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Neurogenomic Mechanisms of Aggression in Songbirds

Donna L. Maney^{*} and James L. Goodson[†]

^{*}Department of Psychology, Emory University, Atlanta, Georgia, USA

[†]Department of Biology, Indiana University, Bloomington, Indiana, USA

Abstract

Our understanding of the biological basis of aggression in all vertebrates, including humans, has been built largely upon discoveries first made in birds. A voluminous literature now indicates that hormonal mechanisms are shared between humans and a number of avian species. Research on genetics mechanisms in birds has lagged behind the more typical laboratory species because the necessary tools have been lacking until recently. Over the past 30 years, three major technical advances have propelled forward our understanding of the hormonal, neural, and genetic bases of aggression in birds: (1) the development of assays to measure plasma levels of hormones in free-living individuals, or “field endocrinology”; (2) the immunohistochemical labeling of immediate early gene products to map neural responses to social stimuli; and (3) the sequencing of the zebra finch genome, which makes available a tremendous set of genomic tools for studying gene sequences, expression, and chromosomal structure in species for which we already have large datasets on aggressive behavior. This combination of hormonal, neuroendocrine, and genetic tools has established songbirds as powerful models for understanding the neural basis and evolution of aggression in vertebrates. In this chapter, we discuss the contributions of field endocrinology toward a theoretical framework linking aggression with sex steroids, explore evidence that the neural substrates of aggression are conserved across vertebrate species, and describe a promising new songbird model for studying the molecular genetic mechanisms underlying aggression.

I. AGGRESSION IN CONTEXT

Biomedical studies of aggression and its genetic basis are most often focused on pathology, yet aggressive behaviors and related agonistic displays are essential, adaptive components of social behavior that enable animals to secure and defend food, mates, and territories. For many species, aggression is also required to protect offspring from would-be predators. Thus, given that effective aggression is often essential for gene propagation, we can expect that it will be under strong selection to meet species-typical and population-specific demands. Further, for any given species, aggression will be adaptive in some contexts but not others, and it may therefore be the case that the neural and neurogenomic mechanisms of aggression vary in relation to the functional goals of the behavior. Numerous findings support this view, including evidence that parental aggression and male–male aggression are regulated by different suites of neuroendocrine mechanisms in rodents (Gammie, 2005; Trainor *et al.*, 2008; Veenema *et al.*, 2007) and that neuropeptides differentially influence territorial aggression and aggressive competition for mates in songbirds (Goodson and Kabelik, 2009). Indeed, the idea that aggression is differentially regulated across distinct

functional contexts, and distinct motivational states, has been around for more than 40 years (Moyer, 1968). This functional perspective suggests that ethological approaches will be particularly useful for identifying integrated suites of neurogenomic mechanisms that regulate aggression in any given context and will provide a powerful framework for distinguishing pathology from normal, adaptive variation (Blanchard and Blanchard, 2003, 2005).

In this review, we focus on aggression in the context of competition for resources, for example, defending a breeding territory or a position in a dominance hierarchy within a social group. This type of aggression, particularly in a reproductive context, is part of a suite of related behaviors that characterize a “life history strategy” maximizing short-term gains as opposed to longer term investments (Maynard-Smith, 1977; Trivers, 1972). Short-term relationships with mates, high aggression among same-sex individuals, and low parental care typify this strategy. At the other end of this continuum is a strategy characterized by commitment to one mate, avoidance of injury, and a high level of parental care. The two strategies are difficult to employ simultaneously, resulting in a trade-off between time spent on territorial aggression versus parenting. This trade-off has become a classic principle in ethology and is universal among vertebrates, including humans (Trivers, 1972).

Disruptive selection that drives the sequestration of territorial and parental behavior into alternative strategies is most likely to act on genes with widespread effects—particularly those with multiple functions. Genes encoding the action or regulation of hormones are obvious examples of such genes (Finch and Rose, 1995; Hau, 2007; Ketterson and Nolan, 1992; McGlothlin and Ketterson, 2007; Miles *et al.*, 2007; Moore, 1991; Nijhout, 2003; Rhen and Crews, 2002; Sinervo and Svensson, 2002). A growing literature suggests that trade-offs between parenting and territorial aggression are associated with gonadal steroids; in many species of fish, birds, rodents, and primates, including humans, high levels of circulating androgens are associated with increased intrasexual competition manifested as aggression or mating effort, whereas low levels are associated with increased parenting effort (e.g., Ketterson and Nolan, 1994; McGlothlin *et al.*, 2007). In humans, paternal care and fatherhood have been repeatedly shown to correspond to low levels of testosterone (T) (Fleming *et al.*, 2002; Gray, 2003; Gray *et al.*, 2002; Storey *et al.*, 2000; Wynne-Edwards, 2001), whereas high T levels are associated with male–male aggression and competition (Bernhardt *et al.*, 1998; Book *et al.*, 2001; Booth *et al.*, 1989). These opposing strategies can be generalized as a tendency to prioritize shorter term goals (mating) versus longer term goals (parental investment); at the former end of this continuum in humans, associations have been reported between T and antisocial activities such as alcoholism, drug use, reckless driving, failure to plan ahead, risk-taking, and assaults (Aromaki *et al.*, 1999; Dabbs and Morris, 1990; Udry, 1990). Strategies to balance effort toward short-term versus long-term goals may therefore involve a limited number of hormones and genes. In this review, we attempt to bring together behavior, reproductive endocrinology, and genetics by focusing on species in which all three have been characterized in some detail.

Since the scientific study of behavior began, birds have been the most commonly studied animals in relation to territoriality, dominance, and agonistic communication. Their popularity primarily reflects their unique accessibility—including location and use of vocal

and visual communication channels—and the fact that territorial birds are readily captured using mist nets and playback of song. For the biomedical researcher attempting to model social behavior in humans, birds may seem to represent a rather distant taxonomic group. But in fact, birds have provided the test bed for some of the most influential theories in the history of aggression research, and it is no exaggeration to say that our understanding of the hormonal mechanisms of aggression in all vertebrates, including humans, has been built in large part upon discoveries that were first made in birds (Archer, 2006; Goodson *et al.*, 2005a,b,c; Konishi *et al.*, 1989). For example, pioneering studies in birds established the theoretical framework currently used by researchers to understand how hormones mediate a trade-off between aggression and parenting in mammals (Wingfield *et al.*, 1990). This theoretical framework, which has been called the “challenge hypothesis,” is based on the idea that the role of gonadal steroids in aggression is modulated by social context. It predicts that when males are challenged in a reproductive context, T levels rise to facilitate territorial aggression and suppress parental behavior. Since it was first proposed by Wingfield *et al.* (1990), the challenge hypothesis has found support in a wide variety of nonavian vertebrate taxa including fish, reptiles, and primates, including humans (reviewed by Archer, 2006). The parallels between songbirds (particularly New World sparrows) and humans with regard to the social modulation of gonadal steroids and their effects on aggressive and parental behavior are voluminous and are summarized in Table 5.1. The underlying neuroendocrine mechanisms are nearly identical in birds and humans and are based on the function of the hypothalamo-pituitary-gonadal (HPG) axis, which is universally recognized as being highly conserved across all vertebrates (reviewed by Adkins-Regan, 2005).

Despite the contributions of avian research to our understanding of human behavior, genomic resources in birds have lagged well behind those in mammals—although this situation is now rapidly changing. In the sections that follow, we first explore the neural and endocrine literature on songbird aggression, and then describe a relatively new research program that is focused on the neurogenomics of territorial aggression in white-throated sparrows (*Zonotrichia albicollis*), a species that exhibits morphological and behavioral polymorphisms associated with a chromosomal inversion (Thomas *et al.*, 2008; Thorncroft, 1975). Importantly, the morphs differ not only in their territorial aggression, but also in parental behavior, and thus this species offers an extraordinary opportunity to examine neurogenomic mechanisms that integrate aggression with other aspects of social phenotype and context-specific behavior.

