection in the female slice. Formation of the connection was blocked in male slices by an inhibitor of the aromatase enzyme necessary for E2 synthesis. These results indicated not only the importance of estrogens for masculinization of this circuit but further suggested that the male slices synthesize estrogen *de novo*. Media from these slice cultures contained E2 and there was more E2 in male cultures than in females (Holloway and Clayton, 2001).

Estrogens may influence masculine growth of the song system by stimulating the synthesis, transport and secretion of BDNF. Expression of BDNF in juvenile zebra finches appears to depend on estrogens. BDNF mRNA begins to be expressed in HVC of males but not females between days 30–35 (Dittrich et al., 1999). Expression of BDNF mRNA could be induced within 24 hr in male HVC by systemic estrogen treatment at days 15 and 20–25 but not at day 10 (Dittrich et al., 1999). This suggests that E2 is naturally responsible for the upregulation of BDNF seen in HVC of males at this time. E2 appears to act even earlier to establish the estrogen-dependent increase in BDNF. BDNF in HVC is not upregulated by systemic E2 in juvenile females, unless they are also treated with E2 shortly after hatching (Dittrich et al., 1999), at ages when estrogens have their most potent and permanent masculinizing effects on song system growth (Adkins-Regan et al., 1994). These results show an important relationship between sex steroids and BDNF, one that may remain important in the adult brain in species that retain significant neural plasticity throughout life.

Steroid and BDNF interactions in the adult song system

Interactions between sex steroids and BDNF in the adult song system have been studied in two contexts. These are 1) the addition of newborn neurons to HVC and 2) seasonal plasticity of the adult song control circuits. These two models identify common principles of steroid and BDNF interactions in adult brains that are similar to those seen in juvenile development.

1. Neuronal addition to the adult song system

One of the most striking forms of plasticity in the song system of adult birds is the ongoing addition of newly born neurons to HVC. As much as 1.5% of the HVC neurons in an adult Canary (*Serinus canarius*) are generated per day (Goldman and Nottebohm, 1983). Treatment of adult female canaries with exogenous T tripled the number of new neurons added to HVC (Rasika et al., 1994). This effect of T on neuronal addition required BDNF (Rasika et al., 1999). T treatment increases both BDNF mRNA and protein in HVC cells (Rasika et al., 1999, Wissman and Brenowitz, 2009). Intracerebral infusion of recombinant BDNF (rBDNF) into HVC increased the number of new neurons by the same amount as did T treatment. Infusion of a BDNF blocking antibody prevented the T-induced increase in new neurons in HVC (Rasika et al., 1999). In adult male canaries the number of new HVC neurons born during the spring decreased by 50% over the next four months (Nottebohm et al., 1994). Infusion of rBDNF into HVC 14–20 days after the birth of new neurons increased the number of neurons that survived for at least 8 months compared with birds that were infused with BDNF either 4–10 or 24–30 days after birth (Alvarez-Borda et al., 2004). Systemic treatment of adult male white-crowned sparrows with T decreased the number of neural progenitors labeled by the thymidine analog 5-Bromo-2'-Deoxyuridine (BrdU) in the ventricular zone (VZ) dorsal to HVC (Larson, Wang, Caras, Gale, Perkel, Thatra, and Brenowitz, unpublished observation). Together these observations suggest that T suppresses cell division in the VZ, that T-induced expression of BDNF increases the survival of newly generated neurons in HVC, and that there is a sensitive period soon after new neurons first reach HVC when BDNF has this effect on neuronal survival.

The interaction between T and BDNF in adult neurogenesis is mediated by local angiogenesis in HVC. T treatment of female canaries upregulated expression of both vascular endothelial growth factor (VEGF) and its receptor Quek1/VEGF-R2 tyrosine kinase within two weeks in HVC (Louissaint et al., 2002). Both the induction of VEGF and Quek1/VEGF-R2 appear to be mediated by the aromatization of T to E2; treatment of cultured HVC endothelial cells with E2 but not 5- α dihydrotestosterone (DHT) increased Quek 1/VEGF-R2 mRNA expression, and systemic treatment of female canaries with E2 induce Quek 1/VEGF-R2 and increased the addition of new HVC neurons *in vivo* (Hidalgo

et al., 1995, Louissaint et al., 2002). T also expanded the microvasculature in HVC, including increases in the number of new endothelial cells, and the diameter, perimeter, and area of capillaries.

