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Commentary

Muscle, a conduit to brain for hormonal control of behavior

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ABSTRACT

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Investigation into mechanisms whereby hormones control behavior often starts with actions on central nervous system (CNS) motivation and motor systems and is followed by assessment of CNS drive of coordinated striated muscle contractions. Here we turn this perspective on its head by discussing ways in which hormones might first act on muscle that then secondarily drive upstream the evolution and function of the CNS. While there is a lengthy history for consideration of this perspective, newly discovered properties of muscle signaling reveal novel mechanisms that may well be captured by endocrine systems and thus of interest to behavioral endocrinologists.

1. Introduction

The vast majority of us who call ourselves behavioral endocrinologists often focus our research on the central nervous system (CNS), especially the brain. This might involve the way sensory and cognitive forces act via the brain to influence pituitary-based axes and the hormones they produce or it involves the way the CNS responds to hormones to influence the processing of sensory information, the motivation to behave, and the motor outputs whose patterning forms the basis of the hormone-dependent behavior.

Of course, a complete understanding of behavior requires an appreciation of the ways that neural circuits control striated muscles that, by their appropriate coordinated contractions, produce all the requisite postural, limb and accessory movements that together form true behavior. For behavior to arise then, selection must minimally shape the neural motor pathways, select skeletal muscles and their fibers and the proprioceptive sensory feedback circuits, from muscle to brain. Although we have been aware for many years that muscles themselves are targets of hormonal action, there is increasing evidence that muscles exert unique forms of control over the CNS that impact behavior. It may well be worth a second look at the striated muscles themselves and how they may modify the CNS in a retrograde or “muscle-up” fashion.

Here we present some lines of research that point to striated muscles as primary sites for guiding evolution (ultimate causation) and

functions (proximate causation) of the CNS and behavioral performance. From the standpoint of hormonal action, we consider two cases that address ways in which androgens target striated muscles that in turn provide a basis, or potential basis, to drive upstream neural control systems and ultimately behavioral performance. First, we describe evidence for selection acting on androgen receptor (AR) expression in muscle as directly guiding the physical complexity underlying the performance of male courtship displays (ultimate causation). Second, we describe evidence from the well-studied spinal nucleus of the bulbocavernosus (SNB) system of rodents whereby androgen actions on muscles per se guide the development of upstream neural systems controlling muscle function (proximate causation).

By what mechanisms might hormones act on muscle to impact the brain? Whereas some mechanisms have been well-described, there are a surprising number of novel ways that muscle use or modified muscle gene expression promotes or modifies CNS function. Because we see this as an exciting emerging field, and one worthy of attention by behavioral endocrinologists, we focus the next section of this article describing some muscle-specific states that drive the CNS and several possible mechanisms that might be captured and utilized by hormones to influence behavior retrogradely. We start this section with a description of new evidence for how modified gene expression in skeletal muscle alone serves to regulate sleep-wake cycles. We conclude with a discussion of several newly identified pathways whereby muscles signal

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to the CNS to influence diverse brain states.

2. Hormonal control of male manakin courtship

We have considerable evidence that selection targeting the expression of androgen receptor (AR) in skeletal muscle is a pioneering event in the evolution of hormone-dependent courtship displays by at least some male vertebrates. Male golden-collared manakins (*Manacus vittellinus*) perform a courtship display that includes significant motor output including lengthy jumps created by contractions of muscles controlling the lower extremities, to powerful wing- and roll-snaps involving specialized contractions and coordinated movements of the upper extremities, all of which are combined with various postural movements and positioning (Schlinger et al., 2013). Many of the bird's individual movements are made at great speed. In most males, the movements are performed with exquisite precision, seen especially clearly when they are video recorded with ultra high-speed cameras (from 125 to 2000 frames per second) with images played back in slow motion (Barske et al., 2011).

This interesting bird belongs to a family of birds found in forests throughout the neotropics. Males of many of these species perform elaborate courtship displays as they engage in little to no parental care and can dedicate their time and energy to trying to attract females for copulations (Prum, 1990, 1994). Thus, physically intensive courtship displays are widespread in males of this clade. But they are not alone in this regard as other species from somewhat disparate bird groups also court females using interesting motor performances. Nevertheless, many of the manakins do stand out for their elaborate displays.