II. HORMONAL MECHANISMS OF AGGRESSION

A. Territoriality in the breeding season

There are about 10,000 species of birds, almost half of which are songbirds. Territoriality runs the gamut, with members of some species nesting colonially or cooperatively, others defending territories of several hectares. Perhaps the best-studied territorial species are the seasonally breeding New World sparrows, which include song sparrows (*Melospiza melodia*), field sparrows (*Spizella pusilla*), white-crowned sparrows (*Zonotrichia leucophrys*), and dark-eyed juncos (*Junco hyemalis*; see Arcese *et al.*, 2002; Chilton *et al.*, 1995; Carey *et al.*, 2008; Nolan *et al.*, 2002 for reviews). In migratory populations, the males

arrive at the breeding grounds a week or so before the females and stake out territories containing food sources and nest sites. The females then arrive, basing their mate choices on the quality of the males as well as their territories. It is therefore important, in fact critical, for males to establish high-quality territories early in the breeding season. Once a male has attracted a mate, she will help defend the territory.

The most ubiquitous and frequent behavior used for territory defense by songbirds is, not surprisingly, song. Each species' song is distinct, and within a species there is enough variation that individuals can recognize each other's songs (Krebs, 1971). Some species sing different types of song in different contexts; for example, the "complex song" of the field sparrow is considered more aggressive than the "simple song" (Carey *et al.*, 2008; Nelson and Croner, 1991), and the chestnut-sided warbler (*Dendroica pensylvanica*) sings a different song to an intruder than he does to a potential mate (Kroodsma *et al.*, 1989; Lein, 1978). Although most of the singing is done by males, females of some species do sing during agonistic encounters (e.g., Baptista *et al.*, 1993; Falls and Kopachena, 2010). Males typically choose a centrally located perch and sing loudly at regular intervals, making their presence known to would-be mates and intruders. In a now-classic study, Krebs (1976) showed that experimental removal of territorial male great tits (*Parus major*) resulted in rapid takeover of the vacated territories by other males; however, broadcasting a former resident's song from a loudspeaker in his territory significantly delayed that takeover (see also Falls, 1988). Although most song is typically sung from a prominent perch in the center, it is also commonly used near territory boundaries, particularly directed at neighbors, as the territory is established. Males learn to recognize their neighbors' songs and will tolerate them at a distance; however, hearing an unfamiliar song will generally trigger an investigation and aggressive response (Falls, 1969; Goldman, 1973, Krebs, 1971; Kroodsma, 1976).

In addition to song, territorial sparrows are likely to exhibit a number of other displays during territory establishment and maintenance. Birds of both sexes may puff out their feathers, in particular raising those on the head to form a crest, or quiver their wings while pointing a closed bill at the intruder (Elekonich, 2000; Nice, 1943). They may peck furiously at nearby objects. If the intruder is unfazed, the resident then resorts to more direct physical threats, flying directly over the intruder, chasing him, and eventually attacking him. Opponents may fly at each other with feet stretched forward and may even fall to the ground as they engage and struggle. Physical fights are rare, however, and generally limited to the early breeding season before territory boundaries are firmly established.

Territorial responses can be studied in the field by observing naturally occurring behavior or by staging a "simulated territorial intrusion" (STI). In this procedure, experimenters place a decoy "intruder," often accompanied by song played through a loudspeaker, onto a resident's territory; the resident's behavioral response is then quantified. Taxidermic or painted models may be used as decoys, or a live, unfamiliar male in cage may be presented. The most robust responses are obtained by presenting both decoy and recorded song so that the resident receives both visual and auditory cues (Wingfield and Wada, 1989). The behavioral data that are collected typically include latency to respond, songs, flights directed at the decoy threat displays (e.g., wing quivers), distance from the decoy at the closest

approach, and the time spent within a certain distance, for example 5 m, of the decoy (Wingfield, 1984b, 1985; Wingfield and Hahn, 1994).

B. Hormones and territoriality

In the 1970s and 1980s, John Wingfield and Donald Farner revolutionized the study of behavior in songbirds by developing methods to measure gonadal hormones in small plasma samples collected from free-living individuals (Wingfield and Farner, 1976). The ensuing research in “field endocrinology” (Wingfield *et al.*, 1990; Walker *et al.*, 2005) elucidated patterns of gonadal hormone secretion over the reproductive cycle in a wide variety of avian species. In general, the stages of breeding associated with high levels of aggression coincide with high plasma levels of T. In song sparrows, for example T peaks during territory establishment when agonistic encounters are most frequent (reviewed by Wingfield *et al.*, 2001), rises again during egg-laying, and slowly declines until the incubation phase when territory disputes are rare (Fig. 5.1A). In some multiple-brooded species, for example house sparrows (*Passer domesticus*), competition for nest holes appears to drive an increase in plasma T during each egg-laying period (Hegner and Wingfield, 1986; Fig. 5.1B).

The temporal correlation between high plasma T and territorial behavior suggests that the two are related, and experimental manipulation of either T or the competitive environment shows that the relationship is bidirectional. Male song sparrows implanted subcutaneously with T-filled silastic capsules during territory establishment showed a more aggressive response to STI than males given empty capsules, and won territories that were twice the size (Wingfield, 1984b,c). Perhaps more interesting, however, was the effect on these males’ neighbors. The residents occupying territories adjacent to the treated males also showed increases in plasma T, suggesting that having to defend their territories against their more aggressive, T-treated neighbors stimulated HPG activity. In a separate study, male song sparrows were removed from their territories, allowing new residents to take over. In this case, both the new residents and their neighbors experienced high T levels compared with unmanipulated controls (Wingfield *et al.*, 1987). Together, these studies show not only that T increases aggression, but also that engaging in agonistic encounters increases plasma T. The HPG response is rapid; plasma T rises significantly within 10 min of STI onset in male song sparrows (Wingfield *et al.*, 1987) and can remain high for several days. This prolonged hormonal response probably heightens vigilance in anticipation of a sustained challenge (Wingfield, 1994a).

C. Aggression outside the breeding season

In seasonally breeding birds, the gonads regress and become quiescent during the nonbreeding season. A testis that measures more than 10 mm across during breeding may shrink to less than 1 mm in the fall. Ovaries likewise regress such that follicles are barely visible. As a result, plasma levels of gonadal steroids fall to low or even nondetectable levels (Wingfield and Farner, 1993). The gonads remain in this state until the long days of spring stimulate photoreceptors in the mediobasal hypothalamus, triggering HPG hormone secretion and gonadal recrudescence (reviewed by Dawson *et al.*, 2001).

For many species, the fall in plasma gonadal steroid levels at the end of the breeding season coincides with abandonment of breeding territories and the onset of flocking behavior. For others, quiescence of the HPG axis does not seem to affect territorial behavior at all. Both of these scenarios are considered below.

1. Aggression in flocks—In species that do not defend territories year-round, the conclusion of territoriality each year may give rise to flocking behavior. In flocks, birds maximize food-finding while minimizing predation risk (Hamilton, 1971). Competition for food, roosting sites, and other resources within flocks creates many opportunities for agonistic interactions, and some of the same behaviors used to defend territories, for example, song and threat displays, are also seen in this context. Other common aggressive displays in flocks include displacements, wherein one individual approaches another and causes it to move away, and hold-offs, wherein an individual refuses to be displaced. Each of these behaviors is easily observed in free-living groups, for example, in the vicinity of a popular food source (e.g., Ficken *et al.*, 1978; Harrington, 1973; Rohwer and Rohwer, 1978), or in captivity (e.g., Schlinger, 1987; Watt, *et al.*, 1984).

