Assessment of Alarm Calling by California Ground Squirrels: The Role of Concomitant Visual Cues, Tonic Effects, and Latent Behavioral Systems

By

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TABLE OF CONTENTS

ABSTRACT

This dissertation explores the role of visual cues, time frames, and latent behavioral systems in California ground squirrel (*Spermophilus beecheyi*) assessment of alarm calls.

Surprisingly little research has examined the communicative effects of cues associated with ritualized signals. Chapter 1 studied the role of visual cues in short-term ground squirrel assessment of repetitive "chatter-chat" anti-predator calling. Visual cues (a bipedal or quadrupedal squirrel model) were paired factorially with repetitive alarm or control (mallard) calling. Unexpectedly, I observed no differential effects of vocal stimuli. In contrast, the quadrupedal model evoked higher levels of visual monitoring than the bipedal model. Additionally, females demonstrated higher vigilance in response to the bipedal than the quadrupedal model, whereas males displayed the opposite pattern. Finally, males showed more relaxation of alarm than females during the second playback minute.

Chapter 2 investigated whether chatter-chat calling and associated visual cues exert a lingering effect on perceivers in the aftermath of these playbacks. The aforementioned playbacks were followed by an approximately nine-minute pause without playbacks. Subsequently, a single alarm chatter was broadcast to "probe" for differential latent effects of the prior playbacks. Most remarkably, the probe revealed latent differential effects of prior vocal playback conditions. Females differentiated more strongly on the basis of previously heard vocal than visual stimuli, but males did the opposite. Prior exposure to repetitive alarm calling suppressed relative mobility in females, whereas males were more responsive to visual cues, relaxing less in the aftermath of the quadrupedal model presentation.

The final chapter extends this discovery of latent processes to the field of animal behavior in general. A literature survey revealed numerous examples of such **latent systems**, in which the underlying cognitive or motivational states brought about by exposure to a given stimulus are sustained even when the behavioral responses they influence are no longer observable. This review of the literature on habituation, imprinting, and bird song development not only documented the importance of latent systems, but also suggested that the probe methodology used here provides a powerful and more sensitive new tool for the analysis of latent processes.

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INTRODUCTION

Animals rely heavily on the information extractable from communicative signals, using them as warning systems about potential predators, as well as sources of information about the presence and receptivity of potential mates, the species or individual identity of nearby animals, and so on. However, ritualized signals are not sufficient information sources for an individual to develop a complete picture of its surroundings (Smith, 1977). This inadequacy is exacerbated by the fact that signals lose their information value as they become degraded or distorted over distance and time (Morton 1975; Wiley and Richards 1978), and can even be misleading when signalers make errors or produce "deceitful" signals in an attempt to manipulate the behavior of others (Dawkins and Krebs 1978). How can individuals build a more complete picture of their surroundings, and compensate for the inadequacy of signals? They must be proactive in their quest for the information needed for adaptive decision making, viewing the signals of others with skepticism (Dawkins and Krebs 1984) and probing their environments for useful information available from both signals and non-signaling sources (Owings and Morton 1998). One category of non-signaling sources involves the cues associated with the production of ritualized signals. For example, the posture (e.g. relaxed versus alert) and orientation of an individual producing an alarm call could give observers clues as to the location and level of threat posed by a potential predator. Nonetheless, surprisingly little research has been conducted on the influence of such cues on communicative interactions (Leger 1993; but see e.g. Leger et al. 1979; West and King 1988a; West and King 1988b; Blumstein and Arnold 1995).

As shown in the previous example, signals and their associated cues need not be accessible via the same perceptual modality. Partan and Marler (1999) summarized a number of ways in which signals produced in different modalities could combine to affect the behavior of perceivers; these included modulation or masking of one component by the other, independent effects of the separate components, and interaction of the components to produce a completely novel response. Partan and Marler considered cues as well as ritualized signals to be components of multimodal signals (Partan and Marler 2005); thus, it might be assumed that combinations of cues and signals would evoke similar patterns of behavioral responses as combinations of different ritualized signals. Hence, in addition to ascertaining what cues animals use in assessing signals, it would be informative to gather more data on how animals combine inputs from cues and signals in their behavioral decisions. In the first chapter of this dissertation, I describe a playback study in which I combined visual cues (squirrel models in different postures and orientations) with audio signals (alarm and control calls) in order to determine how California ground squirrels (*Spermophilus beecheyi*) use cues in their assessment of alarm calls.

Although signals provide an insufficient base for developing a complete picture of one's world, signals and signaling behavior can comprise richer information sources than is typically recognized. Communication research typically has focused on the immediate effects of signals on behavior, but signals can also have more lingering, or **tonic**, effects (although see e.g. Loughry and McDonough 1988; Hersek and Owings 1993; Hanson

2003). Tonic signaling exerts its effects over longer time scales (e.g. tens of minutes, hours, or days as opposed to seconds or minutes; see e.g. Schleidt 1973; Owings and Morton 1998). Owings *et al.* (1986) hypothesized that the repetitive "chatter-chat" vocalizations of California ground squirrels, which often continue for tens of minutes, might play a role in tonic communication. The effects of such repeated signals can accumulate over an extended time scale that serves to increase or maintain vigilance in perceivers. Thus, information may be extractable not only from the structure of individual signals, but also from the patterning of signal repetition. Studies to date have provided limited support for the concept of tonic communication (Loughry and McDonough 1988; LeRoux et al. 2001; Sloan and Hare 2004). However, the aforementioned studies have not considered the possibility that tonic signaling may elicit effects that remain quiescent in perceivers. For example, signal perceivers may maintain an elevated state of preparedness to respond rapidly to subsequent signals; this state would not be detectable until the occurrence of the second signal. My study of California ground squirrels explored the possibility of quiescent tonic effects by introducing a "probe" stimulus, in which a short alarm call was used to elicit behavior that might unmask any lingering effects of an initial repetitive chatter-chat playback. The results of this experiment are discussed in the second chapter of this dissertation.

The potentially latent tonic behavioral effects of signaling raised the possibility that this phenomenon might be common to other aspects of animal behavior as well. Therefore, I conducted a literature search in which I found many other examples of quiescent behavior, involving well-known processes such as habituation, imprinting, and bird song development. These phenomena share the following feature: the persistence of a cognitive or motivational state resulting from exposure to a given stimulus, initially evidenced as an observable behavioral response that subsequently disappears, whose continued presence can be demonstrated by presentation of an appropriate response-triggering stimulus. I refer to these phenomena as **latent systems**, which I describe in detail in the third chapter of this dissertation. Additionally, I discuss the potential importance of using probe stimuli in detecting the presence of latent systems, and the ways in which our understanding of animal communication could be expanded by exploring the role(s) of latent systems.

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CHAPTER 1

Assessment of Anti-Predator Vocalizations by California Ground Squirrels: Roles of Sex Differences and Visual Cues

ABSTRACT

This study explored the role of different visual cues on the reactions of California ground squirrels to playbacks of both alarm calls evoked by mammalian predators and control sounds (mallard “quacking” a.k.a. “hail calls”). Visual cues were manipulated by factorially pairing taxidermic squirrel mounts in two postures, bipedal and quadrupedal, with 3-min audio playbacks of repeated calling. Squirrels were more responsive to visual cues than vocalizations: the quadrupedal squirrel model evoked a strong increase in surveillance behavior in the initial minute of playback, whereas the bipedal model elicited little change. Accompanying vocal playbacks did not produce significant differential effects on squirrel responses. Sex differences strongly influenced the results, and examination of the data across different time frames revealed different behavioral patterns. Females showed a strong surge in surveillance behavior in the initial minute of playback, whereas males exhibited little change in behavior over time. Moreover, the sexes exhibited contrasting patterns of overall vigilance during the initial playback minute: females increased vigilance in response to the bipedal model but decreased it in response to the quadrupedal model; males did exactly the opposite. Finally, males relaxed their state of alarm across the entire observation period whereas females persisted in a state of alarm.

INTRODUCTION

Markl (1985) distinguished *signals*, or *ritualized displays*, from *cues*; *signals* have been acted on by selection for communicative function whereas *cues* consist of other sender traits not so selected. Individuals participating in communication actively extract information from both sources (e.g. Owings & Morton 1998, Leger 1993, Smith 1977). Such proactivity in the pursuit of information is useful to perceiving animals in at least two ways. It serves as a reliability check on the information uncovered in signals and thereby protects the perceiving animal from manipulative use of signals by others (e.g. Jackson & Wilcox 1990, Lloyd 1965, Munn 1986). And, it may "fill in the gaps" left by perception of a signal that is an inadequate source of information, either because it is degraded (Wiley & Richards 1978), or more generally because signals are not selected to be complete sources of the information that other animals need for adaptive decision making (Smith 1986). Signals and the cues that occur in association with them are often detectable through different sensory modalities; consequently, such proactive information extraction is often multisensory, necessitating the integration of inputs from multiple sensory systems (Partan & Marler 1999, West *et al.* 1994).

Studies of the role of cues in communication have proven to be invaluable in elucidating how communication can be complex even when the signal production side of that process seems relatively simple (Leger 1993; Owings and Hennessy 1984). For example, the single-note vocalizations used by California ground squirrels (*Spermophilus beecheyi*) do not have a high level of production specificity (as in Macedonia & Evans 1993); these calls are emitted in response to both predators and agonistic conspecifics (Owings & Hennessy 1984; Leger, Owings & Gelfand 1980). But attention to accompanying cues allows perceivers to tailor their reactions to these vocalizations much more precisely than would be possible if responses were based on the structure of the signal alone. For example, raptor attacks typically elicit calls from multiple individuals whereas agonistic encounters usually involve only a single call, and perceiver response is stronger and more cautious to playbacks of multiple calls (Leger *et al.* 1979). So, attention to signal structure in addition to cues allows a perceiving squirrel to differentiate between a life-threatening situation and one that is much less dangerous and hence not worth spending precious resources on. An additional layer of complexity becomes evident when one considers that animals can obtain information by observing the *outcome* of communicative interactions, and can modify their behavior toward signalers accordingly (e.g. Peake *et al.* 2001). Variables such as the number of animals responding to a signaler and the apparent strength of their responses could serve as cues to an onlooker. Therefore, it is clear that use of cues can be a crucial feature in animal communication. Nevertheless, studies of the influence of cues in communicative interactions have remained rare (but see e.g. Leger *et al.* 1979, West & King 1988a,b, Blumstein & Arnold 1995).

We examined the use of visual cues by California ground squirrels during playbacks of chatter-chat call series elicited by mammalian predators (Owings & Leger 1980). Concomitant contextual cues were presented in the form of taxidermically mounted "calling" squirrels whose vigilance-related postures varied.

METHODS

Subjects

During the spring and summer of 2003 and 2004, we studied California ground squirrels living along the Alameda Watershed at the Frog Pond (Alameda County, CA). The study site was located in an area of oak savanna, and potential predators included coyotes, bobcats, rattlesnakes, golden eagles, and red-tailed hawks. Squirrels were captured from April 20th through July 23rd 2003 and May 8th through July 15th 2004 in Tomahawk live traps baited with black oil sunflower seeds, and then sedated with Ketamine HCl (40 mg/kg, injected intramuscularly), which allowed them to be handled. The animals then were ear tagged with numbered metal bands (National Band and Tag Company, Newport, KY), dye-marked with individually distinctive numbers using Nyanzol-D dye (The ADI Group USA, Jersey City, NJ) applied with a cotton-tipped applicator, weighed, sexed, inspected for reproductive condition and age class (juvenile or adult) and released at the point of capture. All experiments were conducted from May 24th through August 3rd 2003 and May 15th through August 1st 2004 on these free-ranging individuals.

Visual Cue Playback Experiments

Playback Stimuli

The visual stimuli used during playbacks were taxidermic mounts of California ground squirrels, oriented in different directions and positioned in either a bipedal or quadrupedal stance (the quadrupedal stance is more common in California ground squirrels during higher-urgency situations; Leger *et al.* 1979). The bipedal mount was prepared from a

655 g adult male and the quadrupedal mount was of a 565 g adult female; both were captured in the Arboretum at the University of California, Davis. The vocal stimuli consisted of California ground squirrel alarm calls and control sounds; mallard *(Anas platyrhynchos)* "hail," or "quack," calls were chosen as the control stimuli because these birds were common at the study site. The anti-predator calls used for playback were recorded by L.A. Rabin from a nearby population (Los Vaqueros Watershed in the Altamont Pass Wind Resource Area, Alameda County, CA) in August and September 2001 in response to domestic dog presentations. The vocalizations consisted of initial "chatter" calls followed by repetitive "chat" calls. Three alarm call exemplars were used: two from adult females and one from an adult male. Calls were recorded with a Sennheiser K3U/ME-80 shotgun microphone at a distance of 1 -10 m from the vocalizing squirrel. Recordings were digitized by passing the analog signals through a Sony DVMC-DA2 Media Converter into a Macintosh computer, where the BIAS Peak DV program (Berkeley Integrated Audio Software, Inc.) was used to save them as digital wave files. The calls then were filtered using a Butterworth band pass (passing frequencies from 2300 to 15000 Hz, order=40) to minimize ambient noise. Since the desired playback stimulus length was 3 min and two of the recordings were of shorter duration than this (47 s and 61 s), the repetitive chat portions of the calls (41 s and 43 s, respectively) were copied and pasted into sound files to produce 3-min calls.

Three exemplars of mallard "hail" calls (Cornell Laboratory of Ornithology/Interactive Audio 1992; and Files 2286 and 5493, Borror Laboratory of Bioacoustics, Department of Evolution, Ecology, and Organismal Biology, Ohio State University, Columbus, OH, all rights reserved) were used as control sounds. Mallard calls were chosen as control stimuli because the experiments were conducted adjacent to a pond at which mallards were common; moreover, the "hail" call was a fairly loud call (as were the squirrel vocalizations) and did not appear to be particularly threatening or unusual for the squirrels to hear, based on our previous observations.

All audio stimuli were standardized to a mean volume of 93.76 dB (range: 91.3-94.6 dB) using a Brüel & Kjaer sound level meter (set at "Linear" and "Hold-Peak") placed 1 m directly in front of the speaker. Stimuli also were standardized with respect to total amount of continuous sound. This was accomplished by visually inspecting signals' oscillograms in the Peak program, and the duration of sound judged to be above background levels was summed up to arrive at an estimated total signal duration. The average total amount of sound for the three squirrel call tracks was determined to be 9.5 s of sound for the 3 min duration of playback. Thus, the mallard calls were standardized to approximately the same sound duration by repeating a given call (interspersed with silent intervals of random duration) as many times as necessary to produce 9.5 s of total sound; this resulted in 6 call repetitions for two stimulus tracks and 9 repetitions for the third. Audio tracks then were imported into iTunes v 4.7(41) and subsequently burned onto CD-R's for use in playbacks.

Equipment and Set-up

The playback apparatus consisted of a box frame constructed with PVC tubing and covered with green "camouflage"-patterned nylon cloth; the box had a hinged lid atop which was fastened the squirrel taxidermic mount (hereafter referred to as the "model") representing a vocalizing animal. The lid was controlled (using kite string manipulated by the researcher from approximately 15 m away) to pivot such that the squirrel would either be visible above the box or concealed within the box. A speaker (RCA PRO-LX550 75-Watt 2-way bookshelf speaker, with a Linaeum dipole tweeter and a 5" honeycomb woofer) was positioned at one end of the PVC box (under the rostral end of the model). Audio playback stimuli were played on a Sony Discman ESP^2, amplified by a Kenwood KAC-529S car amplifier, and broadcast from the speaker.

Surveyors' flags were positioned at 5 m-intervals from the playback apparatus along the cardinal compass directions, as well as NW, NE, SE, and SW, to a distance of about 30 m from the squirrel box. These flags allowed determination of the distances between focal animals and the playback apparatus, as well as distance(s) to nearest neighbor(s), for narration purposes. At least 30 min prior to the start of playback trials, black oil sunflower seeds were scattered at a radius of approximately 5 m to 10 m from the model/speaker set-up, to lure subjects into the vicinity.

Playback Protocol

Ground squirrels were videotaped by observers sitting within a sunshade "blind" or beneath a large canopy located approximately 15 m from the model and speaker. Focal animals were filmed (using a Panasonic 5100 HS video camera attached to a JVC BR-S405U HiFi SVHS VCR, a Panasonic PV-DV601 digital video camera, a Canon ZR40 digital video camera, and a Sony Handicam Vision CCDTRV58NTSC Hi-8 camera) for a baseline, or "pretrial," period of 2 min prior to the playback; simultaneously, the following additional information was narrated onto the video sound track at 30-sec intervals: 1) distance and direction (N, S, E, W) of the focal animal from the model; 2) whether the focal animal's head was facing into the 180° arc toward or away from the model; and 3) whether the focal animal's head was facing the same or the opposite direction (within 180°) as the rostral end of the model (i.e., displaying the same "direction of gaze"). The number (and if possible, the identity) of other squirrels within a 10-m radius also was narrated. Following pretrial, the squirrel mount was revealed and squirrels were allowed to habituate to the mount for a minimum of 1 min. If the focal animal remained in view and appeared to have habituated to the mount (i.e., it resumed or continued foraging or other activities it had been engaged in prior to model presentation) and no obvious disturbances (such as alarm calling, appearance of a predator, or a vehicle driving past) occurred during that time, the vocal playback was started. Playback continued for 3 min; 30-s narration and videotaping continued throughout this period.

Each subject received four treatments: quadrupedal model/anti-predator call, bipedal model/ anti-predator call, quadrupedal model/control (mallard) calls, and bipedal model/control (mallard) calls. Only one alarm call and one control sound exemplar were played to a given subject, to control for the two vocal conditions between the two model postures. The order of presentation of the four treatments was varied among subjects to counterbalance for order effects, and the playback apparatus was placed in several different locations adjacent to the pond. The squirrel mount was positioned in different compass directions, to minimize the effects of preferential directional orientation by the squirrels. To avoid possible habituation or sensitization effects, we waited at least 1 h between playback trials.

Videotape Analysis

VHS recordings were played back on a Panasonic Video Cassette Player AG-7100 connected to a Panasonic AG-A750 Editing Controller and viewed for analysis on a Panasonic CT-1331Y video monitor. Hi-8 video recordings were burned to a DVD and viewed and analyzed on a Macintosh computer using DVD Player v 3.2. All other trials were imported to a Macintosh computer using iMovie 3.0.3, and behaviors were quantified by viewing trials using QuickTime Player. Pretrial and playback periods were instantaneously sampled at 6-s intervals for the following measures: posture (bipedal, sitting upright, quadrupedal), head elevated or not, feeding, and locomotion (walking or running). Additionally, 30-s narrated data (distance to model, orientation with respect to the model, and orientation with respect to the model's gaze direction) were transcribed from the audio track of the videotapes.

