



ACADEMIC
PRESS

General and Comparative Endocrinology 128 (2002) 153–161

GENERAL AND COMPARATIVE
ENDOCRINOLOGY

www.academicpress.com

Manipulation of visual sympathetic sign stimulus modifies social status and plasma catecholamines

Wayne J. Korzan, Tangi R. Summers, and Cliff H. Summers*

Department of Biology, Biology and Neuroscience Group, University of South Dakota, 414 East Clark Street, Vermillion, SD 57069-2390, USA

Accepted 1 July 2002

Abstract

Darkening of postorbital skin (eyespots) in *Anolis carolinensis* occurs during stressful situations via adrenal catecholaminergic activation of β_2 -adrenergic receptors. The eyespots form more rapidly in dominant males during social interaction. It was hypothesized that eyespot darkening from green to black is a social signal that communicates disposition, resulting from sympathetic activation and determines social hierarchy. To assess the value of the eyespot as a signal influencing social dominance, males were paired according to size and weight, and painted postorbitally; one with green and the other with black paint. Painted pairs interacted with aggressive displays, approaches, and bites. Dominant status was determined by continued displacement of an opponent, superior perching position, and lighter color. Males with eyespots darkened by black paint were dominant in 100% of interactions and more aggressive. Males viewing an opponent with eyespots painted black exhibited higher plasma epinephrine (Epi), norepinephrine (NE), and dopamine (DA) levels than males with eyespots hidden with green paint and isolated controls. Results for behavior were similar, but those for plasma catecholamines contrast with those from recent experiments in which interactions occurred vs. a mirrored reflection. The eyespot is a potent stimulus, acting as a social signal that confers dominant status to its bearer, and activates the sympathetic nervous system in opponents that observe it during aggressive interactions.

© 2002 Elsevier Science (USA). All rights reserved.

Keywords: Aggression; Epinephrine; Norepinephrine; Dopamine; Eyespot; Lizard; *Anolis carolinensis*; Sign stimulus; Social hierarchy; Social status; Visual cue; Dominant; Subordinate

1. Introduction

Visually apparent sign stimuli, such as the red bill spot on herring gulls (Tinbergen and Perdeck, 1950), dramatically influence behavior (Lorenz, 1937). Behavioral and physiological responses are also highly modified by elevated plasma catecholamine levels, often associated with the “fight or flight” response (Cannon, 1914; Cannon and Paz, 1911). In some animals, such as the lizard *Anolis carolinensis* (Korzan et al., 2000a) and the fish *Haplochromis burtoni* (Muske and Fernald, 1987), sympathetic stimulation of adrenal catecholamine release actuates a visually apparent and socially relevant sign stimulus that modifies behavior and has broad implications for the establishment of social dominance

roles. The purpose of this work was to explore the capacity of the sign stimulus of *A. carolinensis* in establishing dominant social status.

Social interaction stimulates elevated sympathetic activity and release of epinephrine (Epi) and norepinephrine (NE) from the adrenal gland and autonomic nerve terminals (Matt et al., 1997; Summers and Greenberg, 1994). These hormones bind to β_2 -adrenergic receptors of the postorbital skin and cause darkening, while the rest of the lizard's body is still green (Fig. 1; Hadley and Goldman, 1969; Vaughan and Greenberg, 1987). Darkening of the postorbital skin produces eyespots that act as a sign stimulus to *A. carolinensis* that influences aggressive behavior during social interactions (Korzan et al., 2000a,b, 2001a). Males that exhibit more rapid eyespot darkening also generate aggression sooner and at higher intensities during an interaction, and characteristically achieve dominant status (Larson and Summers, 2001; Summers, 2002; Summers and

* Corresponding author. Fax: +605-677-6557.

E-mail address: cliff@usd.edu (C.H. Summers). URL: <http://www.usd.edu/~cliff/>.

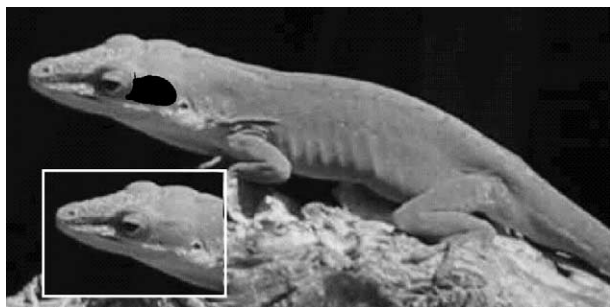


Fig. 1. Image of male *A. carolinensis* with artificially darkened eyespot. Notice the inset of lizard with unaltered eyespot region.

Greenberg, 1994). Eyespot formation occurs under all stressful conditions (Summers and Greenberg, 1994), but in the context of social dominance relationships, eyespots may allow displaying male *A. carolinensis* to communicate neuroendocrine condition without engaging in physical interaction (Greenberg and Noble, 1944). In their natural habitat, they occupy exclusive territories usually keeping long nearest neighbor distances (Jenssen et al., 1995). In concert with agonistic behavior and eyespot color, male *A. carolinensis* also use body color as a signal of aggressive intent and social position. Although body color can be affected by light, temperature, time of day, and humidity (Cooper and Greenberg, 1992), it also reliably changes from green overall to brown, as social status varies from dominant to subordinate (Greenberg and Crews, 1990). Body color and eyespots appear to convey adaptive value by limiting overall aggression (Korzan et al., 2000a,b, 2001a) by visually displaying neuroendocrine condition.

The cichlid fish *H. burtoni* exhibits a similar sympathetically regulated visual sign stimulus, the eyebar, to signal territory ownership and aggressive intent (Muske and Fernald, 1987). Eyebar darkening is controlled by the release of epinephrine to melanophore cells of dominant individuals (Muske and Fernald, 1987). During the establishment of social hierarchy, males of both *H. burtoni* and *A. carolinensis* display these social signals. In male *A. carolinensis*, although both aggressors develop the visual signal, the first male to exhibit darkened eyespots becomes dominant (Larson and Summers, 2001). Eyespots fade on both males when the social hierarchy is established and aggression diminishes. Eyebars are retained on the dominant *H. burtoni*, a chronic advertisement of their social status (Muske and Fernald, 1987).