In some species, winter flocks adopt a highly organized social structure. Many of us are familiar, for example, with the dominance hierarchies, or “pecking order,” established by chickens (*Gallus gallus domesticus*; Allee, 1936, 1942). Similar social arrangements have been observed in wild and captive groups of sparrows, for example, dark-eyed juncos (*Junco hyemalis*; Sabine, 1959), white-throated sparrows (Schneider, 1984; Watt *et al.*, 1984), Harris sparrows (*Zonotrichia querula*; Rohwer, 1975) and to a lesser extent, song sparrows (Nice, 1943). Within a group, each pair of individuals has a stable relationship such that one is dominant to the other. The subordinate will allow the dominant to displace it, deferring access to resources, and the dominant is unlikely to tolerate the close proximity of the subordinate. When all members of the group are considered together, there is in most cases a linear order of dominance; in other words, there is an alpha bird that dominates all others, a beta that dominates all but the alpha, and so on down to a bird that is subordinate to all. Exceptions, such as triangular relationships, are fairly common. Reversals, in which the hierarchy is challenged and altered, do occur, but overall the arrangement is fixed and stable (Sabine, 1959). In abiding by this social structure, the members of the group avoid the potential injuries and high energy expenditure that would result from constant aggressive encounters; when the hierarchy is stable, actual fighting is extremely rare. Only newcomers are subject to aggressive behavior, which subsides as they are assimilated into the group and assume a fixed rank (Tompkins, 1933).

Whereas physical contact and escalated fights are not normally required for the maintenance of a stable hierarchy, its initial establishment is associated with frequent aggressive interactions. When unfamiliar birds are forming a group, or when newcomers arrive, rank is settled by the outcome of agonistic encounters. Age and sex can predict rank in some cases. For example in white-crowned sparrows and related species, males tend to dominate females and older birds dominate younger ones such that the hierarchy within a group follows the general rule: adult males>adult females>immature males>immature females (Keys and Rothstein, 1991). In some species, such as house sparrows and Harris sparrows, plumage characteristics can predict rank; dominant individuals have dark “bibs” that appear to serve

as status signals (Møller, 1987; Rohwer, 1975). Manipulation of bib coloration does not, however, alter status; rather, it results in heightened aggression toward the altered individuals, whose new plumage coloration is inconsistent with their behavior (Rohwer and Rohwer, 1978).

The relationships between dominance rank and HPG function have been explored in many species of birds. This body of work, which spans almost 75 years, collectively shows that dominance rank is predicted by plasma T levels only in groups that are newly forming. In other words, when unfamiliar birds come together to form a social group, their T levels during the establishment of the hierarchy contribute toward their eventual rank. Later, after the hierarchy is settled and stable, rank is unrelated to plasma T (Baptista *et al.*, 1987; Buchanan *et al.*, 2010; Chase, 1982; Ramenofsky, 1984; Schlinger, 1987; Wiley *et al.*, 1999).

Given that engaging in aggressive behavior causes T to rise (Wingfield *et al.*, 1987), we must ask whether high T leads to the acquisition of a high rank, or vice versa. Some authors have reported that short-term treatment with exogenous T can alter stable hierarchies; the newly acquired ranks were maintained in white-crowned sparrows (Baptista *et al.*, 1987) and domestic hens (Allee *et al.*, 1939) but not Japanese quail (*Coturnix coturnix japonica*; Selinger and Bermant, 1967). In the majority of such studies, T treatment affects eventual rank when it is done early during hierarchy establishment. After rank is stable, however, rank is usually unaffected by T administration (Buchanan *et al.*, 2010; Crook and Butterfield, 1968; Lumia, 1972; Mathewson, 1961; Rohwer and Rohwer, 1978; Wiley *et al.*, 1999). The lack of an effect of T treatment on established, stable ranks has been attributed to learning, or “social inertia” (Guhl, 1968; Wiley *et al.*, 1999), in groups of individuals that are familiar with each other. In house sparrows, which do not defend large breeding territories, dominance rank during the nonbreeding season is determined largely by rank at the end of the breeding season when plasma T level is falling (Buchanan *et al.*, 2010). In other words, winners stay winners and losers stay losers (Chase, 1982). HPG activity during the breeding season may therefore contribute toward rank during the nonbreeding season. During both times of year, actual physical aggression is limited to periods of instability during which high-ranking positions up for grabs or otherwise in dispute. After the establishment of social relationships and boundaries, physical aggression is rare and plasma levels of T are much lower.

2. Territoriality in the nonbreeding season—Song sparrows in resident populations, despite being seasonal breeders that undergo gonadal regression in the fall, can maintain essentially the same territories year-round. Whereas the song sparrows studied by Wingfield in New York State (e.g., Wingfield, 1984a,b,c, 1985) abandon their territories and migrate at the conclusion of the breeding season, populations in Western Washington remain on the same territories, molt their feathers, and then, despite having completely regressed gonads and nondetectable levels of T, resume territorial defense (Wingfield and Hahn, 1994). Furthermore, young males just hatched the previous spring can establish new territories in the fall without an increase in T. STI during the fall induces the same behavioral responses as in spring, but without an accompanying HPG response (Soma and Wingfield, 2001; Wingfield and Hahn, 1994), and castration does not interfere with the ability to maintain a territory (Wingfield, 1994b). These results led to the hypothesis that territorial aggression

and gonadal steroid secretion can become uncoupled in this and other species that defend territories outside the breeding season.

To address this question, Soma *et al.* (1999, 2000a,b) tested whether T action is necessary for autumnal aggression in a population of song sparrows in Western Washington. To block the effects of T, they administered androgen receptor antagonists and, because T can also act via conversion to estradiol (E2), blockers of that conversion. They found that blocking the action of aromatase, an enzyme that converts T to E2, reduced territorial aggression even in the fall when plasma levels of gonadal steroids are naturally very low. This result suggested that autumnal aggression is not in fact independent of steroid hormones. This reduction was reversed by treatment with E2 (Soma *et al.*, 2000a).

Because gonads are not required for autumnal territorial defense (Wingfield, 1994b), the E2 that drives this behavior must come from a non-gonadal source. One such source may be the brain itself, which contains high levels of aromatase. In song sparrows, aromatase mRNA is expressed in the brain at all times of year (Soma *et al.*, 2003; Wacker *et al.*, 2010) suggesting a possible source of E2 available to brain tissue year-round. Aromatase activity in the ventral telencephalon, which contains the putative avian homologue of the amygdala, is reduced during molt, at which time aggression is also low (Soma *et al.*, 2003). Brain-generated E2 may thus be an important regulator of territorial aggression in this species. To synthesize E2, the brain may make use of androgen precursors from regressed gonads or the adrenals (see Soma, 2006; Soma *et al.*, 2008 for reviews). Alternatively, some regions of the brain contain all of the enzymes necessary to synthesize E2 *de novo* from cholesterol substrate (reviewed by Soma *et al.*, 2008; Ramage-Healey *et al.*, 2010), obviating the need for a peripheral steroid synthesis altogether. The regions of the brain important for aggressive behaviors, for example the nuclei that control singing behavior, are rich in these enzymes (Ramage-Healey *et al.*, 2010). Aromatase expression is high in the ventromedial nucleus of the hypothalamus (VMH) during all times of year except during molt, when aggression is virtually absent (Wacker *et al.*, 2010). In mice, distinct populations of VMH neurons contribute to fighting and mating behavior (Lin *et al.*, 2011). In Section III, we will explore further the role of this region in aggression in songbirds.

Like androgen release from the gonad, E2 synthesis by the brain appears to be behaviorally regulated. Ramage-Healey *et al.* (2008) recently showed that in zebra finches (*Taeniopygia guttata*), hearing conspecific song increases E2 concentrations in the auditory forebrain within minutes. Pradhan *et al.* (2010) showed subsequently that in nonbreeding, territorial song sparrows, exposure to STI rapidly increases activity of 3 β -hydroxysteroid dehydrogenase, an enzyme necessary to synthesize E2 from androgen precursors. These findings demonstrate that the steroid environment within the brain is dynamic, sensitive to the social environment, and more independent of the gonad than previously thought. These discoveries of socially regulated, brain-generated steroid synthesis challenge the traditional view of hormone-mediated aggression and highlight the importance of songbird models to our understanding of how steroids regulate gene activity in the brain.

