Gonadal androgens are critical for the development and maintenance of sexually dimorphic regions of the male nervous system, which is critical for male-specific behavior and physiological functioning. In rodents, the motoneurons of the spinal nucleus of the bulbocavernosus (SNB) provide a useful example of a neural system dependent on androgen. Unless rescued by perinatal androgens, the SNB motoneurons will undergo apoptotic cell death. In adulthood, SNB motoneurons remain dependent on androgen, as castration leads to somal atrophy and dendritic retraction. In a second vertebrate model, the zebra finch, androgens are critical for the development of several brain nuclei involved in song production in males. Androgen deprivation during a critical period during postnatal development disrupts song acquisition and dimorphic size-associated nuclei. Mechanisms by which androgens exert masculinizing effects in each model system remain elusive. Recent studies suggest that brain-derived neurotrophic factor (BDNF) may play a role in androgen-dependent masculinization and maintenance of both SNB motoneurons and song nuclei of birds. This review aims to summarize studies demonstrating that BDNF signaling via its tyrosine receptor kinase (TrkB) receptor may work cooperatively with androgens to maintain somal and dendritic morphology of SNB motoneurons. We further describe studies that suggest the cellular origin of BDNF is of particular importance in androgen-dependent regulation of SNB motoneurons. We review evidence that androgens and BDNF may synergistically influence song development and plasticity in bird species. Finally, we provide hypothetical models of mechanisms that may underlie androgen- and BDNF-dependent signaling pathways.

**Key words:** androgens, neurotrophin, dimorphic, motoneurons, HVC.

**Contents**

**INTRODUCTION**

Gonadal androgens, including testosterone (T) and its metabolite dihydrotestosterone (DHT), have powerful effects on both the developing and mature nervous system in a wide variety of vertebrate species, thereby regulating many different behaviors. These steroid hormones also regulate the expression of the neurotrophin brain-derived neurotrophic factor (BDNF) in many systems, raising the possibility that BDNF may mediate some of the effects of androgens on development, physiology and regeneration of neural systems in diverse species. In mammals, androgens are crucial for male-specific development and maintenance of numerous neural regions and cell populations in the spinal cord, brainstem, hypothalamus, hippocampus, and amygdala, to name a few (for reviews, see Morris et al., 2004; Foradori and Handa, 2008; Sengelaub and Forger, 2008; McCarthy, 2010). In avian species, androgens are necessary for the male-specific
development and seasonal plasticity of HVC (formerly "high vocal center") and other sexually dimorphic nuclei of the songbird brain (for reviews, see Ball et al., 2004; Wade and Arnold, 2004; Adkins-Regan, 2009; Balthazart et al., 2010).

Similarly, BDNF plays a distinct role in the development and continued functioning of neurons throughout the nervous system. For example, the actions of BDNF and neurotrophins 4 and 5 (NT 4/5) signaling at their shared receptor, tyrosine receptor kinase b (TrkB), promote outgrowth of neurites during development and axonal regeneration following peripheral injury in adulthood (Li et al., 1994; Friedman et al., 1995; Kobayashi et al., 1997; Munson et al., 1997). Additionally, BDNF/TrkB signaling in adulthood regulates both the local trafficking and translation of mRNA in dendrites required for the formation and maintenance of synapses (Tongiorgi et al., 1997, 2004; Righi et al., 2000; Matsumoto et al., 2001; Takei et al., 2004; Brigadski et al., 2005) and the morphology of dendrites themselves (Kojima et al., 2001; Takei et al., 2004).

While the interaction of BDNF with other steroid hormones, such as estradiol, in neuroprotective processes and in learning and memory is well characterized (for reviews, see Miranda et al., 1994; Friedman et al., 1995; Kobayashi et al., 1997; Munson et al., 1997). Additionally, BDNF/TrkB signaling in adulthood regulates both the local trafficking and translation of mRNA in dendrites required for the formation and maintenance of synapses (Tongiorgi et al., 1997, 2004; Righi et al., 2000; Matsumoto et al., 2001; Takei et al., 2004; Brigadski et al., 2005) and the morphology of dendrites themselves (Kojima et al., 2001; Takei et al., 2004).

**BDNF AS A POSSIBLE MEDIATOR OF ANDROGEN ACTION ON SNB MOTONEURONS**

The interaction of BDNF and androgens on SNB motoneurons

Of the many steroid-sensitive neural systems in the central nervous system, the sexually dimorphic motoneurons of the spinal nucleus of the bulbocavernosus (SNB) have yielded a trove of information regarding the interaction of androgenic and BDNF signaling on neural functioning. Found in the ventral horn of the lower lumbar spinal cord, SNB motoneurons of male rodents innervate the bulbocavernosus (BC), levator ani (LA), and the external anal sphincter (EAS) muscles. The striated muscles of the BC/LA attach to the base of the penis, mediating male-specific reflexive copulatory behaviors (Breedlove and Arnold, 1980; Sachs, 1982; Meisel and Joppa, 1994; Cooke et al., 1998). Adult females have a vastly smaller or entirely absent BC/LA complex, and correspondingly, fewer and smaller SNB motoneurons than adult males (Hayes, 1965; Breedlove and Arnold, 1980; Schroder, 1980; McKenna and Nadelhaft, 1986; Tobin and Joubert, 1988) (Fig. 1). Sexual dimorphisms in the SNB system arise during perinatal development. Male and female rats possess the same number of SNB motoneurons until just before the day of birth. On that day, and extending through postnatal day 10, SNB motoneurons of both sexes undergo a wave of apoptotic cell death, but do so in a sexually differentiated, androgen-dependent manner (Nordeen et al., 1985). Many more SNB motoneurons die in females than in males, resulting in the adult sex difference in motoneuron number (Freeman et al., 1996; Forger, 2009). Numerous studies demonstrate the key role androgens play in the perinatal survival of SNB motoneurons. For example, perinatal treatment of female rodents with androgens will prevent the loss of SNB motoneurons (Breedlove and Arnold, 1983a; Nordeen et al., 1985; Sengelaub and Arnold, 1986). Likewise, male rats possessing the testicular feminized mutation (tfm) of the androgen receptor (AR) (substituting a single amino acid) that renders the protein nonfunctional, experience increased perinatal apoptosis of SNB motoneurons and exhibit dramatically fewer SNB neurons in adulthood (Breedlove and Arnold, 1981; Sengelaub et al., 1989).

