Scared and less noisy: glucocorticoids are associated with alarm call entropy

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The nonlinearity and arousal hypothesis predicts that highly aroused mammals will produce nonlinear, noisy vocalizations. We tested this prediction by measuring faecal glucocorticoid metabolites (GCMs) in adult yellow-bellied marmots (Marmota flaviventris), and asking if variation in GCMs was positively correlated with Wiener entropy—a measure of noise. Contrary to our prediction, we found a significant negative relationship: marmots with more faecal GCMs produced calls with less noise than those with lower levels of GCMs. A previous study suggested that glucocorticoids modulate the probability that a marmot will emit a call. This study suggests that, like some other species, calls emitted from highly aroused individuals are less noisy. Glucocorticoids thus play an important, yet underappreciated role, in alarm call production.

Keywords: alarm calls; arousal; communication; noise; nonlinearity; Marmota flaviventris

1. INTRODUCTION

The vocalizations of highly aroused mammals often contain nonlinear vocal attributes that include chaotic noise. Indeed, noise and other nonlinearities increase with arousal in human infant cries [1], dog (Canis familiaris) barks [2], piglet (Sus scrofa) squeals [3], meerkat (Suricata suricatta) alarm calls [4] and highly aroused vocalizations from other taxa (e.g. [5]). These acoustic features are thought to result from ‘over-blowing’ the sound production system, and it is hypothesized that habituating to such nonlinearities is difficult [6,7].

Expanding on Fitch and his colleagues’ idea, Blumstein et al. [8–10] proposed that these nonlinear sounds honestly communicate the sound of arousal and fear. To date, there have been three playback studies which have demonstrated that alarm calls with nonlinearities in them (noise, [9,11]; sub-harmonic intrusions, [12]) are more evocative than calls without nonlinearities or with less noise. A further prediction from the nonlinearity and arousal hypothesis is that glucocorticoids should be associated with acoustic characteristics.

Specifically, individuals that have relatively high glucocorticoid levels should produce alarm calls that are relatively more noisy (or contain other forms of nonlinearities), because they should be more aroused than those with relatively low glucocorticoid levels.

We tested this hypothesis with yellow-bellied marmots (Marmota flaviventris), a 3–5 kg, facultatively social, ground-dwelling squirrel—a good model because much is known about the contexts under which this species emits alarm calls [13]. Previous research has demonstrated that the propensity of adult females to produce alarm calls is correlated with glucocorticoids [14]; females are more likely to call on an occasion when she has relatively higher levels. In addition, there is substantial variation in faecal glucocorticoid levels between individuals and within an individual on different occasions; thus there is the opportunity for variation in glucocorticoid levels to be associated with call structure. Finally, juvenile marmots recently emerged from their natal burrows occasionally produce fear screams that contain noise and other nonlinear vocal attributes [8], but the degree of noise in either juvenile or adult alarm calls has not been formally quantified. We predicted that glucocorticoids would be associated with the degree of noisiness in adult alarm calls.

2. MATERIAL AND METHODS

(a) Subjects and data collection

Marmots were studied at the Rocky Mountain Biological Laboratory during their summer active seasons between 2003 and 2009. We aimed to trap individuals in our study population every other week during routine morning and early evening trapping sessions. When trapped, marmots occasionally called. We recorded alarm calls from marmots contained in live traps using Audix OM-3xb microphones (frequency response: 40 Hz–20 kHz) placed 20–40 cm from calling subjects; these calls were recorded onto digital recording gear (either a Sony PCM-M1 digital audiotape recorder, or a Marantz PMD 660 direct to disk recorder) sampling at 44.1 kHz with 16-bit resolution. Because yellow-bellied marmots communicate risk, not predator type [13,15], recording trapped subjects controlled the context (and presumably the degree of risk) that calling marmots experienced, which allowed us to focus on other potential drivers of acoustic variation.