Forty years of research on free-living sparrows has shown that aggression depends on steroid hormones. Even when at first glance it appears that aggression and steroid hormones have

come “uncoupled,” for example, when resident song sparrows vigorously defend territories despite low plasma T, the evidence shows that low levels are necessary for the expression of territoriality and that the brain itself may produce sufficient amounts. The role of steroid hormones, particularly E2, in aggression seems very similar to their role in sexual receptivity (reviewed by Maney, 2010): plasma levels need not be high, and in fact seasonal peaks in plasma levels need not be associated in time with the behavior. A low level, however, is required for the behavior to be expressed. Because the frequency of aggressive behavior is clearly not always correlated directly with plasma levels of steroid hormones, it is possible that the hormones play a priming, or permissive, role and that other hormones or neurotransmitters are also important.

D. Evolution of aggression and life history strategies

Research on wild song birds has demonstrated a robust, two-way relationship between aggression and HPG activity. What does that finding tell us about the evolution and genetic control of these behaviors? Because steroid hormones affect suites of behaviors, not just aggression, it is helpful to think about this issue in terms of behavioral strategies. As discussed in Section I above, investment in territory defense and mate-finding defines a strategy at one end of a behavioral continuum, with investment in survival and parenting at the other (Trivers, 1972). This trade-off appears to be mediated, at least in part, by HPG activity (Ketterson and Nolan, 1994; McGlothlin *et al.*, 2007; Wingfield *et al.*, 1990). In species with male parental care, T is high during territory establishment but falls during the parental phase (Fig. 5.1A, B). Exogenous administration of T during the parental phase inhibits parental behavior and increases territorial behavior (Hegner and Wingfield, 1987; Schoech *et al.*, 1998; Silverin, 1980). In species without male parental care, for example polygynous species in which females do the bulk of the care, T remains high in males for the duration of the season (Fig. 5.1C). In a study by Wingfield (1984c), T treatment of male song sparrows not only reduced parental care but also induced polygyny in this normally monogamous species. These males spent much of their time singing and acquired huge territories, attracting multiple females. Their failure to provision the young, however, likely reduced their overall reproductive success (Hegner and Wingfield, 1987; Silverin, 1980). Alterations in HPG function could therefore result in large, cascading effects and make an important contribution to variation in social behavior and social strategies (Ketterson and Nolan, 1992; Sinervo and Svensson, 2002). When the effects of a hormone are antagonistic with respect to behavior, for example, in the case of T and parenting versus aggression, “antagonistic pleiotropy” can give rise to behavioral trade-offs (Finch and Rose, 1995). We hypothesize that suites of related genes, the expression of which is tightly governed by social cues, may act hierarchically to organize and regulate the hormonal and neural systems that promote territorial aggression and reduce parental behavior. In the next section, we explore the neural circuits that are involved and consider how evolution has shaped them in species with different behavioral strategies.

III. TRANSCRIPTIONAL ACTIVITY AND NEURAL MECHANISMS OF AGGRESSION IN BIRDS

A. Transcriptional traces of aggression reveal ubiquitous vertebrate themes

Although it has long been known that all vertebrates share some basic features in the organization of the amygdala, hypothalamic nuclei, and associated (limbic) areas of the basal forebrain and midbrain, the extent of these similarities has become much more clear in the past 10 years as investigators have combined genomic data with conventional neuroanatomical and functional approaches (e.g., using lesions and pharmacological manipulations). We now know that birds and rodents exhibit extraordinary similarities in the organization of limbic brain areas (Goodson, 2005; Newman, 1999), including distinct homologies at the subnuclear level (e.g., Goodson *et al.*, 2004a; Kingsbury *et al.*, 2011) and very specific similarities in the topographical patterns of transcriptional response to aggressive interactions (and other social interactions, as well), as established through the experimental induction of immediate early gene (IEG) transcripts and their protein products (Ball and Balthazart, 2001; Goodson, 2005). The IEGs most commonly used for such functional studies are *c-fos* and a gene variably known as *zif-268*, *egr-1*, *NGFI-A*, *krox-24*, or *Zenk* (the latter being a name often used in birds as an acronym for the other four names; Mello *et al.*, 1992).

Experimentally induced increases in IEG mRNA can be detected by *in situ* hybridization (ISH) within 15–30 min. For detection of IEG proteins by immunocytochemistry (ICC), most investigators harvest brain tissue 60–90 min after the experimental manipulation, such as an aggressive interaction. Because the induced IEG proteins are still elevated at 90 min and two half-lives of the protein have passed (Herdegen and Leah, 1998), it is possible to determine whether the experimental manipulation may have decreased IEG activity from control levels (e.g., Bharati and Goodson, 2006; Goodson and Wang, 2006). Most behavioral neuroscientists are primarily interested in IEGs not because of what they do inside the cell, but rather because they provide a proxy marker to indicate that a cell has responded in some way to a stimulus. That response may or may not be associated with action potentials and release of neurochemicals (Herdegen and Leah, 1998), but by labeling for IEG transcripts and proteins, investigators can gain a good idea about the functional properties of different brain areas or cell groups. The actual molecular functions of IEGs are varied, but typically include the regulation of other genes involved in experience-dependent neuroplasticity (Herdegen and Leah, 1998; Mello and Ribeiro, 1998), and thus IEGs probably have the ultimate effect of changing the way that cells function and behave during subsequent behavioral interactions.

In rodents, resident–intruder encounters induce IEG activity in a characteristic pattern that includes the medial amygdala (MeA), medial bed nucleus of the stria terminalis (BSTm, a component of the medial extended amygdala that shares many anatomical and functional properties with the MeA), anterior hypothalamus (AH), ventrolateral lateral septum (LS), ventrolateral subdivision of the ventromedial nucleus of the hypothalamus (VMHvl; or lateral VMH in hamsters), dorsal premammillary nucleus, and the dorsal midbrain periaqueductal gray (Kollack-Walker *et al.*, 1997; Motta *et al.*, 2009). Notably, along with

the medial preoptic area, these same brain areas are central to the regulation of most other social behaviors, including communication behaviors, parental care, pair bonding, appetitive and consummatory sexual behavior, juvenile play, social recognition, and both same-sex and opposite-sex affiliation (Goodson, 2005; Newman, 1999). Despite the similarities, the relative amount of IEG activity across the different nodes of this “social behavior network” is distinctive for each social context, suggesting that functional relationships across the network nodes are dynamic and context-specific. Distinct patterns of correlated activity between network nodes in different social contexts have now been demonstrated in multiple vertebrate classes (Crews *et al.*, 2006; Hoke *et al.*, 2005; Yang and Wilczynski, 2007).

The areas comprising the social behavior network are readily identified in birds and are anatomically and functionally conserved across amniote vertebrates, and in fact, the basic features of this network are present even in fish (Goodson, 2005; Goodson and Bass, 2002). Consistent with this conservation, territorial songbirds housed in captivity exhibit a pattern of IEG activity after STI that is virtually identical to the pattern described for rodents after a resident–intruder encounter (Goodson and Evans, 2004; Goodson *et al.*, 2005b). This work has been conducted in animals housed in their natural habitat, and data for catecholaminergic midbrain areas are even available from animals occupying natural territories (Maney and Ball, 2003). In addition, following exposure to same-sex conspecifics through a wire barrier in a quiet room (which elicits very little overt behavior), territorial finches exhibit relatively greater Fos and/or egr-1 responses than do gregarious species in a pattern similar to aggressive encounters (Goodson *et al.*, 2005a). Hence, at least to an extent, the IEG activity of these brain areas reflects perceptual or motivational processes, not simply activation of aggression.