Behavior, central serotonergic activity, and plasma catecholamine levels influence eyespot coloration (Larson and Summers, 2001; Summers and Greenberg, 1994). Concurrently, eyespots also influence behavior along with central and peripheral monoaminergic activity in opponents viewing them (Korzan et al., 2001a). During aggressive encounters, temporally specific activation and reuptake of 5-HT are highly correlated with eyespot celerity and social status (Larson and Summers,

2001; Summers, 2001, 2002; Summers and Greenberg, 1994; Summers et al., 1998). In addition, significant increases in plasma catecholamines (NE, Epi, and DA) and agonistic behaviors such as biting, aggressive display, approaches, and head nods are stimulated when individuals view themselves in a mirror as a reflected opponent with no apparent eyespots (Korzan et al., 2000a,b, 2001a). In contrast, males viewing a reflected opponent with darkened eyespots, that probably signify dominant status, have significant decreases in plasma catecholamines and agonistic behaviors (Korzan et al., 2000a,b, 2001a). Results from these and other recent experiments suggest that the mechanisms regulating eyespots, behavior, autonomic function, and social status are strongly influenced by central serotonergic activity (Korzan et al., 2001a; Larson and Summers, 2001; Summers, 2002). Consequently, eyespot formation appears to be linked directly with the neuroendocrine machinery involved in establishing social status. We hypothesized that animals with eyespots painted black, but viewing an opponent with eyespots hidden by green paint, would exhibit more agonistic behavior and become dominant in the majority of encounters. That is, we hypothesized that by using paint, we could control which males will be dominant and subordinate and thereby simultaneously affect plasma catecholamine concentration according to each animal's status. Therefore, the purpose of this study was to test the importance of this visual sign stimulus for producing dominant status and influencing plasma catecholamines during interactions between two males of similar size and weight.

2. Materials and methods

2.1. Animals

Adult male (>60 mm snout-vent length) *A. carolinensis* were obtained from a commercial supplier (Marabell's, Gonzales, LA). Each lizard was weighed, measured, and placed individually into one half of a terrarium. Each side (25 cm)³ contained a diagonally placed wooden perch and was separated from the other half by an opaque divider (Korzan et al., 2000a). Animals that were placed adjacently, on opposite sides of the divider, were matched for size (mean weight varied by less than 6 mg and mean snout-vent length varied by 2 mm) to minimize the influence of body size on behavioral interactions (Tokarz, 1985). There were no significant differences in mean initial weights ($F_{2,25} = 0.3$, $P > 0.73$) or snout-vent lengths ($F_{2,25} = 1.4$, $P > 0.27$) between pairs or isolated controls. Lights, temperature (14L32 °C:10D20 °C), and relative humidity (70–80%) were regulated to maintain gonadal activity (Licht, 1971). Lizards were fed live crickets and watered ad libitum.

2.2. Experimental design

Following one week of acclimation, males were tested for reproductive condition. Only males responding with courtship behavior when presented with a female (Greenberg and Crews, 1990) were included in the study. Reproductive state and androgen levels may be associated with aggressive capacity (Greenberg and Crews, 1990; Lovern et al., 2001). The eyespot region of each lizard was manipulated by covering with non-toxic paint from a commercial supplier (Accent Acrylic Paints, Bloomsbury, NJ), or water for isolated controls (Fig. 1). Twenty-four hours before behavioral testing, postorbital skin of one lizard chosen randomly from a matched pair was painted black, to darken the eyespot region, and postorbital skin of the other lizard was painted green ($N = 8$ for each group), covering the area to hide the formation of natural eyespot (Korzan et al., 2000a,b, 2001a). When eyespots are hidden by green paint, the animal is visually similar to a subordinate male in the sense that subordinate males have a significantly longer latency to eyespot darkening (Larson and Summers, 2001; Summers and Greenberg, 1994) and therefore are more often viewed without darkened eyespots. Conversely, black paint mimics the rapid onset of eyespot formation in dominant males by artificially affixing the eyespot, prior to the encounter. One group of males were painted with water (controls; $N = 10$) and never exposed to an opponent, and therefore isolated from behavioral interaction. Behavior was manually recorded for 10 min (Summers, 2002), following removal of the opaque divider between two animals. Observations were made with room lights off and cage lights on (Korzan et al., 2000a,b). Darkened room, cage illumination, and distance of observers from cages (1.5 m) minimized observer effects on lizard behavior (Sugerman, 1990). All behavioral observations were performed in three days between 12:30 and 3:00 p.m. central time. This window of observation was selected because room humidity and temperature were consistent at 32 °C. All procedures were carried out under guidelines of the University of South Dakota IACUC.

2.3. Aggressive behavior

All paired males interacted aggressively. Aggressive displays of *A. carolinensis* have been previously described (Crews, 1975, 1979; DeCourcy and Jenssen, 1994; Evans, 1936; Greenberg, 1977; Greenberg and Noble, 1944). All displays performed in assertion or challenge context (DeCourcy and Jenssen, 1994; Greenberg, 1977) were counted during a 10-min period (Korzan et al., 2000a). Behavioral records included approaches, bites, and extension of dewlap with headnods. Maximally aggressive display behavior (sagittal expansion) includes a combination of lateral compression of the rib cage, nuchal crest expansion, dewlap extension,

sagittal spatial positioning, and headnods. Animals were scored with an approach every time they slowly decreased the distance between themselves and their opponent and the opponent did not move away. Aggressive approach often precedes display and always precedes biting behavior. Winning an antagonistic encounter is accomplished by a greater frequency of and shorter latency to aggressive acts (such as biting) by a given male and by chasing and displacing the opponent. Fight-winning males were confirmed to be socially dominant by realization of the most superior perching position (the highest position directly under the overhead light), continued displacement of the subordinate individual from the superior perch position and from other locations within the cage, and by lighter body coloration.