The underlying neuromuscular substrate for the performance of complex courtship displays is just beginning to be investigated and understood. In the case of the golden collared manakin, we know that one muscle in particular seems specialized for the bird's ability to make their loud wing- and roll-snaps. Wingsnaps are produced singly, in mid-air, as the males jump from sapling to sapling around their display courts. Roll-snaps are produced as a rapid sequence of individual snaps with up to 23 snaps produced in ~300 msces (Fuxjager et al., 2013). These snaps can be heard for considerable distance through the forest and are produced, in all likelihood, to attract females to the male leks (Chapman, 1935). Snaps are produced as the wings are lifted by the supracoracoideus muscles, and then retracted by the scapulohumeralis (SH) muscles (Fuxjager et al., 2017a). This latter SH muscle is unusually enlarged and modified in this manakin (Lowe, 1942; Schultz et al., 2001), and is specialized to contract in an exceptionally fast manner (Fuxjager et al., 2017b).

Androgen action is crucial for performance of male manakin courtship (Day et al., 2006; Chiver and Schlinger, 2017a, b) with actions on skeletal muscles required for appropriate behavioral output. Androgen receptor antagonists that interfere with AR peripherally produce substantial deficits in male courtship (Fuxjager et al., 2013). Manakin skeletal muscles express AR at high levels (Feng et al., 2010) and androgens promote extensive modification in the cohort of genes expressed in forelimb muscles, with significantly greater impact on the SH muscle than on the PEC (Fuxjager et al., 2016). Inhibition of AR-dependent gene expression and muscle function specifically reduces contractile properties of the SH that underlie the muscle's participation in the creation of wing- and roll-snaps (Fuxjager et al., 2017b).

Whereas these data point to a central role for muscle AR function in steering the production of courtship behavior, they do not address the role of the CNS itself. While we do know that AR are expressed in brain of golden collared manakin in places that likely house pre-motor neurons that, when activated, produce the complex and highly coordinated set of behaviors that make-up courtship (Fusani et al., 2014), we know little about the central actions of androgen per se in influencing behavior. Presumably there is significant specialization of the manakin neural circuitry required to produce the behavior we observe in nature. Despite these limitations in the scope of our knowledge about CNS

control of male manakin behavior, we do have evidence that AR expression in skeletal muscle might be the key to the evolution of elaborate male courtship.

Across several species of birds with varying degrees of physical courtship, levels of AR expression in skeletal muscles, and not in the spinal cord, directly predict the complexity levels of that courtship (Fuxjager et al., 2015). Expression levels of ER had no bearing on courtship in muscle or in spinal cord. Moreover, expression levels of AR are independent of circulating testosterone levels as well as sex and age of the manakins (Fuxjager et al., 2018). All of these data support the notion that selection, presumably a result of female mate choice (Barske et al., 2011), has driven the evolution of increasing levels of AR and AR-responsivity specifically in muscle to promote the profound changes in gene expression required to produce contractile and metabolic states needed to produce complex behavior (Fuxjager et al., 2018).

Of course, there are other explanations and more data is needed to confirm this conclusion. Nevertheless, it stands at this point as a viable possibility, and lends support for the overall notion that selection for advanced AR signaling occurs first in muscle, and the neural circuits that drive specific muscles and the patterns of their contractile states arises as a secondary phenomenon. Muscles that are adapted for modified contraction might themselves communicate to central neural circuits acutely, by retrograde signaling, such as with growth factors (see below) or by other signaling mechanisms. Muscles with modified contractile states driven by unmodified neural circuits might produce behavior that is attractive to females. Once these contractile states become a target of female selection, further alteration of neural circuitry might arise to drive those muscles in ways that maximally stimulate the females to promote matings.

The manakin system we describe is not the only example where evolution might first act on peripheral musculature. Skeletal muscle AR expression and function is well-established in a variety of model systems (e.g. Zornik and Kelley, 2011). Muscles that contract to produce the unique foot-waving behavior of some species of frogs might have evolved AR expression first for this behavior to arise for use as an aggressive/territorial signal (Mangiamele et al., 2016). Similarly, AR expression in forelimb muscles of anolis lizards may also predict muscle function in courtship displays (Johnson et al., 2018). While these examples illustrate potential mechanisms for hormonal capture of some complex behaviors, it is indeed hard to imagine, for example, that the broad neural modifications that arose in songbirds to promote song and song learning developed secondarily to heightened AR expression and function in syringeal muscles (Veney and Wade, 2004). Nevertheless, AR are expressed and functional in syringeal muscles of suboscine as well as oscine songbirds (Veney and Wade, 2004; Feng et al., 2010; Fuxjager et al., 2014). Thus, AR expression in these muscles is likely an important target of selection that may form the basis of aspects of the evolution of the neural song system required for production of oscine birdsong.