BDNF expression by HVC cells lagged VEGF expression by one week or more. *In situ* hybridization indicated that many of the BDNF expressing cells were endothelial. Inhibition of VEGF-R2 *in vivo* in HVC prevented both increased vasculature and neuronal addition. Treatment of cultured endothelial cells with T, E2, or DHT increased production of BDNF protein (Louissaint et al., 2002). T induced higher levels of BDNF protein, however, than did either E2 or DHT alone, which may indicate that there is a synergistic effect of androgens and estrogens on BDNF expression. Receptor inhibition also blocked the induction of male-like song normally induced in female canaries by T, and expression of BDNF by plasmid transfection of HVC cells reversed this inhibition and restored song behavior (Hartog et al., 2009). Together these results suggest a serial activation pathway: T is aromatized to E2 which increases vascularization in HVC by increasing VEGF-R2 expression in endothelial cells. These cells are thus sensitized to respond to T-induced VEGF. The endothelial cells in the expanded vascular bed are then stimulated by T to express BDNF, which increases the survival of new neurons and increased song behavior (Louissaint et al., 2002). The interaction between T and BDNF may therefore be indirect.

2. Seasonal plasticity of the adult song system

Seasonal plasticity of brain structure and function has been observed in every vertebrate taxon (Tramontin and Brenowitz, 2000). The song control system provides the most pronounced example of seasonal plasticity in an adult brain, and remains the leading model for study of this phenomenon. Seasonal plasticity of the song system provides an excellent model for studying the interaction between sex steroids and BDNF, with the novel feature that BDNF conveys the transsynaptic trophic effects of T and its metabolites to efferent nuclei.

In arctic, temperate, and subtropical birds, breeding is usually restricted to spring and early summer, and photoperiod is the most common environmental factor that influences activation of the reproductive system. Reproduction may also be seasonal in tropical species in which there are seasonal changes in other environmental factors, such as rainfall, that influence breeding (e.g., Moore et al., 2004). Song behavior occurs most often or only in the breeding season. Song structure also changes seasonally (Nottebohm and Nottebohm, 1978, Smith et al., 1997a).

Seasonal changes in song behavior are accompanied by changes in the morphology of song nuclei in essentially every seasonally breeding songbird species that has been examined (for reviews see Brenowitz, 1997, Tramontin and Brenowitz, 2000). The volumes of HVC, RA, X, and nXIIts increase by up to 200% during the breeding season (Fig. 2). Cellular attributes of song regions also change (Table 1) (reviewed in Brenowitz, 2008). The spontaneous neurophysiological activity of RA neurons is greater in breeding White-crowned Sparrows (*Zonotrichia leucophrys*) and Song Sparrows (*Melospiza melodia*) (Meitzen et al., 2007a, Meitzen et al., 2007b, Meitzen et al., 2009).

Neuron number in HVC also changes seasonally. In wild-caught Song Sparrows, for example, neuron number in HVC increases from about 150,000 in the fall to 250,000 during the breeding season (Smith et al., 1997a). This change in neuron number results from seasonal patterns of cell death and ongoing neurogenesis. At the end of the breeding season, circulating T levels drop and there is an increase in the death of mature HVC neurons (Thompson et al., 2007, Thompson, 2011). There is a subsequent increase in the addition of new neurons to HVC in nonbreeding birds (Alvarez-Buylla et al., 1990, Tramontin and

Brenowitz, 1999). The death of mature HVC neurons creates “vacancies” for the addition of new neurons (Scharff et al., 2000, Thompson and Brenowitz, 2009).

The seasonal changes in the song nuclei are primarily regulated by changes in gonadal T and its metabolites (Brenowitz, 2008). Plasma T levels are high throughout the breeding season and drop to basal levels at the end of breeding (Wingfield and Farner, 1980). The sensitivity of song nuclei to sex steroids varies seasonally. Androgen receptor mRNA and protein in HVC are expressed at higher levels in breeding-condition birds (Fig. 3 Gahr and Metzdorf, 1997, Soma et al., 1998, Fraley et al., 2010). Both androgenic and estrogenic metabolites of T contribute to seasonal growth of the song circuits and song production (Smith et al., 1997b, Soma et al., 2002, Tramontin et al., 2003, Meitzen et al., 2007a).

