Behavioral effects of social challenges and genomic mechanisms of social priming: What's testosterone got to do with it?

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Abstract   Social challenges from rival conspecifics are common in the lives of animals, and changes in an animal's social environment can influence physiology and behavior in ways that appear to be adaptive in the face of continued social instability (i.e. social priming). Recently, it has become clear that testosterone, long thought to be the primary mediator of these effects, may not always change in response to social challenges, an observation that highlights gaps in our understanding of the proximate mechanisms by which animals respond to their social environment. Here, our goal is to address the degree to which testosterone mediates organismal responses to social cues. To this end, we review the behavioral and physiological consequences of social challenges, as well as their underlying hormonal and gene regulatory mechanisms. We also present a new case study from a wild songbird, the dark-eyed junco *Junco hyemalis*, in which we find largely divergent genome-wide transcriptional changes induced by social challenges and testosterone, respectively, in muscle and liver tissue. Our review underscores the diversity of mechanisms that link the dynamic social environment with an organisms' genomic, hormonal, and behavioral state. This diversity among species, and even among tissues within an organism, reveals new insights into the pattern and process by which evolution may alter proximate mechanisms of social priming [*Current Zoology* 60 (6): 791–803, 2014].

Keywords   Social priming, Challenge hypothesis, Aggression, Hormonal mechanism, Testosterone, Genomics

1   Behavior and the Dynamic Environment: What is Social Priming and How Might It Operate?

Most behaviors are plastic traits in that they are expressed in response to stimuli that vary in space or time, and consequently, animals can modify their behavior to environmental conditions that shift over the course of minutes, hours, or seasons. Many behaviors are nonetheless individually consistent, or repeatable (Duckworth, 2010), and include a heritable component (Mackay et al., 2009; Weber et al., 2013). Behavior has long been hypothesized to be at the forefront of evolutionary change (Mayr, 1963), and flexibility in social behavior, in particular, is thought to be a major way by which animals respond to their dynamic world, both immediately and over evolutionary time (Renn and Schumer, 2013; Taborsky and Oliveira, 2012; West-Eberhard, 2003). Many open questions remain, however, regarding the proximate mechanisms linking behavior and the environment (Wingfield, 2012), a pressing issue with implications for understanding how selection acts in the face of ongoing anthropogenic environmental change.

In this article, we focus on aggressive behavior in the context of territorial defense. This behavior is taxonomically widespread, situationally plastic, individually consistent, and has consequences for survival and reproductive success (Nelson, 2006). Importantly, social challenges, or threats from a rival conspecific, can lead to an aggressive response and other changes in behavior and physiology, such as altered metabolism, stress response, or immune function (Wingfield et al., 2001). If these phenotypic effects serve to prepare animals for future competition, then 'social priming' may occur. These priming effects are often studied within the frameworks of the challenge hypothesis or winner effect (Dugatkin, 1997; Wingfield et al., 1990, elaborated below) and focus on the transient rise in testosterone (T) associated with social instability in many male vertebrates. Because socially mediated changes in T usually occur on a slower timeframe than most social challenges (e.g. 10 to 30 minutes, Gleason et al., 2009), and elevated T is not required for an immediate aggressive responses to an intruder (Rosvall et al., 2012b; Soma et

al., 2008), these hormonal changes are thought to prepare animals for *future* social instability by changing the organism's physiological and genomic state (i.e. the challenge hypothesis; Wingfield et al., 1990). While many studies support these socially mediated changes in T (reviewed in Archer, 2006; Hirschenhauser and Oliveira, 2006; Wingfield et al., 1990), emerging evidence suggests this phenomenon may not be as generally applicable as once thought. For example, many recent studies provide only mixed support for the challenge hypothesis (Husak and Lovern, 2014), meta-analyses suggest that in most bird species studied to date, males do *not* elevate T after a social challenge (7 spp elevate T; 14 spp do not; Goymann, 2009), and social elevation of T in female vertebrates may be even less common (Rosvall, 2013). Experimental tests of the hypothesis that social challenges prime, or adaptively prepare, animals for future social instability– without a rise in T– are still rare (see below).

These observations highlight several issues related to whether and how social priming occurs. First, can social priming occur without systemic changes in the hormone long hypothesized to mediate adaptive responses to social challenges? Second, what alternative mechanisms control socially mediated shifts in behavior and physiology? And, finally, how do these mechanisms coordinate diverse organismal processes throughout the brain and body, if not by a change in T levels in the blood that can act systemically?