Although it is intuitive to interpret IEG induction as reflecting a positive relationship between a brain area and behavior, negative correlations between IEG cell counts and aggressive behavior are observed for multiple brain areas. These include the AH, both pallial and subpallial subdivisions of the LS, and the paraventricular nucleus of the hypothalamus (PVN; Fig. 5.2; Goodson *et al.*, 2005b). This pattern of results suggests that aggression is under inhibitory control, at least by some areas. Consistent with this idea, lesions of the LS increase resident–intruder aggression in male field sparrows and pigeons (*Columba livia*; Goodson *et al.*, 1999; Ramirez *et al.*, 1988). Note, however, that such effects are not observed in some contexts, such as aggressive competition for mates in male zebra finches, a highly gregarious species (Goodson *et al.*, 1999).

The PVN is heavily interconnected with the social behavior network and plays an important role in the regulation of autonomic and pituitary activity in relation to behavioral state. A subset of cells in the PVN produce arginine vasotocin (VT; homologue and evolutionary precursor of mammalian arginine vasopressin, VP), and the percentage of those cells that express Fos is also negatively correlated with aggressive response to an STI in male song sparrows (Fig. 5.3; Goodson and Kabelik, 2009). VT and VP are secretagogues for adrenocorticotrophic hormone, and thus the lower IEG activity of the PVN VT cells in more aggressive males likely reflects a lower stress response to the encounter. Virtually identical results for VP–Fos colocalization are obtained in lab mice (Ho *et al.*, 2010). As addressed in Section III.B below, this negative relationship between aggression and VT–Fos

colocalization in the PVN accurately predicts pharmacological effects that vary in relation to the subject's dominance status (Goodson *et al.*, 2009b).

B. Neurochemistry and major modulators

Although territorial aggression in birds has been the focus of hundreds of studies, including many that address proximate endocrine mechanisms (Goodson *et al.*, 2005c; Konishi *et al.*, 1989), a surprisingly small number of experiments have been conducted with the goal of delineating relevant neurochemical circuits in the brain. An early study of whole-brain neurochemistry shows that dopamine, norepinephrine, and acetylcholine are all associated with aggressive behavior in male Japanese quail (Edens, 1987), and catecholaminergic midbrain nuclei show increased IEG activity in response to STI in song sparrows (Maney and Ball, 2003).

Following aggressive competition for a potential mate, male zebra finches exhibit significant increases in the percentages of tyrosine hydroxylase-ir (TH-ir) cells expressing Fos within the “retrosubstantia nigra” area (A8), substantia nigra (A9), ventral tegmental area (VTA; A10), and midbrain central gray (A11), but show a significant decrease in TH–Fos colocalization within the A12 neurons of the tuberal hypothalamus (Bharati and Goodson, 2006). TH is the rate-limiting enzyme for catecholamine synthesis, and all of the cell groups just listed are known to be dopaminergic. Despite these results, treatments with quinpirole, a dopamine D2 receptor agonist, significantly decrease aggression during mate competition. D1 and D4 agonists are without effect, although a modest inhibition is observed with the D3 agonist 7-OH-DPAT, which may reflect weak binding to the D2 receptor (Kabelik *et al.*, 2010). These seemingly contradictory results likely reflect the fact that courtship is displayed at a high rate during the competition tests, and given that TH–Fos colocalization in the central gray and caudal VTA correlates positively with courtship singing (Goodson *et al.*, 2009a), the increased colocalization of TH and Fos following mate competition is likely attributable to directed singing and not the display of aggression. The number of TH-ir cells in the central gray also correlates positively with the average number of songs that male zebra finches sing to females during courtship tests. Notably, territorial finch species exhibit fewer TH-ir cells in the caudal VTA than do gregarious species such as the zebra finch, although this may reflect a lower level of affiliation rather than a negative relationship between this cell group and aggression (Goodson *et al.*, 2009a).

Dopaminergic mechanisms of song have also been examined in European starlings (*Sturnus vulgaris*), which sing in the context of breeding both to attract females and to repel other males. Antagonism of D1 receptors decreases song in breeding-condition males, whereas a dopamine reuptake inhibitor facilitates it (Schroeder and Riters, 2006), and aggressive song correlates negatively with D1 receptor density in numerous areas, including the LS, BSTm, medial preoptic area, and central gray (Heimovics *et al.*, 2009).

The handful of other neurochemical manipulations that have been conducted in studies of avian aggression have focused on neuromodulators such as VT, vasoactive intestinal polypeptide (VIP), and endogenous opioids. Of these, the endogenous opioids have received the least attention, but are known to inhibit aggression in Japanese quail, at least partially via the delta receptor subtype (Kotegawa *et al.*, 1997). These neuropeptides are each produced in

multiple brain areas, and as suggested for VT, it may be the case that the different cell groups have divergent effects on behavior (a possibility that should also be considered in relation to the major neurotransmitters just discussed; Goodson and Kabelik, 2009).

Both VIP and VT exert complex effects on aggression that likely reflect the modulation of stress- and anxiety-related processes. For instance, in territorial male field sparrows housed in aviaries placed in their natural habitat, intraseptal infusions of VT decrease aggression in resident–intruder tests, but selectively facilitate the spontaneous use of an agonistic song type during the dawn song period (Goodson, 1998a). No effects are observed for the multipurpose song type that is used to attract females, and VIP tends to exert an opposite pattern of effects (Goodson, 1998a,b). Interestingly, the VT and VIP systems in the LS are both sensitive to sex steroids. Castration causes down- and upregulation of VT and VIP immunoreactivity, respectively, and T or E2 replacement reverses these effects (Aste *et al.*, 1997; Panzica *et al.*, 2001; Voorhuis *et al.*, 1988; but see Wacker *et al.*, 2008). The VT/VIP systems therefore represent a possible mechanism whereby gonadal steroids may modulate aggression.

In order to determine whether VT modulates stress-related processes, and whether it does so in a manner that is integrated with its effects on agonistic behavior, Goodson and Evans (2004) examined Zenk responses to nonsocial stress alone (capture in an outdoor flight cage and restraint for intraventricular infusions), or the same nonsocial stressors followed by STI. These manipulations were conducted in male song sparrows housed in flight cages placed in their natural habitat. Subjects were infused with either vehicle or a VT V_{1a} receptor antagonist and were sacrificed at the completion of testing for immunolabeling of Zenk and VT. In some brain areas, nonsocial and social stimuli induced Zenk within the same subset of cells, which was discernable because (1) in vehicle-treated animals, the nonsocial stressor induced a significant increase in Zenk-ir cell numbers, and subsequent exposure to the social challenge produced no further increase, but (2) blocking the Zenk response to handling with the V_{1a} antagonist revealed a sensitivity to the social challenge (i.e., by eliminating the ceiling effect). This “integrated” pattern of Zenk response was observed for numerous areas, including the AH, POA, lateral VMH, lateral BST, and most zones of the LS. Notably, in all of these cases, the antagonist exerted more pronounced effects in the subjects that were exposed to the nonsocial stress alone. However, in the BSTm and ventrolateral LS, Zenk responses to the social challenge were significantly greater than to the nonsocial stressor, even in vehicle-treated subjects, indicating that at least some cells in these areas are more selectively activated by social challenge. The BSTm showed particularly selective responses to the social challenge, which were completely blocked by the V_{1a} antagonist (Goodson and Evans, 2004).

Unfortunately, VT-ir cells of the BSTm were not detectable in this study (these neurons are weakly immunoreactive in most vertebrates and may store little peptide relative to the amount being released), but the VT-immunoreactive neurons of the PVN showed significant responses to social challenge and, most interestingly, the VT–Zenk colocalization was reduced by the V_{1a} antagonist only in the animals exposed to the STI (Goodson and Evans, 2004). As assessed in a later study with more robust immunolabeling of VT, VT–Fos colocalization in the BSTm of male song sparrows is not increased by social challenge,

whereas colocalization in the PVN is negatively correlated with aggression (Fig. 5.2; Goodson and Kabelik, 2009).