We used recordings from 46 adult subjects (eight males and 38 females) and extracted three to five 100 ms duration segments that contained alarm calls for a total of 223 calls. We used Sound Analysis Pro (Ofer Tchernichowski, City College of New York) to automatically extract the Wiener entropy associated with each adult alarm call; additional analyses described in the electronic supplementary material quantified entropy in the background environmental noise. A sound with Wiener entropy = 0 would be pure noise; noise is reduced as Wiener entropy decreases (i.e. approaches negative infinity). It is important to note that Wiener entropy does not distinguish between turbulent noise created by wind or microphone movement, and the chaotic noise produced by asymmetrically vibrating vocal folds. Only the latter is relevant for the nonlinearity hypothesis. Thus, we were very careful to select calls with no obvious background noise in them (see the electronic supplementary material). The marmot alarm calls analysed were all whistles that had variable numbers of harmonics; thus, we also counted the number of energy bands in the call (i.e. the fundamental frequency plus all harmonics).

Following Blumstein et al. [14], faeces found in traps when we reached them were collected in a plastic bag, immediately placed on ice, and frozen at −20 °C within 2 h of collection. We assumed that faecal glucocorticoid metabolites (GCMs) reflect levels pooled over time, and, unlike concentrations in blood samples, are not influenced acutely by the capturing procedure. Faecal GCMs were measured with a double-antibody 125I radioimmunoassay kit (MP Biomedicals, Costa Mesa, CA, USA). Details of sample preparation and assay validation are found elsewhere [14].

(b) Statistical analysis

Faecal GCM levels were log_{10} transformed to normalize their distribution. We fitted a linear mixed effects model where we modelled
alarm call entropy as a function of $\log_{10}$ GCM level on the occasion when the caller called, alarm call duration, the total number of energy bands in a call (calls are harmonically structured so this value was the number of harmonics + 1), the time of day in which the sample was collected (AM or PM), the caller’s sex, along with the following two way interactions—sex $\times \log_{10}$ faecal GCM, time of day $\times \log_{10}$ GCM, sex $\times$ energy bands, sex $\times$ call duration and energy bands $\times$ call duration. We also fitted mixed effects models to study the effect of GCM on the total number of energy bands and on the call duration. In both models, we controlled for repeated individual observations.

3. RESULTS

More negative values of Wiener entropy are ‘smaller’ and reflect sounds that are ‘clearer’ or more articulated. After controlling statistically for a number of
There was no relationship between GCM levels and the number of energy bands. Such a relationship would suggest that scared marmots produced louder alarm calls. Since we also found that increased faecal GCM levels were associated with reduced call duration, we suspect that scared marmots are producing shorter and more defined calls, regardless of the number of energy bands produced.

Yellow-bellied marmots communicate risk by varying the rate and amplitude of their alarm calls [15]. When we recorded animals calling in traps, there was quite a bit of amplitude variation. This variation in amplitude sounds, to us, like what we hear naturally in the meadows; not all alarm calls are especially piercing.

Our results suggest that in marmots, and perhaps in other species, highly aroused animals produce piercing alarm calls that are probably the product of selection to have an immediate strong response in receivers. Piglets emit clearer, more piercing calls during castration compared with the less stressful periods before and after castration [16]. In addition, highly aroused isolated goats (Capra hircus) emitted tonal bleats [17]. Unhealthy or physically stressed dogs produce barks that either have lower-than-normal harmonic-to-noise ratios or very high harmonic-to-noise ratios [18]. These results suggest that stressed animals can either emit very noisy calls (low harmonic-to-noise ratio) or, like marmots, exceedingly clear, tonal calls (high harmonic-to-noise ratio). However, even in marmots, evocativeness can be produced by noisy vocalizations. Other vocalizations, such as screams, may be especially evocative because of their noise and other nonlinear attributes. Taken together, this research confirms that glucocorticoids play an important role in alarm call production [19], but risk can be communicated in multiple ways. Scared juveniles scream while scared adults produce less noisy calls.

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