Below, we begin to address these questions by considering the phenotypic, hormonal, and gene regulatory responses to social challenges. With respect to mechanisms, there is a strong foundation upon which to hypothesize that selection ought to favor mechanisms of social priming that operate independently of changes in T in circulation. For one, even though elevated levels of T can shift organismal processes toward greater investment in activity, aggression, and energy metabolism (Ketterson and Nolan, 1999; Wingfield et al., 1990), there are costs associated with elevated T, e.g. in relation to trade-offs with parental care, immune function, fat storage, or stress reactivity (Wingfield et al., 2001). If these costs are especially high, selection should act to modify the mechanisms that dynamically link an organism's social environment with its phenotype in a manner that minimizes these costs. These adaptations should include socially mediated changes in steroid sensitivity, local synthesis, or metabolism in target tissues like the brain, as well as social priming that is mediated via completely different signaling systems (e.g. biogenic amines, see §4).

Some of these alternative mechanisms may sufficiently stimulate the brain to elicit behavioral responses (see below); however, aggressive behavior also requires a full body response. Competitive interactions may involve energetically expensive movements (e.g. chasing, fleeing), they may lead to tissue damage from abrasions or exertion, and they may initiate changes in metabolism, muscle activity, and immune or inflammatory responses. It is essential to consider these peripheral responses to the social environment for a full understanding of the costs and benefits of any one mechanism of social priming. How these peripheral responses might be coordinated is also unclear (Pfaff, 2010), particularly if there is no change in T in circulation. Autonomic regulation may play a role (e.g. dopamine acting on brain and spleen to influence aggression and immune defenses, respectively; Elenkov et al., 2000), and various signaling molecules may change tissue-specific steroid synthesis or sensitivity (Kabelik et al., 2010), thus altering T-dependent phenotypes without adjusting T levels in circulation (Schmidt et al., 2008).

Here, our specific goal is to address the role of testosterone in mediating social priming. We focus on the proximate mechanisms regulating responses to social challenges and the degree to which they are coordinated by changes in T in circulation. We begin by reviewing overlap in the behavioral and physiological responses to social challenges and T, respectively (§2), followed by a synthesis of some of the alternative mechanisms of social priming proposed above, including changes in local steroid synthesis or sensitivity (§3) and other signaling molecules (§4). We close by considering gene regulatory responses to T and social challenges (§5), and we present a novel case study that contrasts the genome-wide transcriptional changes that are induced by social challenges and T in a songbird, the dark-eyed junco *Junco hyemalis*. We highlight these changes in gene expression because they are thought to be an important mechanism of phenotypic plasticity (Aubin-Horth and Renn, 2009; Renn and Schumer, 2013), and T has long been known to have massive effects on gene expression. Although the relationship between transcription and translation is not necessarily 1:1, these shifts in gene regulation are thought to be the primary way by which T influences phenotype (Chang et al., 1995; see also Foradori et al. 2008 for a discussion of the non-genomic effects of T), and there are likewise major genomic and non-genomic effects that occur after T is converted into estradiol as well (Cornil et al., 2006).

We hypothesize that, if social priming is mediated by

T, then the behavioral, physiological, and gene regulatory responses to T and to social challenges will largely overlap. Departure from full overlap at these multiple levels will shed light on the degree to which evolution can co-opt vs. decouple diverse mechanisms regulating responses to the social environment. Our review emphasizes behavioral effects that are likely to be largely mediated via the brain, as well as changes in behavior and physiology that might also be regulated in the periphery. The research we describe spans a wide range of primarily vertebrate groups, including laboratory model organisms as well as a variety of species studied in their natural environment. Thus, these data offer insights into the pattern and process by which evolution can and has altered organismal responses to the social environment.

2 Behavioral and Physiological Effects of Testosterone and Social Challenges

Many of the phenotypes that ought to be advantageous in the face of social instability are also affected by experimental manipulations of T. For example, T implants increase activity, territorial patrolling, and various aggressive behaviors (e.g. Ketterson et al., 1996; Wingfield et al., 1987). T also may reduce anxiety and increase focus (Aikey et al., 2002), allowing animals to concentrate on the immediate needs of a social challenge while de-emphasizing other processes. T implants alter several components of peripheral physiology as well, including muscle hypertrophy, immune function, and metabolism (Staub and DeBeer, 1997).