In territorial estrildid finches, exposure to a same-sex conspecific through a wire barrier actually decreases VT–Fos colocalization in the BSTm, but the same manipulation increases VT–Fos colocalization in gregarious finch species, and the territorial birds do show large increases in VT–Fos colocalization if they are reunited with their pair-bond partner. Conversely, socially induced VT–Fos colocalization in the BSTm is blocked in the gregarious zebra finch if the subjects are intensely subjugated by a dominant bird (Goodson and Wang, 2006). Thus, the VT neurons of the BSTm exhibit an exquisite sensitivity to the valence of social stimuli, and more recent findings suggest that this valence sensitivity is not extended to nonsocial stimuli (Goodson *et al.*, 2009c).

The differential response profiles of the VT neurons in the BSTm and PVN may account for at least a portion of the context-specificity that is observed following central infusions of VT or V_1 receptor antagonists. For instance, in zebra finches, VT promotes aggression in the context of mate competition and a V_{1a} antagonist inhibits aggression (Goodson *et al.*, 2004b). These effects are consistent with the observation that VT–Fos colocalization is increased in the BSTm during mate competition, but not in the PVN. Conversely, resident–intruder aggression is inhibited by VT infusions in territorial species, consistent with the negative correlation between aggression and VT–Fos colocalization in the PVN (Goodson and Kabelik, 2009; Goodson and Wang, 2006). The different effects on mate competition and resident–intruder aggression (or nest defense in zebra finches) can be observed in the same species (Goodson *et al.*, 2009b; Kabelik *et al.*, 2009), as shown for the territorial violet-eared waxbill (*Uraeginthus granatina*) in Fig. 5.4A–B. However, in the violet-eared waxbill, males that are typically dominant do not show a behavioral response to the V_{1a} antagonist in standard resident–intruder tests whereas aggression is facilitated in subordinates (Fig. 5.4C; Goodson *et al.*, 2009b). Thus, perhaps only the subordinate males activate the PVN VT neurons during aggressive encounters and this activation inhibits aggression.

The ability to label IEG products has provided immeasurable insight into the neural basis of aggression, allowing the identification and mapping of specific circuits that respond rapidly to social stimuli. Lacking until recently were powerful genomic methods necessary for a more complete understanding of the complex protein interactions involved in social responses. The sequencing of the zebra finch genome (Warren *et al.*, 2010) has provided unprecedented insight into what happens inside the songbird brain during agonistic encounters. Using tools such as high throughput sequencing and microarray analysis, investigators can now look at the regulation of many hundreds of genes simultaneously. In a recent gene profiling study, Mukai *et al.* (2009) compared the expression of more than 11,500 different gene transcripts in free-living song sparrows responding either to STI or a control intrusion by a heterospecific. For behavioral manipulations conducted during the breeding season, 67 gene transcripts were differentially expressed in the hypothalamus following exposure to an STI compared to control. During the fall, when territorial aggression seems to be independent of gonadal steroid production (reviewed by Soma, 2006; Soma *et al.*, 2008; see also Section II), 173 transcripts were affected (Mukai *et al.*, 2009).

There were significant interactions between season and STI for 88 transcripts (Mukai *et al.*, 2009), which may in part reflect the differential regulation of the pituitary–gonadal axis across seasons. The expression of many of the gene transcripts was not, however, affected by season and therefore may be important for the regulation of aggressive behavior itself rather than endocrine responses to aggressive encounters. This study represents the early days of genomic analysis of social behavior in free-living, natural populations and sets the standard for many more sure to follow. In the next section, we consider a songbird species that because of a natural genetic anomaly is becoming a popular model for studying the genetic mechanisms underlying aggression.

IV. A NATURAL MODEL UNITING SOCIAL BEHAVIOR, HORMONES, AND GENETICS

A. The white-throated sparrow

The underlying genetic basis of variation in social behavior is of intense interest, yet only a handful of genes have been linked to specific social behaviors in vertebrates (reviewed by Robinson *et al.*, 2005). Thus, there is an obvious need to identify populations, human or otherwise, in which there is clear linkage between genes and social behavior. A common wild songbird, the white-throated sparrow, offers such an opportunity. This species, in which socially monogamous pairs defend breeding territories, provision the young with food, and form flocks with stable dominance hierarchies in the winter, is a typical New World sparrow in nearly all respects. What sets it apart from other songbirds is that it exhibits alternative phenotypes, defined by a plumage polymorphism, that differ in their social behavior. Both males and females can be categorized into one of two plumage morphs that differ primarily in the color of the crown stripes (Lowther, 1961; Piper and Wiley, 1989; Watt, 1986; see Fig. 5.5). Behavioral studies conducted in the animals' natural habitat have established that individuals with a white medial stripe (WS) on the crown engage in a more aggressive strategy, whereas birds with a tan medial stripe (TS) are more parental.

The species represents a promising model in which to study the genetic basis of aggression because the plumage pattern segregates with the presence or absence of a structural rearrangement of chromosome 2. WS individuals are heterozygous for the rearranged chromosome ($ZAL2^m$), whereas those of the TS morph are homozygous for the wild-type chromosome ($ZAL2$; Thornycroft, 1975). Once they molt into adult plumage the phenotype is fixed for the lifetime of the individual. Within a population, approximately half of the birds are WS ($ZAL2/2^m$), whereas the other half are TS ($ZAL2/2$; Lowther, 1961; Thornycroft, 1975). This balanced polymorphism is maintained in the population by disassortative mating—WS and TS birds nearly always mate with individuals of the opposite morph (Knapton and Falls, 1983; Lowther, 1961; Thornycroft, 1975; Tuttle, 1993). This mating pattern results in a virtual absence of birds homozygous for $ZAL2^m$, a genotype that Thornycroft (1975) hypothesized may be less viable due to recessive deleterious mutations. Of more than 1000 individuals genotyped, only one was found to be homozygous for $ZAL2^m$ (Maney *et al.* unpublished data; Romanov *et al.*, 2009; Thornycroft, 1975).

The behavioral differences that segregate with the *ZAL2^m* chromosome have been well documented in field studies. Males and females of the WS morph are more aggressive, both in territorial defense and in mate-seeking, than their TS counterparts. WS males sing more in response to STI than TS males (Collins and Houtman, 1999; Kopachena and Falls, 1993a; Horton and Maney, unpublished observations) and are more likely to trespass onto the territories of other males (Tuttle, 2003). Whereas WS females sing and engage in active territorial defense independently of their mates, TS females do so only rarely (Kopachena and Falls, 1993a; Horton and Maney, unpublished observations). TS birds of both sexes feed young more often during the parental phase of the breeding season than do WS birds (Knapton and Falls, 1983; Kopachena and Falls, 1993b). The relative strategies employed by the different morphs of this species therefore fall onto different ends of the behavioral continuum between territoriality and parenting (Trivers, 1972).

B. Endocrine and neuroendocrine correlates of behavioral polymorphism

Because the behavioral trade-off between territorial defense and parenting is clearly mediated at least in part by HPG activity in songbirds (Ketterson and Nolan, 1994; McGlothlin *et al.*, 2007; Wingfield *et al.*, 1990), we should immediately suspect that, in white-throated sparrows, HPG function may vary according to morph. Spinney *et al.* (2006) found that in free-living birds in breeding condition, WS males do have larger testes and higher levels of circulating T than TS males. This phenomenon has also been demonstrated in captive populations (Maney, 2008; Swett and Breuner, 2009). In both the field and the lab, however, the difference in plasma T disappears when birds are not in breeding condition (Maney, 2008; Spinney *et al.*, 2006). Interestingly, morph differences in aggression appear only during the breeding season, mirroring the morph difference in circulating T. In winter flocks and in laboratory-housed birds held on short days, morph is not related to dominance rank or to aggression (Dearborn and Wiley, 1993; Harrington, 1973; Piper and Wiley, 1989; Schlinger, 1987; Schwabl *et al.*, 1988; Watt *et al.*, 1984; Wiley *et al.*, 1999). In contrast, when birds are held on long days and undergo gonadal recrudescence, WS birds engage in significantly more aggression than their TS cage-mates and tend to outrank them (Fig. 5.6; see also Watt *et al.*, 1984). Morph differences in dominance and aggression may therefore depend on season and thus perhaps on differences in HPG function.