2.4. Plasma catecholamines assay

Within 5 s of completion of the experiment, animals were decapitated and blood was taken immediately from the body and head into capillary tubes. The tubes were then centrifuged to separate blood cells and plasma. Determination of catecholamine concentration from small volumes of plasma (25–100 μ l) was achieved using a modification of the method by Lin et al. (1984). An internal standard, DHBA (100 ng/ml), was added along with 25–100 μ l plasma into a 1.5 ml syringe-filter cartridge (EG&G WALLAC/AKRON) with 50 mg acid-washed aluminum oxide (BAS). Immediately upon the addition of 1 ml Tris buffer (1.86 M, pH 8.65), samples were vortexed and capped, then rotated for 10 min and vortexed again. The supernatant was aspirated through the filter and the alumina was then washed and vortexed four times with 1 ml H₂O to which a small volume of pH 7 buffer was added and aspirated to near dryness each time. A 200 μ l microcentrifuge tube was placed on the cartridge as a receiver tube and centrifuged to remove any residual fluid. A new receiver tube was placed on the cartridge and 100 μ l of 0.1 N perchloric acid (HClO₄) was added to the sample. At this time, the samples were vortexed for 30 s, allowed to stand for 3–5 min, and vortexed again. The cartridge and receiver tube were centrifuged until no fluid remained in the cartridge. Sixty microliters HClO₄ extract was injected directly into a HPLC system (Waters) and analyzed electrochemically with an LC-4B potentiostat (BAS). The electrode potential was set at +0.6 V with respect to an Ag/AgCl reference electrode. Mobile phase consisted of 14 g citric acid, 8.6 g sodium acetate, 110 mg 1-octanesulfonic acid (sodium salt), 150 mg EDTA disodium salt, and 100 ml methanol in 1 L deionized water. Flow rate was maintained at 1.0 ml/min.

2.5. Body coloration

Typical body coloration of healthy *A. carolinensis* is bright green. The initial body color of each male was

recorded and verified every minute during the entire interaction. Coloration was ranked from 1 (bright green), 2 (part green/part brown), 3 (all visible pigmented body surfaces a light to moderate brown) to 4 (dark brown; modified from Greenberg and Crews, 1990). Males that completed agonistic social interactions were usually either bright green or dark brown. The coloration of individuals after aggressive interaction was representative of social status (Greenberg and Crews, 1990). Males that turned brown were usually subordinate and retention of bright green coloration was consistent with dominant social status.

2.6. Statistical analyses

Comparisons of the frequency of aggression or plasma catecholamine concentrations between eyespot painted groups were performed by a Student's paired *t* test and comparisons of either group with controls were carried out by Mann–Whitney *U* (in some cases, variances were not homogeneous). Two-way repeated measures ANOVA (followed by Dunnett's post hoc analysis) was used to analyze the time course of body color change, with comparisons made between dominant and subordinate males at a given time using Student's paired *t* test. An examination of the expected frequency of dominant and subordinate status was performed using Fisher's exact test.

3. Results

3.1. Behavior

All paired males responded behaviorally to one another during the timed interaction. The presence or absence of eyespots strongly affected agonistic behavior between the two combatants. Males confronting an opponent with hidden eyespots exhibited increased agonistic behavior. Conversely, males challenged by an opponent with eyespots permanently darkened with black paint demonstrated fewer agonistic behaviors. For example, aggressive displays in which full sagittal expansion was achieved (Greenberg, 1977) were significantly inhibited in males with opponents having darkened eyespots when compared to males in which the opponents' eyespots were hidden by green paint ($t_p = 4.4$, $P < 0.003$; Fig. 2 top). Thus, males viewing an opponent with hidden eyespots completed significantly more aggressive displays than males viewing opponents with darkened eyespots.

Along with displays, combatants also physically interacted by biting each other. Paired *Anolis* often resort to biting when intense displaying behavior fails to identify the victor. Similar to displays, males viewing and opponent with hidden eyespots bit significantly

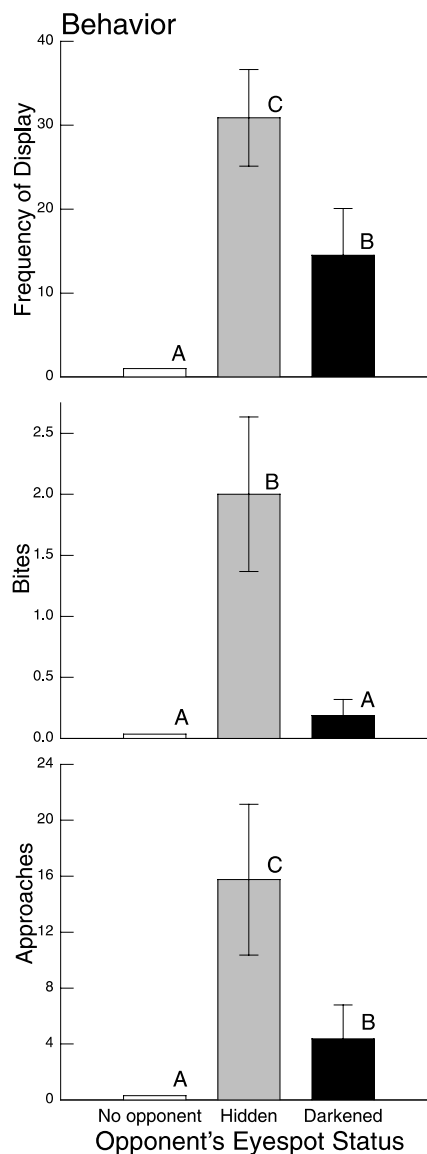


Fig. 2. The mean (\pm SEM) number of aggressive displays with full sagittal expansion (top; $t_p = 4.4$, $P < 0.003$), bites (middle; $t_p = 3.3$, $P < 0.01$), and aggressive approaches (bottom; $t_p = 2.4$, $P < 0.05$) during a 10-min period were significantly more frequent among males viewing an opponent without eyespots (i.e., hidden by green paint) compared with (paired *t* test) those viewing opponents with eyespots darkened by black paint. A male viewing an opponent with hidden eyespots has had his postorbital skin painted black and one viewing an opponent with darkened eyespots has had his eyespot regions hidden with green paint. Means with no common superscript letters (e.g., A vs. B or B vs. C) are significantly different. Social interaction, regardless of the eyespot paint color, produced significantly more aggression than that observed in isolated animals (which did not exhibit any behavior), with the exception that subordinate animals (viewing opponent with dark eyespots) did not have significantly more bites (Mann–Whitney $U = 30.0$, $P > 0.1$).