Experimental manipulations of the social or competitive environment affect some of these same phenotypes. For example, residency status, resource availability, and the density, rank, or sex of nearby conspecifics can influence the expression of aggression and the outcome of a social challenge (Desjardins et al., 2012; Enquist and Leimar, 1987; Fuxjager et al., 2010b; Saltz, 2013). Prior winning experiences also can influence future aggression and the likelihood of winning a subsequent social challenge (i.e. the winner effect, Dugatkin, 1997; Gleason et al., 2009; Hsu et al., 2006). In some species, fighting itself is sufficient to elicit these behavioral and hormonal effects (Dijkstra et al., 2012), but in others, it is winning, rather than fighting per se, that seems to control priming effects (Hirschenhauser et al., 2008; Oliveira et al., 2005). Males in some species are so attuned to dynamic shifts in the social environment that they increase androgen concentrations in the bloodstream from merely observing a social challenge (e.g.

cichlid fish *Oreochromis mossambicus*, Oliveira et al., 2001), and bystanders behave more aggressively in subsequent social interactions (e.g. fighting fish *Betta splendens*, Clotfelter and Paolino, 2003).

Species that vary seasonally in the degree to which social challenges elicit a systemic elevation of T provide a natural experiment with which to test whether socially mediated changes in T have priming effects on future behavior. Male song sparrows *Melospiza melodia*, for example, are territorial year-round, and they respond equally aggressively during a simulated territorial intrusion (STI) in the spring breeding season as they do in the winter. What differs, though, is their aggressive behavior immediately *after* the STI (Wingfield, 1994). After STI during the spring, males continue to behave aggressively (e.g. with heightened activity, singing, and close proximity to the vicinity of the challenge), whereas these same behaviors drop off sharply following the STI in the winter. Critically, males elevate T in response to STI in the spring but not the winter, and T implants rescue this heightened post-STI aggression in the winter (Wingfield, 1994). Thus, social elevation of T appears to enhance future territorial aggression and vigilance, consistent with T-mediated social priming in the spring and alternative mechanisms of aggression in the winter (see §3).

Combined manipulations of the social environment paired with pharmacological treatments are an especially informative method for investigating social priming, and although they are the only direct way to tease apart the effects of the social challenge from those of the hormone itself, they are relatively rare, an observation that proves problematic in light of recent evidence that socially mediated changes in T in circulation do not necessarily occur. Following a victory in their home cage, for example, male California mice *Peromyscus californicus*, are more likely to win again in the future (Fuxjager et al., 2009). Prior winning experiences and post-victory elevation of T *both* contribute to this winner effect, which lasts into the next day (Trainor et al., 2004). The congeneric white-footed mouse *P. leucopus*, on the other hand, lacks both a socially induced T elevation and a winner effect, but post-victory treatment with T induces the winner effect (Fuxjager et al., 2011), highlighting T as a mediator of social priming in this genus. The black redstart *Phoenicurus ochruros* is a songbird species in which males are physiologically capable of elevating T during the breeding season (i.e. in response to injection with exogenous gonadotropin-releasing hormone), but they do not do so in re-

sponse to a STI (Apfelbeck and Goymann, 2011). Experimental treatment to block the local effects of T does not decrease overall aggressive response to STI, but it does shift behavioral responses away from vocal signals of aggression, and towards other non-vocal aggressive behaviors (Apfelbeck et al., 2013). Thus, some behavioral responses to social challenges may be fine-tuned by T to some degree, though not via socially mediated changes in T in circulation (see §3 for more examples).

3　Socially Mediated Changes in Local Steroid Sensitivity or Synthesis

Several lines of evidence suggest that T may mediate organismal responses to social challenges via changes in the local production or response to T in target tissues, rather than via changes in T levels in the bloodstream that are regulated by production/release from the gonads. Male California mice, for example, experience a socially induced increase in androgen receptor (AR) mRNA and protein expression in areas of the brain known to regulate aggression (Fuxjager et al., 2010a), meaning that socially challenged males have a greater neural capacity to bind and process T. These shifts in apparent neural sensitivity to T may operate via changes in reward processing, as they are also highly dependent on context: up-regulation of AR in reward centers of the brain only occurs in response to winning a fight in a male's home cage, whereas changes in AR expression in other areas of the social behavior network of the brain occur regardless of residency status. Because California mice socially modulate AR in the brain alongside change in T in circulation, though, this finding does not fully tease apart whether these local changes in steroid sensitivity can occur independently of systemic changes in T.