The forces of selection on AR expression we describe above, point to androgen activation of muscles states to produce behavior. It stands to reason that for hormones to act on muscles to direct, upstream, the proper neural circuitry to drive behavior, that hormone action might occur developmentally. The well-established neuromuscular system controlling the bulbocavernosus muscle of rodents illustrates clearly how hormones act developmentally on AR-dependent muscle to create appropriate neural circuitry.

3. The SNB system

The SNB system has held profound and sustained interest for those studying sexual differentiation of the nervous system (Sengelaub and Forger, 2008; Swift-Gallant and Monks, 2017). There are a number of reasons for this, not least of which being that the SNB is methodologically tractable for site of androgen action studies. Here we discuss 2 main features of the SNB that are relevant for the present discussion: 1)

sexually dimorphic aspects of the SNB are essentially modular, being extremely androgen dependent and functionally specialized and 2) the SNB has direct functional relevance for sexual selection and for sexually-dimorphic development of the CNS.

The SNB is a neuromuscular system that mediates genital responses including intense erections and ejaculation (see Hull and Meisel, 2002 for review). Although the SNB system is profoundly sexually dimorphic in all mammals studied to date with the exception of the naked mole rat (Seney et al., 2006), our discussion will focus on the SNB of mice and rats, in which we have a more thorough mechanistic understanding of its function and sexual differentiation.

SNB motoneurons form 2 distinct pools that co-mingle in a dorso-medial position in the 2 posterior lumbar segments of the spinal cord (McKenna and Nadelhaft, 1986). One of these pools innervates the *bulbocavernosus* and *levator ani* (BCLA) muscles, whereas the other pool innervates the external anal sphincter (EAS). None of these striated muscles attach to the skeleton. The EAS resides at the terminus of the gut and regulates defecation. The *bulbocavernosus* muscle surrounds and attaches to the base of the phallus and *levator ani* muscle attaches to this structure and encircles the rectum. Despite the suggestive anatomy and name, the *levator ani* muscle does not have a discernable role in excretion (Holmes and Sachs, 1994), but rather in males the BCLA muscles work together to increase pressure within the primarily *corpus spongiosum* tissues in the base of the penis which mediates ejaculation of semen and causes intense engorgement of the glans. In rats and mice contraction of the BCLA muscles that occurs during penile erection results in the glans acquiring a cup-like shape. These functions of the BCLA are critical for fertility in mice and rats as surgical excision of these muscles impairs male fertility and is associated with loss of intense erections and inefficient deposition and removal of seminal plugs (Sachs, 1982; Wallach and Hart, 1983; Elmore and Sachs, 1988). Despite the clear functional specialization of the BCLA and EAS motoneurons, it is unclear the degree to which they may contract independently.

There are 2 major upstream “modules” that drive the sexually dimorphic elements of the SNB system. These include 1) multi-segment spinal neural networks that coordinate autonomic tone in the erectile tissues of the phallus with genital sensation and perineal muscle contraction and 2) supra-spinal afferents that mediate contextual control of genital arousal (reviewed in Hull and Meisel, 2002). In males, these modules can be studied in a dissociated manner either using distal cues that trigger erections (e.g. the non-contact erection paradigm) or using lesions that disrupt supra-spinal afferents (including those to SNB motoneurons). Complete thoracic spinal cord transection, which removes the supra-spinal afferents but maintains the spinal module, greatly facilitates reflexive erections triggered by retraction of the prepuce (Hart, 1967). These observations lead both to the conclusion that the spinal module is sufficient for reflexive erections typical of neurally intact animals, and also to the conclusion that supra-spinal afferents exert primarily inhibitory control on genital arousal. More discrete brain lesions can also facilitate reflexive erections in rats (Monaghan et al., 1993). These supra-spinal afferents are thought to regulate the correct contextual expression of erection, for example by facilitating genital arousal when estrous odors are present. The SNB system is then engaged reflexively when genital stimulation occurs in the context of copulation.

Mirroring the functional specialization of BCLA and EAS motoneurons is a distinct degree of androgen sensitivity and response. Motoneurons innervating the BCLA are dramatically sexually dimorphic, express high levels of AR (Jordan, 1997) and are androgen dependent in many morphological and functional respects. In contrast, motoneurons that innervate the EAS are sexually monomorphic, express low levels of AR (Jordan, 1997) and are not androgen dependent. Several lines of evidence support the notion that androgen responsiveness and sexual dimorphism originate primarily from androgen action on the target musculature as opposed to being an intrinsic

property of the SNB motoneurons (reviewed in Monks and Holmes, 2018; Swift-Gallant and Monks, 2017). Nonetheless, neural AR has also been implicated in mediating several morphological androgenic responses (e.g. Watson et al., 2001; Raskin et al., 2012).