Because a number of different observers analyzed videotapes, a subset of the videos was reanalyzed by a naïve observer in order to determine the reliability of observations. This observer was given an explanation of the criteria defining each behavior, without being supplied with any information on the situations in which behaviors typically occurred and without any knowledge of treatment conditions or the purpose of the study. The observer analyzed 25% of the videos from data averaged over the entire 3 min of playback as well as initial minute data for 2004 (15.9% of the trials subjected to ANOVA's): one trial from each of seven focal animals, with the treatment type chosen at random (two samples of each treatment type were examined, with the exception of quadrupedal control, for which only one trial was used). Three trained observers had analyzed the 2004 videotapes; all were represented in this sample. The naïve and trained observers' measurements then were compared using the index of concordance, or the total number of agreements divided by the sum of agreements and disagreements (Martin & Bateson 1993). For behaviors analyzed to obtain the 3-minute playback averages (2 min. pretrial and 3 min. playback quantified at 6-s intervals), the naïve observer's scores matched the trained observers' scores, on average, 89.2% of the time (range: 80.5-94.6%). When matching for each of the ten behavioral categories was examined independently, it was determined that matching occurred 76.2% to 97.3% of the time for the individual behaviors. For the first minute of playback, the naïve observer's measurements showed 89.0% matching (range: 74.8-95.2%) with those of the trained observers. Comparing the measurements individually by behavior, 72.6% to 97.3% of the naïve and trained observers' scores matched. Therefore, the reliability of the measurements was robust, since the naïve observer's measurements showed 89% agreement overall.

Statistical Analysis

Principal components analysis

All statistical analyses were performed using SPSS Graduate Pack 11.0 for Mac OS X. Principal components analysis (PCA) was performed on mean data (averaged over the two minutes of pretrial, the initial minute of playback, and the second minute of playback, respectively) for the ten initial behavioral measures obtained from videotape analysis. Although squirrel responses had been filmed for three minutes following the start of playback, many of the animals disappeared from the observers' view during the third minute of playback for at least one of their four trials; consequently, the third minute was not included in subsequent analyses. Components (subsequently referred to as "factors") with eigenvalues greater than 1.0 then were subjected to analysis by general linear model, using a mixed repeated measures/between-subjects analysis of variance (ANOVA). Tabachnick & Fidell (1983) recommend consideration of loadings with values of 0.30 or greater; thus, behavioral measures meeting this criterion were determined to contribute to PCA factors.

ANOVA's were used to investigate playback effects for two time periods: the first minute of playback (to reveal initial responses) and pretrial through minute 2 (to ascertain possible changes in behavior over time). Visual cue and vocal stimulus (and playback minute, for the minute-to-minute analysis) were used as the within-subjects factors in the ANOVA's; sex was used as the between-subjects factor. For significant ANOVA interactions, *t* tests were run to determine whether individual comparisons were significant. Paired samples *t* tests were used when comparisons involved the same individuals under different treatment conditions; independent samples *t* tests were performed for other comparisons. For independent samples *t* tests, if Levene's test for equality of variances produced significant results, the "equal variances not assumed" *t* test results were reported; otherwise, the "equal variances assumed" values were reported. When the ANOVA within-subjects tests that adjusted for non-sphericity of data (Greenhouse-Geisser, Huynh-Feldt, and lower-bound) produced different p-values than did the unadjusted within-subjects test, Mauchly's test of sphericity was consulted to ensure that the assumptions of equal variance and independence of the residual errors were met. Since no significant results were found for Mauchly's test, and because the Greenhouse-Geisser, Huynh-Feldt, and lower-bound test results also gave significant results in these cases, only the unadjusted (sphericity assumed) ANOVA results are reported.

To ensure that significant effects of the within- and between-subjects factors were not simply attributable to differences in behavior during the pretrial period, the same procedure described for the analysis of minute 1 data was followed for pretrial data. Additionally, to determine whether the potential confounding factors of time of day at which trials began or the order in which treatments were presented (trial order) displayed an effect, repeated measures ANCOVA's were conducted using trial order or trial start time as covariates, and sex as the between-subjects factor, for the initial minute and pretrial-through-minute 2 analyses. In addition, data were examined for between-years differences using mixed repeated measures/between-subjects ANOVA (with visual and vocal stimuli as within-subjects factors, trial minute as a within-subjects factor for the minute-to-minute analysis, and sex and year as between-subjects factors).

RESULTS

Overview of Results

Sex differences played a very strong role in determining squirrel responses to playback: females and males demonstrated different patterns of behavior for all three of the behavioral categories examined (vigilance, relaxation of alarm, and surveillance), with females appearing to display a higher level of responsiveness overall. The visual cues of quadrupedal versus bipedal model posture also exerted strong differential effects on behavior: the quadrupedal model evoked a strong increase in surveillance behavior early on in the playback, whereas the bipedal model elicited only weak effects. However, the effect of model posture on vigilance depended on the sex of the subject, with females responding more strongly to the bipedal model and males showing a greater response to the quadrupedal model. Moreover, squirrels exhibited significant changes in surveillance behavior over time, with response strongest during the first minute of playback but tapering off during the second minute (these temporal effects were associated with sex differences, as well as with differences in model posture).

Playback Results

Principal Components Analysis

Principal components analysis (PCA) performed on data for ten initial behavioral measures extracted four components with eigenvalues greater than 1.0 (Table 1.1). Stevens (2002) suggests as a guideline that those factors be retained that are needed to account for a minimum of 70% of the total variance; here, the four factors account for

72.1% of total variance. The loadings of the ten initial behaviors onto these factors are shown in Table 1.2. ANOVA's were performed on individual scores derived from these principal components, using visual cue and vocal stimulus as within-subjects factors and sex as a between-subjects factor. These analyses revealed strong associations between experimental treatments and three of the factors, 1, 2, and 3. Factor 4 will not be discussed further for the following reasons: it explained a smaller percentage of the variance in the data (11.5%) than the other three factors, provided no significant effects for either the initial minute or the pretrial-through-minute 2 analyses, and the behavioral responses associated with it (bipedal stance, walking, not sitting) did not fall into any obvious category.

Factor 1: Vigilance

Factor 1, which explained 28.6% of the total variance, was associated with the following behaviors (with loadings of at least 0.30, listed in decreasing order of loading weight, and with the descriptors "increased" and "decreased" specifying the sign of the factor loading): increased time with head elevated, decreased time in quadrupedal stance, increased distance to speaker, increased time in bipedal stance, increased time sitting upright, decreased time feeding, decreased time walking, and increased time running (Table 1.2). These behaviors are consistent with increased vigilance in an attempt to localize the threat (e.g. by elevating the head, and decreasing routine maintenance activities, such as eating) after fleeing to a safe place for these activities (e.g. running, in the opposite direction from the speaker). In subsequent discussion, this suite of behaviors will collectively be referred to as "vigilance."

During the initial playback minute, females and males displayed strongly divergent response patterns to the two visual stimuli: females exhibited stronger vigilance in response to the bipedal than the quadrupedal model, whereas males did exactly the opposite, demonstrating higher vigilance to the quadrupedal than the bipedal model (Figure 1.1; visual*sex interaction: $F_{1,9}=5.829$, $p=0.039$). Females' vigilance in response to the bipedal model was significantly higher than toward the quadrupedal model, toward which they showed a relative decrease in this measure (Figure 1.1; paired samples *t* test: $t=-2.283$, df=9, $p=0.048$). There were no significant effects of sex, vocal stimulus, or visual stimulus alone on vigilance, nor were there significant interactions among these factors. Time of day at which trials began, order in which treatments were presented, and year in which the trials were run did not significantly affect responses to playbacks.

To ensure that the differential behavior observed during the first playback minute was not due to differences that had carried over from the pretrial period, an ANOVA was run on the factor 1 pretrial scores. We found no significant differences for the pretrial period with regard to the variables that differed during the first playback minute (visual stimuli, sex differences, and interactions between these or among these and the vocal stimuli), nor with regard to the potentially confounding factors of time of day, trial order, and year. This test did reveal a significant difference with regard to (future) vocal condition: squirrels demonstrated significantly lower vigilance scores prior to hearing the alarm calls than they did before control (mallard) call presentation ($F_{1,9}=10.083$, $p=0.011$; alarm mean=-0.39, s.e.=0.17; control mean=-0.08, s.e.=0.18). However, since no vocal stimuli were broadcast during the pretrial period, and no aspect of the playback apparatus reflected differences in which stimulus was to be broadcast, it appears this finding was due to sampling error. In order to further explore how this differential response might have affected initial responses to playback, we examined the change in vigilance between pretrial and minute 1, but found no significant change (independent samples *t* test, equal variances not assumed: t=1.186, df=34.936, p=0.244; mean (± s.e.) increase in factor 1 score: alarm, 0.505±0.248; control, 0.160±0.153).

To determine whether there were detectable changes in vigilance over time, factor 1 scores were compared across the three time periods (pretrial, playback minute 1, and playback minute 2). No significant effect of model posture, vocal stimulus, time period, sex, or any interaction among these four factors was detected.

Factor 2: Relaxation

The behavioral measures loading most heavily onto factor 2, which explained 17.8% of total variance, were (in order of decreasing contribution): decreased time spent running, increased time feeding, increased time sitting upright, and decreased time in the quadrupedal stance (Table 1.2). The tendency not to run implies a lack of alarm; the fact that animals were sitting upright (as opposed to a more upright bipedal stance) and feeding suggests that although they still were somewhat alert, they were relaxed enough to continue or resume feeding. Thus, this cluster of behaviors seems to imply that the squirrels were beginning to relax from an initial state of alarm, and will be referred to as "relaxation."

Averaged across all 3 minutes (pretrial-through-minute 2), males demonstrated significantly higher levels of relaxation than females (Figure 1.2; $F_{1,8}$=10.010, p=0.01). Females, however, showed a strongly negative mean factor 2 value (implying increased running, decreased feeding, decreased sitting upright, and increased time quadrupedal), suggesting that they maintained an elevated state of alarm. This sex difference did not depend on visual or vocal stimulus, nor were changes seen between time periods. No other significant main effects or interactions were seen for the within- and between-subjects factors, nor did the potential confounding factors of time of day, trial number, or year noticeably affect the results. The squirrels' levels of relaxation were not affected by any of the within- or between-subjects factors or possible covariates during the initial minute of playback, nor were differences detected during the pretrial period alone.

Factor 3: Surveillance

The behavioral measures loading most heavily onto factor 3, which explained 14.2% of total variance, were (in decreasing order of loading weight): increased time facing toward the speaker, increased time facing in the direction in which the model was oriented, and decreased time spent walking (Table 1.2). Because the first two measures relate to visual information acquisition, and the decrease in walking implies that the animal has stopped moving (arguably to allow it to focus on its visual surroundings), factor 3 will be referred to as "surveillance."

Subjects' surveillance behavior clearly was affected by the posture of the squirrel model presented during playback: their responses showed different temporal patterns in response to the quadrupedal versus the bipedal model (Figure 1.3; visual*time: $F_{2,16}=4.282$, $p=0.032$). Surveillance in response to the quadrupedal model was significantly higher during the first minute of playback than during pretrial (paired samples *t* test: $t=-2.569$, df=21, $p=0.018$) and dropped off during the second minute, whereas response to the bipedal model was low initially and decreased even more during the second minute (Figure 1.3).

Moreover, males and females showed different patterns of behavior over time, with females displaying the highest levels of surveillance during playback minute 1 but dropping to lower-than-pretrial levels in minute 2; males, on the other hand, showed little change in surveillance from pretrial to minute 1, but a slight increase during the second minute (Figure 1.4; sex*time: $F_{2,16}=5.468$, $p=0.015$). Surveillance by females during minute 1 was significantly higher than during minute 2 (Figure 1.4; paired samples *t* test, $t=2.541$, df=19, $p=0.020$) and also higher than male pretrial surveillance (Figure 1.4; independent samples *t* test, equal variances not assumed: $t=2.442$, df=24.699, $p=0.022$).

For the minute-by-minute analysis, there were no main effects of visual cue, vocal stimulus, or sex, and there were no further interactions. Time of day, trial order, and trial year were tested and found not to affect the outcome. Additionally, although the pretrial-through-minute 2 analysis found strong interactions among factors across the three time periods, no significant effects of visual stimulus, vocal stimulus, sex, or any interactions among the three were detected for the initial playback minute or the pretrial period.

DISCUSSION

Our study indicates that the vigilance-related postures adopted by calling California ground squirrels are used as important visual cues by perceivers of the calling squirrel. These cues not only influenced overall vigilance levels and visual surveillance behavior, but also accounted for more variation in response than the vocal stimuli with which they co-occurred. Vocal stimuli did not exert significant differential effects on any of the three behavioral categories studied (vigilance, relaxation, and surveillance). In addition, males and females often showed very different patterns of response, reacting in opposite ways to a given stimulus; this may have been due to differing time budgets between the sexes. A calling squirrel standing quadrupedally evoked a strong increase in surveillance during the first minute of playback; a bipedal squirrel elicited little or no response. On the other hand, females exhibited higher vigilance in response to the bipedal model and a relative decrease in response to the quadrupedal model during the first minute of playback, whereas males did precisely the opposite. Our results also emphasize the importance of examining behavior over different time frames: the differential vigilance response to visual stimuli by the two sexes was detectable only during the first minute of playback, whereas sex differences in relaxation and variation in patterns of surveillance were discovered only by following behavior throughout the course of the experiment, from pretrial through the second minute of playback.

Clearly, model posture was a visual cue that ground squirrels found particularly relevant in their assessment of vocal signals. Why did squirrels show an increase in surveillance in response to the quadrupedal model during the first minute of playback, but display little or no change in reaction to the bipedal model? Leger *et al.*'s (1979) playback study found that in higher risk situations, squirrels tended to freeze in a quadrupedal as opposed to a bipedal stance; thus, the stationary quadrupedal posture of the model paired with vocal signals might have implied a high level of threat. Therefore, the increased level of surveillance in reaction to the quadrupedal "caller" may have reflected this perception of greater danger.

However, another factor that profoundly influenced the experimental outcomes involved sex differences in responses. The aforementioned strong increase in surveillance elicited by the quadrupedal model was driven primarily by females; male surveillance remained at a low level. Additionally, males showed significantly higher relaxation of alarm responses during the course of the experiment than did females, who appeared to maintain an elevated state of alarm throughout. Why was there such a discrepancy? This striking difference in behavior would be consistent with the hypothesis that males and females have different strategies for dealing with potentially threatening situations: females appear to act in a more risk-averse fashion, whereas males seem to be more risk-prone. That is, females tend to act with more caution (e.g. increasing vigilance and surveillance) whereas male strategies show higher variance, increasing vigilance only when danger seems imminent.

Male California ground squirrels use site-specific aggression to defend their territories against intruding males (Boellstorff & Owings 1995; Owings *et al.* 1977). In the present experiment, we baited the area in an approximately 10-m radius around the playback apparatus in order to attract squirrels into the vicinity. This bait attracted a large number of squirrels to the area, including individuals that, judging from trap records, came from neighboring territories. Conceivably, resident males switching to a predator-surveillance mode could suffer greater costs in terms of territorial invasions than females, which would explain why males showed higher levels of relaxation than females and little or no surveillance behavior. Conversely, females may benefit more from nepotistic surveillance behavior in defense of offspring (e.g. Leger & Owings 1978, Owings & Leger 1980) and other female relatives in the area (females are less likely than males to undergo natal dispersal; Boellstorff & Owings 1995, Fitch 1948, Holekamp 1984). These differing strategies would explain the sex differences in relaxation and surveillance. From our casual observations, it seemed that the greatest number of agonistic acts were initiated by a few males; however, such behavior often occurred outside of experimental trials and was not quantified. Data collected during playback trials were inconclusive, as only two animals, one male and one female, were recorded to have engaged in any agonistic behavior during these periods (chasing another squirrel, on one occasion apiece). Sex differences in vigilance have been described in other mammalian species (e.g. Fragaszy 1990, Burger & Gochfeld 1994, Rose & Fedigan 1995), although many of these studies have shown higher vigilance levels in males than females. However, Rose and Fedigan (1995) reported that higher vigilance in white-faced capuchin (*Cebus capucinus*) males seems to be directed more at other males than at potential predators. Moreover, the aforementioned studies did not involve an influx of male intruders, as did the present study. Nor did these studies activate a clear predatory situation, as did our playbacks. Therefore, it is unclear whether the males in our study demonstrated "atypical" behavior.

Sex differences in risk sensitivity also could explain the opposite patterns of vigilance elicited by the two caller postures during the initial minute of playback: female vigilance increased in reaction to the bipedal model but decreased in response to the quadrupedal model, while males behavior was exactly the opposite. Consider first the female response: their mean reaction to the quadrupedal model was a negative value. One way to interpret this finding is by changing the "signs" associated with the behavioral measures contributing to vigilance (PCA factor 1; Table 1.2). The five measures with the highest loading values, with their signs reversed, are as follows: decreased time with head elevated, increased time quadrupedal, decreased distance to playback speaker, decreased time bipedal, and decreased time sitting upright. Other than distance to the speaker, these measures all seem to indicate lowering of the animal's profile, making it less conspicuous to potential predators. Leger *et al.* (1979) explained similar variation in ground squirrel responses to anti-predator calls by proposing that there is a trade-off between information acquisition and self-preservation. In the present study, females near the self-preservation extreme of the spectrum might have inferred a higher level of threat upon seeing the quadrupedal model (as suggested by Leger *et al.*'s 1979 findings) and thus lowered their heads and torsos to make themselves less conspicuous. Conversely, the bipedal model, although representing potentially threatening circumstances, may have signified a lesser degree of danger and thus induced a shift toward the information-acquisition end of the continuum; squirrels consequently were more apt to sit upright and scan their surroundings to determine the cause of disturbance.

Males, on the other hand, appeared to use a different strategy: their vigilance levels rose in response to the quadrupedal caller but dropped when seeing the bipedal one. In this case, males may have taken advantage of the presence of the “vigilant” bipedal model and the numerous females recruited into vigilance (as suggested by Owings & Hennessy 1984) by decreasing the amount of time they spent in more elevated, vigilant postures, and only increasing vigilance themselves when the more evocative quadrupedal model was presented.

Investigating the different time frames over which responses to playback occurred was crucial in detecting various patterns of behavior. Owings and Coss (1991) stressed the importance of examining multiple organizational levels within which animal behavior occurs, pointing out time-frame differences in particular. In the present study, differences in vigilance were detectable only in the initial minute of playback; treatment effects on relaxation and surveillance were apparent only when the entire period between pretrial and the second minute of playback was examined. Female surveillance increased between pretrial and minute 1 and appeared to drop off during the second minute. Female differentiation between visual stimuli was detectable only during the first minute of playback. Thus, at least in females, surveillance and perhaps vigilance peak in the initial minute and subsequently taper off. Leger and Owings (1978), in alarm playbacks to California ground squirrels, likewise found a strong increase in upright posturing, increased running, and generally decreased walking in the first minute following playback; these effects declined over the 3-min post playback period. In the present study, the vocal stimuli were presented repeatedly throughout the two minutes for which responses were analyzed. It is possible that the reason females showed decreased surveillance and vigilance over time is that they habituated to the playback stimuli; in other words, their response declined with repetitive presentation of a given stimulus. This phenomenon is very common in animal behavior, and has been described in a wide variety of species in response to playbacks (e.g. Searcy *et al.* 1982, Cheney & Seyfarth 1988, Searcy *et al.* 1994, Hare 1998, Bee & Gerhardt 2001).