Seasonally breeding species with year-round territoriality can help to rectify this potential confound because aggressive responses often persist in the non-breeding season even when the gonads are regressed and blood T levels are essentially undetectable. For example, during the non-breeding season, male song sparrows respond to STI with an aggressive response and a concomitant increase in neural activity of the enzyme 3-β-hydroxysteroid dehydrogenase (3βHSD), which converts the adrenally derived prohormone DHEA into androstenedione, an intermediate along the pathway to produce T (Pradhan et al., 2010; Soma et al., 2008). Male white-crowned sparrows *Zonotrichia leucophrys pugetensis* likewise respond to STI during the late breeding season

(when T levels are also quite low) with shifts in estradiol concentrations in behaviorally relevant areas of the brain (Charlier et al., 2011), further suggesting social effects of aggressive encounters on local steroid micro-environments. In these sparrows, therefore, as well as other birds and rodents, non-breeding aggression appears to be facilitated by a combination of seasonal changes in sex steroid sensitivity in the brain, adrenal prohormones that are metabolized in the brain into more active sex steroids, and de novo synthesis of steroids in the brain (Soma et al., 2014). Evidence suggests that these changes do not necessarily depend upon a winning experience or physical interaction with a rival. For example, merely hearing another male's song leads to rapid and significant local synthesis of estradiol in the auditory forebrain in male zebra finches (*Taeniopygia guttata*, Remage-Healey et al., 2008).

In sum, social challenges and/or winning experiences can alter the hormonal microclimate in specific areas of the brain, but the available behavioral data do not yet fully support the hypothesis these local changes can engender lasting behavioral effects without a concomitant change in T in circulation (Wingfield, 1994).

If socially mediated changes in steroid sensitivity or synthesis can engender social priming, then another prediction is that these effects ought to operate locally within peripheral tissues, similarly to how they operate in the brain. Consistent with this view, male song sparrows respond to STI in the non-breeding season with a socially induced increase in locally synthesized DHEA in the liver, an effect that may serve to power the metabolic needs of an aggressive encounter (Newman and Soma, 2011). Likewise, the acrobatic display behaviors used by male golden-collared manakins *Manacus vitellinus* are significantly diminished by treatment with an antiandrogen that selectively acts on the periphery, even though vocal components of the display are not affected (Fuxjager et al., 2013). Although this last example comes from a different sort of social behavior (courtship), these data suggest that some behavioral responses to the social environment are indeed regulated at the periphery, here within skeletal muscle. Skeletal muscle is a major regulator of behavior, in that movement clearly cannot occur without it. Skeletal muscle is also known to express androgen receptors (Feng et al., 2010) and steroidogenic enzymes (e.g. 3βHSD), which can change in response to muscle expenditure (Aizawa et al., 2008). Thus, it is feasible that a physical interaction with a competitor occurs alongside muscle-specific shifts in the binding and processing of T. Androgen re-

ceptors are also expressed in spleen, liver, and fat (Benten et al., 2002; Delic et al., 2010; Veilleux et al., 2009), and a number of peripheral immune tissues synthesize steroids de novo from cholesterol (Schmidt et al., 2008), again demonstrating the potential for local, peripheral changes in steroid binding/processing as a mechanism of social priming.

4 Alternative Mechanisms of Social Priming

Other than testosterone and its metabolites, there are many signaling molecules that might facilitate social priming of both brain and body. For one, some species that do not elevate T to social challenges instead elevate corticosterone (CORT, Baird et al., 2014; Landys et al., 2007). Although sometimes thought of as a 'stress hormone,' this adrenally-synthesized steroid is primarily a metabolic hormone that can increase activity and cardiovascular function, mobilize energy reserves, and suppress sickness behavior (Ashley et al., 2009; Breuner et al., 1998; Haller et al., 2008; Landys et al., 2004; Sapolsky et al., 2000) – all phenotypic response that ought to be adaptive in the face of an intense aggressive interaction or more prolonged periods of social instability. CORT receptors and local CORT synthesis have also been documented in a variety of neural and peripheral tissues (Lattin et al., 2012; Schmidt et al., 2008), laying the potential for local CORT-related processes to control responses to social challenges, even without a change in CORT in circulation. Consistent with this view, male song sparrows see an increase in CORT concentrations in liver and muscle tissue in response to STI during the non-breeding season, even though plasma CORT is unaffected by social challenge (Newman and Soma, 2011).

Several neuropeptides likewise have well-established links with aggression and may regulate responses to social challenges. Here, we only touch upon some briefly, as they have been detailed in other thoughtful and recent reviews (Curley et al., 2011; Hsu et al., 2006; Nelson and Trainor, 2007; O'Connell and Hofmann, 2011). Biogenic amines, including serotonin, dopamine, epinephrine, and norepinephrine, are known to rapidly respond to changing social stimuli and mediate behavioral responses. For example, visual interaction with a rival selectively stimulates dopaminergic neurons in male cichlid fish (*Astatotilapia burtoni*, O'Connell et al., 2013). Like T, secretion of biogenic amines appears to be highly sensitive to the specifics of the social environment, as evidenced by differential release of norepi-

nephrine and serotonin in relation to the level of competition in the social environment in birds, fish, and rodents (Bell et al., 2007; Hall et al., 2011; Salvante et al., 2010; Sewall et al., 2013). The nonapeptides, i.e. members of the arginine vasopressin/oxytocin family, also may mediate responses to the social environment (Goodson and Thompson, 2010; Kabelik et al., 2010). For instance, repeated social defeat leads to reduced expression of vasopressin and a persistent enhancement in anxiety-like behaviors in mice (Curley et al. 2011), and Lincoln's sparrows *Melospiza lincolnii* exposed to more challenging songs show reduced vasotocin labeling in the brain, compared to males exposed to less challenging songs (Sewall et al., 2010).