($t_p = 3.3$, $P < 0.01$) more often than males with opponents having darkened eyespots (Fig. 2 middle).

Aggressive approaches toward an opponent are also important because they usually precede display and

always precede bites and tail whipping between male *A. carolinensis*. Males that shorten the distance between the combatant and themselves are increasing the intensity of the interaction. Approaches were most frequent ($t_p = 2.4$, $P < 0.05$) from males viewing an opponent with eyespots hidden by green paint (Fig. 2 bottom).

3.2. Body color

After an average of 4 min of aggression, during the 10 min interaction between two combatants, body color in one group began to darken. Males viewing an opponent with eyespots hidden by green paint (became dominant animals) were the most aggressive and maintained bright green body coloration (Fig. 3). Although both groups began with the same mean body color, after 1 min of social interaction, animals viewing opponents with darkened eyespots (became subordinate males) began to have darker body color. Body color in this group became progressively darker, until it was significantly darker due to treatment (eyesspots hidden by green paint, viewing dark eyespot opponent; $F_{1,140} = 12.5$, $P < 0.003$; two-way repeated measures ANOVA), time ($F_{10,140} = 6.4$, $P < 0.001$), and there was an interaction of treatment by time ($F_{10,140} = 5.8$, $P < 0.001$). Specifically, although there were no significant ($F_{10,70} = 1.1$, $P > 0.38$) differences over time among males viewing opponents without eyespots (hidden), animals that viewed opponents with darkened eyespots had significantly ($F_{10,70} = 7.2$, $P < 0.001$) darker body color by 4 min in comparison with their initial body color. When comparing the two treatment groups, the

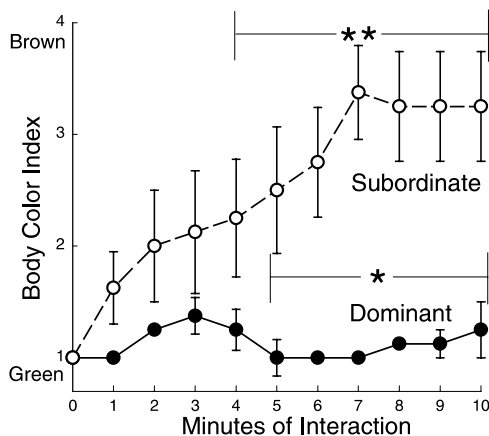


Fig. 3. The body color of males viewing an opponent with eyespots darkened (by black paint), i.e., subordinate males (see Fig. 5), changes from bright green to dark brown (1 = bright green, 2 = part green/part brown, 3 = all light/moderate brown, and 4 = dark brown) over the duration of the interaction. Darkening of the skin is a typical characteristic of subordinate status. Subordinate males are significantly darker than their original color after 4 min ($F = 7.2$, $P < 0.001$; identified by **), significantly darker than dominant males after 5 min of interaction ($t_p = 2.7$, $P < 0.033$; identified by *), and remain dark for the duration of the interaction.

overall body color of the group facing opponents with dark eyespots was significantly ($t_p = 2.7$, $P < 0.033$ at 5 min) and continuously (e.g., $t_p = 5.7$, $P < 0.001$ at 7 min; and $t_p = 4.0$, $P < 0.005$ at 10 min) darker than that of their opponents after 5 min of interaction. From that point on, males combating an opponent with dark eyespots exhibited decreased aggression and a chromatically darkened body (Fig. 3).

3.3. Plasma catecholamines

Concentrations of NE were approximately 10-fold higher than Epi, and 100× higher than DA in the plasma of *A. carolinensis* (Fig. 4). These results are consistent with the relative levels reported in previous studies (Korzan et al., 2000a; Matt et al., 1997; Summers and Greenberg, 1994), suggesting that NE is the predominant plasma catecholamine in *A. carolinensis*. Aggressive interaction of paired males affected all plasma catecholamines, dependent on eyespot coloration. Plasma concentrations of NE (Fig. 4 top) were significantly ($t_p = 2.6$, $P < 0.04$) elevated in males viewing opponents with darkened eyespots, as compared to those viewing opponents without eyespots (hidden) or isolated controls. In addition, animals viewing opponents with darkened eyespots also had significantly elevated plasma Epi ($t_p = 2.6$, $P < 0.05$) and DA ($t_p = 3.3$, $P < 0.02$; Fig. 4 middle, bottom) when compared to males viewing opponents without eyespots (hidden) or isolated controls.

3.4. Social status

Anoles with darkened eyespots and confronting opponents without eyespots (hidden) exhibited elevated aggression with normal body color and plasma catecholamine levels. It is reasonable that this group should have a majority of males that attained social dominance. In this experiment, 100% of males with artificially darkened eyespots became dominant ($P < 0.00016$; Fig. 5). Although pairs were matched for size they were not exactly equal, and in 87.5% of encounters, males that became dominant were lighter in weight than those that became subordinate.