Androgens prevent perinatal apoptosis of SNB motoneurons not by acting directly on them, but by acting on their target BC/LA muscles. Supporting this notion, local treatment of the BC/LA musculature with the androgen antagonist hydroxyflutamide interferes with androgen-mediated sparing of SNB motoneurons from apoptosis in perinatal development more than an equal dose delivered systemically (Fishman and Breedlove, 1992). Furthermore, while both adult SNB motoneurons and BC/LA muscles express AR at high levels, only the BC/LA complex expresses AR protein by birth while SNB motoneurons do not appear to possess AR until after motoneuron death has ended (Fishman et al., 1990; Jordan et al., 1991, 1997). Thus, the BC/LA muscles, and not SNB motoneurons, are positioned to respond directly to androgens during the period of cell death. Additional evidence that androgens act on the BC/LA muscles comes from a mosaic rat model in which approximately half of the motoneurons express normal AR and half express the nonfunctional tfm allele of the receptor. In this model, perinatal androgen treatment rescued SNB motoneurons from death independent of whether they expressed a functional AR or the nonfunctional tfm mutation (Freeman et al., 1996). While androgens do not act directly on SNB motoneurons to ensure their survival, it is less clear what cell type critically mediates the androgen-regulated survival of the SNB system. Androgens can act directly on the muscle to keep it alive independent of the motoneurons (Fishman et al., 1990). However, relatively recent data based on a transgenic mutant male rat model in which functional ARs are expressed only in skeletal muscle fibers, suggest that ARs in muscle fibers per se are not sufficient to prevent the system from dying (Niel et al., 2009). While these data could be interpreted as indicating that muscle fibers are not the target of androgen action, it is also possible that in addition to muscle fibers, androgens may act on other cell populations in muscle to maintain the SNB
system during perinatal development. Other candidate cell populations in skeletal muscle are fibroblasts and endothelial cells (Jordan et al., 2002; Monks et al., 2004). In any case, it seems likely that muscle-derived neurotrophic factors mediate the androgenic rescue of SNB motoneurons during development. Indeed, blocking TrkB receptor locally in the developing BC/LA muscles reduces the number of SNB motoneurons that survive (Xu et al., 2001), suggesting that muscle-derived BDNF normally plays a role in the retrograde signaling that keeps SNB motoneurons alive. Again, because the neurotrophins NT 4/5 share the TrkB receptor, more research is required to resolve which neurotrophin, or combination of neurotrophins, mediate androgens' sparing effect on SNB motoneurons during development.

In adulthood, androgens continue to maintain normal SNB system function in rats and mice, as castration of adults leads to a reduction in the size of SNB somata and BC/LA muscle fibers and a retraction of the extensive dendritic arbors of the motoneurons. Androgen replacement prevents these regressive effects if provided immediately after castration, and can reverse them if provided 1–2 months after castration (Breedlove and Arnold, 1981; Forger and Breedlove, 1986; Kurz et al., 1986; Wee and Clemens, 1987; Goldstein et al., 1990; Forger et al., 1992; Rand and Breedlove, 1995; Watson et al., 2001; Zuloaga et al., 2007). The BC/LA complex also seems to mediate at least some of the effects of androgen on SNB morphology in adulthood. For example, castration-induced dendritic retraction in SNB motoneurons can be reversed by local treatment of the BC/LA muscle complex with androgens. In contrast, local administration of an anti-androgen to the muscles leads to significant decreases in the dendritic arborization of the SNB motoneurons innervating them (Rand and Breedlove, 1995). As described below, there is accumulating evidence that androgens and BDNF may act synergistically to maintain the morphology of SNB motoneuron in adulthood.

The role of BDNF in the recovery of AR expression in axotomized SNB motoneurons

The first studies linking BDNF signaling to androgen action on the SNB system was in axotomized motoneurons. For neuromuscular systems in general, loss of contact with their target musculature by peripheral axotomy leads to a number of physiological and morphological changes in motoneurons (Grafstein, 1978; Semagor et al., 1986; Titmus and Faber, 1990; Bisby and Tetzlaff, 1992; Brannstrom et al., 1992a). For example, peripheral axotomy of motor axons causes the dendrites of the affected motoneurons to regress (Brannstrom et al., 1992a; O’Hanlon and Lowrie, 1995). SNB motoneurons are also affected by axotomy in a similar fashion, causing both cell bodies and dendrites to atrophy (Yang and Arnold, 2000b; Yang et al., 2004). Axotomy also leads to a significant reduction in AR expression in SNB motoneurons and in their ability to accumulate T and metabolites (Al-Shamma and Arnold, 1995; Yang and Arnold, 2000a), implicating the BC/LA target muscles as a source of regulatory factors that influence the androgen sensitivity of SNB motoneurons. This notion is supported by studies demonstrating that many of the atrophic effects of axotomy on motoneuronal dendrites and somata are alleviated following reinnervation of the target muscles (Sumner and Watson, 1971; Bisby and Tetzlaff, 1992; Brannstrom et al., 1992b; O’Hanlon and Lowrie, 1995; Bodo and Rissman, 2008).