Because levels of gonadal steroids differ between the morphs, the behavioral polymorphism may be driven by the effects of these steroids on the brain. To evaluate this hypothesis, Maney *et al.* (2005) compared the morphs with respect to the VT and VIP neuropeptide systems, which are highly steroid dependent (Aste *et al.*, 1997; Panzica *et al.*, 2001; Voorhuis *et al.*, 1988). WS birds had higher levels of VT-immunoreactivity in the BSTm and ventrolateral LS than TS birds. Since T is higher in WS males, this result is consistent with the idea that T may be engaging the VT system in the LS. Central administration of VT in the closely related white-throated sparrow induces agonistic song (Maney *et al.*, 1997), suggesting that engagement of this system may be directly related to aggressive behaviors. Central administration of VIP, in contrast, reduces agonistic song in field sparrows (Goodson, 1998a); immunoreactivity for this peptide was higher in the ventrolateral LS of the TS (less aggressive) morph. VIP immunoreactivity in this region is inversely

proportional to T levels (Aste *et al.*, 1997) again supporting a possible role for gonadal sex steroids in the control of aggression in this species.

C. Causality and “phenotypic engineering”

Looking for morph differences in endocrine variables in unmanipulated individuals is an important endeavor, in that significant correlations can help illuminate possible physiological causes of aggression. Such correlations alone, however, can provide only limited information on causal mechanisms. The morph difference in plasma T, for example, could be a consequence, rather than a cause, of polymorphic behavior. Either scenario would explain the observed correlations between T and social behavior (Spinney *et al.*, 2006). As discussed in Section II above, experiments involving manipulation of T or of social contexts in songbirds have revealed causal effects in both directions. Recall that in other songbird species, free-living males treated with T defend larger territories, engage in more aggression, acquire more mates, and provide less parental care than untreated males (Hegner and Wingfield, 1987; Schoech *et al.*, 1998; Silverin, 1980; Wingfield, 1984b,c). Territorial intrusion or the presence of receptive females, however, cause release of endogenous T (Dufty and Wingfield, 1986; Moore, 1983; Wingfield and Hahn, 1994; Wingfield and Monk, 1994). A one-way causal effect of T on aggression and parenting may not completely explain polymorphic behavior in white-throated sparrows.

Some authors have suggested that the role of hormones in alternative phenotypes is best studied by performing hormonal manipulations, or “phenotypic engineering” (Ketterson and Nolan, 1992; Miles *et al.*, 2007; Zera and Harshman, 2001). To test whether morph-dependent variation in territorial behavior in male white-throated sparrows is attributable entirely to variation in T, Maney *et al.* (2009) eliminated morph differences in T and then compared WS and TS responses to STI in the lab. Males in nonbreeding condition received silastic implants containing T, so that plasma levels in the WS and TS groups were high and equal. When presented with audio playback of conspecific male song, WS males sang significantly more often than TS males. This result suggests that WS males respond more aggressively to a territorial challenge than TS males, even when T levels are experimentally equalized between the morphs.

If morph differences in social behavior are not caused simply by differences in plasma T, then our search for causal factors should turn to other aspects of HPG function, for example, steroid binding or metabolism. The list of such factors is long and includes a large number of receptors, enzymes, and binding globulins. Comparative genomic approaches are required to conduct large-scale comparisons of gene expression as well as detailed analysis of the genetic differences between the morphs.

D. Mapping the ZAL2^m

The early genetic work in the white-throated sparrow, done more than 35 years ago (Thornycroft, 1975), showed definitively that morph differences are associated with a clear, tractable chromosomal rearrangement. The ZAL2^m chromosome thus offers a powerful starting point for understanding the mechanisms underlying aggressive behavior in birds and other vertebrates. In essence, nature has created a genetic manipulation that allows us to

identify genes that are affected by the rearrangement and therefore potentially causal for heightened aggression in the WS individuals.

The identification of such genes first requires mapping of the ZAL2^m rearrangement; genes that map within it can then be evaluated as likely candidates. Using a comparative genomic approach, Thomas *et al.* (2008) (see also Davis *et al.*, 2011; Huynh *et al.*, 2010a,b) began the initial modern genomic characterization of the ZAL2^m rearrangement. By taking advantage of the genomic resources available for two other avian species, the chicken and the zebra finch, they established a comparative map of ZAL2^m and found that the chromosome contains a complex rearrangement involving not one but at least two inversions around the centromere (Fig. 5.7). The two inversions may have occurred in succession, with the second completely contained within the first. Alternatively, ZAL2 and ZAL2^m may each represent rearranged versions of an ancestral chromosome 2, having undergone rearrangement at different times. The rearrangement now spans the majority of the chromosome and could contain as many as 1000 protein-coding genes (Davis *et al.*, 2011; Thomas *et al.*, 2008). Thus, although the region containing the rearrangement is large, this work has delineated a finite set of genes linked to the behavioral and plumage polymorphisms in this species.

Exactly how does the architecture of ZAL2^m affect the expression of the genes inside the rearrangement and the proteins they encode? Inversions are hypothesized to affect gene and protein function in two main ways. First, genes at or near the breakpoints may be physically disrupted or otherwise directly affected by the breakage and subsequent change in position. So far, sequencing efforts have identified no genes physically disrupted by the ZAL2^m breakpoints (Davis *et al.*, 2011); however, position effects may have led to functionally distinct alleles for those nearby. For example, a cluster of genes encoding bitter taste receptors has been separated by one of the breakpoints and now maps to different arms of the chromosome (Davis *et al.*, 2010). This separation, which appears to have led to nonsynonymous variants detected between the ZAL2 and ZAL2^m, may have implications for diet and habitat selection but is unlikely to explain morph differences in aggression and parenting.

The behavioral polymorphisms in social behavior in this species are more likely related to a second important consequence of pericentric inversions, which is the suppression of recombination and subsequent genetic differentiation of the inverted region. Thorneycroft (1975) observed that pairing in the ZAL2/2^m bivalent during meiosis was limited to one arm of each chromosome. Cytogenetic mapping efforts (Davis *et al.*, 2011; Thomas *et al.*, 2008) suggest that single recombination events elsewhere in the chromosome would give rise to gametes with large duplications and deletions, thereby effectively preventing the inheritance of the recombined chromosomes. ZAL2^m may therefore be largely isolated from ZAL2. Population genetics studies have confirmed differentiation between the ZAL2 and ZAL2^m over the entire rearranged region as a result of suppression of recombination (Huynh *et al.*, 2010a; Thomas *et al.*, 2008). Alleles are shared between the haplotypes only at the tip of the short arm of ZAL2/2^m, which is outside the rearrangement (Fig. 5.7). The rearrangement itself contains a unique set of alleles that are not shared with ZAL2—an estimated 3000 fixed differences (Davis *et al.*, 2011), and this set is inherited together. Thus, the lack of gene flow between the ZAL2 and ZAL2^m has provided opportunity for the evolution of

functionally distinct alleles that are restricted to one arrangement or the other. In the continuing analysis of the rearrangement, we should expect to find a series of genes, inherited together as a unit in WS birds, that are functionally distinct from the ZAL2 alleles with regard to either their protein products or patterns of expression. Given the important role of the HPG axis in aggression in this and other species, the strongest candidates will be closely related to HPG function.