Field studies of wild birds show that growth of the song system occurs rapidly once plasma T levels first start to rise as day length increases in late winter, and precedes full seasonal reproductive development. (Smith et al., 1997a, Tramontin et al., 2001). In a laboratory study of captive White-crowned Sparrows implanted with a systemic T implant and exposed to a long day photoperiod to mimic breeding conditions, HVC grew to its full breeding size and neuron number increased from 90,000 to 150,000 within 7 days. This addition of 60,000 neurons to an adult brain within such a short time period is unprecedented. HVC's efferent targets, RA and area X, grew more slowly and were not significantly larger until 20 days after T implant (Tramontin et al., 2000). These studies indicate that the song control circuitry grows rapidly and sequentially in response to seasonal hormonal cues.

Given the pronounced and rapid effects of steroid hormones on seasonal growth of the song circuits, we asked which nuclei within this circuit are directly targeted to initiate such growth; steroid receptors or their mRNA are present in all of the major song nuclei (Fig. 1). A clue was provided by the observation that HVC grows rapidly and its efferent targets RA and X grow more slowly (Tramontin et al., 2000). The rapid growth of HVC and slower growth of its efferent targets suggested that T initially acts directly on HVC, which subsequently stimulates growth of RA and X transynaptically. We have conducted several studies to test this hypothesis. In one study we lesioned HVC on one side of the brain in nonbreeding-condition White-crowned Sparrows and then exposed them to breeding T and photoperiod conditions for 30 days (Brenowitz and Lent, 2001). The unlesioned side of the brain acted as an internal control. The unilateral HVC lesions completely blocked the growth of the ipsilateral RA and X, but the contralateral RA and X, as well as the contralateral HVC, showed normal growth. In a second study we placed small T implants unilaterally in the brain near HVC or RA in male sparrows in nonbreeding condition for 30 days (Brenowitz and Lent, 2002). The T implant near HVC produced significant growth of the ipsilateral (but not contralateral) HVC, RA, and X, and increased neuronal number in the ipsilateral HVC. The T implant near RA, however, did not produce growth of the ipsilateral RA, HVC, or X. The failure of this latter implant to stimulate growth of RA is noteworthy given that this nucleus has abundant AR (Fraley et al., 2010). In a third study unilateral intracerebral infusion of DHT plus E2 adjacent to HVC produced significant growth of neurons, and increased spontaneous activity, in ipsilateral but not contralateral RA (Meitzen et al., 2007a). These results together indicate that T and its metabolites induce seasonal growth by acting directly on HVC, which in turn provides some permissive or trophic support of growth to its efferent targets RA and X.

T-induced trophic support provided by HVC to its efferent targets requires the release of BDNF, and the release of and/or response of efferent neurons to BDNF is dependent on the electrical activity of neurons in these nuclei. It is not yet known whether the release of BDNF from HVC neurons occurs at axon terminals following anterograde transport, or by exocytosis from extrasynaptic sites. Both mechanisms of BDNF release have been observed

in other neural systems (von Bartheld et al., 1996, Kohara et al., 2001, Trueta and De-Miguel, 2012). The release of BDNF by HVC neurons stimulates growth and increased electrical activity of postsynaptic neurons. In a cDNA microarray hybridization study we found that the gene for BDNF is significantly upregulated in HVC following the transition of White-crowned Sparrows from nonbreeding to breeding conditions (Thompson et al., 2012). *In situ* hybridization confirmed that BDNF mRNA is expressed widely throughout HVC of sparrows exposed to breeding conditions and that expression increased over the 7 days following exposure to systemic T (Fig 4 Wissman and Brenowitz, 2009). BDNF mRNA is not expressed in RA in either breeding or nonbreeding condition sparrows. TrkB mRNA is expressed in RA, but does not vary seasonally (Wissman and Brenowitz, 2009). Infusion of rBDNF protein into RA of nonbreeding condition sparrows stimulates growth of RA neurons (Wissman and Brenowitz, 2009) and increased spontaneous activity to breeding-typical levels (Wood, Lent, Perkel, and Brenowitz, unpublished observation). Infusion of tetrodotoxin (TTX) near RA inhibits the electrical activity of HVC axon terminals and RA neurons and this blocks the neurotrophic effects of a systemic testosterone implant. Co-infusing rBDNF with TTX near RA rescues the growth of RA neurons (Brenowitz and Lent, unpublished observation).