4. Discussion

4.1. Social behavior and eyespots

The presence of eyespots, whether artificially or naturally obtained, effectively influences agonistic behavior, plasma catecholamine activity, and consequently, determination of social status in male *A. carolinensis* (Figs. 2, 4, and 5; Korzan et al., 2000a,b, 2001a; Larson and Summers, 2001; Summers and Greenberg, 1994). Although male *A. carolinensis* partition the natural habitat

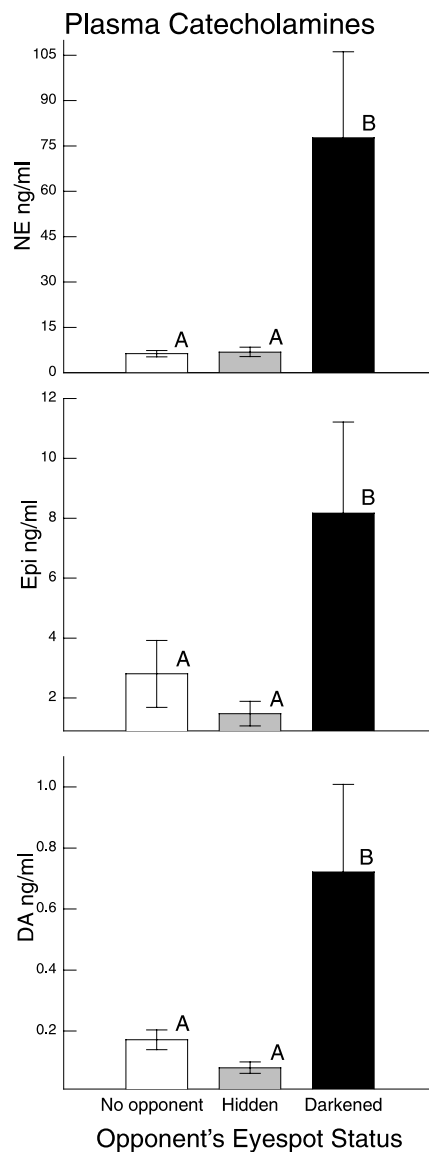


Fig. 4. The mean (\pm SEM) concentrations of plasma norepinephrine (top; $t_p = 2.6$, $P < 0.04$), epinephrine (middle; $t_p = 2.6$, $P < 0.05$), and dopamine (bottom; $t_p = 3.3$, $P < 0.02$) were significantly greater (paired t test) in males viewing an opponent with darkened eyespots (painted black) compared with those viewing opponents with eyespots hidden by green paint. Means without common superscript letters (e.g., A vs. B) are significantly different. In all cases, subordinate animals (viewing opponents with dark eyespots) had elevated catecholamines compared with isolated controls (Mann–Whitney U for NE = 2.0, $P < 0.004$; for Epi = 6.0, $P < 0.05$; and for DA = 7.0, $P < 0.015$), but in no case was plasma catecholamine concentration elevated in dominant (viewing opponents without eyespots) animals. Means with common superscript letters (e.g., A vs. A) are not significantly different.

by means of intense territorial contests and enduring rivalry (Jenssen et al., 2001), social antagonism between closely adjacent males is rarely observed (Jenssen and Nunez, 1998; Jenssen et al., 1995). Similarly, wild males that might become subordinate under captive conditions quickly leave the proximity of a more dominant resident male (Jenssen and Nunez, 1998; Jenssen et al., 1995). It

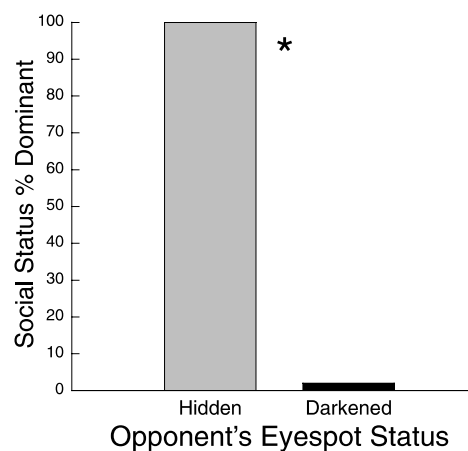


Fig. 5. Males viewing an opponent with eyespots hidden by green paint achieved dominant status in 100% of agonistic interactions. Comparison of dominant and subordinate status was accomplished using Fisher's Exact test ($P < 0.00016$; identified by *).

is just these sorts of distant or short-term interactions in which eyespots of *A. carolinensis* may have the most social value. For a species like *A. carolinensis* in which male territories generally do not overlap (Jenssen and Nunez, 1998; Ruby, 1984; Nunez et al., 1997), eyespot formation could provide a relatively remote indicator of internal neuroendocrine function representative of putative social status (Summers, 2001, 2002) and thereby reduce the number of social aggressive interactions (Korzan et al., 2000a,b, 2001a) that are necessary. This might explain the limited number of social interactions observed in recent field studies (Jenssen and Nunez, 1998; Jenssen et al., 1995) for this highly volatile and stress sensitive species (Summers, 2002; Summers and Greenberg, 1994). Although eyespots as a social signal influence plasma catecholamines and dominant-subordinate relationships expressly in *A. carolinensis*, it is likely that other social signals, such as body language, influence both neuroendocrine output and social status, regardless of the species involved.

4.2. Eyespots are a part of integrated behavioral, neural, and endocrine responses that produce social status

Individuals that achieve dominant status during an aggressive social interaction have different hormonal (Summers, 2001, 2002) and central neurochemical patterns, particularly serotonergic, than subordinate animals (Korzan et al., 2000b, 2001a; Summers, 2001). Furthermore, in male *A. carolinensis* changes in serotonin (5-HT) concentration can alter social status (Larson and Summers, 2001). In addition, peripheral stress hormones influence serotonergic activity (Summers et al., 2000). The behavioral, monoaminergic, hormonal, and autonomic responses in *A. carolinensis* appear to be linked to a visual indicator of social status and stress: eyespots. The results reported here from socially aggressive interactions

between two males corroborate previous work with single males interacting with their mirror image (Korzan et al., 2000a,b, 2001a) and suggest that sympathetically stimulated dark eyespots are a potent sign stimulus that inhibits aggression and influences plasma catecholamines in an opponent. As a visual sign stimulus, eyespots stimulate a cascade of neurochemical and behavioral responses to prepare the individual for a subordinate role. Previous experiments have demonstrated that during aggressive interactions between two males, the first to achieve eyespots becomes dominant in virtually all encounters and that elevated 5-HT delays eyespot formation (Larson and Summers, 2001; Summers and Greenberg, 1994). The results of this experiment suggest that male *A. carolinensis* use the eyespots as a visual signal of the physical condition of their opponent, decreasing physical contact and possible injury in determining social hierarchy. In their natural habitat, male *A. carolinensis* have large territories, maintained by continuous monitoring, patrolling, and frequent territorial displays (Jenssen et al., 1995). Artificially darkened eyespots consistently produce dominant social status in captive animals (Fig. 5), perhaps naturally darkened eyespots function to help maintain social distance in the wild.