Importantly, experiments using genetic or pharmacological manipulation of AR in skeletal muscle have revealed a primary muscular site of action in androgens determining dendritic extent (Rand and Breedlove, 1995; Huguenard et al., 2011). It is worth noting, however, that evidence for neural AR contributing to the dendritic extent of SNB neurons also exists, as mice with conditional deletion of AR in neurons due to Nestin-Cre driver have reduced dendritic length (Raskin et al., 2012). These findings may provide a mechanism whereby the muscular effectors can be efficiently integrated or disintegrated from the neural networks that mediate the behavior as a whole.

It is also worth noting that unlike many neural systems that have critical or sensitive developmental periods, the SNB system requires little sensory stimulation to sexually differentiate and function normally, but rather sexual differentiation seems to depend primarily on androgen signaling. In rats, sexually differentiated maternal care manifests in increased anogenital licking of male pups, and disrupting this selective anogenital licking by rendering dams anosmic reduces masculine copulatory responses related to penile insertion (reviewed in Moore, 1992). Pups of anosmic dams similarly have reduced numbers of SNB motoneurons, but this reduction in neuron number occurs equally in males and females (Moore et al., 1992). More to the point, dendritic length of SNB neurons of adult males raised by anosmic dams is also reduced in the region in which sensory afferents from the perineal skin send projections and in which interneurons express FOS following perineal tactile stimulation (Lenz and Sengelaub, 2006). Furthermore, when pups are raised artificially and anogenital tactile stimulation is determined experimentally during the first 3 postnatal weeks, not only do low levels of perineal anogenital stimulation reduce dendritic length and copulatory behavior associated with penile insertion, but also reduce the appearance of cups in reflexive erection tests, providing direct evidence for reduced SNB function per se (Lenz et al., 2008). Taken together, these studies provide evidence that sensory stimulation of the perineum in early life contributes to the morphological developmental and sensorimotor integration of the SNB system, but it is important to keep in mind that even under extreme sensory deprivation (e.g. artificial rearing with essentially no anogenital tactile stimulation), the SNB system remains qualitatively masculine and functional.

When considering the ultimate biological reasons for modularity in the SNB system it remains important to consider how sexual and natural selection might operate on this system. It is difficult to explain sexually dimorphic elements of the SNB system without invoking sexual selection. Genital reflexes are directly related to fertility, and in the case of rodents, where SNB dimorphism is especially pronounced, there is evidence for intense selection pressure on genital morphology and function. One area where this link has been made convincingly is in induction of a luteal phase by coital stimulation. The exact nature of the coital stimulation required for “pseudopregnancy” varies somewhat between species, but in mice it is a feature of ejaculatory behavior other than the formation of an ejaculatory plug, as males that do not produce a seminal plug due to excision of the seminal vesicles nonetheless retain the ability to induce pseudopregnancy with ejaculation (McGill et al., 1968). Although it is plausible that the SNB system is responsible for this, as surgical excision of the BCLA of adult male mice results in loss of their ability to induce pregnancy but not pseudopregnancy in females (Elmore and Sachs, 1988). Regardless, we know that the BCLA are important for male fertility, but less clear is the relationship between variability in the structure and/or function and reproductive fitness. That is, we know that: 1) species that have promiscuous mating strategies also have a more pronounced dimorphism in the SNB (rats and mice) than those with monogamous (prairie voles – Holmes et al., 2009) or eusocial (naked mole rats – Seney et al., 2006) strategies, 2)

that reproductive activation within individuals (e.g. by season) activates SNB systems (Hegstrom and Breedlove, 1998; Forger and Breedlove, 1987), 3) that variations in circulating androgens contribute to these state differences (e.g. as postulated by Hart, 1983), and 4) that the BCLA muscles contribute to male fertility. Whereas we lack evidence that more masculine SNB phenotypes are causally linked to increased reproductive fitness, it is a plausible hypothesis and one which argues that it is the expression of AR in the BCLA muscles that promotes fitness, hence, selection has acted on this muscle AR expression as a first step in promoting the outstanding sexual dimorphism of this neuromuscular circuit.