Al-Shamma and Arnold (1997) sought to identify the factor(s) produced by BC/LA muscles that influence AR expression in SNB motoneurons. In an elegant series of experiments, they delineated a number of synergistic androgen- and BDNF-dependent properties of SNB motoneurons and BC/LA target muscles. First, the authors discovered that disruption of retrograde transport from BC/LA muscles to SNB motoneurons using vinblastine reduced AR immunoreactivity in the neural soma, while tetrodotoxin-induced paralysis of the musculature had no effect. Systemically administered

Fig. 1. Sections from the lumbar segment of the rat spinal cord. Thionin-stained transverse sections from (a) male and (b) female fifth lumbar segments, respectively. The arrow in (a) points to the left spinal nucleus of the bulbocavernosus (SNB) of the male. Note the virtual absence of the SNB in the female spinal cord. Scale bar = 400 μm. Reproduced from Breedlove and Arnold (1980).
vinblastine also inhibits orthograde transport in neurons, but because target BC/LA musculature was injected in this experiment, presumably only retrograde transport by SNB motoneurons was disrupted. These results suggest that BC/LA muscles supply some retrogradely transported trophic factor(s) to SNB motoneurons to maintain normal AR expression, and that muscle activity per se did not have a role. Next, the authors sought to identify potential trophic factors that might be retrogradely provided to SNB motoneurons to influence their expression of AR. Several potential neurotrophic factors were applied to axotomized SNB motoneurons, including ciliary neurotrophic factor (CNTF), glial-derived neurotrophic factor (GDNF), neurotrophin-4 (NT-4), and BDNF. Only BDNF application to severed axons was able to reverse the effects of axotomy on AR expression in SNB motoneurons, while GDNF exacerbated the reduction in AR expression (Al-Shamma and Arnold, 1997). Later studies demonstrated a distinct dose–response effect of BDNF administration to severed SNB motoneuron axons on the recovery of AR expression (Yang and Arnold, 2000a).

Initial studies describing the ability of BDNF to reverse the effects of axotomy on AR expression in SNB motoneurons were performed on gonadally intact rats (Al-Shamma and Arnold, 1997; Yang and Arnold, 2000a). Like axotomy, castration leads to a significant decrease in AR protein expression and androgen accumulation in intact SNB motoneurons, and this response can be reversed by steroid replacement (Breedlove and Arnold, 1980, 1983b; Freeman et al., 1995; Matsumoto et al., 1996; Jordan et al., 1997). Thus, questions remained as to whether androgens might also play a role in regulating BDNF-mediated recovery of AR expression in SNB motoneurons following axotomy. A rat model that incorporated both castration and axotomy revealed a more complex relationship between androgens and BDNF in the maintenance of AR expression (Yang and Arnold, 2000b). In this study, BDNF treatment of axotomized SNB axons in castrated animals, by itself, did not rescue AR expression in SNB motoneuron soma. Importantly, AR expression in axotomized SNB motoneurons of castrated rats only recovered to control levels if they received both T-replacement and BDNF treatment. In other words, AR expression in SNB motoneurons seems to respond to T only if they are provided BDNF, either exogenously supplied in the case of axotomized SNB cells, or via connection to their target musculature. Thus, it is possible that SNB motoneurons normally receive BDNF from their target muscles, which allows them to respond to T by boosting their expression of AR. AR expression in SNB motoneurons of T-treated, axotomized animals not receiving BDNF recovered somewhat, but did not reach the levels achieved when both T and BDNF were provided (Fig. 2). Thus, BDNF-mediated recovery of AR expression in SNB motoneurons is dependent on the presence of androgens, whereas BDNF enhances, but is not absolutely necessary, for the androgen-induced expression of the receptor.

Clues about the specific mechanisms by which androgens and BDNF synergize to maximize AR expression in SNB motoneurons is suggested by evidence that nuclear localization of AR in SNB motoneurons is differentially affected by castration and axotomy (Yang and Arnold, 2000b). Castration drastically reduces nuclear localization of AR immunoreactivity in SNB motoneurons. T-replacement restores nuclear protein in intact SNB motoneurons, or axotomized SNB motoneurons if they are provided with exogenous BDNF. Without BDNF, nuclear AR remains low in axotomized SNB motoneurons of T-treated castrates. The obvious implication of these data is that BDNF may have a critical role in the transcriptional activity of AR in SNB motoneurons.

Multiple androgen response elements (AREs) are located in the upstream regulatory region of the AR gene and are involved in androgen-dependent upregulation of receptor mRNA (Grad et al., 1999). Thus, initial increased AR immunoreactivity in axotomized SNB motoneurons in castrated animals given T may be simply due to autoregulation of the AR gene via the induction of transcriptional activity by the AR receptor itself. However, maximal levels of nuclear AR, comparable to that seen in intact SNB motoneurons in normal males, may require multiple downstream effectors of BDNF signaling. BDNF activation of the associated TrkB receptor induces a number of signal transduction cascades, including the phospholipase C gamma (PLCγ) – Ca²⁺/calmodulin-dependent kinase II (CaMKII) pathway, the phosphoinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway, and the Raf/MEK/ERK (mitogen-activation kinase) pathway. Continuous stimulation of the Raf/MEK/ERK pathway can enhance and sustain ARE-inducible gene transcription (Carey et al., 2007) via the regulation of AR coactivators such as cAMP response element binding protein (CREB), steroid receptor coactivator-1 (SRC1), and p300 (Rowan et al., 2000; Debes et al., 2002; Unni et al., 2004). Perhaps this same pathway is induced by BDNF in SNB motoneurons (Fig. 3).