4.3. Darkened eyespots inhibit aggressive behavior

Although behavioral interactions and establishing social status between male *A. carolinensis* have been described in detail in many studies (Crews, 1975, 1979; DeCoursey and Jenssen, 1994; Evans, 1936; Greenberg, 1977; Greenberg and Noble, 1944), it has only more recently been reported that the latency to eyespot formation is tightly correlated with dominant status (Larson and Summers, 2001; Summers and Greenberg, 1994). In addition, recent studies suggest that manipulating the color of the eyespots has a profound effect on aggressive behavior and plasma catecholamines in lizards interacting with a mirror image (Korzan et al., 2000a,b, 2001a). Studies involving social behavior and aggression between two males of similar size typically record intense aggression from both individuals at the beginning of the interaction (Summers, 2001, 2002). In contrast, males perform noticeably fewer aggressive behaviors when viewing an opponent with eyespots darkened (Fig. 2), with the frequency of aggressive behaviors declining over the course of the encounter. On the other hand, males visually observing decreased aggression and absence of eyespot formation in an opponent with eyespots hidden by green paint may be persuaded that their opponent is a subordinate animal.

4.4. Role of 5-HT: eyespots and social interaction

Recent studies suggest that the neural machinery responsible for coordination of eyespots, behavior, auto-

nomic function, and social status is profoundly affected by central serotonergic activity (Korzan et al., 2001a; Larson and Summers, 2001; Summers, 2002). During an aggressive encounter, temporally specific activation and reuptake of 5-HT in an individual are highly correlated with eyespot celerity and social status (Larson and Summers, 2001; Summers, 2001, 2002; Summers and Greenberg, 1994; Summers et al., 1998). Levels of central 5-HT, at least in some parts of the brain such as raphe, appear to be inversely correlated with aggression (Korzan et al., 2001a). When central serotonin is manipulated via 5-HT reuptake inhibitors, such as sertraline or fluoxetine, the result is behavioral and physiological changes that influence the outcome of aggressive interaction (Deckel, 1996; Deckel and Jevitts, 1997; Larson and Summers, 2001). Dominant males treated with sertraline have a longer latency to eyespot formation and dominant animals given sertraline have an approximately fifty percent chance of becoming subordinate (Larson and Summers, 2001). Therefore, the same central serotonergic mechanisms that influence the celerity of eyespot formation also appear to influence social status.

4.5. Plasticity of social status

Whatever the behavioral or neural mechanism involved, artificially darkening eyespots of one animal in an evenly matched pair produce dominant social status for 100% of the males with blackened eyespots (Fig. 5). In addition, although size is often considered the principal factor in determining status (Tokarz, 1985), among this set of closely matched males, the smaller male (by weight), with eyespots darkened, became dominant in 87.5% of trials. This suggests that animals are not exclusively predetermined by genetic makeup to have a particular social status and that even neuroendocrine mechanisms predisposing a particular outcome can be modified by an artificially applied potent sign stimulus.

4.6. Other species have potent sign stimuli

Social sign stimuli are not uncommon in the animal kingdom. However, visual sign stimuli that project sympathetic activation are rare. Two species that exhibit this characteristic are *A. carolinensis* and *H. burtoni*. Both species use chromatic skin darkening of regions of the head, eyespots and eyebars, to visually announce sympathetic state and/or aggressive intent. Although the social value of these two sign stimuli is similar, the mechanics of formation are slightly different. *H. burtoni* darkens an area ventral to each eye by means of direct innervation from the facial nerve. No direct innervation is apparent for *A. carolinensis* (Kleinholz, 1938). In contrast, the skin just behind each eye is darkened more indirectly via sympathetic stimulation of adrenal chromaffin release of hormone. The postorbital skin dark-

ening response for each species is still ultimately stimulated by norepinephrine. Eyespots or bars are potent signals, but other animals also produce behavioral visual signals, often associated with fight or flight, when plasma catecholamine secretion is elevated. Our results suggest a general vertebrate model with neural circuitry adapted to integrate both input and output of signals, behavior, and neuroendocrine condition, resulting in adaptive responses.

4.7. Eyespots and behavior influence plasma catecholamines

Artificially manipulated eyespot coloration has been demonstrated to influence catecholamine secretion and behavior in previous experiments using aggression toward mirror images (Korzan et al., 2000a, 2001a) and, in the currently reported experiment, with paired animals (Figs. 2 and 4). More than one factor, such as viewing aggression and eyespots simultaneously, are likely to be involved in stimulation of norepinephrine secretion into plasma. Exposure of paired *A. carolinensis* to repeated viewing of videotaped aggression subsequently stimulates increases in agonistic behavior between pairs of male *A. carolinensis* (Yang et al., 2001). In contrast, our study suggests that viewing aggression from males with darkened eyespots reduces aggressive behaviors and increases plasma norepinephrine. Previous work suggests that increased catecholamine secretion stimulates aggressive acts (Baenninger, 1968; Korzan et al., 2000a; Marrone et al., 1966). In wild-type rats, aggressive coping strategies for social and non-social stressors are found in animals with high sympathetic-adrenomedullary activation (Sgoifo et al., 1996). Conversely, aggression has been shown to stimulate secretion of catecholamines (Matt et al., 1997). Therefore, seeing aggression and/or behaving aggressively stimulate increased norepinephrine secretion. The eyespots' signal caused by increased catecholamines is also a potent stimulus influence to further subsequent catecholamine secretion in the opponent.