4. Skeletal muscle gene expression and brain driven sleep-wake cycles

By what mechanisms might hormones modify properties of muscle that, in turn, then modify the CNS? The previous two examples have focused on the action of androgens on muscle and how selection for androgen-muscle action might modify the CNS. Because androgens regulate transcription, how might changes in the muscle transcriptome influence the neural control of behavior? Insight into this possibility has recently emerged through investigation of the control of circadian rhythms and sleep in mammalian model systems. Biological timing mechanisms associated with modified gene expression in skeletal muscle appears to directly influence brain-driven processes.

Circadian timing in mammals is regulated by a master circadian clock located in the brain. Although most peripheral tissues express the molecular components necessary for circadian timekeeping, and have endogenous circadian oscillators, those oscillators are under the direct control of the clock in the suprachiasmatic nucleus (SCN) of the hypothalamus (Donaldson and Stephan, 1982). Skeletal muscle fibers also have endogenous clocks that, while capable of keeping daily time independently, are generally seen as under the control of that same master clock in the SCN (McCarthy et al., 2007).

Skeletal muscle involvement in the synchronization of circadian rhythms was first considered when it was seen that daily exercise provided physiological feedback to the central circadian timing system (Edgar and Dement, 1991; Buxton et al., 2003). Nevertheless, the basis of the circadian processes in skeletal muscle and the mechanisms for their communication with the brain's clock were not understood. Molecular timing processes in skeletal muscle are mediated by the regulatory influences of the transcription factor Bmal1 on MyoD, a molecular regulator of myogenesis (Andrews et al., 2010). In addition, in most mammalian tissues, Bmal1 is a core regulator of circadian clocks (Bunger et al., 2000). Its expression in the SCN is necessary to drive circadian rhythms throughout the body (McDearmon et al., 2006).

Recently, it has been shown that circadian factors in skeletal muscles have direct effects on the sleep-wake cycle (Ehlen et al., 2017) with Bmal1 playing a crucial role. The mammalian sleep-wake cycle is under direct control of the circadian timing system. Prior to this study, it was assumed that the central clock was exclusively responsible for regulating sleep and wake patterns. Ehlen et al. (2017) showed that manipulation of Bmal1 expression in skeletal muscle impacted the sleep-wake cycle with a direct regulatory role on some brain-driven sleep processes. Whole-body knockout (KO) of Bmal1 was known to produce hypersomnia in mice and the inability to rebound from sleep loss. The investigators created several mouse lines (McDearmon et al., 2006) that allowed the tissue-specific rescue of Bmal1 expression in Bmal1 KO mice. Surprisingly, rescue of Bmal1 in brain of Bmal1 KO mice had negligible effects on sleep. By contrast, when the gene was selectively rescued in skeletal muscle, most sleep impairments were eliminated and normal sleep-wake patterns were restored.

To determine if the effects of Bmal1 in skeletal muscle were necessary for the normal manifestation of sleep-wake states in mice,

Bmal1 was selectively deleted in skeletal muscles. A prior study reported that this muscle-specific deletion of Bmal1 alters circadian timing (Hodge et al., 2015), so there was already evidence that this peripheral mutation could influence brain-driven processes. The skeletal muscle-specific KO of Bmal1 in mice recapitulated the sleep impairments observed in the whole-body KO. This finding demonstrates that Bmal1 expression in skeletal muscle is necessary for normal sleep-wake states in mice. However, these findings did not eliminate the possibility that these effects on sleep were driven by peripheral pleiotropic mechanisms of the rescue in the skeletal muscle, instead of by acting directly on sleep regulatory mechanisms in the brain.

To confirm a primary role of the brain in these sleep impairments, individual mice from each of the models were sleep deprived and sleep-wake dynamics during sleep recovery were examined. The ability to recover from sleep loss is a brain-driven process called sleep homeostasis. When too little sleep is detected, this homeostatic mechanism generates a sleep rebound that is characterized by deeper sleep and more sleep during recovery. Although the physiological components and functions of this mechanism are poorly understood, it is a reliable and phylogenetically-conserved mechanism, and in mammals it is driven by neuronal signaling. The ability to recover from sleep loss is impaired in mice with whole-body KO of Bmal1, and it is restored in Bmal1 muscle rescue mice but not in Bmal1 brain-rescue mice. Skeletal muscle-specific KO of the Bmal1 gene in mice recapitulated the same homeostatic impairments observed in the whole-body Bmal1 KO mice. These findings demonstrate that although sleep is indeed a brain-driven process, sleep homeostasis requires Bmal1 expression in skeletal muscle.