One intriguing finding of this study is related to the fact that squirrels increased surveillance in response to the quadrupedal model. The behavioral measures contributing to PCA factor 3, which we referred to collectively as "surveillance," were increased time facing toward the model (the model was placed just above the speaker), increased time facing in the direction in which the model was oriented, and decreased time spent walking. This suggests that the squirrels might have been following the caller's gaze direction, and that they looked toward this animal more often at the beginning of a calling bout (presumably to gather information about a potential threat). Other non-human species are known to follow gaze direction (e.g. primates: Tomasello *et al.* 1998; domestic dogs: Miklosi *et al.* 1998; bee-eaters: Watve *et al.* 2002). Captive South African fur seals *(Arctocephalus pusillus)* could follow the direction of a human experimenter's head turn, but not gaze direction alone (Scheumann & Call 2004). It is unknown whether squirrels can follow gaze direction, or whether they pay more attention to other cues, such as body orientation, instead. It is possible that one factor contributing to the higher surveillance level elicited by the quadrupedal model was that observers were better able to determine its orientation, since its silhouette provided cues that allow observers to distinguish the head from the horizontally projecting tail. Since the bipedal model looked more nearly cylindrical and held its tail at substrate level, orientation might have been more difficult to judge. However, this study was not able to tease out the possible effects of uncertainty about orientation from the relative level of threat represented by the contrasting model postures.

Curiously, the squirrels did not behave differentially in response to the vocal stimuli (alarm call versus mallard call). Our inability to detect such differences may have been due to confounding effects that were in play prior to initiation of playback, since vigilance levels were found to be higher prior to control playback than before alarm playback. However, because the potential confounding factors we examined (time of day, trial order, and year in which trials were conducted) did not influence these results, the question of what caused these differences and the larger question of whether squirrels would otherwise have responded differentially toward the vocal stimuli remains unanswered. The alarm calls used in this study were a subset of a larger pool of recordings, some of which were used in a separate playback experiment (Rabin 2005); call exemplars used in that study were found to evoke alert behavior in ground squirrels. Therefore, it seems reasonable to assume that the alarm calls used in the present study also were evocative.

A potential confounding factor that may have had a bearing on the differentiation between models was the (unintentional) use of male and female squirrels to prepare the taxidermic mounts (for the bipedal and quadrupedal postures, respectively). However, standard taxidermic forms were used to prepare the mounts; these should have neutralized anatomical differences between the two. Therefore, it is unlikely that model sex had a bearing on the results.

In conclusion, these results demonstrate the necessity of considering the cues co-occurring with ritualized displays, as well as differences in individual characteristics that influence the assessment systems of a signal recipient and contribute to that individual's eventual response. We cannot understand the true complexity of communication systems and their impacts on individuals and their environments without a thorough examination of these crucial sources of information.

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TABLES AND FIGURES

Table 1.1. Percentage of variance explained by components resulting from PCA extraction on ten initial measures of ground squirrel response to playback experiments. Behavioral responses entered into the PCA first were averaged over the pretrial period (2 min), the initial minute of playback, and the second minute of playback. Measures for 11 squirrels were used to generate the components. PCA components with eigenvalues greater than 1.0 (factors 1 through 4) were selected for further analysis.

Total Variance Explained

	Initial Eigenvalues			Extraction Sums of Squared Loadings		
Component	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	2.858	28.583	28.583	2.858	28.583	28.583
2	1.781	17.812	46.395	1.781	17.812	46.395
3	1.416	14.160	60.555	1.416	14.160	60.555
4	1.153	11.529	72.083	1.153	11.529	72.083
5	.809	8.088	80.171			
6	.615	6.155	86.326			
7	.543	5.431	91.757			
8	.440	4.397	96.153			
9	.311	3.110	99.263			
10	7.366E-02	.737	100.000			

Extraction Method: Principal Component Analysis.

Table 1.2. Contributions of the ten initial measures of ground squirrel responses, averaged separately over the pretrial period (2 min), the initial playback minute, and the second playback minute, to the four PCA components selected for further analysis. Separate ANOVA's then were performed on these PCA factors to investigate effects during the initial minute of playback, as well as minute-to-minute changes over the course of the experiment.

Component Matrix[a]

	Component			
	1	2	3	4
Distance to Speaker	.678	-9.720E-02	-.144	-6.990E-03
Facing Toward Model	-.109	-.100	.842	-6.530E-02
Facing Direction of Model Gaze	-.287	.163	.710	.259
Bipedal	.499	.139	-3.126E-02	.684
Sitting	.487	.659	-6.621E-04	-.406
Quadrupedal	-.780	-.472	-.153	-.154
Head Up	.858	-.108	9.943E-02	3.718E-02
Feeding	-.446	.688	-.197	-.198
Walking	-.410	2.474E-02	-.326	.575
Running	.326	-.758	-5.460E-02	-.233

Extraction Method: Principal Component Analysis.

[a] 4 components extracted.

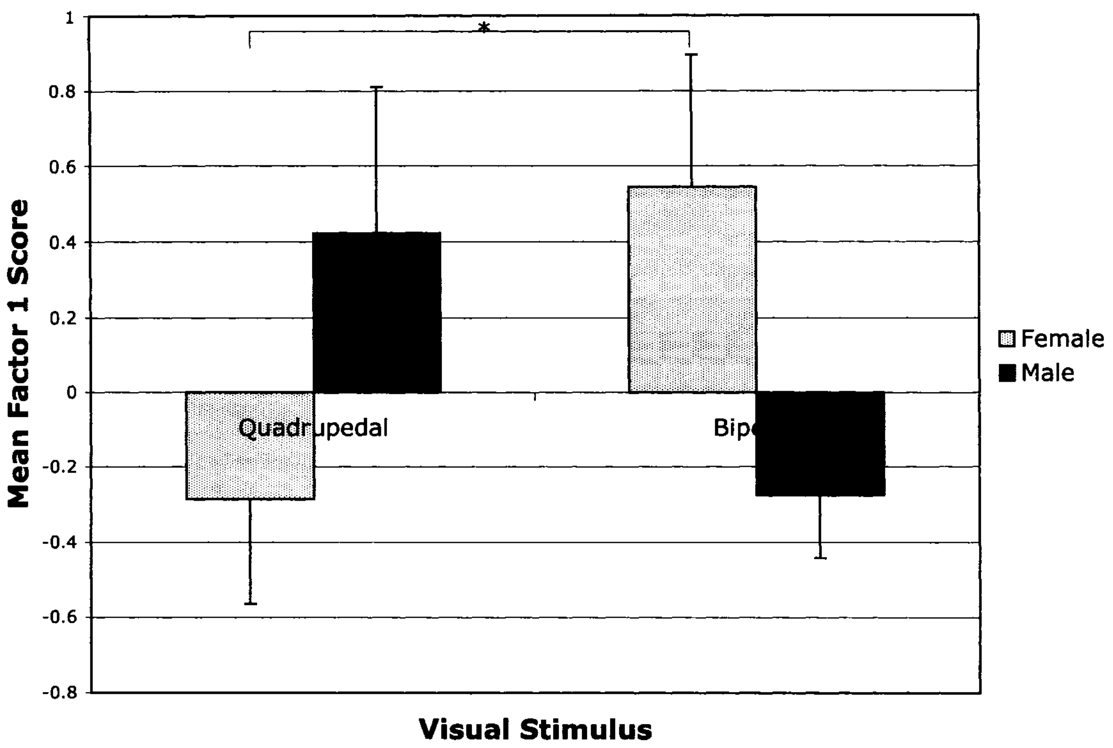

Figure 1.1. Sex differences in response to visual stimulus (model posture) for mean (±s.e.) PCA factor 1 scores averaged over the initial minute of playback (visual*sex interaction: $F_{1,9}$=5.829, p=0.039; N=11: 5 females, 6 males). Factor 1 was associated with vigilance. Females showed significantly higher responses to the bipedal than the quadrupedal model (paired samples *t* test: *t*=-2.283, df=9, p=0.048); males displayed the opposite response pattern.

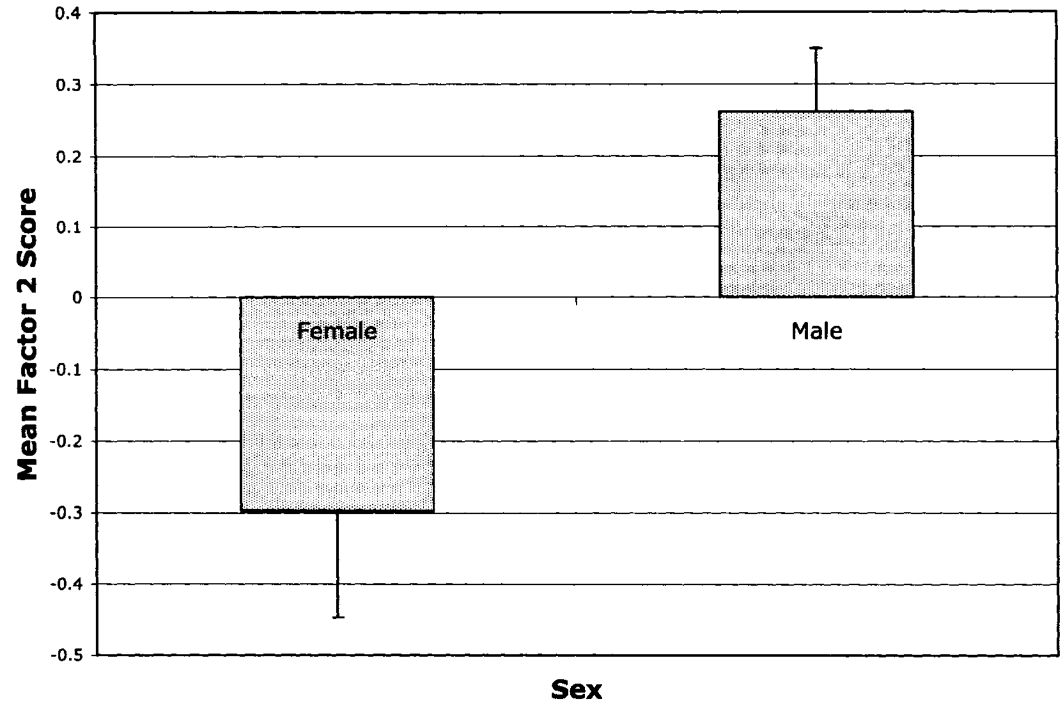

Figure 1.2. Sex differences in mean (±s.e.) PCA factor 2 scores examined from the pretrial period through the second minute of playback ($F_{1,8}$=10.010, p=0.013; N=10: 5 females, 5 males). Factor 2 was associated with relaxation of alarm. Males showed a higher level of relaxation than females, whose mean response on this measure was a negative value and therefore implies a continued state of alarm.

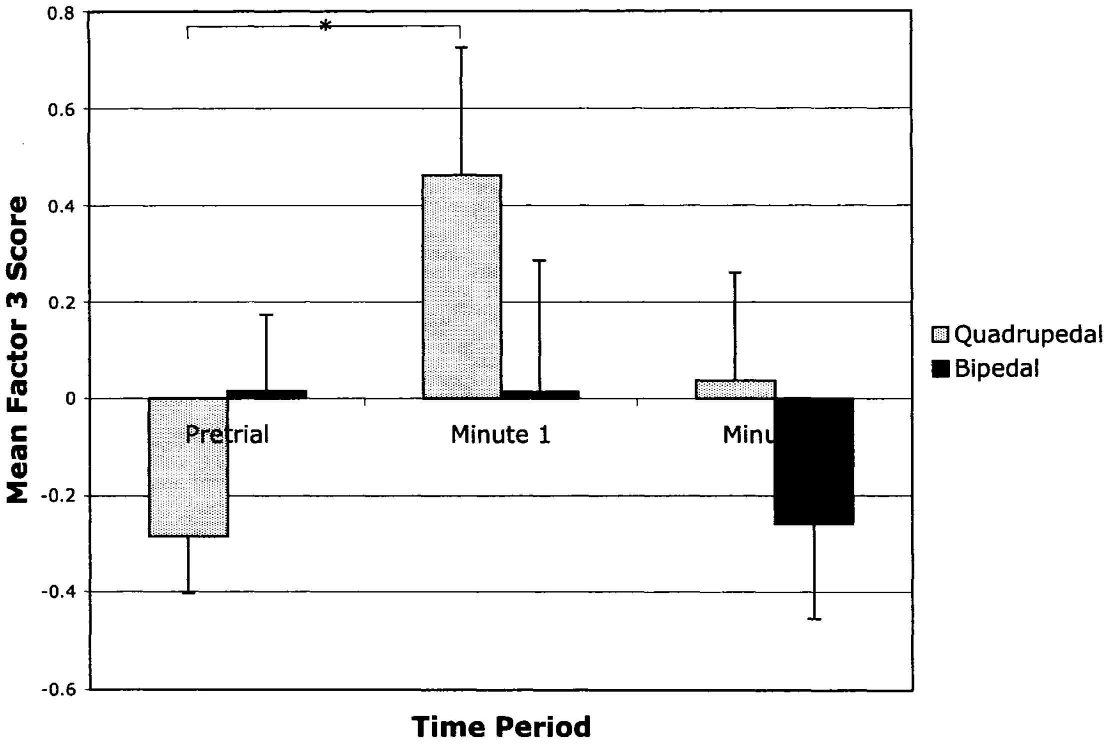

Figure 1.3. Differential changes in response to the quadrupedal versus the bipedal model over the course of the experiment in mean (±s.e.) PCA factor 3 (visual*time interaction: $F_{2,16}=4.282$, p=0.032; N=10). Factor 3 was associated with surveillance behavior. Surveillance in response to presentation of the quadrupedal model increased significantly between pretrial and minute 1 (paired samples *t* test: *t*=-2.569, df=21, p=0.018).

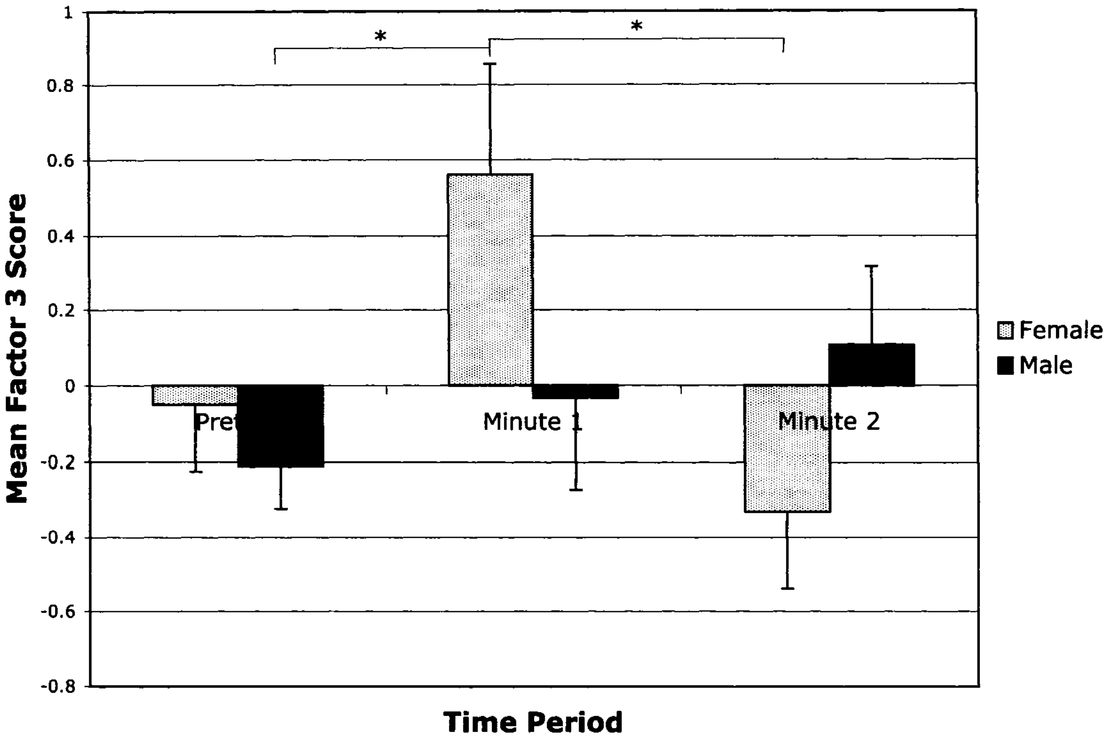

Figure 1.4. Sex differences and change over the course of the experiment in mean (±s.e.) PCA factor 3 scores (sex*time interaction: $F_{2,16}=5.468$, p=0.015; N=10: 5 females, 5 males). Factor 3 was associated with surveillance. Female surveillance during minute 1 was significantly higher than during minute 2 (paired samples *t* test, *t*=2.541, df=19, p=0.020) and also higher than male pretrial surveillance (independent samples *t* test, equal variances not assumed: *t*=2.442, df=24.699, p=0.022).

CHAPTER 2

Tonic Effects of Repetitive Alarm Calling and Caller Posture On Subsequent California Ground Squirrel Responses to an Alarm Call

ABSTRACT

This study examined whether repetitive anti-mammalian-predator calls and associated postural cues exert a lingering, or "tonic," effect on California ground squirrel behavior. The tonic communication hypothesis predicts that vigilance will increase or be maintained in response to repetitive signal inputs. The evidence of tonic effects has been limited in earlier research, but these previous studies were not designed to detect either latent effects of repetitive signaling or the impact of contextual cues that typically accompany such calling and may enhance the effects of calling. Therefore, we sought such tonic effects by using a "probe" playback (a brief, non-repetitive chatter alarm call) in the aftermath of playbacks involving both visual and vocal stimuli. Squirrels were initially exposed to a taxidermic mount of a bipedal or quadrupedal squirrel that "produced" a series of either repetitive alarm calls or control sounds (mallard quacking). Following a delay after completion of these initial stimulus presentations, squirrels then were exposed to the probe playback, which was the same for all initial playback conditions. Surprisingly, although vocal stimuli had elicited no immediate differential effect on squirrel behavior, they exerted a delayed influence that was revealed upon probe presentation. Females initiated movement more often during the initial post-probe minute if they previously had heard the control than the alarm call. This finding implies suppressive effects of repetitive alarm calling on subsequent mobility. However, unlike females, who differentiated more strongly on the basis of previously heard vocal stimuli, males demonstrated more discrimination on the basis of prior visual stimuli. Males showed less relaxation of the alarm response during the second post-probe minute when

they previously had seen the quadrupedal than the bipedal model. Squirrels demonstrated vigilance following the probe stimulus, but did not show differential responses on the basis of previously experienced vocal or visual stimuli. No statistical interaction effects between prior visual and vocal stimuli were observed for any of the behavioral measures. The squirrels' tendency to sit upright appeared to be differentially affected by prior visual stimuli, although delay times between initial playback and probe and the number of neighboring squirrels influenced this measure.

INTRODUCTION

Schleidt (1973) defined tonic communication as "discrete signs...repeated by the transmitting animal in more or less regular intervals, and in that way have a continual effect on the receiving animal, reflected in a gradually changing or steadily maintained behavioral output." Such repetitive signals might not produce recognizable changes in perceiver behavior with each input, but may have a substantial influence on temporally extended behavioral states.