Interestingly, not only BDNF, but also androgens themselves have also been shown to rapidly and transiently activate the Raf/MEK/ERK pathway in an AR-dependent manner (Nguyen et al., 2005). In this model, synergistic AR- and BDNF-induced transcription would upregulate several target genes, including AR itself. Thus, it seems that while androgens are necessary for high levels of AR expression in SNB motoneurons, BDNF signaling likely also plays a role. Nuclear localization of AR in SNB motoneurons depends on the presence of androgens and also on their connections to target muscles, which may supply BDNF as a critical regulator (Yang and Arnold, 2000b).

Overlapping signaling pathways between androgen and BDNF may explain this phenomenon as well. For example, the AR protein contains several phosphorylation sites that affect its localization and transcriptional activity (Gioeli et al., 2002; Wong et al., 2004). Specifically, dephosphorylation of Ser-650 of the AR protein by protein phosphatase 1 (PP1) significantly

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increases the nuclear localization of the receptor in cell culture (Chen et al., 2009). Protein kinase C-alpha (PKCα), a potential downstream effector of BDNF/TrkB signaling, enhances PP1 activity in cardiac cells (Braz et al., 2004; Nicolaou and Kranias, 2009). Future studies could test this model (Fig. 4), by asking whether
BDNF-induced PKC activity enhances Ser-650 dephosphorylation and nuclear localization in SNB motoneurons. Other signal transduction cascades mediated by BDNF/TrkB signaling may also influence AR trafficking in SNB motoneurons. For example, in prostate cancer cells, calmodulin (CaM) is known to form complexes with AR (Cifuentes et al., 2004) and, in turn, recruits kinases such as the BDNF/TrkB effector Akt, which phosphorylates and influences its stability and nuclear localization (Lin et al., 2001; Sun et al., 2003; Reddy et al., 2006).

The interaction of androgens and BDNF in the regulation of SNB motoneuron morphology

BDNF from target BC/LA musculature is implicated in morphological changes in SNB motoneurons following castration and axotomy. For example, BDNF treatment of severed axons maintains the size of SNB somata in both castrated and gonadally intact animals, but androgens alone do not (Yang and Arnold, 2000b). These data indicate that the mechanisms mediating the trophic effects of androgens and BDNF on SNB soma size are partially separate. However, at least some overlap of signal transduction pathways of BDNF and androgens seems likely given the interdependence of BDNF and androgens in restoring nuclear AR in axotomized SNB cells (Yang and Arnold, 2000b; see previous section). Consequently, target-derived BDNF may enhance AR-mediated gene expression by maximizing nuclear localization of the receptor (Fig. 4), providing a greater trophic effect on SNB soma size.

This notion is supported by studies that indicate skeletal muscle expresses both BDNF mRNA and protein (Koliatsos et al., 1993; Kust et al., 2002; Saka et al., 2007; Matthews et al., 2009; Garcia et al., 2010; Ogborn and Gardiner, 2010).

The dendritic arbor of axotomized SNB motoneurons shows similar dependencies on nuclear AR, requiring both systemic androgens and BDNF to cut axons for full maintenance (Yang et al., 2004). The idea that muscles may supply a trophic factor to maintain SNB dendrites was first suggested by studies locally treating SNB target muscles with androgens and showing that this caused dendrites to grow compared to anti-androgen application to the muscles (Rand and Breedlove, 1995). However, the signaling mechanisms and precise role that androgens and BDNF play in the trophic maintenance of SNB dendrites are not well understood.

At the molecular level, loss of androgens after 2 weeks of castration led to a significant decrease in mRNA for both BDNF and its TrkB receptor in SNB motoneurons, but not in neighboring motoneurons in the sexually monomorphic retrodorsolateral nucleus (RDLN; Ottem et al., 2007). Castration also reduced the amount of BDNF protein in the proximal dendrites of SNB motoneurons (Ottem et al., 2007). In agreement with these studies, others found significantly reduced TrkB immunoreactivity in SNB motoneurons 3 weeks after castration (Osborne et al., 2007). Furthermore, TrkB immunoreactivity also decreased in the less androgen-responsive motoneurons innervating the quadriceps muscles at this time point (Osborne et al., 2007). It is possible that androgens regulate TrkB in all

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**Fig. 4.** Proposed mechanism by which BDNF increases nuclear localization of AR in SNB motoneurons. BDNF binding to the TrkB receptor leads to the activation of phospholipase c gamma (PLCγ) which liberates diacylglycerol (DAG), which then targets protein kinase c alpha (PKCa). A downstream effector of activated PKCa is protein phosphatase -1 (PP-1), which targets Serine-650 (Ser-650) of AR for dephosphorylation. Dephosphorylation at Ser-650 of AR has been shown to increase nuclear localization of the receptor. Free inositol triphosphate targets receptors of the endoplasmic reticulum, which leads to an efflux of Ca²⁺ into the cytoplasm, activating Ca²⁺/calmodulin-dependent kinase (Ca²⁺/CaM). Ca²⁺/CaM kinase can complex with AR and recruit activated AKT kinase. Phosphorylation of AR by AKT influences stability and nuclear localization of the receptor. AKT is also a downstream target of BDNF signaling via phosphoinositide 3-kinase (PI3K).
motoneuronal populations but that it takes longer to emerge in populations that express less AR than SNB motoneurons.