Overexpression of Bmal1 in skeletal muscle was also found to influence sleep homeostasis. Unlike the KO model, this manipulation had no detectable influence on circadian timing or any other ancillary or pleiotropic phenotypes that could influence the sleep-wake cycle. However, overexpression of Bmal1 in skeletal muscle reinforced sleep homeostatic processes making mice resistant to sleep loss. Mice overexpressing Bmal1 in skeletal muscle exhibited more vigilance and they were easier to keep awake during sleep deprivation procedures. Moreover, they had lower basal NREM slow wave activity, a measure of sleep pressure. These findings show that although Bmal1 is primarily considered a circadian regulatory gene, its expression in skeletal muscle is an important regulator of brain-driven sleep regulatory processes, and its influences on sleep are not dependent on circadian regulation of sleep states.

Although the mechanism that regulates sleep homeostasis is not well understood, it is known that cholinergic signaling in neurons in the basal forebrain are crucial (Hanlon et al., 2011). Most of these neurons express adenosine receptors and adenosine in the basal forebrain is an important regulator of sleep homeostasis. The Bmal1 skeletal muscle-overexpression model provides important clues about how this process may be working. Mice that overexpress Bmal1 in skeletal muscles exhibit higher levels of basal FOS immunoreactivity in cholinergic basal forebrain neurons. FOS is an immediate early gene that serves as a marker of neuronal activation. Sleep deprivation produces larger FOS increases in cholinergic neurons of the basal forebrain in Bmal1 skeletal muscle-expression mice than in wild-types. These findings suggest that Bmal1 expression in skeletal muscles directly signals cholinergic neurons of the basal forebrain through an as yet unidentified pathway. Thus, the results of these sleep studies add to the growing body of evidence that skeletal muscle signaling plays a critical role in modulating what are normally seen as brain-dependent processes.

5. Novel mechanisms through which muscles communicate with the CNS

The ways that muscle might communicate with and modify the CNS are diverse, with some already well-studied. For instance, afferent

signaling from contracting skeletal muscles has been shown to regulate release of neurotransmitters in the brainstem (Potts et al., 1999), pituitary gland (Gosselink et al., 2000), and midbrain (Li and Mitchell, 2003; Li et al., 2001). Both motor and proprioceptive neurons require the presence of their striated muscle targets for their proper survival, growth and differentiation (Kablar and Rudnicki, 1999, 2002). Considerable research has established the role of a variety of neurotrophic factors, likely produced in the target muscles, as responsible for the maintenance and growth of these peripheral neuronal systems (Angka et al., 2008).

Many sexually dimorphic features of the SNB system originate from androgen action on target muscles (reviewed in Swift-Gallant et al., 2012), with androgenic regulation of neurotrophic factor production being thought to mediate these effects (see Sengelaub and Forger, 2008, Verhovshek et al., 2013 for review). Although identification of the neurotrophic factor(s) responsible for developmental rescue of SNB motoneurons remains elusive, manipulations of neurotrophic factor signaling is effective in altering SNB motoneuron survival in neonatal rats (Forger et al., 1993; Xu et al., 2001). In adulthood, there is considerable evidence for brain-derived neurotrophic factor (BDNF) produced by BCLA muscles retrogradely mediating androgenic regulation of dendritic extent (Yang et al., 2004) and androgen receptor expression (Al-Shamma and Arnold, 1997) of SNB motoneurons. Curiously, it may be the case that BDNF is a negative regulator of dendritic length, as antagonism of BDNF signaling with trkB IgG is effective in preventing reduction of SNB motoneuron dendritic length following castration of male rats (Verhovshek and Sengelaub, 2010).

BDNF has gained wide-acceptance as a crucial muscle-derived factor that promotes proper function of the neuromuscular junction (Hurtado et al., 2017). Moreover, exercise and muscle contractions promote release of BDNF systemically, with increasing evidence for trophic actions at distant CNS targets, specifically the brain (Gomez-Pinilla et al., 2008).