Many of these neuropeptides have peripheral targets that mediate important homeostatic processes. For example, vasopressin regulates water retention and blood pressure as well as liver, kidney, and cardiovascular function (Thibonnier et al., 2001). Catecholamines can be locally synthesized in the spleen (Kubovcakova et al., 2001) and influence immune processes (Felten and Olschowka, 1987). Furthermore, many of these signaling molecules are known to be regulated by sex steroids. For example, castration increases methylation in the promoter region of the vasopressin gene in male rats, leading to decreased neural gene expression of vasopressin; treatment with T reverses these effects (Auger et al., 2011). Experimental manipulations of T or estradiol also can increase serotonin receptor gene expression in certain brain areas (Sumner and Fink, 1998), suggesting that cross-talk among various signaling molecules is yet another layer of complexity in mechanisms of social priming.

5 Gene Regulatory Responses to T and to Social Challenges

5.1 Review of the literature

With the growth of high-throughput sequencing and expression profiling in recent years, we have learned a lot about gene regulatory responses to a range of pharmacological and environmental manipulations in a variety of species, providing new insights into understanding how animals respond to changes in T or the social environment. For example, dark-eyed juncos treated with T implants show changes in the expression of many genes in the brain, including several that have been linked to aggression, metabolism, sexual behavior, and activity (Peterson et al., 2013). T-treated males have greater expression of melanocortin receptor 4 (MC4R)

in the hypothalamus. MC4R binds to α-melanocyte sti-mulating hormone (αMSH), a peptide hormone best known for its influence on coloration, but also for its pleiotropic effects on appetite, metabolism, sexual func-tion, and aggressive behavior (Rushton and Templer, 2012). Neural AROM expression is also up-regulated in response to T implants in juncos. Because of the estab-lished links between AROM and several sexual and aggressive behaviors (Cornil et al., 2006; Rosvall et al., 2012a), these findings suggest that an increase in sys-temic T may co-occur with local neural changes in sex steroid metabolism as well as changes in other behavi-orally relevant signaling systems.

Not surprisingly, experimental treatment with sex steroids influences gene expression in the periphery as well. Laboratory mice treated with androgens show major changes in liver gene expression, particularly related to the breakdown of fatty acids and the metabo-lism of lipids and steroids (van Nas et al., 2009). These changes in gene expression correlate well with a variety of metabolic traits, including body weight and levels of cholesterol and insulin in blood (van Nas et al., 2009). Immune function also may be affected by T, and indeed laboratory mice given repeated T injections over the course of several weeks show shifts in the expression of several immune-related genes in the liver, including various chemokines, cytokines, and components of in-nate immunity (Krucken et al., 2005). In male juncos treated with T, the liver also responds with shifts in hundreds of genes related to growth and metabolism, e.g. insulin receptor 4, follistatin (Peterson et al., 2014). Androgens also act on skeletal muscle to bring about various changes linked to muscle hypertrophy and ac-tivity in golden-collared manakins (*Manacus vitellinus*, e.g. insulin-like growth factor 1, parvalbumin, Fuxjager et al., 2012) and several hundred genes related to energy metabolism in dark-eyed juncos (Peterson et al., 2014), including those regulating insulin metabolism and cel-lular growth. Importantly, the direction of these shifts in gene regulation are largely consistent with T-enhanced breakdown of energy stores paired with T-induced mus-cle growth and energy availability – all effects that ought to be well suited for responding to a persistent social challenge.

Experimental manipulations of the social environ-ment also reveal widespread dynamism across the ge-nome. Honey bees *Apis mellifera* exposed to a phero-mone released during colony defense show changes in neural gene expression that include up-regulation of genes involved in sensory processing as well as up-regulation of various biogenic amine precursors and associated receptors, i.e. invertebrate analogs of some of the neu-rotransmitters described in §4 (Alaux et al., 2009). These shifts occur alongside widespread decreases in various metabolic functions, particularly oxidative phosphorylation, suggesting that bees experience social priming at the expense of certain self-maintenance func-tions while they are responding to a threat.