In the BDNF-containing dendrites of SNB motoneurons of gonadally intact rats, TrkB is concentrated at synapses, both postsynaptically in the dendrites and presynaptically in glutamatergic terminals (Ottem et al., 2007). Fig. 5 offers a hypothetical model for how this synaptic arrangement might act to maintain glutamatergic synapses (Tongiorgi et al., 1997, 2004; Righi et al., 2000; Matsumoto et al., 2001; Takei et al., 2004; Brigadski et al., 2005) and the physical structure of the arbor (Kojima et al., 2001; Takei et al., 2004). Thus, androgen deprivation downregulates dendritic BDNF protein and TrkB expression in SNB motoneurons, which may trigger dendritic retraction. Moreover, loss of BDNF from target musculature of SNB motoneurons may also underlie dendritic retraction following axotomy. Recall that local androgen treatment of BC/LA muscles can restore SNB dendritic arborization following castration (Rand and Breedlove, 1995), and that both BDNF treatment of severed axons and androgen replacement are required to restore the SNB dendritic arbor following axotomy and castration (Yang et al., 2004). Taken together, these studies suggest the tantalizing idea that androgens regulate the amount of BDNF protein in SNB motoneurons in two ways: by regulating BDNF gene expression in the motoneurons themselves, and also by regulating the expression of TrkB receptors needed to retrogradely transport muscle-derived BDNF to the cell bodies (Ottem et al., 2007). This proposition is also consistent with recent findings that castration significantly increases BDNF protein in BC muscles (Verhovshek et al., 2010). The plausible explanation put forward by this group is that a loss of androgens following castration may disrupt retrograde transport from BC muscles to SNB motoneurons, causing an accumulation of BDNF in the muscles. Such retrograde transport of BDNF is activity-dependent in other systems (Watson et al., 1999) and requires endocytosis of the BDNF–TrkB complex, which is then moved retrogradely in transport vesicles by dynein motor proteins (Watson et al., 1999; Bhattacharyya et al., 2002). Because castration decreases TrkB expression in SNB motoneurons (Osborne et al., 2007; Ottem et al., 2007) and leads to diminished neural activity (Holmes and Sachs, 1992; Osztályi et al., 2000).

Fig. 5. Potential BDNF signaling pathways for maintaining glutamatergic synapses on SNB dendrites. (Left) BDNF in SNB dendrites may be released and act in an autocrine fashion to activate TrkB receptors, which phosphorylate NMDA receptors and enhance excitatory the response to glutamate. (Middle) Prolonged BDNF–NMDA receptor signaling may increase recruitment and translation of postsynaptic density proteins, thereby strengthening synapses and dendritic integrity. (Right) BDNF in SNB dendrites may also act as a retrograde signal, binding TrkB receptors on afferent glutamatergic appositions, thereby activating presynaptic TrkB receptors to enhance glutamate release subsequently, which would also boost activity of postsynaptic glutamate receptors. Because TrkB protein is localized both in SNB dendrites and in their afferents, these potential pathways are not mutually exclusive.
Fargo et al., 2003), altered retrograde transport could lead to an accumulation of BDNF in BC muscles and reduced accumulation in SNB motoneurons. Supporting this idea is the finding that a polyglutamine expanded AR linked to Kennedy’s disease disrupts retrograde transport in motoneuron axons (Katsuno et al., 2006; Kemp et al., 2011). This transport defect was associated with decreased gene expression of the dynemin binding protein, dynactin 1 (Katsuno et al., 2006), essential for efficient retrograde axonal transport (Deacon et al., 2003; Schroer, 2004). Future studies should determine whether castration decreases dynactin 1 expression in SNB motoneurons, which in addition to deficits in TrkB receptors might also contribute to the reduced retrograde transport of BDNF protein from BC/LA muscles. A second, likely parallel, source of BDNF loss in SNB motoneurons following castration is at the level of gene expression in the motoneurons themselves. Following castration, BDNF protein is significantly decreased in SNB dendrites (Ottem et al., 2007). Androgen loss leads to a significant decrease in BDNF transcripts, specifically those containing the non-coding exon VI (Ottem et al., 2010), which we will discuss shortly. Thus, reduction of BDNF in SNB motoneurons after castration may arise from two separate consequences of androgen loss: a cell-autonomous reduction in BDNF expression and a loss of BDNF from the target muscles.

Non-coding exons of BDNF

The BDNF gene is unique in that it contains eight non-coding exons and a ninth coding exon (Aid et al., 2007). During transcription, a single non-coding exon (I–VIII) is spliced to exon IX (the coding exon), and many studies suggest that the specific arrangements of these non-coding exons dictates the cellular compartment to which the mRNA will be trafficked (Pattabiraman et al., 2005; Chiaruttini et al., 2008; Tongiorgi, 2008; Baj et al., 2011). The “spatial code” provided by the non-coding exons is emerging in recent studies where BDNF transcripts containing non-coding exons I and IV tend to be restricted to the soma (Pattabiraman et al., 2005; Chiaruttini et al., 2008; Tongiorgi, 2008; Baj et al., 2011), while BDNF transcripts containing non-coding exons II and VI are trafficked to dendrites in an activity-dependent manner (Pattabiraman et al., 2005; Chiaruttini et al., 2008; Tongiorgi, 2008; Baj et al., 2011) where their translation leads to spatially restricted, local activation of TrkB receptors (Baj et al., 2011). Strikingly, the loss of BDNF protein from SNB dendrites following castration (Ottem et al., 2007) coincides with a significant decrease in mRNA for exon VI (Ottem et al., 2010), the exon which directs the transcript to dendrites in other systems. Reduced dendritic BDNF protein in SNB neurons due to decreased dendritic-directed transcripts (Ottem et al., 2010), combined with reduced retrograde transport of mature BDNF protein from BC target muscles (Verhovshek and Sengelaub, 2010), could explain the drastic decline in TrkB activation, and therefore length of SNB dendrites, following castration.