BDNF's neurotrophic actions can occur directly within the CNS itself, with muscle-secreted factors apparently reaching the brain to promote this local signaling. For example, the metabolic byproduct β -hydroxybutyrate and the myokine cathepsin B both increase systemically following exercise, they both cross the blood-brain-barrier and both are linked to increases hippocampal BDNF expression and/or neuroplasticity (Sleiman et al., 2016; Moon et al., 2016). PGC-1 α (peroxisome proliferator-activated receptor gamma coactivator 1- α) activation stimulates release of the muscle-derived peptide irisin into circulation (Bostrom et al., 2012). Irisin, a relatively newly identified hormone (myokine) induces BDNF expression in the hippocampus (Wrann et al., 2013) which has been implicated as a mediator of a variety of neural functions including sleep (Kushikita et al., 1999). Thus, muscle secreted protein hormones or fuel metabolites can signal to the brain to influence neural function and behavior.

Other metabolic byproducts appear also able to form a cross-talk linkage between muscle and brain. Kynerinine, a metabolite of tryptophan, can promote depressive-like symptoms, increased stress responses, neuroinflammation and memory loss while also reducing neuroplasticity (Kobilo et al., 2011, 2014). Muscles release kynerinine or kynurenic acid, with the later metabolite unable to cross-the blood-brain-barrier and thus having little impact on brain and behavior. Up-regulation of kynerinine amino transferases, enzymes that converts kynerinine to kynurenic acid in skeletal muscle alone, can reduce plasma levels of kynerinine, thus limiting many of its detrimental effects on brain (Agudelo et al., 2014).

In two of these examples, the muscle to brain signaling system involves a metabolic byproduct. Whereas muscle use is viewed as the primary mechanism causing secretion of these factors, might circulating hormones, by modifying the muscle transcriptome, directly or indirectly influence the expression of enzymes that form brain-acting

factors? In manakins, androgens have marked effects on many genes regulating metabolism in addition to genes that influence contraction states (Fuxjager et al., 2016, 2017a). By their actions on the manakin musculature, it is possible that androgens modify the male's behavioral performance by the release of specific metabolites into the vasculature? Such ideas remain to be tested.

These few examples for muscle-derived factors that modify the CNS may indeed be just the tip of the iceberg as a large number of proteins have been identified in both mice and humans that are likely secreted and likely function as intercellular signaling molecules (Lindskog, 2015; Uhlen et al., 2010; Zhou et al., 2017). Some of these may be secreted by muscle and while it is likely they could alter the functions of diverse tissues (with tissues and processes involved in metabolism the most likely), some may also be found to alter properties of the brain. Perhaps some of these are also influenced by hormones acting on muscle to promote neural development, function and behavior. We also cannot eliminate the possibility that novel factors may be hormone-dependent and secreted by muscle for actions on the CNS. For example, muscle-derived nitric oxide has been shown to enhance hippocampal neurogenesis (Deng et al., 2009); miRNAs have also been found to be released systemically as a mechanism for tissue cross-talk (Thomou et al., 2017).

6. Conclusions

In summary, selection for enhanced hormonal sensitivity, and in particular to androgen action, may be a primary event in the evolution of behavior. By targeting the musculature, neural circuits may arise that then drive those muscles to produce behaviors upon which selection acts. We already know that hormones can protect muscles developmentally to promote survival of their neural afferents and this serves as a first-step model for elaboration of additional neural control systems. The mechanisms by which hormones may modify the musculature to, in turn, the brain. It is quite evident that changes in the muscle transcriptome alone can guide neural function and behavior. A variety of factors, some well-described and others newly identified, may serve as the messengers for communication between muscle and brain.

Such findings also raise new questions. For example, we might ask which comes first: a) when a specific behavior is favored by selection, is that behavior modified at the outset by the building of new neural circuits that ultimately target specific musculature from which a new behavior then arises, or b) does selection first target muscles and establish specialized muscle contraction states that respond to extant neural circuits, or c) does selection first target muscles and those specialized muscles are worthy targets of subsequent afferent neural modification? Perhaps behavior is shaped by selection acting simultaneously on muscle and the CNS. Behavior could be shaped by selection acting on HUB genes (highly connected nodes in gene regulatory networks) that drive the evolution of muscle and CNS-specific gene networks simultaneously that, only together then produce appropriate behavior? Are muscle and brain genes co-localized within the genome such that chromosomal mutations disrupt cohorts of genes that simultaneously modify neural and muscular underpinnings of behavior?

From the standpoint of behavioral endocrinologists, one can ask many of the same questions concerning the timing and site of actions of hormones. Do hormones activate or tune behavior acutely first by their actions on muscle or first by actions on the CNS? We capture the model systems described as well as some of the ideas discussed in Fig. 1. We hope this will serve as a template to motivate additional “muscle-up” research by behavioral endocrinologists. In all likelihood, evolution and endocrine control work through each of these mechanisms, and likely others, to achieve the vast collection of hormone-dependent behavioral states observed in nature.