4.8. Comparison of mirror and paired interactions

When compared to previous work, data from this study support the premise of eyespots as a sign stimulus. Recent experiments manipulating the eyespot region with paint suggest that the eyespots of *A. carolinensis* act as a visual cue during agonistic interaction with an opponent in a mirrored reflection (Korzan et al., 2000a). In contrast, this study incorporates the use of paired males with one male having darkened eyespots and the other having eyespots hidden with paint. Behaviorally, these two studies are very similar in that animals viewing an opponent with darkened eyespots exhibited decreased bites, aggressive display, and approaches. Males viewing an opponent with hidden eyespots displayed increased

aggressive behavior. Conversely, the level of aggression observed by a combatant is not similar between mirror and paired interaction experiments. A reflected opponent without eyespots (hidden) appears very aggressive, but a real opponent without eyespots, either naturally (low plasma NE/Epi) or artificially hidden, is not overly aggressive (Fig. 2; Korzan et al., 2000a; Summers and Greenberg, 1994). In addition, the plasma catecholamine levels exhibited opposite patterns. Males viewing a reflected opponent without eyespots (hidden) had significantly elevated levels of plasma catecholamines compared to isolated controls or males viewing a reflected image with eyespots darkened (Korzan et al., 2000a). In contrast, in experiments using paired males, a male viewing an opponent with darkened eyespots had significantly elevated levels of plasma catecholamines when compared to isolated controls or males viewing an opponent with hidden eyespots (Fig. 4). These differences in plasma levels of catecholamines may be partially explained by body coloration change of the males viewing an opponent with darkened eyespots (Fig. 3). Males viewing a reflected opponent with darkened eyespots exhibit no noticeable color change (they stayed green). Although mirror and paired experiments were designed very similarly, differences in plasma catecholamine concentrations might also be due to differences in completion of social interaction, because aggression against a mirrored reflection never produces dominant or subordinate standing.

4.9. Conclusions

These experiments suggest that early or artificial eyespot presence potentially influences the outcome of establishing social hierarchy between two combatants, as suggested previously (Korzan et al., 2000a; Larson and Summers, 2001; Summers and Greenberg, 1994). Males viewing opponents with darkened eyespots had significantly fewer agonistic behaviors and significantly higher levels of plasma catecholamines. Visual signaling of autonomic state is not an exclusive trait in *A. carolinensis*. Other visual cues such as body coloration also seem to influence behavior and status acquisition. These experiments support the mounting evidence that latency to eyespot formation may be a crucial factor for establishing social hierarchies among *A. carolinensis*. In addition to influencing socially adaptive behavior, the presence of darkened eyespots in an opponent also stimulates plasma norepinephrine secretion. This result is the opposite of that from recent experiments using mirror images to stimulate aggressive behavior, in which animals viewing no eyespots (hidden) had elevated plasma NE. A comparison between these two ethological techniques should yield important advances for behavioral studies and help resolve the role of catecholamines in aggressive behavior (Korzan et al., 2001b).

Acknowledgments

We thank Mark Drymalski, Meredith Flynn, Maria Martin, Holly Miner, Christie Nicholas, and Kenneth Renner for the technical assistance. We thank Mark Drymalski additionally for photography. We also thank Loren Buck, Whitney Meyer, Øyvind Øverli, and Ken Renner for critical reading of the manuscript. This work was supported by NIH Grant 1 F31 MH64983-01, Sigma Xi Grants-in-aid and NSF EPSCoR graduate fellowship granted to W.J.K.; and by NIH Grant P20 RR15567.