Although tonic processes have received relatively little attention, researchers studying anti-predator behavior in ground squirrels have made inroads into understanding the significance of behavioral patterning in tonic time frames. For example, ambush-hunting rattlesnakes pose a more tonic local threat than gopher snakes, which hunt by ranging more widely; correspondingly, rock squirrels (*Spermophilus variegatus*) exhibit more persistent caution and anti-snake behavior after encounters with rattlesnakes than gopher snakes (Hanson 2003). California ground squirrels (*S. beecheyi*) are also sensitive to the temporally extended threat posed by rattlesnakes; during the season that rattlesnakes hunt in their colony, these squirrels move into a sustained state of anti-rattlesnake vigilance, tonically repeating the snake-specific signal of tail flagging (Hersek & Owings 1993). In response to mammalian predators, California ground squirrels call repetitively, beginning with a multi-note "chatter" and transitioning to a variable number of single-note "chats" that can continue for tens of minutes (Owings *et al.* 1977, Owings & Virginia 1978). Owings *et al.* (1986) proposed that such repetitive calling reflects tonic communication, with a cumulative effect over an extended time scale that serves to increase or maintain vigilance in perceivers. Tests of this hypothesis have been conducted in various rodent species: *S. beecheyi* (Loughry & McDonough, 1988); Richardson's ground squirrel, *S. richardsonii* (Sloan & Hare, 2004); and Brants' whistling rat, *Parotomys brantsii* (LeRoux *et al.*, 2001); these studies have reported limited support for the tonic hypothesis.

However, the aforementioned studies did not explicitly test the possibility that some effects of tonic signals may be latent. Tonic processes involve persistence of a state even in the absence of immediate external stimulation, and this persistent state may not be reflected in outwardly visible behavior. Because of this, a quiescent state may exist that is not detectable simply through observation; a more proactive approach is necessary. The present study was designed to extend our understanding of the role of tonic processes in mammalian anti-predator communication by using a method that may be especially sensitive to the latent effects of tonic processes. We presented a "probe" stimulus (a single, non-repetitive alarm chatter) following an initial playback to help reveal lingering effects of repetitive alarm calling.

Moreover, because vocal signaling naturally occurs in association with visual stimuli (e.g. different caller postures and orientations), vocal playback alone might be less likely to induce tonic effects than a combination of visual and audio stimuli. Our playbacks paired visual cues (squirrel models in different postures) with repetitive call playback; this potentially increased the sensitivity of our behavioral assay. This design also allowed us to look for possible interactive effects of visual and auditory stimuli on tonic responses. Partan and Marler (1999) proposed that multimodal signals with nonredundant components could affect behavioral outcomes in the following ways: one component could modulate the effect of the other; one component could dominate or mask the effects of the other; the components could have completely independent effects on behavior; or the components could interact to produce a completely new response. Our playback design allowed us to test whether any of these mechanisms exert their effects over a tonic time frame.

METHODS

Subjects

In the spring and summer of 2003 and 2004, California ground squirrels living along the Alameda Watershed at the Frog Pond (Alameda County, CA) were captured with Tomahawk live traps, sedated with intramuscular injection of Ketamine HCl (40 mg/kg), weighed, sexed, inspected for reproductive condition and age class (juvenile or adult), ear tagged (National Band and Tag Company, Newport, KY), and dye-marked with individually distinctive numbers using Nyanzol-D dye (The ADI Group USA, Jersey City, NJ). All experiments were conducted from May 24th through August 3rd 2003 and May 15th through August 1st 2004 on these free-ranging individuals. The study site was located in an area of oak savanna, and potential predators included coyotes, bobcats, rattlesnakes, golden eagles, and red-tailed hawks.

Probe Experiments

Probe trials consisted of the following phases: initial playback of repetitive vocal signals ("chatter-chat" alarm calls or a control sound) paired with visual stimuli (3 min), followed by a delay of at least 2 min in which no visual or vocal stimuli were presented, followed by the probe test, which consisted of playback of a very brief, non-repetitive alarm call (chatter call only); squirrel responses were filmed for 2 min following the probe.

Playback Stimuli

Two vocal and two visual stimuli were utilized in the initial playback period preceding the probe test. The visual stimuli used during playbacks were taxidermic mounts of California ground squirrels positioned in either a bipedal or quadrupedal stance (quadrupedal vigilance is more common in California ground squirrels during higher-urgency situations; Leger *et al.* 1979). The bipedal mount was prepared from a 655 g adult male and the quadrupedal mount was of a 565 g adult female; both were captured in the Arboretum at the University of California, Davis. The vocal stimuli consisted of California ground squirrel alarm calls and control sounds; mallard *(Anas platyrhynchos)* "hail," or "quack," calls were chosen as the control stimuli because these birds were common at the study site.

The alarm calls, which consisted of initial chatter calls followed by repetitive chat calls, were recorded by L.A. Rabin from a nearby population (Los Vaqueros Watershed in the Altamont Pass Wind Resource Area, Alameda County, CA) in response to domestic dog presentations. Three exemplars of alarm call series were used: two from adult females and one from an adult male. Three exemplars of mallard hail calls (Cornell Laboratory of Ornithology/Interactive Audio 1992; and Files 2286 and 5493, Borror Laboratory of Bioacoustics, Department of Evolution, Ecology, and Organismal Biology, Ohio State University, Columbus, OH, all rights reserved) were used as control sounds. A speaker (RCA PRO-LX550) was positioned under the rostral end of the model, and vocal stimuli were played on a Sony Discman ESP^2, amplified by a Kenwood KAC-529S car amplifier, and broadcast from the speaker. Vocal stimuli were standardized to a mean volume of 93.76 dB at 1 m from the speaker (flat frequency response setting) and a mean total sound duration of 9.5 s, over a total playback duration of 3 min. Additional details about the recordings are reported elsewhere (Miyasato *et al.*, in prep).

Initial Playback Protocol

The details of the initial playback experiment preceding the probe trials have been presented in greater depth elsewhere (Miyasato *et al.*, in prep) but will be summarized briefly here. Free-ranging, marked squirrels were drawn into filming range by scattering black oiled sunflower seeds throughout the testing area. Each subject was presented, on different occasions, with four different treatments in a 2 x 2 factorial design: a vocal stimulus (alarm call or control call) paired with a visual stimulus (quadrupedal or bipedal squirrel model). These paired stimuli were presented to subjects for 3 min, after which the audio track was turned off and the squirrel model was hidden from view. Only one alarm call and one control sound exemplar were played to a given subject, to control for the two vocal conditions between the two model postures. The order of presentation of the four treatments was varied among subjects to counterbalance for order effects, and the playback apparatus was placed in several different locations adjacent to the pond. The squirrel mount was positioned in different compass directions, to minimize the effects of preferential directional orientation by the squirrels. Experiments were conducted between 8:00 am and 3:00 pm (median start time 10:00 am).

Probe Trial Protocol

The probe trial consisted of the broadcast of a single chatter vocalization (the initial multi-note component of a repetitive chatter-chat call series); this probe stimulus was identical to the first call of the repetitive call series broadcast to subjects during initial playbacks. To distinguish between the initial phase of repetitive call playback and the subsequent brief probe playback, the two types of vocal stimuli will be referred to as a "string" or "probe", respectively. Three chatter exemplars were used for probes, corresponding to the three audio strings used for initial playbacks. Whereas the string stimuli (either the chatter-chat call or control mallard call) were presented over a period of 3 min, the probe stimulus lasted no more than 1.3 s. Also unlike the initial playback period, the visual stimuli (quadrupedal and bipedal squirrel mounts) were not presented during the probe trial. Thus, any differentiation between visual or vocal stimuli during the probe trial was due solely to lingering effects from the preceding period.

In 2003, the probe trials were started following a 2-5 min delay after the ending of the playback experiment, when focal animals were still in view. However, because a large proportion of the animals escaped down burrows or left the area during or immediately after playbacks, this condition was modified so that probe trials could be started at time delays greater than 5 min (i.e., as soon as focal animals emerged from burrows or returned to the testing area; mean ± se: 9.27 ± 1.28 min; range: 2 – 31 min). Thus, when the focal animal was in view of the cameras and within a radius of approximately 20 m from the speaker, and at least 2 min had elapsed since completion of the initial playback, the probe trial was started.

During probe trials, ground squirrels were videotaped by observers sitting within a sunshade "blind" or beneath a large canopy located approximately 15 m from the model and speaker. Focal animals were filmed (using a Panasonic 5100 HS video camera attached to a JVC BR-S405U HiFi SVHS VCR, a Panasonic PV-DV601 digital video camera, a Canon ZR40 digital video camera, and a Sony Handicam Vision CCDTRV58NTSC Hi-8 camera) for 2 min following the probe stimulus, and the number of other squirrels within a 3-m radius of the focal squirrel was narrated at 30-s intervals.

Videotape Analysis

VHS recordings were played back on a Panasonic Video Cassette Player AG-7100 connected to a Panasonic AG-A750 Editing Controller and viewed for analysis on a Panasonic CT-1331Y video monitor. Hi-8 video recordings were burned to a DVD and viewed and analyzed on a Macintosh computer using DVD Player v 3.2. All other trials were imported to a Macintosh computer using iMovie 3.0.3, and behaviors were quantified by viewing trials using QuickTime Player. Probe trials were instantaneously sampled at 6-s intervals for the following measures: posture (bipedal, sitting upright, quadrupedal), head elevated above the line of the back or not, feeding, and locomotion (walking or running).

Statistical Analysis

Because most of the subjects from 2003 did not complete all four probe treatments due to the 5-min window in which they had to be present following the playback trial, analysis was conducted instead on a subset of two trials per animal. Six animals had completed three of the four experimental treatments, and another six had received all four treatments. The animals that had received three treatments were compared on the basis of what vocal strings they had heard. Since they had completed either two alarm or two control treatments, they were accordingly grouped either into "alarm" or "control" categories for analysis. The remaining six animals then were randomly assigned to alarm or control categories until there were 6 animals in either category (maintaining an equivalent number of males and females between categories). This held the vocal string stimulus constant for a given animal and allowed within-subjects comparisons of the effects of visual stimuli.

All statistical analyses were performed using SPSS Graduate Pack 11.0 for Mac OS X. Principal components analysis (PCA) was performed on raw data collected for the two minutes following probe playback for the seven behavioral measures obtained from videotape analysis. Components with eigenvalues greater than 1.0 then were subjected to analysis by general linear model, using a mixed repeated measures/between-subjects analysis of variance (ANOVA). Visual stimulus (and minutes elapsed, for the analysis of minutes 1-2) was treated as a within-subjects factor, and vocal string and sex were treated as between-subjects factors. ANOVA's were conducted on mean PCA factor scores for three time frames: the average for the initial minute following the probe stimulus, the average of the second minute, and average values for minutes 1 and 2 examined together. To determine whether the assumptions of equal variance and independence of the residual errors were met, Mauchly's Test of Sphericity was examined for each ANOVA performed. Since the assumptions were met for all tests, only the “Sphericity Assumed” results are reported. For significant ANOVA interactions, independent samples *t* tests were run to determine whether individual comparisons were significant. Although some comparisons (i.e., those involving interactions with visual stimuli) might more appropriately have been tested using paired samples *t* tests, SPSS recommends using independent samples *t* tests when paired samples correlation coefficients are low and their corresponding significance values are high. Since this was almost always the case, independent *t* tests were run for all samples.

To determine whether the potential confounding factors of time of day, delay between end of playback and beginning of probe trial, and number of squirrels within 3 m of the focal animal affected the PCA factor scores, separate ANCOVA’s were conducted on these variables. Because SPSS would not test for the effects of covariates corresponding to each level of within-subjects factors, covariates instead were tested by treating visual stimulus (and minutes elapsed, for combined minutes 1-2) as a between-subjects factor; vocal string and sex also were (appropriately) tested as between-subjects factors. To make the results of these analyses more comparable to the mixed repeated measures/between-subjects ANOVA’s described above, results for time of day and delay between playback and probe are reported both as they were computed, and also with degrees of freedom adjusted to treat visual stimulus and minutes elapsed as within-subjects factors. Due to the fact that the data set for number of neighboring squirrels was incomplete (only three of the twelve squirrels had neighbor data for all time points, and data were unavailable for three animals), this analysis is presented only to give a rough estimation of effects of group size. Moreover, the "group" designation is somewhat artificial, as squirrels were induced to enter the testing area by the introduction of food bait. Due to the low sample size, corrected degrees of freedom are not presented for the analysis of neighboring animals.

RESULTS

Overview of Results

Despite the fact that vocal stimuli had elicited no immediate differential effect on behavior, they exerted a delayed influence that was revealed upon probe presentation: females initiated movement more often during the initial post-probe minute if they previously had heard the control than the alarm string. This finding implied inhibitive effects of repetitive alarm calling on subsequent mobility. Sex differences played a substantial role in ground squirrel responses to probe stimuli: whereas females differentiated more strongly on the basis of previously heard vocal strings, males demonstrated more discrimination on the basis of prior visual stimuli. Males exhibited less relaxation of alarm during the second post-probe minute when they previously had seen the quadrupedal than the bipedal model. Both female discrimination between prior vocal strings and male differentiation between prior visual stimuli also were observed across the entire two minutes following probe playback, although these effects were less pronounced than the aforementioned ones. Squirrels' tendency to sit upright also appeared to be affected by prior visual stimuli, although the confounding factors of differing delay times between vocal string and probe playback and variation in number of neighboring squirrels may have influenced this result.

Principal Components Analysis

Principal components analysis (PCA) performed on probe data for seven initial behavioral measures extracted four components with eigenvalues greater than 1.0 (Table

2.1). These four components account for 86.5% of total variance, which exceeds the 70% guideline recommended by Stevens (2002). Because the Varimax rotation produced a better separation of the behaviors into individual components than the unrotated solution, the rotated values (referred to below as "factors") were chosen for further analysis. The loadings of the seven initial behaviors onto these factors are shown in Table 2.2. As suggested by Tabachnick (1983), loadings with values of 0.30 or greater were considered to contribute substantially to the factors, and hence are discussed below. PCA factor values were examined under three different time frames: averaged over the first minute following the probe, to determine initial responses; averaged over the second minute after the probe, to reveal more delayed effects; and the average values for minutes 1 and 2 examined together, to ascertain whether responses differed between the two minutes. ANOVA's performed on these factors using visual cue (and minute following the probe, for the minute 1-2 analysis) as within-subjects factors and sex and vocal string as a between-subjects factors revealed strong associations between experimental treatments and 3 of the 4 factors.

PCA factor 1, which had an eigenvalue of 2.22 and explained 31.72% of total variance, was associated with the following behaviors (listed in decreasing order of loading weight; "increased" or "decreased" indicate whether the sign of factor loading was positive or negative, respectively): increased time spent in the bipedal posture, increased time with head elevated, decreased time in the quadrupedal stance, and decreased walking. These behaviors are consistent with alertness or vigilance. However, there were no significant effects of prior visual stimulus or vocal string on factor 1 scores, nor were there any changes between minutes 1 and 2 following the probe stimulus, nor did the sexes differ in their responses. Therefore, only the remaining three PCA factors, for which significant treatment effects were detected, will be discussed below.

Initial (First Minute) Responses to Probe Playbacks

Factor 2: Movement

Factor 2, with an eigenvalue of 1.43 (explaining 20.39% of total variance), was associated with increased running, increased walking, and increased time spent in the quadrupedal posture (Table 2.2). These behaviors indicate initiation of movement, and will be referred to collectively as "movement."

Females' patterns of movement during the first minute after the probe were strongly affected by the vocal string they had heard in the preceding period; conversely, males showed little differentiation on the basis of vocal strings. Hence, a strong interaction was observed between sex and the lingering effect of repetitive audio stimuli (Figure 2.1; $F_{1,8}=15.816$, $p=0.004$): females showed a much greater degree of movement if they had heard the control sound than if they had heard the chatter-chat alarm ($t_{10}=-2.290$, $p=0.045$), whereas males exhibited a trend toward less movement after hearing the control than the alarm. Females' movement in the aftermath of the control sound was significantly higher than males' ($t_{10}=2.749$, $p=0.021$). Moreover, the overall level of movement differed significantly between the sexes: females initiated movement more often than males (Figure 2.2; $F_{1,8}=14.330$, $p=0.005$). Furthermore, there was a significant effect of vocal string alone, with less movement in the aftermath of the alarm than the control string (Figure 2.3; $F_{1,8}$=6.502, p=0.034). Both the sex difference and the audio effect appear to have been driven by females' strong tendency to initiate movement after being subjected to the control string.

In contrast to the strong effects that the vocal string had on movement, visual stimuli neither influenced movement ($F_{1,8}$=0.204, p=0.663) nor interacted with subjects' sex or vocal stimulus ($F_{1,8}$=0.141, p=0.717; $F_{1,8}$=0.153, p=0.706, respectively). The time of day at which experiments were run did not affect the outcome ($F_{1,15}$=0.451, p=0.512; adjusted df: $F_{1,7}$=0.451, p=0.523), nor did time delay between the preceding playback period and start of the probe trial ($F_{1,15}$=0.010, p=0.922; adjusted df: $F_{1,7}$=0.010, p=0.923). Likewise, the number of satellite squirrels within 3 m of the focal animals did not affect the results ($F_{1,6}$=0.190, p=0.678).

Second-Minute Responses to Probe Playback

Factor 3: Relaxation of Alarm

The third factor had an eigenvalue of 1.21 and explained 17.29% of the total variance. Factor 3 was strongly associated with increased feeding and with increased time in the quadrupedal stance. Since squirrels typically ceased feeding when they were alarmed, and alert animals often fed while sitting on their haunches or positioned bipedally, feeding while quadrupedal implied a more relaxed state; thus, these behaviors will be referred to as "relaxation of alarm" or simply "relaxation."

Visual cues presented during the preceding period strongly interacted with subjects' sex in affecting the degree of relaxation: males showed less relaxation when they had seen the quadrupedal model than the bipedal model; females differentiated to a much lesser degree, with slightly higher relaxation after having seen the quadrupedal model (Figure 2.4; $F_{1,6}=10.784$, $p=0.017$). For males who had seen the quadrupedal model, the mean value for factor 3 was actually negative. If the "signs" of the behavioral measures contributing to factor 3 are reversed, the contributing measures become decreased feeding and decreased time quadrupedal. Decreased time spent quadrupedal implies an increased amount of time spent in more upright postures; combined with a decrease in feeding, these measures suggest increased alertness. Thus, males appeared to be more alert if they previously had been exposed to the quadrupedal model. On the other hand, vocal string did not affect relaxation, either as a simple effect or in interactions (audio: $F_{1,6}=0.003$, $p=0.961$; audio*visual: $F_{1,6}=1.338$, $p=0.291$; audio*sex: $F_{1,6}=1.608$, $p=0.252$).