These observations, together with those discussed above for HVC neuronal addition, suggest the following scenario of interactions between T and BDNF in the regulation of seasonal plasticity. T acts on HVC to increase the synthesis of BDNF by endothelial cells as well as by neurons. BDNF acts locally and rapidly to increase the survival of new neurons added to HVC during seasonal growth of the nucleus. In addition, BDNF synthesized by HVC neurons is released on to postsynaptic RA neurons where it binds to trkB receptors to increase activity and induce neuronal growth. The release of BDNF from HVC and/or response of RA neurons are activity dependent. Given the constitutive expression of trkB mRNA in RA cells, the seasonal growth and increased activity of RA neurons reflects an increase in synthesis of the BDNF ligand by HVC cells, rather than increased sensitivity to the protein in RA neurons. A novel component of this model is that while T increases BDNF synthesis locally in HVC, BDNF exerts its effect on RA transsynaptically following axonal transport and synaptic release, or by extrasynaptic release, and binding to receptors on efferent neurons. In this system, therefore, neurons in the target nucleus RA respond only indirectly to T through the downstream effector BDNF.

Summary and Conclusions

Interactions between T and BDNF are important for the development and adult plasticity of the avian song control system. The nature of the interaction and the cellular effects of T-activated BDNF expression are similar in these two contexts, providing another example of development and adult plasticity exploiting similar molecular and cellular mechanisms (Tropea et al., 2009, Knafo and Esteban, 2012).

The interaction between T and BDNF may be direct, with the hormone/receptor complex binding to a hormone response element on the BDNF gene to regulate its expression (Scharfman and MacLusky, 2006). T and BDNF may also interact indirectly, with T acting through intermediary molecules, such as VEGF, to regulate BDNF expression (Louissaint et al., 2002). Examples of both modes of interaction are seen in the song system.

T and BDNF often interact locally within the same brain region to exert their trophic effects. An example is the increased survival of new HVC neurons by T-induced BDNF expression within this nucleus. The interaction between T and BDNF's actions may also occur across synapses. T increases the local synthesis of BDNF, and the protein is then released on to postsynaptic cells where it has trophic effects. An example is the T-induced increase in

BDNF expression in HVC which is released on to post-synaptic RA neurons to influence neuronal size and activity. This anterograde mode of action compliments the classic retrograde, target-derived mode of action of neurotrophins as shown for NGF (Levi-Montalcini, 1998). Given that steroids of gonadal or neural origin can have organizational and/or activational effects on neural circuits (Simerly, 2002), the anterograde mode of interaction between steroids and BDNF that we observe in the song system may occur in other neural systems as well. Future research should test this prediction.

A question of interest is what adaptive value is conferred by steroid interactions with BDNF. This entire special issue is devoted to examples of these interactions, showing how common they are in the brain. But one can ask why these different classes of molecules came to be functionally linked with each other through evolution.

Steroid biosynthesis is evolutionarily ancient, occurring in bacteria, fungi, plants, and animals, and it therefore arose billions of years ago. Nuclear steroid receptors are of more recent, though still early, origin (Thornton, 2001). Several taxa of marine invertebrates, including echinoderms, crustacea, and cephalochordates have androgen receptor signaling systems, suggesting that they arose at least one billion years ago (Wray et al., 1996, Köhler et al., 2007). BDNF, by contrast, is of more recent evolutionary origin; BDNF and NGF diverged from a common ancestral molecule ca. 600 million years ago (Gotz et al., 1992, Tettamanti et al., 2010). BDNF is only found in vertebrate animals. It is therefore intriguing to speculate about how these very different classes of molecules came to be functionally related in the brain. Some sense of this evolutionary convergence might come from first considering the functional contexts in which these classes of molecules act separately.