V. FUTURE DIRECTIONS

Genetic research with comparative models will ultimately show how key genes, the molecular functions of which are conserved across evolutionary divergence, relate to complex and highly derived social behaviors such as aggression. The mechanisms that underlie social behaviors in less accessible species, such as humans, are best studied in species that live in societies, particularly those that can be studied in their natural habitats or under naturalistic conditions (Insel and Fernald, 2004). The existing database on avian social behavior is unparalleled—for example, for over 4000 species, we know whether they are territorial or colonial, socially monogamous or polygynous, migratory, or sedentary. We have high-quality recordings of their vocalizations. No other group of animals, invertebrate or vertebrate, has been studied with such passion and intensity. This collective database, although it could provide profound insight into the neuroendocrine basis of diverse social behaviors, is underutilized by neuroscientists because the availability of genomic tools has, until recently, been limited. The recent sequencing of the zebra finch genome (Warren *et al.*, 2010) now makes possible unprecedented advances in our understanding of social behavior because the resulting tools are applicable to all songbirds.

Advances in genomic technology, together with conservation of underlying mechanisms, will make it more and more feasible to bridge from well-characterized data-rich lab organisms, such as mice, to phenomena-rich wild species. These species, which include fish, lizards, songbirds, and voles, are proving to be rich resources for the analysis of social behavior and for the development of general principles (Robinson *et al.*, 2005). Studies with these model organisms have demonstrated the power of a comparative approach—looking for neural or genetic differences among individuals with known behavioral differences (Bullock, 1984; Robinson, *et al.*, 2005). The songbird model is preferable to more typical laboratory species in the study of social behavior because of the greater parallels with humans regarding societal structures and hormonal bases of behavioral strategies, as well as the potential to study free-living populations under natural conditions. Our work and that of others is making these natural and powerful models of vertebrate behavior feasible for genomic and neuroendocrine analysis.

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Figure 5.1.

Plasma testosterone (T) profiles over the breeding season in males of three passerine species. (A) In song sparrows, T peaks during territory establishment (prebreeding) and again during laying of the first clutch when females are receptive (sexual), and then falls during incubation and feeding (parental). (B) In house sparrows, T peaks during periods of intense competition for nest sites, prior to each of multiple broods. (C) In red-winged blackbirds (*Agelaius phoeniceus*), males provide little parental care and spend more time on territorial defense; T remains relatively high until the end of the breeding season. Redrawn from data in Wingfield (1984a) and Hegner and Wingfield (1986).

Figure 5.2.

(A–E) Correlations between aggressive behavior and Fos-immunoreactive (-ir) cell counts in the subpallial (ventral, ventrolateral) and pallial (dorsal) zones of the caudal lateral septum (LSc.v, LSc.vl, and LSc.d; A–C, respectively), paraventricular hypothalamus (PVN; D), and anterior hypothalamus (AH; E) of male song sparrows exposed to STI ($n = 16$). The intruder's cage and a speaker broadcasting song were placed adjacent to the subject's cage. Subjects showed selective flights to the cage wall adjoining the intruder, providing a good measure of aggressive response. Data are shown as the natural log (ln) of the number of contacts with the wire barrier during a 10-min test. (F–I) Correlations between barrier contacts and Zenk-ir cell counts in the rostral LS (LSr; F), LS.vl (G), lateral zone of the LSc (LSc.l; H), and PVN (I). Cell counts are shown as the number of immunoreactive nuclei per 100 μm^2 . Modified from Goodson *et al.* (2005b).

Figure 5.3.

The percentage of arginine vasotocin (VT) neurons in the PVN that express Fos after a 10-min STI is negatively correlated with aggression (ln, the number of contacts with the cage wall adjoining the intruder's cage; see Fig. 5.2 caption; $n = 16$) in song sparrows. Modified from Goodson and Kabelik (2009).

Figure 5.4.

(A) Peripheral injections of a novel V1a antagonist that crosses the blood–brain barrier have no effect on resident–intruder aggression in male violet-eared waxbills that are aggressive and typically dominant, but aggression in the context of mate competition is significantly reduced by the antagonist in the same males (B). (C) In males that are typically subordinate, resident–intruder aggression is disinhibited by the same treatments. Modified from Goodson *et al.* (2009b).

Figure 5.5.

Plumage polymorphism in white-throated sparrows. (A) Individuals of the white-stripe (WS) morph have alternating black and white stripes on the crown, bright yellow lores, and a clear white throat patch. (B) Individuals of the tan-stripe (TS) morph have alternating brown and tan stripes on the crown, duller yellow lores, and dark bars within the white throat patch. Photos by Allison Reid. Reprinted from Maney (2008).

Figure 5.6.

Medians, IQR, and ranges for (A) aggression scores (number of aggressive acts initiated per hour) and (B) individual ranks (as percent opponents dominated) within social groups. Males were introduced in single-sex groups of six birds (three WS and three TS per group) in indoor aviaries. Aggression scores and ranks were determined 10–14 days later by observing interactions and constructing dominance matrices. During spring-like day lengths (16L:8D), WS males were (A) more aggressive and (B) outranked TS males. Rank was unrelated to morph on short days (8L:16D). The long- and short-day experiments were conducted on different individuals. Data from Horton and Maney, unpublished.

Figure 5.7.

Model for the $ZAL2^m$ rearrangement. A minimum of two pericentric inversions, represented by the pairs of dashed lines, are hypothesized to have led to the $ZAL2/2^m$ polymorphism. $ZAL2$ (top) and $ZAL2^m$ (bottom) are shown along with a hypothetical chromosomal arrangement (middle) that could be either ancestral to both the $ZAL2$ and $ZAL2^m$ or an intermediate arrangement. Centromeres are represented by filled circles. Dark and light boxes represent segments originating on the short and long arms of the presumed ancestral chromosome, respectively. Free recombination between the $ZAL2$ and $ZAL2^m$ is limited to the tip of the short arm (hatched boxes).

Table 5.1**Evidence of Shared Mechanisms of Competitive Aggression in Birds and Humans**

Prediction	Evidence in New World sparrows	Evidence in humans
Males respond to competition with increased plasma T	Wingfield (1985), Wingfield and Hahn (1994), Wingfield and Wada (1989), Wingfield <i>et al.</i> (1990)	Meta-analysis of 23 studies in Archer (2006)
The plasma T response to challenge increases aggression	Archawaranon <i>et al.</i> (1991), Wingfield (1984b, 1994b)	Meta-analysis of 11 studies in Archer (2006)
Plasma T levels are lower among paternal males	Wingfield (1984a), Wingfield and Farner (1978), Wingfield and Goldsmith (1990), Wingfield <i>et al.</i> (1990)	Berg and Wynne-Edwards (2001), Fleming <i>et al.</i> (2002), Gray <i>et al.</i> (2002), Storey <i>et al.</i> (2000)
Aggressive dominance is correlated with plasma T levels	Archawaranon and Wiley (1988), Schlinger (1987), Wiley <i>et al.</i> (1993)	Meta-analysis of 13 studies in Archer (2006)
Plasma T is associated with alternative life history strategies regarding territoriality versus parenting	Hau (2007), Ketterson and Nolan (1992), McGlothlin <i>et al.</i> (2007), Schoech <i>et al.</i> (1998), Spinney <i>et al.</i> (2006), Wingfield (1984a,b,c)	Dabbs and Morris (1990), Dabbs <i>et al.</i> (1997), Daitzman and Zuckerman (1980), Gray <i>et al.</i> (2002), Julian and McHenry (1989)

The endocrine underpinnings of competitive aggression are broadly similar in New World sparrows (here limited to the *Zonotrichia*, *Melospiza*, and *Junco* genera) and humans (based primarily on Archer, 2006). Only a fraction of the relevant literature is cited here.