Multiple sources of BDNF and multiple signal transduction cascades

More recent evidence calls into question the specific roles of BDNF signaling at its receptor and the trophic interaction of androgen-dependent pathways in the maintenance of SNB somata and dendrites. Systemic treatment of castrated rats with a TrkB receptor antibody averts the reduction of both soma size and dendritic arbor in SNB motoneurons (Verhovshek and Sengelaub, 2010), suggesting that BDNF activity normally drives regression of SNB motoneurons. Systemic treatment with the TrkB antibody also significantly increased the length of SNB dendrites in gonadally intact animals (Verhovshek and Sengelaub, 2010). That local treatment of these perineal (genital region) muscles with a TrkB antibody, but not systemic treatment, blocks the rescue of SNB motoneurons in androgenized females (Xu et al., 2001) suggests the reagent is acting upon the target muscles. While both observations suggest that target-derived BDNF is functionally significant to SNB neurons, they differ in terms of whether the BDNF promotes maintenance or regression of dendrites.

Clues to a potential resolution to the dichotomy come from studies of cultured hippocampal neurons. In these neurons, BDNF signaling at the synapse induces rapid endocytosis and retrograde axonal transport of the neurotrophin–TrkB receptor complex to the cell body of the presynaptic neuron (Heerssen et al., 2004). In a seminal study, pharmacological inhibition of endocytosis blocked BDNF-induced internalization of the neurotrophin–TrkB receptor complex (Zheng et al., 2008). Blocked endocytosis inhibited the neurotrophin-activated PI3K/Akt pathway in the presynaptic neuron specifically, without affecting other BDNF-induced systems, including the MEK/ERK and PLCγ2 pathways, or associated immediate early gene activation. Thus, local application of TrkB antibodies to BC/LA muscles may alter the balance of pathways normally stimulated by BDNF, and may not reflect the overall impact of BDNF that is normally supplied by target muscles and the motoneurons themselves. It is also possible that the antibodies to TrkB triggered a compensatory response in the motoneurons themselves to boost BDNF. In that case, the increased SNB dendrites and somata following local TrkB antibody treatment may have resulted from a net increase in available BDNF. In short, there is ample evidence that androgens tap BDNF-signaling systems to regulate not only the development of the SNB system, but also the plasticity of the system in maturity.

ANDROGENS, BDNF, AND SONG DEVELOPMENT IN SONGBIRDS

Another model system provides insight into the synergistic signaling between androgens and BDNF. Songbirds are excellent models for identifying the endocrinological mechanisms that underlie neural circuit formation and maintenance, with recent attention focused on locally-produced steroids and their non-
genomic actions (for review, see Saldanha et al., 2011). In close-ended song learners like the zebra finch (*Taeniopygia guttata*), song behavior is regulated by a highly sexually dimorphic network of interconnected nuclei. The vocal control nuclei HVC, the robust nucleus of the arcopallium (RA) and its target, and the caudal portion of the motor nucleus of the hypoglossal nerve, specifically the tracheosyringeal portion (nXIIIts, whose cells innervate muscles of the syrinx), are much larger in males than females (Nottebohm and Arnold, 1976). Syrinx volume is greater in males than females (Nottebohm and Arnold, 1976), as is the size of the muscle fibers (Wade and Buhlman, 2000). From post-hatch day 30 (d30) to approximately d65, young male zebra finches learn their song from a male tutor and rehearse it until they can perform a stable version that will attract females (who normally do not sing) during courtship, which begins at about d100 (for review, see Nordeen and Nordeen, 1997). During this sensitive period, the nuclei of the song control circuit increase in neuronal size and number, and axonal projections begin to form between them (Akutagawa and Konishi, 1994; Mooney and Rao, 1994). While steroid hormones do not appear to control the sexual differentiation of these regions in zebra finches in the same way that mammalian differences develop (Wade, 1999), without question androgens contribute to the development of a fully masculine song system. More neurons are observed in RA in adult females treated with DHT during development (Gurney, 1982), and treatment of juvenile males with a 5α-reductase inhibitor results in a decrease in RA neuron number (Grisham and Arnold, 1995). Additionally, chronic exposure to T during song learning severely disrupts song acquisition (Korsia and Bottjer, 1991). Androgens may therefore be responsible for closing the critical period by eliminating the plasticity of the song control circuits (Marler, 1984). In the brainstem of zebra finches, motoneurons of nXIIIts contain androgen receptors (Arnold et al., 1976; Arnold and Saltiel, 1979), and the nucleus grows by about a third in females given T in adulthood (Gurney, 1982; DeVoogd et al., 1991). Muscles of the syrinx also increase in total mass and fiber size in T-treated females (Wade and Buhlman, 2000). Interestingly, there is no sex difference in AR mRNA early in development (d3, d10, d17) in the syrinx musculature (Veney and Wade, 2005), but it becomes sexually dimorphic (male dominant) by adulthood (Veney and Wade, 2004). In a seasonally breeding bird (an open-ended learner that can modify its song from year to year), the Gambel’s white-crowned sparrow, systemic administration of T increases the volume of nXIIIts, which occurs even when the ipsilateral HVC is lesioned and when a T pellet is placed on or near HVC (Brenowitz and Lent, 2002). Thus, as previously hypothesized (Brenowitz and Lent, 2002), cells of nXIIIts may receive retrograde support from the muscles these neurons innervate, in a manner not unlike that indicated in the hypothetical model of an axo-dendritic synapse in Fig. 5.