Neither time of day nor delay between playback and probe trial affected these results (time of day: $F_{1,11}=0.003$, $p=0.957$; adjusted df: $F_{1,5}=0.003$, $p=0.958$; delay: $F_{1,11}=0.529$, $p=0.482$; adjusted df: $F_{1,5}=0.529$, $p=0.500$), nor did the number of squirrels within 3 m of the focal animal ($F_{1,4}=1.272$, $p=0.322$).

Combined Minutes 1 and 2

Factor 2: Movement

As discussed for minute 1, factor 2 was associated with movement. Similarly to minute 1, results examined across minutes 1 and 2 revealed interacting effects of vocal string and sex on movement: females initiated movement much more often when they previously had heard the control than the alarm string, whereas males showed little difference in mobility with respect to vocal string (Figure 2.5; $F_{1,6}=7.378$, $p=0.035$). Female movement in the aftermath of control calls was significantly higher than in males (Figure 2.5; $t_{5.995}=2.615$, $p=0.040$). The level of female differentiation between vocal strings for minutes 1 and 2 was less than that observed during the initial minute alone, implying decay in the evocativeness of the audio treatment over time. Nevertheless, a direct comparison of factor 2 scores between minutes 1 and 2 did not yield significant results ($F_{1,6}=2.945$, $p=0.137$), nor did examination of possible interactions between minutes elapsed and visual stimulus or minutes elapsed and vocal string ($F_{1,6}=0.043$, $p=0.842$; $F_{1,6}=0.033$, $p=0.863$, respectively). Similarly to results for the initial minute, visual stimuli presented during the preceding period did not affect movement (visual: $F_{1,6}=0.486$; $p=0.512$; visual*audio: $F_{1,6}=0.467$, $p=0.520$; visual*sex: $F_{1,6}=0.012$, $p=0.915$).

There were no effects of time of day ($F_{1,27}=0.149$, $p=0.702$; adjusted df: $F_{1,5}=0.196$, $p=0.715$) or delay between playback and probe ($F_{1,27}=1.113$, $p=0.301$; adjusted df: $F_{1,5}=1.113$, $p=0.340$) on movement levels. The number of satellite squirrels within 3 m did not affect mean factor 2 scores ($F_{1,11}=1.484$, $p=0.249$).

Factor 3: Relaxation of Alarm

Factor 3, which corresponded to relaxation of alarm and which was affected by the interaction between visual cues and sex for minute 2, showed similar interaction effects for minutes 1 and 2 examined together: males demonstrated substantially less relaxation (or higher alertness) in the aftermath of exposure to the quadrupedal model than the bipedal model; conversely, females showed higher levels of relaxation following presentation of the quadrupedal model than the bipedal model, although this discrimination was less pronounced than in males (Figure 2.6; $F_{1,6}$=8.300, p=0.028). Although these effects followed similar patterns to what was observed for minute 2, the effects were somewhat weaker when minutes 1 and 2 were combined, thus suggesting that visual effects had a delayed effect on relaxation of alarm. However, there was not a significant difference in factor 3 scores between minutes 1 and 2 ($F_{1,6}$=0.507, p=0.503); likewise, there were no significant interactions between minutes elapsed and visual stimulus or minutes elapsed and vocal string ($F_{1,6}$=0.240, p=0.642; $F_{1,6}$=0.349, p=0.576, respectively). Vocal strings had no effect on relaxation (audio: $F_{1,6}$=0.150, p=0.712; audio*visual: $F_{1,6}$=1.780, p=0.231; audio*sex: $F_{1,6}$=0.099, p=0.764).

Time of day had no effect on these results ($F_{1,27}$=0.640, p=0.431; adjusted df: $F_{1,5}$=0.640, p=0.460), nor did delay between the playback and probe periods ($F_{1,27}$=0.305, p=0.585; adjusted df: $F_{1,5}$=0.305, p=0.605) or the number of squirrels within 3 m of the focal animal ($F_{1,11}$=0.256, p=0.623).

Factor 4: Sitting

Factor 4 had one behavior with loading greater than 0.30: increased time sitting upright (loading=0.965). Although the fact that only one behavior contributed substantially to this factor implies that it may not have been suitable for factor analysis, ANOVA's conducted on this factor are presented here mainly to illustrate patterns that are consistent with results obtained for factor 3. Visual stimuli interacted with sex differences to cause a significant lingering effect on sitting: males sat upright more frequently when they previously had been presented with the quadrupedal model than the bipedal model; females showed a weaker response, sitting upright more often following exposure to the bipedal versus the quadrupedal model (Figure 2.7; $F_{1,6}$=12.339, p=0.013). As with factor 3 (corresponding to relaxation of alarm), males were the sex that tended toward stronger differentiation on the basis of prior visual stimuli. Vocal strings did not significantly influence sitting (audio: $F_{1,6}$=0.364, p=0.568; audio*visual: $F_{1,6}$=0.338, p=0.582; audio*sex: $F_{1,6}$=0.096, p=0.767). The comparison of factor 4 scores between minutes 1 and 2 post-probe did not reveal significant results (minute: $F_{1,6}$=0.371, p=0.565; minute*visual stimulus: $F_{1,6}$=0.056, p=0.821; minute*audio stimulus: $F_{1,6}$=0.403, p=0.549).

There was no effect of time of day on factor 4 scores ($F_{1,27}$=0.984, p=0.330; adjusted df: $F_{1,5}$=0.984, p=0.367). However, the duration of delay between playback and probe trials influenced the results, with the highest factor 4 values occurring primarily for delays of 5 min or shorter (Figure 2.8; $F_{1,27}$=5.424, p=0.028; adjusted df: $F_{1,5}$=5.424, p=0.067). When factor 4 was examined with delay as the sole covariate (treating visual stimulus, audio stimulus, sex, and minutes elapsed as between-subjects factors), the interaction between visual stimulus and sex vanished ($F_{1,27}$=0.001, p=0.982; adjusted df: $F_{1,5}$=0.001, p=0.976). Moreover, the number of neighboring animals within 3 m of the focal squirrel affected mean factor 4 scores (Figure 2.9; $F_{1,11}$=6.267, p=0.029); inclusion of this covariate likewise diminished the visual stimulus-by-sex interaction ($F_{1,11}=3.256$, $p=0.099$), although it should be noted that data on neighboring animals were not available for all subjects. Nevertheless, when both the number of neighboring squirrels and delay between playback and probe were tested as covariates simultaneously, the interaction between visual stimulus and sex “regained” significance ($F_{1,10}=8.049$, $p=0.018$).

Figure 2.10 illustrates the relationship between time delay and number of neighboring squirrels. Two patterns seem to emerge: for the longest delay times (more than 10 min), the number of neighboring squirrels tended to be low (0-2); on the other hand, for short delay times (2-5 min), there was a lot of variation in the number of neighbors present (0-8). With respect to subjects’ sex, most female delay times were 5 min or shorter and seemed to show little association with number of neighbors; conversely, males tended to have longer delay times associated with fewer neighbors.

DISCUSSION

Our results demonstrated that past exposure to vocal and visual stimuli affect the manner in which California ground squirrels respond to subsequent conspecific alarm calls, even after a substantial delay. We also verified that our probe methodology was sensitive enough to detect previously latent behavior. Our most startling discovery was that vocal stimuli exerted a differential effect on female mobility in a tonic time frame, despite the lack of any behavioral differentiation during initial vocal string playback. This effect was not observed in males. On the other hand, males displayed more discrimination on the basis of prior visual stimuli: they relaxed their state of alarm less when they previously had seen the quadrupedal model than the bipedal model, whereas females showed little differentiation. This relaxation effect was most pronounced in the second minute following the probe.

We did not observe any interactions between previously presented visual stimuli and vocal strings, suggesting that for the aforementioned behaviors, different sensory inputs appeared to have separate, discrete effects on the perceivers. This is consistent with Partan and Marler's (1999) category of "independence" of nonredundant components of a multimodal signal. One unusual finding of our study was that although ritualized signals typically are considered to be the primary drivers of perceivers' behavior, the "signal" portion of our playback (the vocal strings) seemed to have little effect on male responses. Our findings emphasize the importance of considering stimuli impinging on different sensory modalities, as well as differences in perceiver characteristics, in explaining the resulting behavioral responses.

During the initial playback portion of this experiment, we observed differential effects of visual stimuli on vigilance and visual surveillance behavior; however, no effects of vocal stimuli were detectable during the initial playback period (Miyasato *et al.,* in prep). The present findings suggest that the reason for lack of an initial vocal effect may be that the vocal strings exerted their influence primarily over a more extended time scale. Owings *et al.* (1986), studying California ground squirrel vocalizations, noted that in contrast to what they observed for non-repetitive alarm calls, repetitive calls were produced fairly late in predator encounters, did not vary seasonally with the emergence of pups, and did not always evoke immediate responses from perceivers. Hence, these authors suggested that repetitive calling might have a cumulative effect over a longer time scale; this could serve to increase or maintain vigilance in other conspecifics. They used the term "tonic communication" (after Schleidt, 1973) to refer to this phenomenon.

Other ground squirrel studies have compared shorter versus longer, repetitive calls and have tried to determine whether repeated calls produce tonic effects; however, results thus far have been inconclusive. Loughry and McDonough (1988), also studying California ground squirrels, found total (bipedal and quadrupedal) vigilance higher in the first 5 min of repetitive calling than in 5 min following non-repetitive calling and cited this as support for the tonic communication hypothesis, but also reported that bipedal vigilance dropped off during the second half of repetitive calling bouts, suggesting that perceivers were "losing interest," which the authors stated would not be expected under the predictions of the tonic hypothesis. Harris *et al.* (1983) conducted playbacks of "hollow chirps," either as a single chirp or repeated over the course of one minute, to Columbian ground squirrels. They found that perceivers spent more time alert in response to repeated calls than to a single call, consistent with tonic communication predictions. Sloan and Hare (2004), testing the effects of repetitive calls on Richardson's ground squirrels, presented short (6-syllable) or long (12-syllable) "monotonous" calls (i.e., call elements repeated at a constant rate) versus short or long calls with more variable repetition rates. These researchers found that longer calling bouts did not lead to higher vigilance in the three minutes following playback, but that monotonous calls did; thus, they concluded that their findings contradicted the tonic communication hypothesis, and instead supported the hypothesis that the degree of monotony of repetitive calls conveys the level of certainty about predatory threat.

The present study suggests a possible reason for the previously mixed support for the tonic communication hypothesis in ground squirrel repetitive calling. Previous studies have, for the most part, measured vigilance (primarily as evidenced by upright postures) in response to repetitive calls and have noted that such behavior decays over time, regardless of call duration. Such findings have been construed as a "failure" of the tonic communication hypothesis. However, these studies have not recognized that signals may be eliciting latent tonic effects even when 1) animals do not outwardly display a heightened state of alertness and 2) alertness does not persist for an exceptionally long time. Regarding the former point, the current study demonstrates that the use of a probe stimulus can reveal details of an individual's internal state of elevated alertness that otherwise might not be detectable. For example, our results indicate that female squirrels that earlier had experienced repetitive alarm calling were less likely to initiate movement than if they had heard a control call. Leger and Owings (1978) similarly found that California ground squirrels decreased walking in the first minute following playback of chatter-chat calls. Walking then increased over the subsequent two post-playback minutes. The present study suggests that repetitive calling had more prolonged suppressive effects on movement that were revealed only through the use of a probe.

Previous studies also have assumed that tonic signals must elicit unusually prolonged effects on perceivers' behavior. This view implies that the signaler is freely able to manipulate the behavior of those hearing the signal. However, as argued by Dawkins and Krebs (1978), the signal perceiver generally will be resistant to such manipulation. For example, an individual hearing a repetitive alarm call would be unlikely to find it in his or her best interest to maintain an elevated level of vigilance over an extended period of time. Therefore, a tradeoff is likely: the signaler may succeed in evoking a brief heightened state of alarm before the perceiver's self interest favors a decline in this behavioral state. This tradeoff may explain the relationship between delay times and sitting upright (factor 4) in our study: the highest factor 4 scores tended to occur for delays of 5 min or shorter. Signalers may benefit from an increased number of vigilant animals sitting upright and scanning for predators, but signal perceivers, after determining that there is no immediate threat, might benefit more by returning to foraging or other maintenance behaviors. Thus, a continuous process of behavioral management (by the signaler) and assessment (by signal perceivers) would be expected to occur (Owings & Morton, 1998), and therefore an unusually extended period of vigilance would be unlikely.

Another way in which the current experiment differs from the aforementioned studies is that it looked not only at the lingering effects of repetitive alarm calls, but also of visual stimuli presented simultaneously with the vocal strings. In the second minute of the present study, differentiation on the basis of prior visual stimulus was evidenced in animals' relaxation of alarm: males relaxed (i.e., began feeding and tended to adopt the quadrupedal posture) much less after seeing the quadrupedal model than the bipedal model. The audio playback study of Leger and Owings (1978) observed squirrel responses in the 3 min following chatter-chat playback, and noted that although feeding initially decreased, it eventually increased again, and animals dropped down from initial upright postures. This implies that in the present study, males took a longer time to reach a relaxed state following the probe test if they previously had been exposed to the quadrupedal model.

Although it may seem counterintuitive that males would act with more caution in response to a quadrupedal versus a bipedal "caller," Leger *et al.* (1979) played back whistle calls (generally produced in response to raptors) to California ground squirrels and observed that quadrupedal freezing increased with the number of whistles broadcast, whereas upright postures became less likely. The authors speculated that this was due to a tradeoff between "safety" and "information-obtaining" behavior: although upright postures facilitated surveillance of the surroundings, they also made individuals more conspicuous to predators; conversely, standing quadrupedally made an animal less conspicuous but less able to acquire information from its environment. If in fact the quadrupedal posture is a response to more acute threats, males in the present study who saw the quadrupedal model may have shown less relaxation in accordance with a perceived higher level of threat. This does not explain why females did not show a similar response. However, Leger and Owings' (1978) chatter-chat playback demonstrated that females with litters showed more upright postures and a trend toward greater decreases in feeding. The playbacks for the current study were conducted later in the season, when pups were less vulnerable. Thus, the females might have shown more relaxation overall than they would have earlier in the year.

The present study initially sought to ascertain treatment effects only after a delay of two to five minutes; however, subjects' tendency to disappear from view following the initial playback precluded this possibility. Hence, we tested squirrels over a wide range of delay times (mean ± s.e.: 9.27 ± 1.28 min; range: 2-31 min). Serendipitously, this showed that lingering vocal effects on movement and differential visual effects on relaxation of alarm were present across a wide range of delay times (as there were no significant effects of delay time on these behaviors).

A surprising finding of this study was that, although we discovered treatment effects on squirrels' movement and relaxation patterns, we did not find differential effects on vigilance. In our study, the first PCA factor (explaining 31.72% of the total variance in our behavioral measures) corresponded to alertness or vigilance. However, this vigilant behavior was not significantly different across any of our treatment categories (visual stimulus, vocal string, minutes elapsed following probe) or between sexes. Thus, vigilance may not be as sensitive a measure of change due to repetitive calling as are other measures. Alternatively, our other behavioral categories may reflect distributed aspects of vigilance (e.g. sitting upright, for factor 4, or negative values for factor 3, relaxation of alarm, reflecting increased vigilance).

One potential confounding factor that may have influenced our results is the fact that we scattered seeds about the study site to attract squirrels. We felt that this was necessary in order to attract a sufficient number of animals to the playback site, as well as to repeatedly attract the same individuals in order to obtain repeated measures on them; however, as a consequence, squirrel density appeared to be unusually high. Nunes *et al.* (1997), who conducted longer-term (several-month duration) food provisioning experiments on Belding's ground squirrels (*Spermophilus beldingi*), found that population density was higher in these areas than in unprovisioned areas, and that pregnant and lactating females showed higher levels of aggression and vigilance than did similar control females in unprovisioned areas. Therefore, it is possible that squirrels in our study exhibited elevated overall levels of aggression or vigilance due to our short-term food provisioning. Alternatively, the squirrels may have decreased their level of alertness in order to devote more time to eating. In any case, there were no significant effects of number of immediate neighbors on our measures of movement or relaxation of alarm.

Nonetheless, squirrel density may have impacted our ability to detect changes in measures such as anti-predator vigilance.

Overall, our results suggest that adult female California ground squirrel behavior is strongly influenced by previously heard vocal signals, whereas males are more attuned to differences in prior visual stimuli. The finding that females appear to be more attentive to audio stimuli is complementary to the observation by Swaisgood *et al.* (1999) that maternal females participated more in vocal signaling in response to rattlesnakes than did other adults. However, this contrasts somewhat with our previous findings (Miyasato *et al.*, in prep), which showed that both sexes were more responsive to visual stimuli during the initial playback period, and no significant effects of vocal string were detectable. Nonetheless, it is possible that females are more sensitive to the tonic effects of repetitive alarm calls, whereas they tend to rely more on visual cues initially.

Thus, our study provides support for the hypothesis that the California ground squirrel repetitive calling produced in response to mammalian predators is an example of a tonic signal, which acts over an extended timescale (beyond the immediate occurrence of the signal) to enhance the degree of alertness in perceivers. Our results also demonstrate that the use of "probes" is a powerful method of revealing latent behavioral states. Furthermore, we determined that visual cues accompanying repetitive calls had a lingering effect on behavioral patterns. However, our use of stationary squirrel models as visual stimuli are only a first step in simulating visual cues that would be produced by a signaling animal; changes in position and posture also would be expected, as well as movements that occurred concurrently with vocalizations (such as chest or tail movements). Further investigation of the effects of tonic signals and the stimuli associated with them is necessary to enhance our understanding of tonic communication processes.

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TABLES AND FIGURES

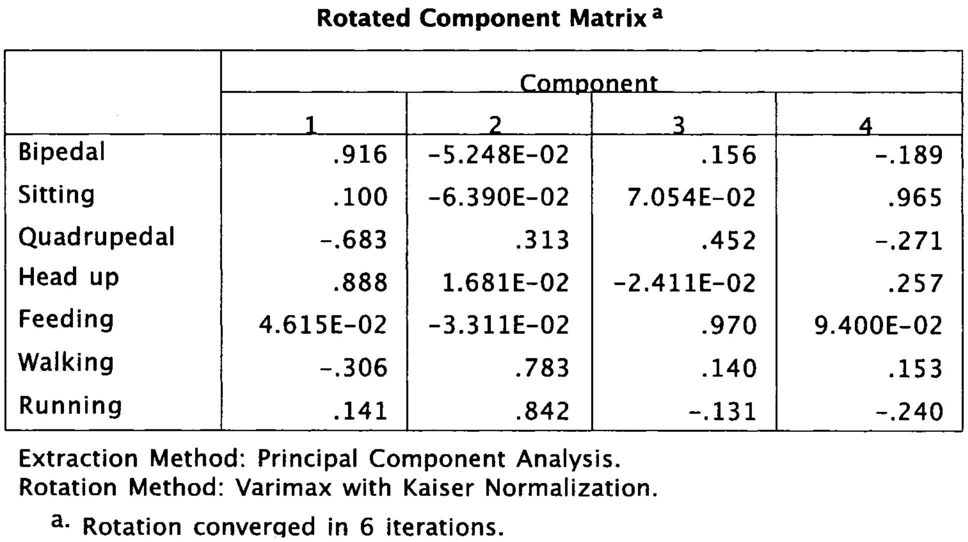
Table 2.1. Percentage of variance explained by components resulting from Principle Components Analysis (PCA) extraction on seven initial measures of ground squirrel response to probe playbacks. Behavioral responses entered into the PCA first were averaged over each individual minute following the probe stimulus (2 min total). Measures for 12 squirrels were used to generate the components. Varimax-rotated PCA components with eigenvalues greater than 1.0 (factors 1 through 4) were selected for further analysis.