Thirty minutes after STI, male sticklebacks *Gaste-rosteus aculeatus* also show changes in the expression of hundreds of genes in various areas of the brain (Sanogo et al., 2012). For example, challenged males upregulate expression of a number of genes with known links to aggression, including POMC (the precursor to αMSH and other peptide hormones), prolactin, and genes encoding precursors to thyroid-stimulating and luteinizing hormones. Like the socially challenged bees above, challenged sticklebacks also experience several transcriptional shifts consistent with less investment in various self-maintenance functions, including immune function (Sanogo et al., 2012). Challenged male song sparrows also experience socially induced changes in the expression of genes whose products regulate thyroid hormone and dopamine synthesis/release in the brain, as well as a number of epigenetic modifiers that provide a mechanism by which social challenges might leave a lasting mark on future behavior (Mukai et al., 2009). Social defeat in laboratory rodents affects many of these same systems (e.g. dopaminergic systems, immune and inflammatory responses) in both the brain and body (Berton et al., 2006; Merlot et al., 2004), pointing to some transcriptional shifts that may represent taxonomi-cally widespread responses to the social environment.

Notably, though, genes related to the local synthesis, metabolism, or binding of T and its metabolites are *not* on the lists of socially sensitive genes for any of these genome-wide analyses of transcriptional responses to social challenges described above. Based on these indi-rect comparisons of studies of T implants and social challenges, it appears that social challenges may acti-vate gene regulatory pathways that are not necessarily dependent on T action. Thus, T and social challenges may influence similar organismal processes, though via different mechanisms. We are not aware, however, of any direct comparison of the genes or gene functions affected by T and social challenge within the same spe-cies.

5.2 A case study from the dark-eyed junco

Here, we provide new analyses that represent a first pass at filling this gap by comparing two studies we

have conducted in the dark-eyed junco: one study in which we assessed the genome-wide transcriptional response to T implants (Peterson et al., 2013; Peterson et al., 2014) and another in which we quantified transcriptional responses to a social challenge (Rosvall et al. in prep., Rosvall et al., 2012b; Rosvall et al., 2014a). Critically, male juncos do not elevate T in response to social challenges under most circumstances (Rosvall et al., 2014b), and the males in the social challenge experiment described here did not alter systemic T levels (Rosvall et al., 2012b), thus allowing us to tease apart responses to systemic changes in T from other social effects on gene regulation.

The full methodological details of these studies are described elsewhere, and so we only briefly summarize them here. Both studies took place at or near the Mountain Lake Biological Station in the Jefferson National Forest, Virginia, USA during the early to mid-breeding season. The T-implant study occurred in early June 2010 using 12 males that were captured, implanted with either T or control implants ($n = 6$ each), and held in a semi-naturalistic outdoor aviary for 26 days prior to euthanasia and tissue harvesting (Peterson et al., 2013; Peterson et al., 2014). This implant regimen induces T levels at the high of the natural range of variation as well many changes in T-mediated behavioral and physiological phenotypes (Ketterson et al., 1991; Ketterson et al., 1996). The social challenge experiment occurred from late April to late May 2011, and it includes socially challenged males ($n = 10$) that were captured and euthanized on their territories roughly 35 minutes after a social challenge in the form of song playback. Control males ($n = 10$) were captured on their territories without such a challenge, and all males were euthanized within 2 min of capture, minimizing any artifacts related to handling (Rosvall et al., 2012b).

Here, we focus our analyses on liver and pectoral muscle samples collected from these males. Both studies measured gene expression on a custom Nimblegen microarray, and although arrays for each study were processed separately, we focus only on those genes expressed in both experiments measured by the same probes. Genes were determined to be expressed following established guidelines based on setting a threshold of fluorescence that exceeds that of 95% of all random probes on the array (Peterson et al., 2012). We calculated a global q-value to standardize the p-value cutoff across tissues and studies in order to ensure consistent correction for false positives following established protocols (Peterson et al., 2013). We then determined which genes were significantly affected by both T-treatment and social stimulus, and calculated a Fisher's exact test to determine if more genes were affected by both experiments than expected by chance.

5.2.1 Results

After global correction for false discovery rate, there were 434 genes affected by T-treatment in muscle, 84 genes affected by social stimulus in muscle, 283 genes affected by T-treatment in liver, and 736 genes affected by social stimulus in liver. Of these genes, 8 were affected in both T-treatment and social stimulus in muscle (Table 1), which is significantly more than expected by chance (expect 3.6 genes to overlap; Fisher's exact test $P = 0.027$). However, only 1 of the 8 was affected in the same direction in both experiments (12%), indicating divergent responses to T and social challenges in pectoral muscle.