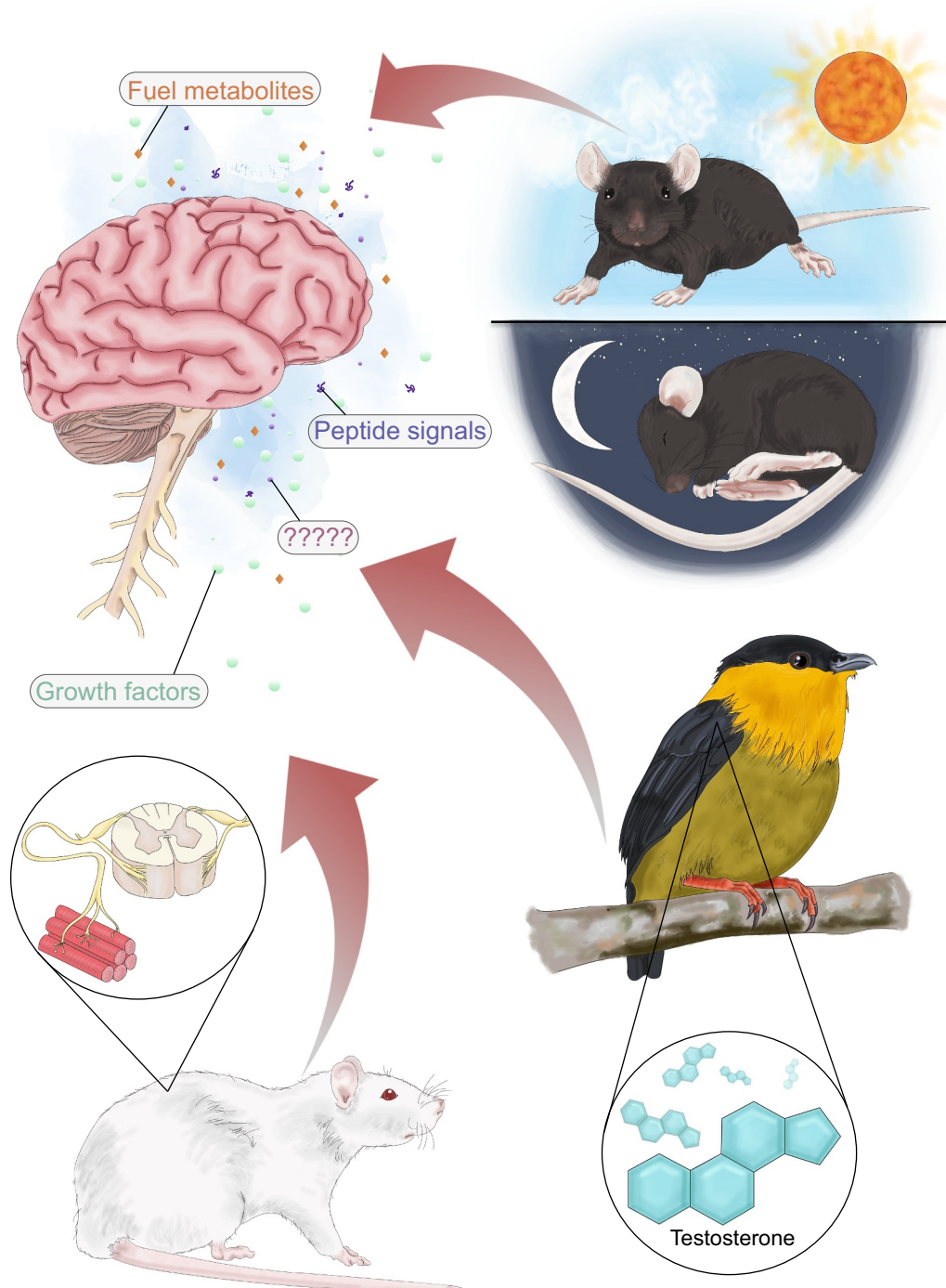


Fig. 1. Several established and putative signaling mechanisms through which striated muscles communicate with and influence brain and behavior. Illustrated also are three examples whereby striated muscle systems appear to drive CNS development or function (counter clockwise): androgen action on the bulbocavernosus muscle of developing male rats; testosterone action on androgen receptors in skeletal muscles determines complexity of courtship behavior in male golden-collared manakin; skeletal muscle Bmal1 expression is required for normal CNS-controlled sleep-wake cycles in mice.

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