Total Variance Explained

	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
Component	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	2.484	35.486	35.486	2.484	35.486	35.486	2.220	31.721	31.721
2	1.337	19.105	54.591	1.337	19.105	54.591	1.427	20.391	52.112
3	1.227	17.534	72.125	1.227	17.534	72.125	1.211	17.294	69.406
4	1.005	14.361	86.486	1.005	14.361	86.486	1.196	17.080	86.486
5	.515	7.363	93.849						
6	.285	4.076	97.925						
7	.145	2.075	100.000						

Extraction Method: Principal Component Analysis.

Table 2.2. Contributions of the seven initial measures of ground squirrel responses, averaged over each individual minute of probe trials (2 min total), to the four Varimax-rotated PCA components selected for further analysis. Separate ANOVA's then were performed on these PCA factors to investigate treatment effects during the initial minute after probe playback, during the second minute after the probe, and across the entire 2-min period.



Rotated Component Matrix[a]

	Component			
	1	2	3	4
Bipedal	.916	-5.248E-02	.156	-.189
Sitting	.100	-6.390E-02	7.054E-02	.965
Quadrupedal	-.683	.313	.452	-.271
Head up	.888	1.681E-02	-2.411E-02	.257
Feeding	4.615E-02	-3.311E-02	.970	9.400E-02
Walking	-.306	.783	.140	.153
Running	.141	.842	-.131	-.240

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.

[a] Rotation converged in 6 iterations.

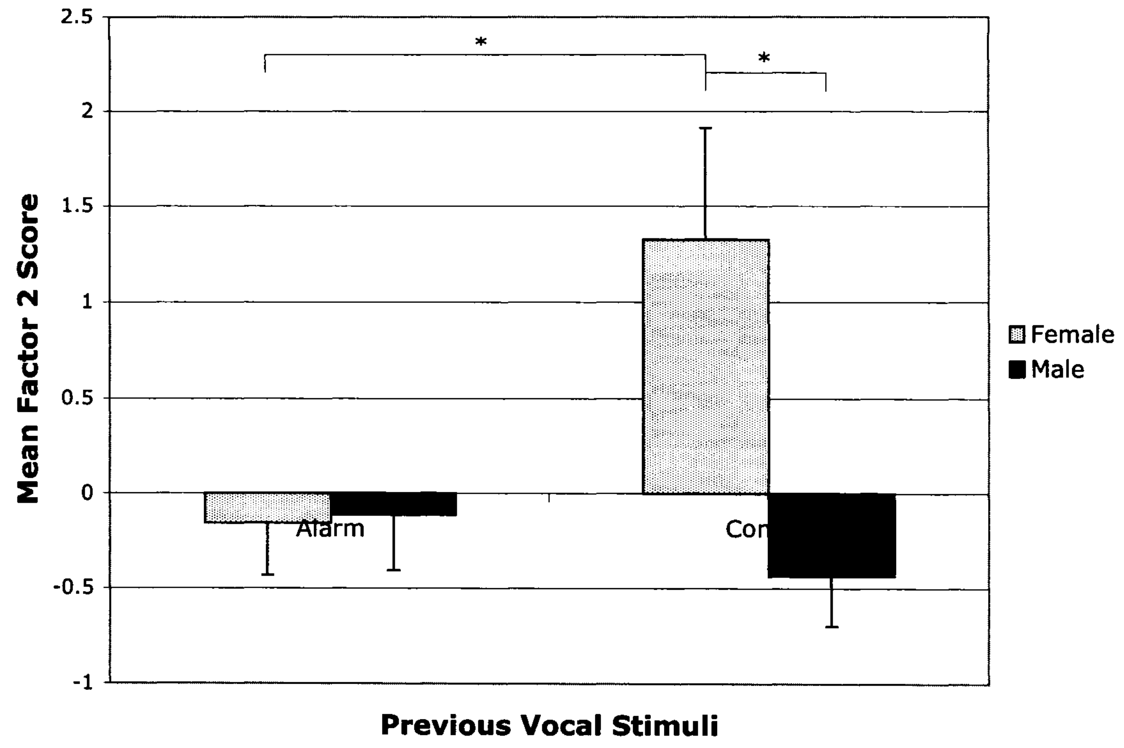


Figure 2.1. Association between past vocal stimuli and subjects' sex on mean (±s.e.) PCA factor 2 scores during the initial minute following probe playback (vocal*sex interaction: $F_{1,8}=15.816$, $p=0.004$; $N=12$: 6 females, 6 males). Factor 2 was associated with initiation of movement (increased running, increased walking, and increased time in the quadrupedal stance). Females showed a greater degree of movement if they heard the control stimulus in the period preceding the probe than if they heard the alarm ($t_{10}=-2.290$, $p=0.045$); females also showed more movement in response to the control than did males ($t_{10}=2.749$, $p=0.021$).

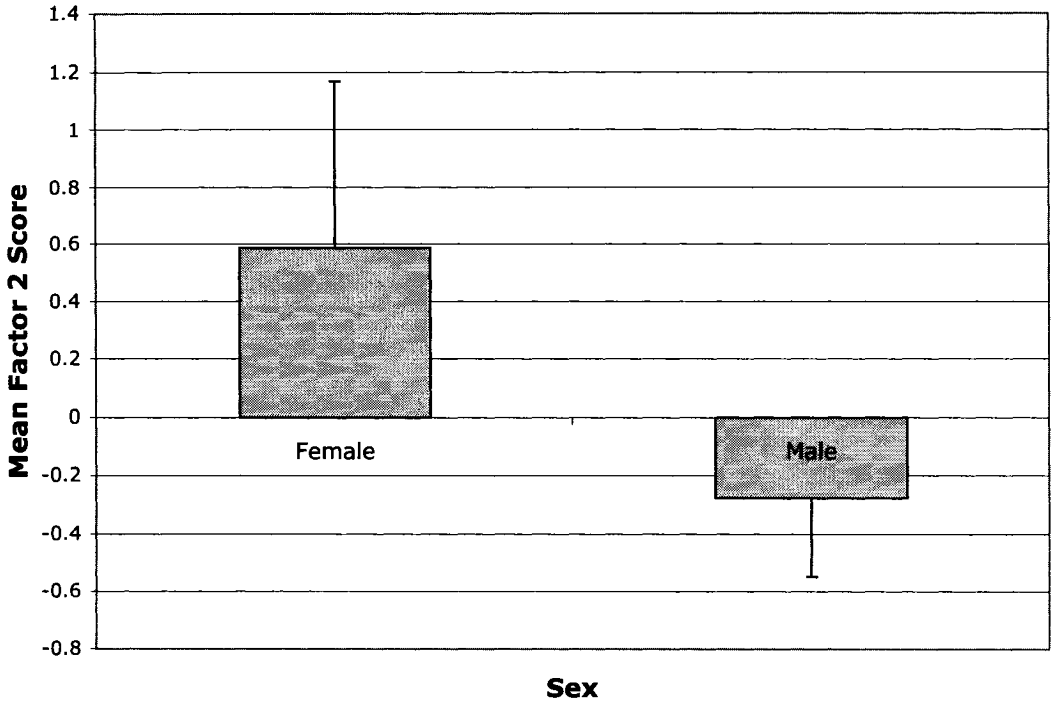


Figure 2.2. Sex differences in mean (±s.e.) PCA factor 2 scores during the initial minute following probe playback ($F_{1,8}$=14.330, p=0.005; N=12: 6 females, 6 males). Factor 2 was associated with movement (increased running, increased walking, and increased time in the quadrupedal stance). Females initiated movement more often than males.

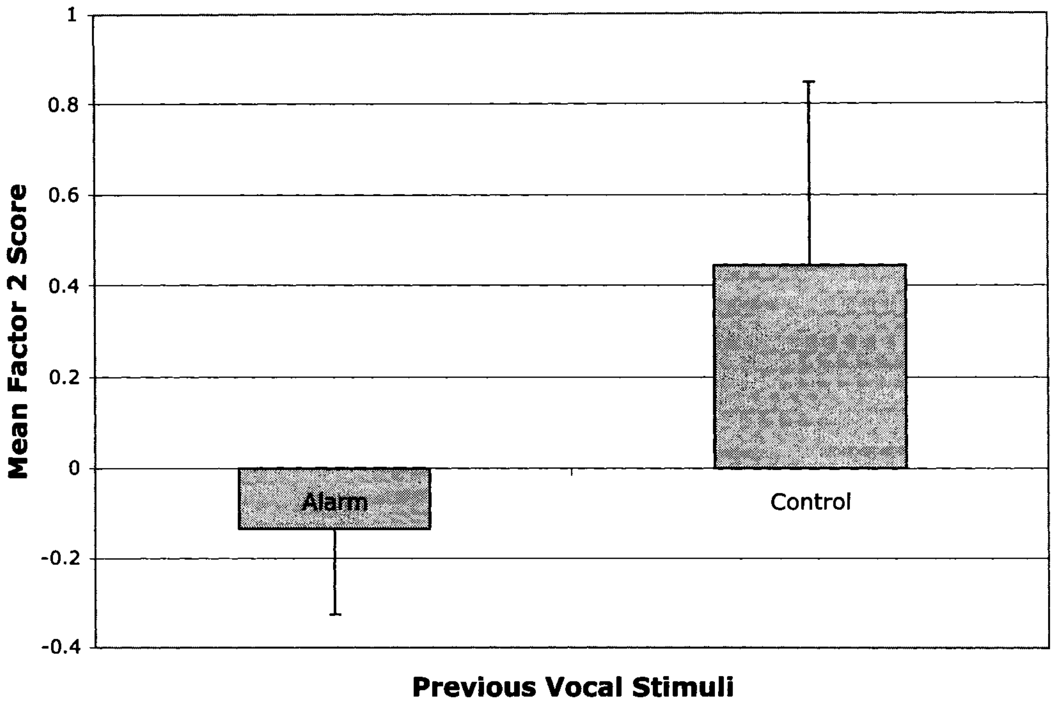


Figure 2.3. Effects of past vocal stimuli on mean (±s.e.) PCA factor 2 scores during the initial minute following probe playback ($F_{1,8}$=6.502, p=0.034; N=12). Factor 2 was associated with movement (increased running, increased walking, and increased time in the quadrupedal stance). Squirrels that heard control calling during the preceding period showed more movement following probe presentation than did squirrels that heard alarm calling previously.

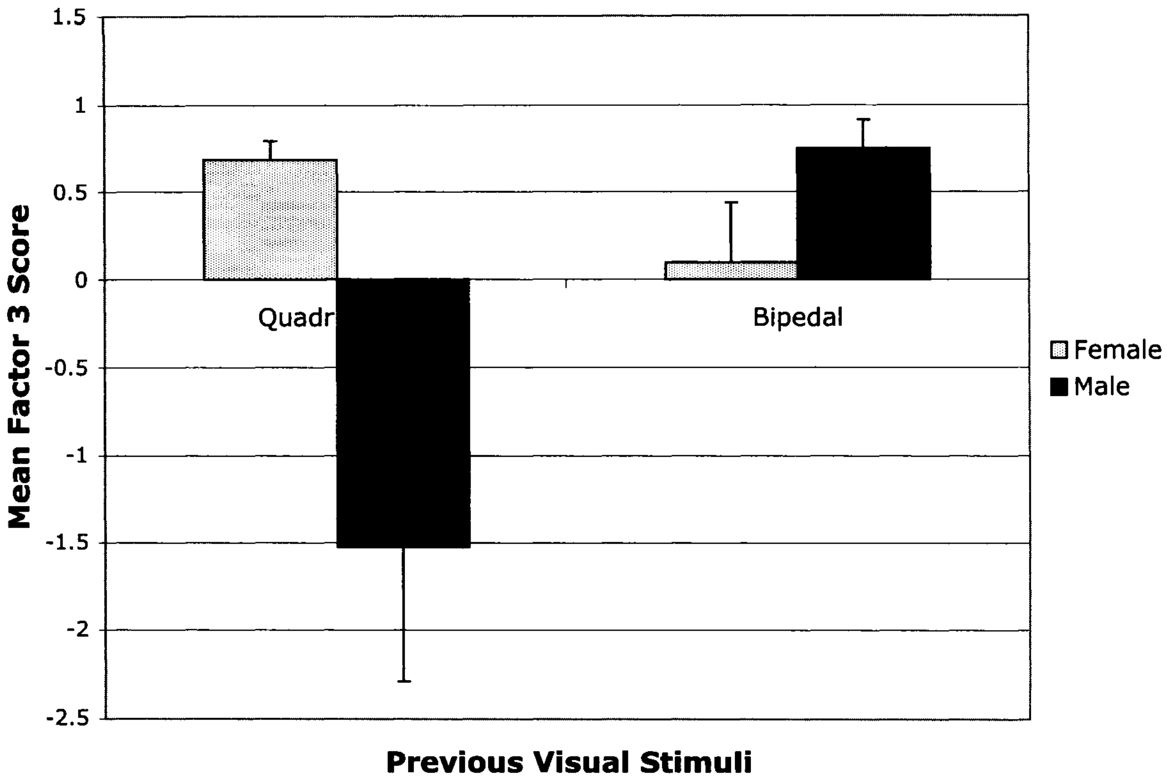


Figure 2.4. Association between past visual stimuli and subjects' sex on mean (±s.e.) PCA factor 3 scores during the second minute following probe playback (visual*sex interaction: $F_{1,6}$=10.784, p=0.017; N=10: 6 females, 4 males). Factor 3 was associated with relaxation of alarm (increased feeding and increased time in the quadrupedal stance). Males showed much lower levels of relaxation if they had seen the quadrupedal model during period preceding probe playback than if they had seen the bipedal model; conversely, females displayed more relaxation after seeing the quadrupedal model.

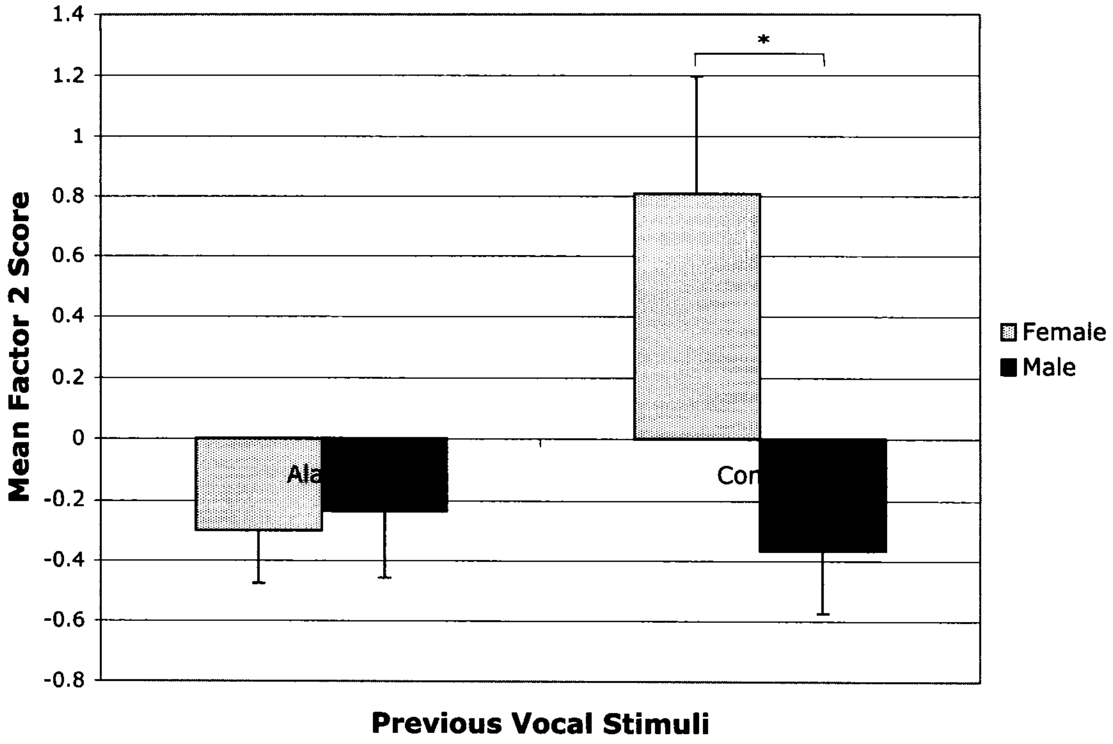


Figure 2.5. Association between past vocal stimuli and subjects' sex on mean (±s.e.) PCA factor 2 scores across the two minutes following probe playback (vocal*sex interaction: $F_{1,6}$=7.378, p=0.035; N=10: 6 females, 4 males). Factor 2 was associated with initiation of movement (increased running, increased walking, and increased time in the quadrupedal stance). Females that heard the control stimulus in the period preceding the probe showed a greater degree of movement than males that heard the control sound ($t_{5.995}$=2.615, p=0.040).

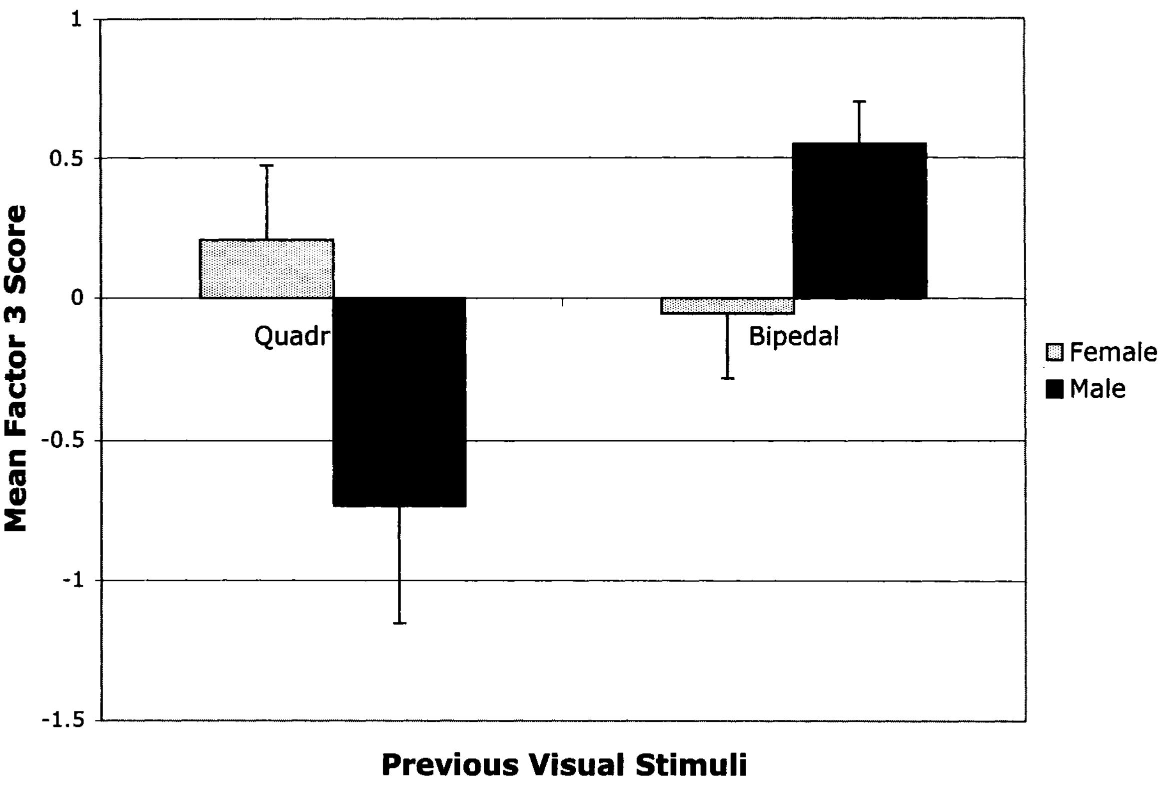


Figure 2.6. Association between past visual stimuli and subjects' sex on mean (±s.e.) PCA factor 3 scores across the two minutes following probe playback (visual*sex interaction: $F_{1,6}$=8.300, p=0.028; N=10: 6 females, 4 males). Factor 3 was associated with relaxation of alarm (increased feeding and increased time in the quadrupedal stance). Males demonstrated less relaxation in response to the quadrupedal model than to the bipedal model; females' responses were weaker and in the opposite direction.