In liver, 21 genes were affected by both T-treatment and social stimulus (Table 2), an overlap not significantly different from that expected by chance (expect 18.4 genes; Fisher's exact test $P = 0.54$). Among the overlapping genes, 10 were affected in the same direc-

Table 1   Genes significantly affected by both testosterone and social challenge in pectoral muscle

Gene ID	Accession # for best hit	Abbreviation	Name of human ortholog	Direction of effect of	
				testosterone	social challenge
isogroup03969	AAF81786.1	SMTN	smoothelin-C	DOWN	UP
isogroup04988	XP_419000.2	MYO10	myosin-X	DOWN	UP
isogroup03683	XP_002188806.1	TMEM60	transmembrane protein 60	UP	DOWN
isogroup13088	XP_002196431.1	RBBP6	retinoblastoma binding protein 6	UP	DOWN
isogroup15578	XP_005489420.1	GPCPD1	glycerophosphocholine phosphodiesterase	UP	DOWN
isogroup19091	XP_005492200.1	PIK3R1	phosphoinositide-3-kinase regulatory subunit 1 (alpha)	UP	DOWN
isogroup10523	NA			UP	DOWN
isogroup12998	NA			DOWN	DOWN

Gene IDs refer to the junco transcriptome. Gene names were assigned based on the best BLAST hit in the non-redundant protein database, and we report names and abbreviations for human orthologs here. NA = no BLAST hit to any annotated gene.

Table 2 Genes significantly affected by both testosterone and social challenge in liver

Gene ID	Accession # for best hit	Abbreviation	Name of human ortholog	Direction of effect of	
				testosterone	social challenge
isogroup06069	XP_002187324.1	KIAA0196	KIAA0196, or strumpellin	DOWN	UP
isogroup11114	XP_002193863.1	AFF2	AF4/FMR2 family member 2	DOWN	UP
isogroup13858	XP_005486373.1	RAPGEF2	rap guanine nucleotide exchange factor 2	DOWN	UP
isogroup00606	NA			DOWN	UP
isogroup07917	NA			DOWN	UP
isogroup10698	NA			DOWN	UP
isogroup16410	NA			DOWN	UP
isogroup19224	NA			DOWN	UP
isogroup21266	NA			DOWN	UP
isogroup16893	NA			UP	DOWN
contig90135	NA			UP	DOWN
isogroup06257	XP_004175949.1	EIF4B	eukaryotic translation initiation factor 4B	DOWN	DOWN
isogroup06294	XP_002190448.1	COL4A1	collagen, type IV, alpha 1	DOWN	DOWN
isogroup10350	XP_002194613.1	OGFR	opioid growth factor receptor	DOWN	DOWN
isogroup13914	XP_002188093.1	GMPR	guanosine monophosphate reductase	DOWN	DOWN
isogroup15116	NP_001006250.1	FKBP4	FK506 binding protein 4, 59kDa	DOWN	DOWN
isogroup17569	XP_002199651.1	CWC22	CWC22 spliceosome-associated protein homolog	DOWN	DOWN
isogroup19870	XP_002186943.1	EXOG	endo/exonuclease (5'–3'), endonuclease G-like	DOWN	DOWN
isogroup21777	XP_002193795.1	PLEKHA8	pleckstrin homology domain-containing family A member 8	DOWN	DOWN
isogroup06066	NA			DOWN	DOWN
isogroup05647	NA			UP	UP

Gene IDs refer to the junco transcriptome. Gene names were assigned based on the best BLAST hit in the non-redundant protein database, and we report names and abbreviations for human orthologs here. NA = no BLAST hit to any annotated gene.

tion in both experiments (47.6%). Because this overlap in the liver is essentially random, we conclude that the gene regulatory responses to T-treatment have no bearing on those responses to a social challenge, and we draw no further functional conclusions from this gene set.

5.2.2 Discussion

These results demonstrate that the immediate gene expression response to a social challenge is not the same as the gene expression response to elevated T, with some degree of tissue-specificity. In the liver, social challenges appear to use different mechanisms than those initiated by T to cause shifts in phenotype. In muscle in contrast, there were slightly more genes in common than expected by chance, though most (7 of 8) were affected in opposite directions, suggesting that T-treatment and social challenges affect *similar* genes, but in *opposing* directions, with a number of additional responses that are unique to T and social manipulations, respectively.

This conclusion becomes clearer when we consider the specific genes affected by the two experimental manipulations. In muscle, SMTN and MYO10, for exam-

ple, are up-regulated in response to social challenge but down-regulated in response to T-treatment, and they are both cytoskeletal components associated with increased contraction (Gerthoffer, 1991; van Eys et al., 2007). Their up-regulation in response to social challenge may reflect the intense behavioral activity characterizing the moments before tissue sampling (e.g. flights, aggressive approach, etc.). Their down-regulation in response to T, however, may be related to the known effects that androgens have on increasing muscle cell size and number (Herbst and Bhasin, 2004), which could reduce the density of such contractile elements.