In vertebrate brains steroid hormones are important cues in the differential development of neural circuits between the sexes. In adults, steroids serve various functions: 1) they activate neurons in sexually dimorphic brain regions to produce sex-typical behaviors; 2) hormones provide a means of restricting neural activity to appropriate environmental and physiological conditions, as seen in the T-induced growth of the song system in birds early in the breeding season; 3) secretion of steroids by the gonads and transport to the brain can coordinate neural and behavioral activation with reproductive physiology. An example of this latter role is the vernal enhancement of song production by T in male birds to attract and stimulate mates at the time of year when the reproductive axis is optimized for breeding in both sexes.

BDNF has a wide range of effects on target cells in the brain, including proliferation, differentiation, survival, growth of processes, neuronal activation, neurotransmission, and synaptic plasticity. In neural systems where it would be maladaptive to have constitutive trophic activity, such as in developmental, sexual, or seasonal contexts, it is important to regulate BDNF expression and to do so in coordination with other molecular and physiological processes. Sex steroids such as the androgens and estrogens are well suited to providing the timing cue to initiate the trophic activity of BDNF in these contexts. Given the recent evolutionary origin of BDNF, it appears to have “captured” pre-existing steroid-receptor complexes as regulators of its own expression, an example of molecular exploitation (Bridgham et al., 2006). From this perspective, the functional linkage of steroids and BDNF through evolution is of adaptive value in sexually dimorphic and reproductively relevant contexts. The advantages conferred by the interaction between hormones and BDNF have led to it becoming a fundamental aspect of vertebrate brain organization.

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I show that sex steroid hormones interact with BDNF in the avian song control system

I show that steroid-BDNF interactions are important for sexually dimorphic development

I show that steroid-BDNF interactions are important for adult neurogenesis and seasonal plasticity

I show that these interactions may occur transsynaptically as well as locally

I show that the phylogenetically ancient steroid-receptor complex captured the more recently evolved neurotrophins

Figure 1.

Simplified schematic sagittal view of the avian song control system showing the distribution of steroid receptors. *Black arrows* connect nuclei in the main descending motor circuit, and *gray arrows* connect nuclei in the anterior forebrain circuit. Abbreviations: AM- nucleus ambiguus; *DLM*, Dorsolateral nucleus of the medial thalamus; *IMAN*, lateral portion of the magnocellular nucleus of the anterior nidopallium; *nXIIIts*, the tracheosyringeal portion of the hypoglossal nucleus; *RA*, the robust nucleus of the arcopallium; *RAm* – nucleus retroambigualis; *syrinx*, vocal production organ; *V*, lateral ventricle; *X*, area X of the medial striatum.

Figure 2.

Song nuclei of Spotted Towhees represented in 3-D, from a caudal perspective. HVC, RA, and area X are each larger in 3-dimensional extent in breeding-condition birds (shown in gray) than in non-breeding-condition birds (shown in white). Figure adapted from (Bentley and Brenowitz, 2002).

Figure 3.

Seasonal changes in androgen receptor expression in HVC of Song Sparrows. Bottom panel: AR mRNA is expressed at higher levels in HVC of breeding birds (adapted from Fraley et al., 2010). Scale-bar = 0.5 mm. Top panel: The density and immunostaining intensity of cells positive for AR-like protein are greater in HVC of breeding sparrows. Note that nonbreeding birds have fainter staining within cell nuclei (adapted from Soma et al., 1998). Scale bar = 20 μ m.

Nonbreeding

Breeding

Figure 4. Dark-field photomicrographs of *in situ* hybridization showing seasonal change in BDNF mRNA expression in HVC of White-crowned Sparrows. BDNF is expressed at higher levels in HVC of breeding birds. Arrows delineate the ventral border of HVC. Scale bar, 300 μ m.

Table 1

Attributes of song system that change seasonally

Volumes of HVC, RA, area X, and nXIIts
Neuronal number in HVC
Neuronal soma size in RA, area X, and LMAN
Neuronal density in RA and area X
Synaptic/dendritic traits in RA
Metabolic capacity of neurons in HVC, RA, and X
Spontaneous neurophysiological activity of RA neurons ^I
Incorporation of new neurons into HVC
Song stereotypy, duration, and rate of production

^I(Park et al., 2005). See reviews by (Ball, 2000) and (Tramontin and Brenowitz, 2000) for other references