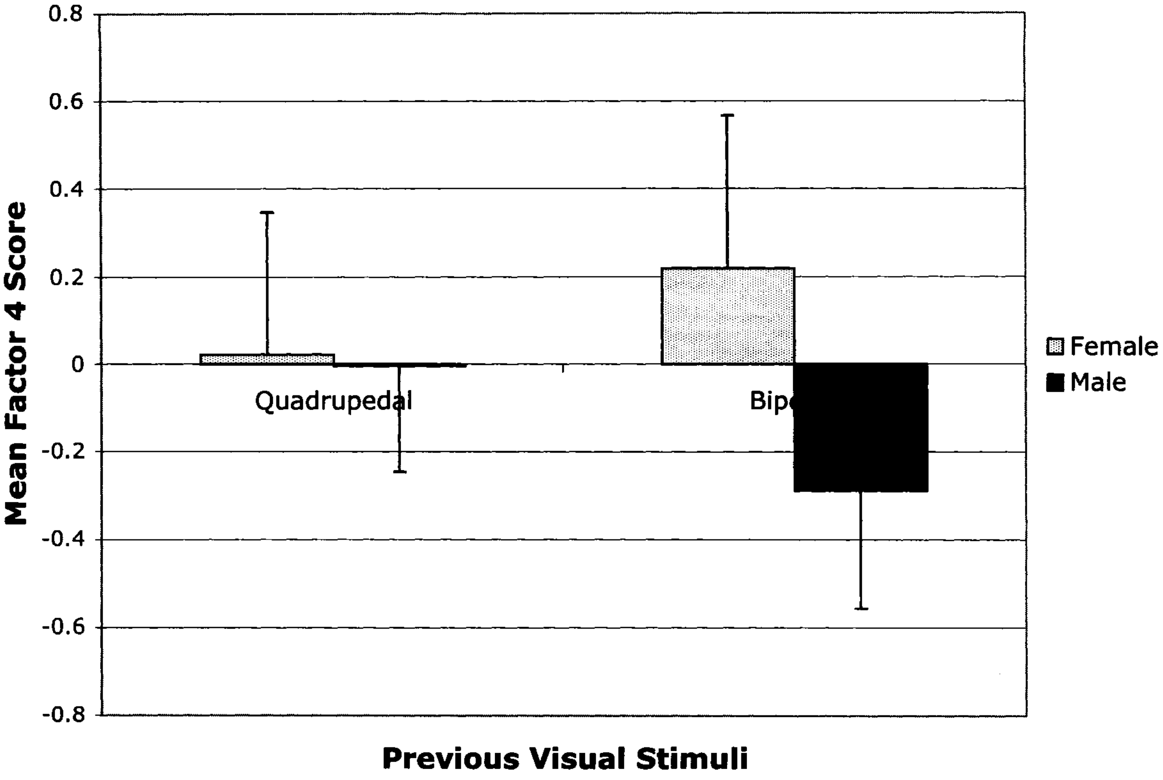


Figure 2.7. Association between past visual stimuli and subjects' sex on mean (±s.e.) PCA factor 4 scores across the two minutes following probe playback (visual*sex interaction: $F_{1,6}$=12.339, p=0.013; N=10: 6 females, 4 males). Factor 4 was associated with sitting upright. After being exposed to the bipedal model in the period preceding the probe, males were less likely to sit upright than were females. The quadrupedal model had little effect on sitting behavior in either sex.

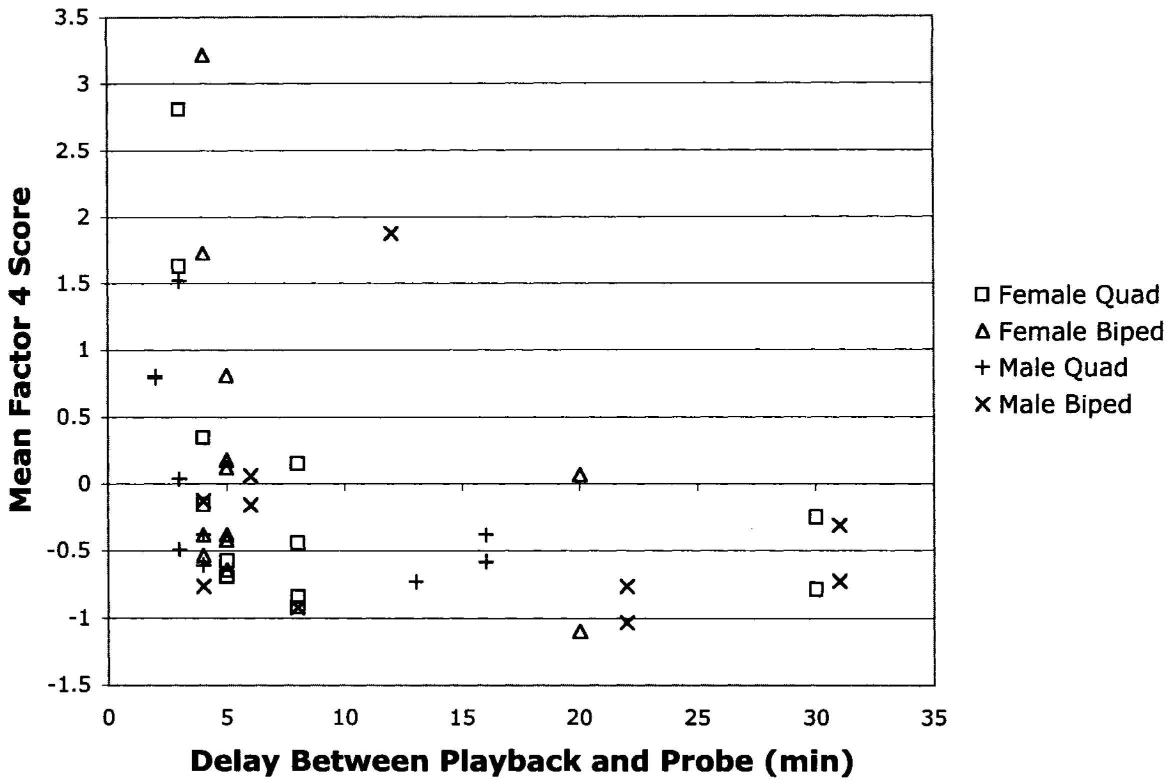


Figure 2.8. Effect of delay duration between the end of the initial playback period and the beginning of the probe trial on mean factor 4 scores across the two minutes following probe playback ($F_{1,39}$=5.108, p=0.029; N=10: 6 females, 4 males). Factor 4 was associated with sitting upright. The highest levels of sitting upright tended to occur when delay times were fairly short.

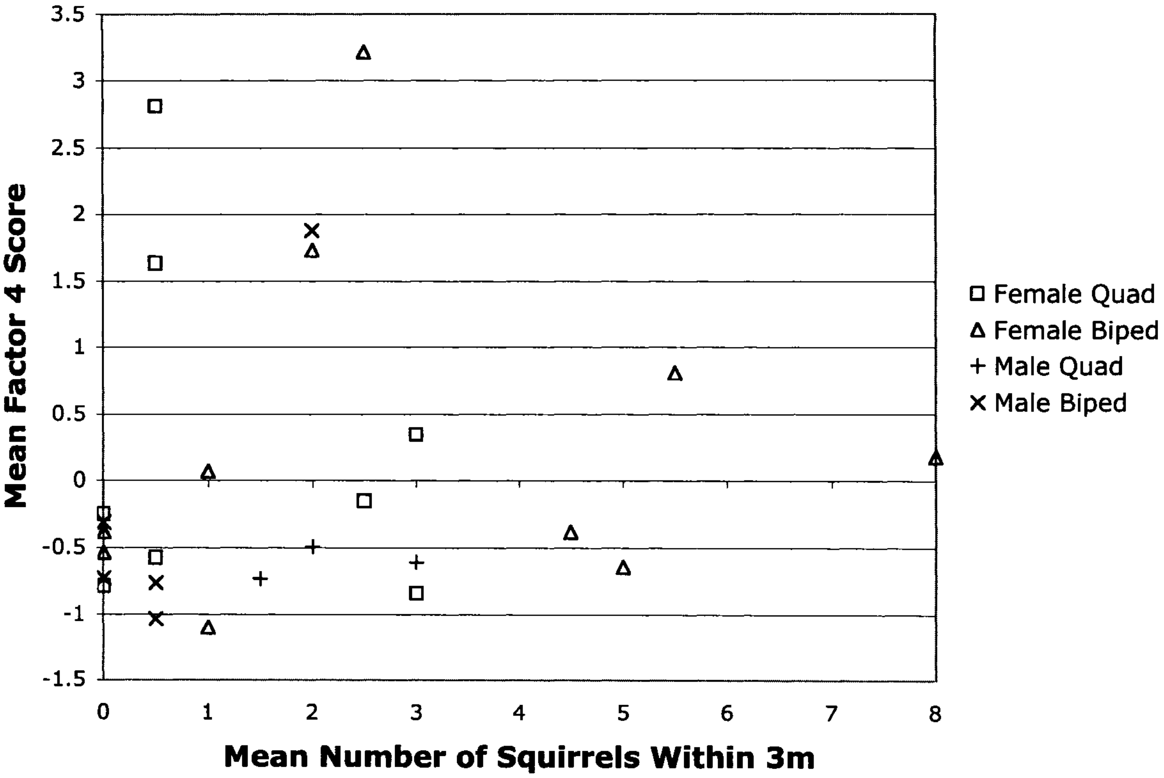


Figure 2.9. Effect of neighboring squirrels within 3 m of the focal animals on mean factor 4 scores across the two minutes following probe playback ($F_{1,11}$=6.267, p=0.029; N=10: 6 females, 4 males). Factor 4 was associated with sitting upright. The highest levels of sitting upright tended to occur when fewer animals were in the vicinity of the focal animals.

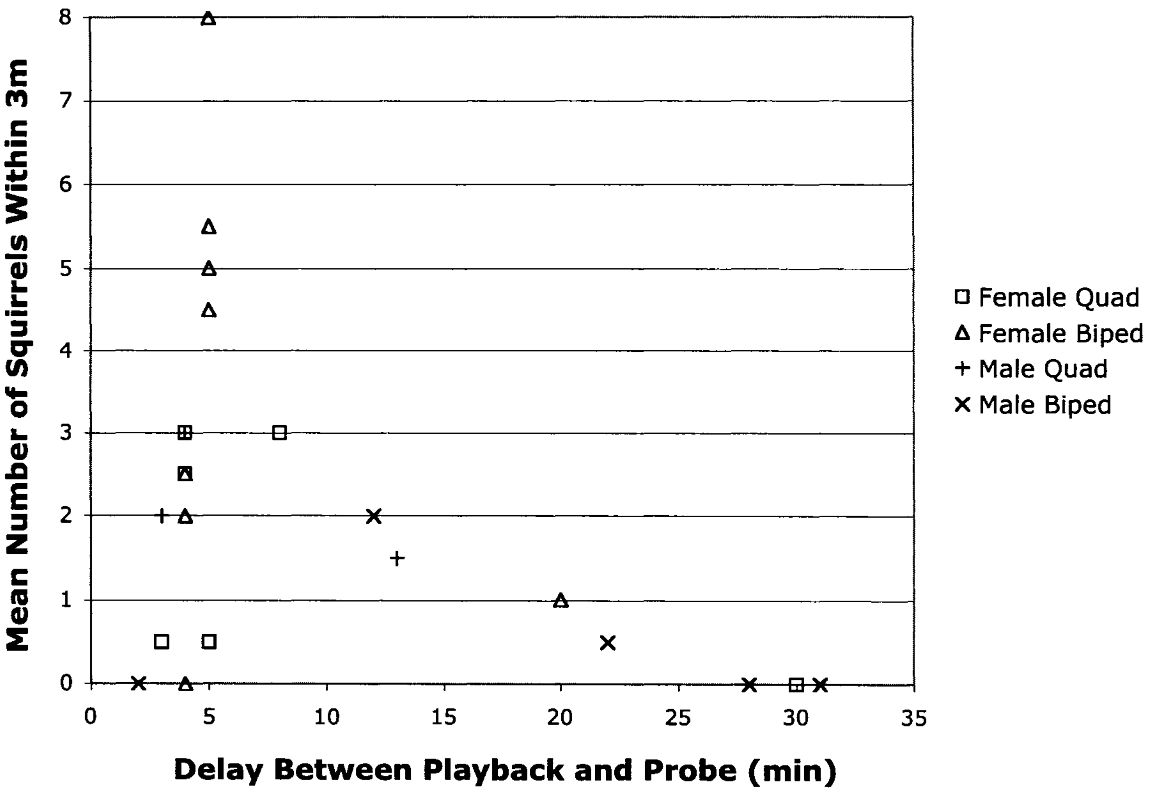


Figure 2.10. Association between mean number of neighboring squirrels within 3 m of the focal animal and delay between the end of playback and beginning of the probe trial.

CHAPTER 3

The Role of Latent Systems in Animal Behavior

ABSTRACT

Animal behavior seldom is considered in terms of behavior that is not readily observable, yet a review of the literature shows that such quiescent systems are fairly common. A **latent system** can be defined as *persistence of a cognitive or motivational state resulting from exposure to a given stimulus, initially evidenced as an observable behavioral response that subsequently disappears, whose continued presence can be demonstrated by presentation of an appropriate response-triggering stimulus*. During the latent phase, behavior related to the original stimulus is unexpressed and may even be actively suppressed; behavioral responses then can be "reactivated" by reappearance of the initial stimulus, possibly coupled with lifting of suppression, or appearance of a novel stimulus that relates in some way to the initial stimulus. Examples of processes associated with latent behavioral potential include habituation, imprinting, and bird song development. Finally, ways in which animal communication research could benefit from taking a latent systems perspective are highlighted, and results from a study of California ground squirrel communication are presented to illustrate one approach to revealing these systems.

INTRODUCTION

Animals begin life facing a startling array of unknown stimuli; however, they also enter the world already equipped with much vital knowledge. The "known" can include features associated with caregivers (some of which can be learned even before birth or hatching), as well as "innate" responsiveness to looming stimuli in their environment. These known features can be paired with novel stimuli in the process known as associative learning. An example of unfamiliar stimuli is the alarm calls produced by members of other species; animals are known to respond to these "foreign" stimuli. Might such unknown stimuli become linked with known predator-related stimuli via associative processes? In order to address this question, Shriner (1999) conducted an experiment in which a novel auditory stimulus was broadcast to golden-mantled ground squirrels (*Spermophilus lateralis*) while a hawk model was flown overhead. The squirrels rapidly developed vigilance responses to the new sound that were comparable with their responses to conspecific alarm calls. Hence, Shriner proposed that such associative learning processes might be the mechanism by which this species learned appropriate responses to heterospecific alarm calls.

However, another equally likely mechanism could have generated such selective responsivity to heterospecific vocalizations: the refined anti-predator systems of adults might be the result of a winnowing process in which the broad responsiveness of young animals is narrowed by suppressing behavior toward irrelevant stimuli. Evidence from a number of species suggests that juveniles typically show more generalized anti-predator responses than adults do. For example, infant vervet monkeys (*Cercopithecus aethiops pygerythrus*) produce alarm calls in response to significantly more species than their elders (including harmless stimuli, such as a pigeon or even a falling leaf; Seyfarth and Cheney 1980). Newly emerged Belding's ground squirrels (*Spermophilus beldingi*) respond for longer periods of time to alarm calls than do older juveniles or adults, and initially respond about equally to alarm versus non-alarm control calls (Mateo 1996a). California ground squirrels *(Spermophilus beecheyi*) show more generalized responses than adults to predators: they respond about equally to aerial predators and dogs, and appear to differentiate less between whistle and chatter alarms than adults do (Hanson and Coss 1997; Hanson and Coss 2001b). Juvenile bonnet macaques (*Macaca radiata*) flee as often as adults upon hearing adult alarm calls, but respond more strongly than adults to juvenile alarm calls and a control sound (a motorcycle engine)(Ramakrishnan and Coss 2000a). Even at ages when young animals might be too uncoordinated to show "appropriate" anti-predator behavior, their responsiveness to alarms has been demonstrated using physiological measures, such as heart rate in pre-emergent Belding's ground squirrels (Mateo 1996) and tail piloerection in California ground squirrels (Hanson and Coss 2001a). Thus, the tendency for young animals either to perform a given response to a greater range of stimuli or show higher levels of responsiveness than adults appears to be fairly widespread. Hence, it is reasonable to hypothesize that the more refined anti-predator responses of adults develop through suppression of inappropriate reactions.

Heterospecifics as well as conspecifics are often useful sources of cues about important events. This may be especially true when sympatric species overlap ecologically because they are vulnerable to the same predators. Hence, it should not be surprising that animals often are responsive to heterospecific alarm calls. For example, golden-mantled ground squirrels and yellow-bellied marmots (*Marmota flaviventris*) are as responsive to each others' alarm calls as to their own (Shriner 1998), and downy woodpeckers *(Picoides pubescens*) increase vigilance upon hearing titmouse and chickadee alarm calls (Sullivan 1984). Such use of heterospecific calls can reach a surprising level of refinement, involving for example appropriate differentiation between each others' aerial predator versus general alarm calls, as seen in two sympatric lemur species, sifakas (*Propithecus verreauxi verreauxi*) and redfronted lemurs (*Eulemur fulvus rufus*) (Fichtel 2004). These interspecies communicative associations do not necessarily involve closely-related species; for example, vervets respond appropriately to aerial versus terrestrial superb starling *(Spreo superbus*) alarm calls (Seyfarth and Cheney 1990), and bonnet macaques react appropriately to playbacks of sambar deer (*Cervus unicolor*) alarm calls (Ramakrishnan and Coss 2000b). Infant vervets, in fact, can distinguish between starling alarm calls and song, and respond appropriately to starling terrestrial predator alarm calls beginning at about three to four months of age (Hauser 1988).