Numerous studies have attempted to determine the steroid-induced mechanisms responsible for the development and maintenance of these sexually dimorphic regions. Neurotrophins are an obvious candidate, and these proteins and their receptors have been found in specific nuclei in the song circuit and are differentially expressed between juvenile and adult stages of development. BDNF has received considerable attention in avian systems in the regulation of neuronal survival and differentiation, and appears to play a significant role in the genesis and maintenance of neurons or circuits. BDNF immunoprotein is detectable in the male canary HVC but little to none is detected in the same region in females (Rasika et al., 1999). In zebra finches, BDNF immunoreactivity is widespread throughout many different areas (Akutagawa and Konishi, 1998). HVC and its surrounding tissue, but not RA, stood out in both the number and density of BDNF-labeled cells. In contrast to d20 birds, there was a sharp increase in BDNF staining in HVC of d45 males, and RA showed moderate BDNF labeling. At d65, few BDNF immunoreactive cells could be detected within HVC, but RA was strongly labeled. The brains of adult (>d90) birds showed low levels of BDNF immunoreactivity in both of the abovementioned song nuclei. Interestingly, no BDNF staining was identified in nXIIIts (Akutagawa and Konishi, 1998; Dittrich et al., 1999). The functional significance of this pattern of expression correlates with the behavioral changes during various periods in development and suggests that the initial BDNF expression in HVC promotes differentiation of the rest of the song circuit (Akutagawa and Konishi, 1994; Mooney and Rao, 1994). Because all song structures examined display BDNF immunoreactivity during the phase in which song begins to be crystallized (d65), which decreases to low levels in adulthood, this finding suggests a role for BDNF in song stabilization.

Potential sites of BDNF action, via TrkB, have been determined in a few studies. In the canary, both males and females express high levels of TrkB immunoreactivity in HVC (Rasika et al., 1999), even though females show little to no BDNF expression in that nucleus. In zebra finches, Wade (2000) mapped the distribution of TrkB immunoreactivity from d30 to d60. In males, TrkB immunoreactive cells were detected in HVC on d30, d45 and d60, and in females at d45 and d60. In RA, TrkB immunoreactive cells were present in d30, d45 and d60 males, but only at d60 in females. Cells expressing mRNA for TrkB were detected in HVC and RA in male and female zebra finches at d20 and d30, the only developmental time points analyzed (Dittrich et al., 1999).

A variety of studies involving biochemical and autoradiographic measures of sex steroid binding, immunohistochemical studies of steroid receptor protein, and analysis of steroid receptor mRNA expression have identified several structures in and around song nuclei that express steroid receptor protein or message (Balthazart et al., 1992; Gahr et al., 1993; Jacobs et al., 1996), resulting in the song circuit being highly steroid-sensitive. Male brains express more AR than those of females by d9, and this sex difference in AR mRNA expression appears to develop independent of the
action of steroid hormones (Gahr and Metzdorf, 1999), as HVC slices in vitro express AR mRNA in a sex-specific manner. The volume of HVC, as determined by levels of AR mRNA to define its boundaries, increases significantly at d20, and becomes roughly 5× greater in adult males than females.

While there are similarities in the time course of expression of AR and BDNF/TrkB, no study to date has examined expression in the same animals at the same time points, or ideally examined the co-localization of these proteins within the same cells. However, several studies, especially in songbirds that breed seasonally, indicate similar effects of T or BDNF injections, and/or that androgenic effects in the song circuit are mediated by BDNF. Much like the effects of increased T on song stability in zebra finches indicated above, infusions of BDNF in the RA of adults result in song characteristics typical of juveniles and induces an increase in the density of synapses within the region (Kittelberger and Mooney, 2005). In canaries, HVC continues to receive new neurons in adulthood, with a majority of these cells projecting to RA (Alvarez-Buylla and Nottebohm, 1988); the cells that are born in fall before the next breeding season survive in significantly greater amounts than those born in the spring during it (Nottebohm et al., 1994). BDNF infusions into the adult male HVC 2–3 weeks after the peak in spring neurogenesis promote the survival of those neurons for at least 8 months (Alvarez-Borda et al., 2004). In females, T increases the size of HVC neurons, their survival, as well as the number that become RA-projecting (Rasika et al., 1999). T treatment also increases BDNF expression in the female HVC, triples the number of HVC neurons (an effect blocked by concurrent delivery of an antibody to BDNF), and increases the level of BDNF protein in HVC (Rasika et al., 1999). Also in canaries, Li et al. (2000) showed that singing males had three times more BDNF mRNA expression in HVC than did non-singing birds, and a strong correlation was found between the number of songs produced and BDNF expression. In other words, the more songs a bird sang, the higher the levels of BDNF in HVC. In addition, the number of bromodeoxyuridine (BrdU)-labeled neurons in the HVC of singing birds was significantly higher than that of the non-singing birds (Li et al., 2000). Comparably, in seasonally-breeding white-crowned sparrows, BDNF mRNA in HVC is upregulated by the long days typical of spring, while levels of TrkB in the nucleus did not differ across season (Wissman and Brenowitz, 2009). Further, BDNF infusions into RA of white-crowned sparrows during the nonbreeding season stimulated breeding-season-like morphology, whereas interfering with BDNF binding reduced those changes (Wissman and Brenowitz, 2009). In canaries, levels of vascular endothelial growth factor (VEGF) and its endothelial receptor tyrosine kinase in HVC in females are increased by T treatment, followed a week later by an increase in BDNF mRNA (mostly in endothelial tissue) in HVC. What is more, inhibition of the receptor decreases neurogenesis and angiogenesis within the region (Louissaint et al., 2002). Systemic inhibition of VEGF is sufficient to block T-induced singing in females, an effect that can be reversed by in vivo transfection of BDNF into HVC, which also resulted in new neuron incorporation into the region (Hartog et al., 2009).