References

- Baenninger, R., 1968. Catecholamines and social relations in Siamese fighting fish. *Anim. Behav.* 16, 442–447.
- Cannon, W.B., Paz, D., 1911. Emotional stimulation of adrenal gland secretion. *Am. J. Physiol.* 28, 64.
- Cannon, W.B., 1914. The emergency function of the adrenal medulla in pain and in major emotion. *Am. J. Physiol.* 33, 356.
- Cooper, W.E., Greenberg, N., 1992. Reptilian coloration and behavior. In: Gans, C., Crews, D. (Eds.), *Biology of the Reptilia. Physiology E: Hormones, Brain and Behavior*, vol. 18. University of Chicago Press, Chicago, pp. 298–422.
- Crews, D., 1975. Inter- and intraindividual variation in display patterns in the lizard, *Anolis carolinensis*. *Herpetologica* 31, 37–47.
- Crews, D., 1979. The hormonal control of behavior in a lizard. *Sci. Am.* 241, 180–187.
- Deckel, A.W., 1996. Behavioral changes in *Anolis carolinensis* following injection with fluoxetine. *Behav. Brain Res.* 78, 175–182.
- Deckel, A.W., Jevitts, E., 1997. Left vs. right-hemisphere regulation of aggressive behaviors in *Anolis carolinensis*: effects of eye-patching and fluoxetine administration. *J. Exp. Zool.* 278, 9–21.
- DeCoursey, K.R., Jenssen, T.A., 1994. Structure and use of male territorial headbob signals by the lizard *Anolis carolinensis*. *Anim. Behav.* 47, 251–262.
- Evans, L.T., 1936. A study of social hierarchy in lizard, *Anolis carolinensis*. *J. Genet. Psychol.* 48, 88–111.
- Greenberg, B., Noble, G.K., 1944. Social behavior of the American chameleon (*Anolis carolinensis*). *Physiol. Zool.* 17, 392–439.
- Greenberg, N., 1977. A neuroethological study of display behavior in the lizard *Anolis carolinensis* (Reptilia, Lacertilia, Iguanidae). *Am. Zool.* 17, 191–201.
- Greenberg, N., Crews, D., 1990. Endocrine and behavioral responses to aggression and social dominance in the green anole lizard, *Anolis carolinensis*. *Gen. Comp. Endocrinol.* 77, 1–10.
- Hadley, M.E., Goldman, J.M., 1969. Physiological color change in reptiles. *Am. Zool.* 9, 489–504.
- Jenssen, T.A., Greenberg, N., Hovde, K.A., 1995. Seasonal shifts in the activity of male *Anolis carolinensis* in the field. *Herp. Monogr.* 9, 41–62.
- Jenssen, T.A., Lovorn, M.B., Congdon, J.D., 2001. Field-testing the protandry-based mating system for the lizard, *Anolis carolinensis*: Does the model organism have the right model? *Behav. Ecol. Sociobiol.* 50, 162–172.
- Jenssen, T.A., Nunez, S.C., 1998. Spatial and breeding relationships of the lizard *Anolis carolinensis*: evidence of intrasexual selection. *Behaviour* 135, 981–1003.
- Kleinholz, L.H., 1938. Studies in reptilian color change. III. Control of light phase and behavior of isolated skin. *J. Exp. Zool.* 15, 492–499.
- Korzan, W.J., Summers, T.R., Ronan, P.J., Summers, C.H., 2000a. Visible sympathetic activity as a social signal in *Anolis carolinensis*: changes in aggression and plasma catecholamines. *Horm. Behav.* 38, 193–199.
- Korzan, W.J., Summers, T.R., Summers, C.H., 2000b. Monoaminergic activities of limbic regions are elevated during aggression: influence of sympathetic social signaling. *Brain Res.* 870, 170–178.
- Korzan, W.J., Summers, T.R., Ronan, P.J., Renner, K.J., Summers, C.H., 2001a. The role of monoaminergic perikarya during aggression and sympathetic social signaling. *Brain Behav. Evol.* 57, 317–327.
- Korzan, W.J., Summers, T.R., Summers, C.H., 2001b. Aggression: towards a reflected image or live combat. *Horm. Behav.* 39, 335–336.
- Larson, E.T., Summers, C.H., 2001. Serotonin reverses dominant social status. *Behav. Brain Res.* 121, 95–102.
- Licht, P., 1971. Regulation of the annual testis cycle by photoperiod and temperature in the lizard, *Anolis carolinensis*. *Ecology* 52, 240–252.
- Lin, P.Y.T., Bulawa, M.C., Wong, P., Lin, L., Scott, J., Blank, C.L., 1984. The determination of catecholamines, indoleamines and related enzymatic activities using three micron liquid chromatography. *J. Liq. Chromatogr.* 7, 509.
- Lorenz, K.Z., 1937. The companion in the bird's world. *Auk* 54, 245–273.
- Lovern, M.B., McNabb, F.M., Jenssen, T.A., 2001. Developmental effects of testosterone on behavior in male and female green anoles (*Anolis carolinensis*). *Horm. Behav.* 39, 131–143.
- Marrone, R.L., Pray, S.L., Bridges, C.C., 1966. Norepinephrine elicitation of aggressive display responses in *Betta splendens*. *Psychol. Sci.* 5, 207–208.
- Matt, K.S., Moore, M.C., Knapp, R., Moore, I.T., 1997. Sympathetic mediation of stress and aggressive competition: plasma catecholamines in free-living male tree lizards. *Physiol. Behav.* 61, 639–647.
- Muske, L.E., Fernald, R.D., 1987. Control of a teleost social signal. I. Neural basis for differential expression of a color pattern. *J. Comp. Physiol. A* 160, 89–97.
- Nunez, S.C., Jenssen, T.A., Ersland, K., 1997. Female activity profile of a polygynous lizard (*Anolis carolinensis*): evidence of intersexual asymmetry. *Behaviour* 134, 205–223.
- Ruby, D.E., 1984. Male breeding success and differential access to females in *Anolis carolinensis*. *Herpetologica* 40, 272–280.
- Sgoifo, A., de Boer, S.F., Haller, J., Koolhaas, J.M., 1996. Individual differences in plasma catecholamine and corticosterone stress responses of wild-type rats: relationship with aggression. *Physiol. Behav.* 60, 1403–1407.
- Sugerman, R.A., 1990. Observer effects in *Anolis sagrei*. *J. Herp.* 24, 316–317.
- Summers, C.H., Greenberg, N., 1994. Somatic correlates of adrenergic activity during aggression in the lizard, *Anolis carolinensis*. *Horm. Behav.* 28, 29–40.
- Summers, C.H., Larson, E.T., Summers, T.R., Renner, K.J., Greenberg, N., 1998. Regional and temporal separation of serotonergic activity mediating social stress. *Neuroscience* 87, 489–496.
- Summers, C.H., Larson, E.T., Ronan, P.J., Hofmann, P.M., Emerson, A.J., Renner, K.J., 2000. Serotonergic responses to corticosterone and testosterone in the limbic system. *Gen. Comp. Endocrinol.* 117, 151–159.
- Summers, C.H., 2001. Mechanisms for quick and variable responses. *Brain Behav. Evol.* 57, 283–292.
- Summers, C.H., 2002. Social interaction over time, implications for stress responsiveness. *Int. Comp. Biol.* 42, 591–599.
- Tinbergen, N., Perdeck, A.C., 1950. On the stimulus situation releasing the begging response in the newly hatched herring gull chick (*Larus a. argentatus* Ponstopp). *Behavior* 3, 1–38.
- Tokarz, R.R., 1985. Body size as a factor determining dominance in staged agonistic encounters between male brown anoles (*Anolis sagrei*). *Anim. Behav.* 33, 746–753.
- Vaughan, G.L., Greenberg, N., 1987. Propranolol, a β -adrenergic antagonist, retards response to MSH in skin of *Anolis carolinensis*. *Physiol. Behav.* 40, 555–558.
- Yang, E.J., Phelps, S.M., Crews, D., Wilczynski, W., 2001. The effects of social experience on aggressive behavior in the green anole lizard (*Anolis carolinensis*). *Ethology* 107, 777–794.