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Research Report

Differences in forebrain androgen receptor expression in winners and losers of male anole aggressive interactions

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ABSTRACT

Size matched male green anoles (*Anolis carolinensis*) were paired in a neutral setting and allowed to engage in aggressive displays. Winners and losers were apparent in each pair within 90 min, resulting in stable dominant/subordinate dyads. Androgen receptor (AR) expression was assessed at three time points after the initial pairing, 2 h, 3 days, and 10 days in dominants, subordinates, and two groups of control males housed alone or with a female for an equal period of time. Expression was quantified in three forebrain areas that have been implicated in aggression and reproductive social behavior in this species, the preoptic area (POA), the anterior hypothalamus (AH), septal area (SEP), and ventromedial nucleus of the posterior division of the dorsal ventricular ridge ($PDVR_{VM}$). There were significant overall group differences in AR mRNA expression in the POA and AH that appeared to result from higher POA AR expression in dominant males compared to other groups, and generally lower AR expression in subordinate males. Pairwise comparison revealed that dominants' AR mRNA expression in the POA was significantly higher in the 2 h and 3 day groups compared to that of subordinates, with a similar, but nonsignificant, difference in the 10 day group. Dominants had significantly higher AR mRNA expression in the AH compared to that of subordinates in the 2 h group, but differences were not significant at later times. The results suggest that POA and AH sensitivity to androgens is increased in dominants compared to subordinates, and that the difference can be seen soon after the agonistic interaction establishing winners and losers.

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1. Introduction

Male aggression is a common feature of vertebrate social behavior. The endocrine modulators and neural substrates of aggression have been widely studied. Testosterone (T) and/or its metabolites modulate male aggression, and the relationship between testosterone and male aggression has been evaluated in many species in many contexts (e.g., Adkins and Schlesinger, 1979; Crews et al., 1978; Wingfield et al., 2001; Wingfield, 2005). Seasonal fluctuations in aggression track seasonal changes in androgens (in lizards: Crews, 1975; Klukowski and Nelson, 1998; in birds: Wingfield et al., 1987; Wingfield et al., 1990). Inter-male aggression is decreased by castration and restored by subsequent testosterone treatment (in lizards: Adkins and Schlesinger, 1979; Crews et al., 1978; Denardo and Licht, 1993; Denardo and Sinervo, 1994; Moore, 1988; Weiss and Moore, 2004). In addition, androgen receptor (AR) antagonists or other antiandrogens successfully block the effect of androgens on aggression in a variety of vertebrates (in birds: Beletsky et al., 1990; Schwabl and Kriner, 1991; Soma et al., 1999b; in lizards: Tokarz, 1987; Tokarz, 1995; in rats: Taylor et al., 1984). Although it is the case that aggression can be expressed in the absence of T (e.g., Greenberg et al., 1984; Moore, 1987), it has nearly always been found that elevated T leads to higher levels of male social aggression.

Just as elevated T can increase aggression, engaging in successful aggression can elevate circulating levels of T. A "T-surge," or rapid elevation of circulating testosterone (Hirschenhauser and Oliveira, 2006; Wingfield et al., 1990), has been documented in several species in winners of aggressive contests consisting of either overt fighting or ritualized displays. For example, in green anoles increases in T levels were observed in winning animals one hour after an agonistic interaction while T levels decreased in losers (Greenberg and Crews, 1990). Similar findings have been reported in other species (California mice: Oyegbile and Marler, 2005; peacock blenny: Oliveira et al., 2001; Oliveira et al., 2009a; cichlids: Dijkstra et al., 2012; humans: Oliveira et al., 2009b). Testosterone differences may remain apparent for several days. Anoles that were exposed to aggressive encounters for three consecutive days had increased T levels compared to animals with no such encounters (Yang and Wilczynski, 2002).

The behavioral and endocrinological consequences of winning aggressive contests can be persistent, particularly when aggression is used to determine position in a dominance hierarchy. Dominant males tend to be more aggressive than subordinate males toward both their former opponent and to novel challengers. Although the literature on hormone differences in dominants and subordinates is not always consistent, when an endocrinological difference has been identified it is always the case that higher androgen levels are seen in the dominant individuals (Borowski et al., 2014; DuVal and Goymann, 2011; Greenberg and Crews, 1990; Parikh et al., 2006; Taves et al., 2009). Socially-linked elevated androgens may play some role in maintaining dominant status or a dominant's ability to win agonistic contests (reviewed in Eisenegger et al., 2011; Gleason et al., 2009). For example, a previously dominant male rat loses its dominant role after castration, but if T treatment is given after castration the male continues to behave aggressively toward intruders (Albert et al., 1986). In green anole lizards, intact males always won and held dominant status when paired with castrated males (Greenberg et al., 1984).

Several areas of the "social brain" have been implicated as having a particularly important role in mediating both aggression and courtship behavior. Steroid hormone receptors are concentrated in these brain regions across vertebrates (Morrell et al., 1979; O'Connell and Hofmann, 2012; Rosen et al., 2002). In *Anolis carolinensis* these areas include the anterior hypothalamus (AH), preoptic area (POA), septum (SEP) and ventromedial nucleus of the posterior division of the dorsal ventricular ridge ($PDVR_{VM}$). Lesions in the anterior hypothalamus and the preoptic area diminish courtship and aggressive behaviors (Farragher and Crews, 1979; Wheeler and Crews, 1978). Similar findings have been made concerning the septal area; lesions in that area result in deficits in intermale aggression (Crews, 1979). The ventromedial nucleus of the PDVR in reptiles is considered part of the homolog of the mammalian amygdala (Northcutt, 1981), and it has been implicated in mediating reproductive behavior and aggression. Lesions to the anterior $PDVR_{VM}$ decrease or eliminate male courtship behavior (Greenberg et al., 1984), and decrease intermale aggression to low levels (Crews, 1979). The activity marker cytochrome oxidase is elevated in the SEP, POA and Amygdala, as well as several other forebrain limbic and sensory areas after five days of staged aggressive interactions, and the intercorrelation of activity among these areas as well as among forebrain visual areas also changes (Sakata and Crews, 2004; Sakata et al., 2005; Yang and Wilczynski, 2007). Social behavior results in elevated immediate early gene FOS levels colocalized with vasotocin (sexual behavior) and dopamine (sexual and aggressive behavior) populations in the POA in the brown anole (*Anolis sagrei*) (Kabelik et al., 2013; Kabelik et al., 2014).

Androgen levels have been shown to up- or down-regulate androgen receptor (AR) expression depending on brain area, context, and developmental stage (Kerr et al., 1995; Lu et al., 1999; Pfannkuche et al., 2011; Wu and Gore, 2010). Therefore, just as changes in testosterone may occur after aggressive encounters, or in dominant vs. subordinate males, it is also possible that changes in brain AR expression might emerge after winning or losing a social contest. Forebrain androgen receptors are known to vary seasonally in forebrain song areas and the preoptic area-hypothalamus in birds (Soma et al., 1999a; Wacker et al., 2010), or over a reproductive cycle (Rhen et al., 2003). Their level has been reported to be higher in dominant vs. subordinate fish (Burmeister et al., 2007) and birds (Cordes et al., 2014), as well as in winners compared to losers of aggressive contests in *Peromyscus* mice (Fuxjager et al., 2010). Differences in androgen receptor number in key brain areas could influence the sensitivity with which circulating androgens modulate aggression behavior and therefore could add to the differences in aggression and courtship seen after winning or losing a social contest. In this study, we used in situ hybridization to quantify and compare androgen receptor expression levels in forebrain areas known to be involved in aggressive behavior in the green anole lizard,