Additional work suggests that these effects of T are due to its aromatization to estradiol. BDNF mRNA expression in the HVC of juvenile birds can be induced shortly (24 h) following systemic estradiol treatment, and the expression is higher in d20–25 birds treated with estradiol than control birds. This BDNF expression was downregulated by up to 90% following treatment with the aromatase inhibitor fadrozole (Dittrich et al., 1999). However, because HVC and RA decrease in volume in castrated song sparrows, an effect prevented by either androgens (T or DHT), estradiol, or a combination of both (DHT plus estradiol), the seasonal increase in the volume of song nuclei may also be mediated, at least in part, by androgenic metabolites (Tramontin et al., 2003).

Overall, our understanding of the interactions of BDNF and androgens in the development of song and the song system is largely incomplete. The time course of neurotrophin expression, and the effect of androgens on that expression, are very suggestive, however. Because BDNF and its high-affinity receptor are expressed in song nuclei at key points in development, and can be modulated by the presence or absence of gonadal hormones, it seems likely that BDNF may mediate some of androgens’ effects, playing a crucial role in differentiation of the song control circuit and song learning and memory. How this may occur has been suggested by several studies in birds, particularly those examining the variety of proteins regulated by song exposure (Pinaud et al., 2008), which detail the experience-dependent modifications in the proteome of cells in steroid-sensitive regions, events that overlap strongly with those in the mechanism proposed in Fig. 3. Given their need for learning song during a critical period in development (close-ended learners), or their ability to modify learned song across seasons (open-ended learners), songbirds serve as “ecologically valid” models to address the roles and interactions of steroid hormones and neurotrophins: song is highly selected for over evolution as its expression is critical for reproductive success. The well-defined brain regions and neuromuscular circuit underlying song, and the ease with which its stereotyped behavioral output can be manipulated and measured in the laboratory, make songbirds an excellent model system for determining the biochemical changes resulting from androgen–BDNF interactions, and how these promote the development and maintenance of song behavior.

CONCLUSIONS

One important conclusion these studies of androgens and BDNF in sexually dimorphic systems offers is the concept that the same hormone, and the same neurotrophic factor, can be interacting at more than one level of a neural system. Both SNB motoneurons and their target muscles possess ARs, and mRNA for BDNF, and in
both sites androgen manipulation affects the neurotrophin. What is more, there is evidence that the two factors interact in both tissues to affect the structure, and therefore presumably the function, of the system. While the details of how the two factors interact differ in the two tissues, they work toward the same goal of masculinizing the system, at least structurally. There are other AR-containing neurons in the same spinal segments as the SNB, and for all we know androgens interact with BDNF in those cells, too, to regulate SNB structure or function. However, it is now empirically clear that BDNF interacts with the AR-positive motoneurons of the SNB system. What is lacking, at present, is some method to dissociate such effects of spinal interneurons.

It may well be that this aspect of the system, responding to steroids and neurotrophins in several different parts of the circuit, is not found solely in the SNB system and birdsong system, but is present in other steroid-sensitive systems as well. If so, then that considerably complicates scientists' efforts to tease apart these molecular mechanisms. Perhaps interactions of ARs and BDNF at several different levels of the birdsong system are hindering our efforts to get a clear view of those mechanisms in songbirds. One way to parse these various actions of the two factors in different levels of a neural system is to use genetic tools to selectively eliminate one signaling mechanism or the other in particular tissues, and/or at particular times in development, to see how that affects the system and the behavior mediated by it. We have reviewed a few examples of that approach in the SNB system above, and it seems likely that more such work will arise in the coming years.

Another lesson is that natural selection appears to have hit upon steroidal regulation of BDNF systems to regulate neural systems several times, as it seems likely that SNB motoneurons in mammals have diverged considerably from neurons controlling birdsong. Presumably all vertebrate neurons have the capacity to evolve steroid-sensitive systems to regulate BDNF signaling. If so, then perhaps these two factors interact to regulate many different hormone-sensitive behaviors in many different species. Doubtless there are other neurotrophic factors at play in the two systems reviewed here, to say nothing of other systems in other species. At least a part of our understanding of the role of BDNF is due to the availability of tools and reagents to study this neurotrophin. We have been looking where the light is. As our capacity to monitor and regulate other neurotrophic factors grows, we will likely find additional mechanisms at work, fine tuning the reproductive behaviors mediated by these and other systems. To be sure, the interaction of androgen and BDNF signaling in the development and maintenance of sexually dimorphic neural systems remains a topic with more questions than answers.

REFERENCES


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