Four other annotated genes were all down-regulated in response to social challenge and up-regulated by T in muscle tissue, and they all reflect differences in muscle cell growth and metabolism. RBBP6 blocks the activity of a known tumor-suppressor (Motadi et al., 2011), and so its up-regulation in response to T is likely to be permissive to cell division. Similarly, PIK3R1 plays a role in insulin-signaling and associated growth pathways (Engelman et al., 2006), further suggesting increased muscle growth in response to T, but a depression of

growth and insulin uptake in immediate response to social challenge. The direction of these changes in RBBP6 and PIK3R1 expression suggests that T-treatment causes increased cell division (and muscle growth), while social challenge may reduce growth and associated energy expenditure. Functional information regarding GPCPD1 is slim, though other phosphodiesterases have been associated with muscle atrophy (Okazaki et al., 2010), suggesting that GPCPD1 response to T and social challenge is consistent with other effects on growth and metabolism.

Exactly why T and social treatments have largely opposing effects on pectoral muscle is not yet clear, though the temporal difference between the two studies warrants emphasis (i.e. response to roughly 3 weeks of T implant vs. 30 min of social challenge), particularly since long-term T implants can lead to unpredictable effects on some T-sensitive processes due to negative feedback. Future work is needed to directly test whether immediate responses to social challenges have longer term priming effects on the periphery. Unfortunately, the one gene affected in the same direction in both experiments is not annotated, and so its shared function in both contexts in the muscle is unknown.

In the liver, the overlap between T and social challenges is not statistically significant, though a handful of the affected genes bear mentioning, as they hint at the alternative mechanisms of social priming we detailed above. For example, KIAA0196 and RAPGEF2 both interact with adrenergic receptors (Freeman et al., 2013) and are regulated in opposite directions in the two experiments, suggesting catecholaminergic regulation of some of the divergent responses to T and social challenges. Along similar lines, the tissue-specificity in the degree of overlap between T and social treatments also speaks to local modulation of the effects of T and social challenges, again highlighting the local, peripheral effects of an organism's hormonal and social environment as a priority for future research.

6   Conclusions and Future Directions

Our literature review reports many cases in which T and social challenges have qualitatively similar effects on organismal physiology and behavior, though the available data on the gene regulatory mechanisms that likely mediate these effects instead suggest that T and social challenges employ different genes or different pathways to alter phenotype, possibly in different ways. Our own work on T-implanted and socially challenged male juncos underscores this view – that social challenges bring about widespread changes in gene expression, only some of which operate via mechanisms that respond to T treatment.

Regarding the question of whether social priming can occur without a systemic change in T, the literature is slim with direct tests of this hypothesis, and more research is clearly warranted, particularly experiments that use combined pharmacological and social manipulations that tease apart the effects of an animal's hormonal state from its social environment. T does seem to play a key role in influencing future aggressive behavior and success in subsequent social challenges (Fuxjager et al., 2011; Wingfield, 1994). However, because the brain can socially modulate its own steroid microenvironment without changing T in circulation (§3), and certain phenotypes are clearly influenced by peripheral responses to steroid hormones (Fuxjager et al., 2013; Newman and Soma, 2011), we propose that the question of whether social priming can occur without a systemic change in T will boil down to the degree to which the peripheral effects of T and other signaling molecules alter the animal's physiological and genomic state in ways that are adaptive for future social instability. In light of the growing number of exceptions to the challenge hypothesis in songbirds, including the junco example we presented above, we urge greater attention to these peripheral, tissue-specific responses, as they may hold the key to understanding why some animals socially modulate T and others do not.

As evolutionary biologists at heart, we cannot help but note the variety of paths by which evolution might be able to alter how animals respond to the social environment. Much greater phylogenetic representation is needed in this line of research before we will be able reconstruct ancestral states to determine whether mechanistic responses to social challenges and to T are shared or not shared due to co-option of existing regulatory pathways vs. decoupling of T from other socially responsive mechanisms. As habitat loss and climate change force animals into new habitats, altered densities, and greater proximity to anthropogenic stressors, animals are faced with new changes to their social environment. Moving forward, understanding how physiology and behavior respond to these changes from an organismal perspective – one that includes both local and systemic, and neural and peripheral processes – may lead to better predictions of how selection might act on social behavior in the face of continued global change. Much further work is still required, but we hope these perspectives will help to shape approaches to these questions in the future.

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