If such heterospecific signal recognition is not the product of associative learning, what mechanism might account for it? One potential explanation is that critical features of the alarm calls of different species are very similar, as is the case with certain avian "hawk alarm" calls (Marler 1955). An intriguing complementary possibility was suggested by researchers studying anti-predator behavior in harbor seals (*Phoca vitulina*) (Deecke et al. 2002). Killer whales (*Orcinus orca*) in the same vicinity display two different feeding strategies, some specializing on marine mammals, including harbor seals, and the other group feeding on fish but not marine mammals. These harbor seals respond more strongly to playbacks of vocalizations of local seal-eating whales than fish-eating whales. Apparently this discrimination is based on habituation to the sounds of the local fish-eating whales and not to any consistent call differences between fish-eating and mammal-eating whales. When the researchers played back sounds from an unfamiliar fish-eating population from Alaska, the seals responded as strongly to these calls as they had to calls from locally occurring seal-eaters.

Deecke *et al.*'s seals may have been suppressing rather than forgetting or losing initial inappropriate responses. Such suppression could occur in other cases of signal recognition as well: in another investigation of anti-predator responses, vervet monkeys that had learned to ignore playbacks of a given alarm call from an "unreliable" caller also ignored other call exemplars in the same category (i.e., leopard vs. eagle calls) when given by the same individual (Cheney and Seyfarth 1988); the authors argued that the animals were showing habituation to these calls. Vervets still responded to the given call type produced by other individuals, as well as calls with different referents produced by the "unreliable" caller (Cheney and Seyfarth 1988). One of the hallmarks of habituation is that even when response to the initial stimulus has declined, it can regain its strength with presentation of a new stimulus (Thompson and Spencer 1966); thus, suppression rather than muscle fatigue or sensory adaptation is responsible for the decreased responsiveness.

Thus, it appears that behavioral suppression is a means by which animals refine their responses to environmental stimuli. The harbor seal example suggests that, during early development, seals show broad responsiveness to predator calls, but then actively suppress responses to calls that turn out to be irrelevant at that point in time. This brings us back to the example of juvenile animals' development of appropriate anti-predator responses. As J. M. Mateo stated, "Despite their vulnerability, juveniles may not emerge with fully formed associations between alarm calls and responses if the local predator context changes over time, thus favouring plasticity in the response repertoire" (Mateo 1996a). One potential means of maintaining such plasticity is through broad initial responsiveness to potentially dangerous stimuli, and subsequent refinement via suppression of responses that would not be adaptive in a given situation. This idea of behavioral suppression, however, is not one that is commonly applied to discussions of animal communication. Moreover, response suppression belongs to an even broader category of latent behavioral phenomena, or ***latent systems***.

A latent system can be defined as follows: *persistence of a cognitive or motivational state resulting from exposure to a given stimulus, initially evidenced as an observable behavioral response that subsequently disappears, whose continued presence can be demonstrated by presentation of an appropriate response-triggering stimulus*. The conditions that manifest a latent system can include reappearance of the initial stimulus, possibly coupled with lifting of suppression, or appearance of a novel stimulus that is sufficiently similar to (or in some way relevant to) the initial stimulus to trigger the behavior. Moreover, during the period when the behavior is latent, it is possible for the underlying state to undergo modifications, due to changes in the animal's situation (e.g. further learning, changes in its environment, maturation, etc.). In addition, different stimuli may be capable of triggering the same memory system. Furthermore, the behavior that emerges will depend on the context in which it is elicited. Thus, when behavior is again expressed, it may not take the same form as the initially expressed behavior.

As this review will demonstrate, numerous examples of latent systems exist in the literature; however, there are many areas from which discussion of such systems is noticeably lacking. Therefore, the goals of this review are to illustrate the wide variety of contexts in which the effects of latent systems on behavior have been demonstrated, to emphasize the importance of using appropriate eliciting stimuli in the study of latent systems, and to demonstrate how the concept of latency could contribute to studies in the field of animal communication by providing a new framework within which communicative interactions can be investigated. Such an approach may well uncover patterns of communicative behavior not yet described by investigators.

ACTIVELY SUPPRESSED BEHAVIOR

Habituation

The phenomenon of habituation can be described as "response decrement as the result of repeated stimulation" (Harris 1943); it has been studied extensively in an enormous variety of taxa (e.g. Harris 1943; Peeke and Herz 1973a; Peeke and Herz 1973b). Thompson and Spencer (1966) derived nine major principles of habituation from an extensive review of the literature; those that are most pertinent to latent systems will be discussed below.

Several major characteristics of habituation noted by Thompson and Spencer indicate that habituation involves active suppression rather than loss of the ability to respond (e.g. due to sensory adaptation or muscle fatigue). Withholding the eliciting stimulus for a period of time results in a recovery of response strength (*spontaneous recovery*); presentation of another stimulus (particularly a strong one) produces an increase in response strength (*dishabituation)*; and the effects of habituation can continue to accumulate even after detectable responses have vanished (*below-zero habituation*). Such features of habituation have important implications for latent systems: they indicate that the systems underlying behavior not only can persist in unexpressed form, but can also continue to adapt during the period of latency.

Habituation has been identified as a potentially important source of selection on the singing behavior of songbirds. Hartshorne (1956), seeking to explain the great variability in singing behavior of many species, proposed the *monotony-threshold* hypothesis; he began by stating, "What stimulates animal organisms is change; what deadens response is sameness, or persistent repetition." Hence, Hartshorne speculated that the function of highly variable singing behavior is to limit the loss of impact of singing on listeners through habituation. Or, as the hypothesis was elucidated by Kroodsma, it is the degree to which successive portions of a singing performance contrast with each other that prevent habituation from taking place (Kroodsma 1982). One way in which birds can increase their singing variability is through the use of different song types, or song repertoires.

Data supporting the hypothesis that song repertoires can prevent habituation in listeners was provided by Searcy and colleagues (Searcy et al. 1982), who played back bouts of a single song type versus bouts containing four different song types to male and female swamp sparrows (*Melospiza georgiana*). Neither males nor females responded differentially to the different song types presented singly. However, females differentiated between single and multiple song-type playbacks: copulation solicitation dropped off in response to the repeated single song type, but recovered during the four-song playback with each transition from one song type to another.

An anti-habituation effect was also observed in male red-winged blackbird (*Agelaius phoeniceus*) responses to playbacks of dissimilar songs (Searcy et al. 1994). In response to territorial intrusion, male red-winged blackbirds perform a "song spread," an aggressive display in which they spread their wings to display red "epaulets" while singing. Males were exposed to playbacks of a given song repeatedly, followed by an abrupt switch to repetition of a second song recorded from the same male that either was similar or dissimilar to the original song. At the beginning of playbacks, males performed a strong song spread, but this response decreased in intensity with song repetition (i.e., habituation occurred). When the song change was to a dissimilar song, response strength recovered significantly, but no such recovery was observed when the second song was similar to the first. Thus, repertoires in red-winged blackbirds appear to overcome habituation in listeners.

Some features of operant and classical conditioning parallel characteristics of habituation. *Extinction* is the decrease in the conditioned response that occurs either when the conditioned stimulus that originally elicited the response is presented repeatedly in the absence of the unconditioned stimulus (classical conditioning), or when the operant response is no longer followed by a reinforcer (operant conditioning). For example, if an animal has learned to associate a new stimulus with a particular reward but the stimulus is subsequently presented repeatedly without the reward, the animal's response level will decrease. Like habituation, extinction involves repeated presentation of a stimulus; moreover, the conditioned response can reappear even after it has undergone extinction (this also is known as spontaneous recovery). These similarities between habituation and extinction are among the observations that led McSweeney and Swindell (2002) to propose that similar processes underlie both phenomena, and to provide evidence from a large number of studies to support their hypothesis. Thus, the properties of extinction, like those of habituation, provide evidence that changes in overt behavior involve active suppression and potential recoverability of behavior and therefore latent systems.

Imprinting: Latent Effects of Early Experience

Imprinting is another well-studied phenomenon that provides further evidence for latent systems. This is a rapid and relatively permanent type of learning that generally occurs in young animals during a sensitive period, in which an animal "imprints" on particular characteristics of a stimulus. Bolhuis (2005) noted that Lorenz's declaration that imprinting is irreversible has led to the mistaken belief that once a young animal has imprinted on an object, it will never show social attachment toward a new object. Bolhuis argued instead that although animals can form subsequent attachments to novel objects, their original attachment is not lost; or, as he cited from Lorenz, "'the recognition response cannot be 'forgotten'!'" This "weaker" form of irreversibility was nicely illustrated in a study by Bolhuis and Bateson (1990) of day-old domestic chicks, in which the birds were first exposed to a cylinder of one color, followed by presentation of a cylinder of another color. The chicks' initial preference for the first stimulus was found to switch to the subsequent stimulus, demonstrating reversibility. However, in another experiment, chicks were presented with one of two more complex stimuli for three days, then the second stimulus for three days, then both simultaneously for three days. The results of this experiment showed that, although the chicks initially reversed preferences from the first to the second stimulus, their preference for the first stimulus reemerged upon simultaneous presentation, regardless of the relative properties of the two stimuli.

This reemergence clearly illustrates that the chicks' initially acquired social preference was retained in a latent state until the appropriate eliciting stimulus appeared.

Bateson (1987) proposed a *competitive exclusion model* that could explain the aforementioned results; this model hypothesizes that "a particular member of a class of inputs from the environment gains access to the systems responsible for executing the relevant patterns of behavior." That is, there are different systems for recognition of environmental stimuli and for carrying out appropriate behavioral responses. However, the output system has a limited input capacity; when it becomes linked to a particular input, it no longer can accept other inputs from the recognition system. So if a novel imprinting stimulus captures enough of the output system from the original stimulus, a reversal occurs; nonetheless, the original stimulus retains connections, albeit a minority of those, within the larger recognition system. Consequently, under the appropriate conditions, the initial imprinting preference can reappear. Thus, neural traces of some proportion of environmental inputs will be present within an animal's nervous system, but remain unexpressed unless a change in context elicits a "rewiring" with respect to the behavioral output system.

Selection-Based Learning in Songbirds

Learning plays a crucial role in songbird vocal development. According to the widely accepted *auditory template* model of song learning (see e.g. Marler 1970), newly hatched birds have a coarse neural template of general features of their species' song. During a sensitive period in development, the bird memorizes songs it hears that are consistent with this template while discarding non-matches; the new input is integrated into the neural representation to create an exact template. The individual then rehearses song, refining output through feedback-based comparisons of output with template, until arriving at the final "crystallized" song.

Evidence of latent systems in song development was provided by Nelson and Marler (1994), who proposed a mechanism for the formation of regional song dialects in white-crowned sparrows. They hypothesized that young males acquire local dialects after dispersing to new breeding territories through selection of particular song types from a pre-existing repertoire of "plastic" songs (i.e., pre-crystallized song produced during the rehearsal phase – the *selective attrition* hypothesis), rather than through memorization of novel song types from neighbors in adulthood (the *instructive* hypothesis). To test this hypothesis, young hand-reared sparrows were allowed to hear a specific set of song types in their first few months of development. Subsequently, as these birds started producing plastic song in their first spring, they were played either 1) song types in their plastic repertoire, or 2) novel songs that they had previously neither heard nor produced. The instructive hypothesis would predict that birds in group 2 should be able to crystallize the novel song types; the selective hypothesis would predict that these birds would instead produce only previously memorized and produced plastic songs. Results were consistent with a selection-based model of dialect maintenance in white-crowned sparrows. The first group crystallized song types matching those they had heard during the second phase of the experiment, whereas the second group crystallized previously produced plastic song rather than the novel songs heard during phase two.

Similar results were revealed for white-crowned sparrows in the wild. Nelson (2000) recorded individual males during plastic and crystallized song production, and found that upon arrival to the breeding ground, many of the males produced two song dialects. However, the majority then retained only one dialect, which was almost always the one that best matched their neighbors' songs. Nelson then conducted a playback to males that sang two dialects, matching one of the songs in their repertoires. These males subsequently increased production of the matching song. Thus, males initially "overproduce" song dialects and eventually discard one; however, the fact that birds can be induced to sing the "lost" dialect indicates that it is suppressed rather than forgotten.

Geberzahn and colleagues (2002) tutored hand-reared nightingales (*Luscinia megarhynchos*) with songs presented in distinct groups (i.e., different song types were presented together in time, in association with each other but not with song types belonging to different song groups). The birds' subsequent plastic and crystallized songs were recorded. The researchers then conducted an interactive playback experiment in which one of four categories of songs were played: 1) crystallized tutored songs; 2) tutored songs produced as plastic but not crystallized song; 3) tutored songs that were not imitated; and 4) novel (never heard) songs. Subjects frequently responded by song matching: they either sang the same song that was broadcast, or another song from the same song group. Although they never matched the novel songs, they produced tutored songs that they had not been observed to produce previously, as well as songs that had been produced as plastic song but apparently had been "discarded" during crystallized singing. Thus, early tutored songs were stored as latent memories, whether or not the birds ever produced them; playback stimuli successfully elicited these stored memories. Moreover, song group matching demonstrated that the birds retained not only tutored song types, but also the particular sequence in which those songs were presented.

EVOLUTIONARY PERSISTENCE OF BEHAVIOR

The aforementioned examples of latent systems all have dealt with persistent traces of experience that were established sometime during a particular individual's lifetime. However, if the concept of latent systems is extended across evolutionary time scales, we find further fascinating examples of this phenomenon. As with the previous cases, these evolutionary examples deal with behavior that previously was expressed, but then subsequently seemed to disappear, until an appropriate eliciting stimulus was presented. However, the behaviors to be discussed in the next section became quiescent in the ancestors of the animals in question, due to loss of response-triggering stimuli at some point in time that we can trace back with relative certainty. As we shall see, the systems underlying behavior remained intact.

Ground Squirrel Anti-Snake Behavior

Coss (1999) noted that "behavioral characters are sometimes retained for long periods of time after selection has been relaxed." Relaxed selection includes, for example, cases where animals entered new habitats where their former predators (or parasites) were absent or later disappeared. In the case of California ground squirrels (*Spermophilus beecheyi*), some populations currently face northern Pacific rattlesnake (*Crotalus oreganus*) and gopher snake (*Pituophis melanoleucus*) predation, whereas others have been free from snake predation for approximately 70,000 to 300,000 years (Goldthwaite 1989). The anti-snake behavior of six populations of squirrels subject to snake predation were compared to five populations where snakes were absent ("relaxed-selection" populations), using presentations of live gopher snakes and rattlesnakes enclosed in wire mesh cages. Typically, ground squirrels faced with snakes will engage in staring, "tail-flagging," using their forepaws to throw substrate at the snakes, and biting. Amazingly, populations under relaxed selection showed even stronger tail piloerection responses (an indication of physiological arousal) than the snake-selected populations, and spent more time facing snakes. No differences were found between the groups in the number of substrate-throwing acts or tail-flagging cycles. Moreover, both snake-selected and relaxed-selection squirrels could differentiate between the two snake species. Thus, it appears that snake recognition is still very strong, despite the likelihood that anti-snake behavior has not been employed in these populations of squirrels under natural conditions for 70,000-300,000 years.

Retained Responses to Brood Parasitism

Coss (1999) also presented several examples of relaxed selection in bird species that historically had been victims of interspecific brood parasitism but since had been freed from such parasitism. Under these conditions of relaxed selection, various species showed lowered likelihood of rejecting unfamiliar eggs than would be the case in parasitized populations. Thus, egg rejection behavior seemed to break down under relaxed selection. However, Rothstein (2001a) argued that retention of egg rejection behavior is more common than has been recognized in the literature. He conducted artificial brown-headed cowbird egg-introduction experiments on Bermuda grey catbirds (*Dumella carolinensis*) and California loggerhead shrikes (*Lanius ludovicianus*); the former had mainland ancestors that were parasitized by brown-headed cowbirds but currently are not under brood-parasitism pressure, and the latter is unlikely to have suffered much, if any, brood parasitism by brown-headed cowbirds. Both species showed high rejection rates (although the Bermuda catbirds did not eject eggs as rapidly as five mainland populations).

Rothstein speculated that the shrikes retained egg-rejection behavior from their Old World relatives, which are parasitized by cuckoos (Cuculinae). Thus, he presented these findings as support for the hypothesis that the retention of egg rejection might have minimal costs and that California passerines that show high rejection rates and currently are parasitized by cowbirds may have possessed this rejection behavior prior to coming into contact with cowbirds. Consistent with Rothstein's view, Baltz and Burhans (1998) found that gray kingbirds (*Tyrannus dominicensis)* in the Bahamas ejected artificial shiny cowbird (*Molothrus bonariensis*) eggs in 17 of 20 nests within 48 hours, although the cowbird was first recorded in the Bahamas in 1993. Regardless of whether this hypothesis holds true, these findings illustrate that at least under some circumstances, egg rejection behavior can persist under conditions of relaxed selection. However, such "relic" behavior would not have been identified without the artificial addition of "foreign eggs."

These illustrations of "relic" behaviors show that latent systems can persist over evolutionary time scales, as well as during the lifetime of a single individual. The mechanisms involved in the maintenance of these systems across different time frames may or may not be the same, and therefore is an area that begs for further study.

APPLYING A LATENT SYSTEMS APPROACH TO ANIMAL COMMUNICATION

Eavesdropping

Animals always seek to actively extract information from their surroundings, and use this information to update their knowledge base in order to inform their decisions about the best ways to behave (Owings and Hennessy 1984; Owings and Morton 1998). Eavesdropping, in which individuals not directly participating in a communicative interaction can intercept information not intended for their ears (or other sensory organs), is an excellent example of this phenomenon. The eavesdroppers, therefore, "silently" (or even "sneakily") acquire valuable knowledge about their environment; researchers, for their part, have devised ingenious methods of determining the information that has been gleaned by these individuals. These approaches to studying eavesdropping parallel the methods by which latent systems may be studied, as both deal with unexpressed behavior. However, in order to be detected, latent systems require an even more proactive approach (i.e., the use of probes), as they cannot be detected simply through observation. Nevertheless, techniques used in revealing eavesdroppers' hidden knowledge should prove invaluable in developing probes for latent systems; several of these will be discussed below.

Great tit (*Parus major*) males assess territorial neighbors by eavesdropping on aggressive encounters (Peake et al. 2001; Peake et al. 2002). By setting up playback experiments using songs of different individuals, the researchers were able to artificially create "winners" and "losers" of audio "interactions"; they then broadcast these individuals' songs to the potentially eavesdropping subjects. Subjects responded by reducing song output in response to the apparent losers of the prior interactions (possibly corresponding to a lower perceived level of threat; Peake et al. 2001); moreover, when they had prior direct (playback) experience with one of the audio interactants, they were able to assess their own relative rank versus the individual interactants (Peake et al. 2002). Similarly, female domestic canaries (*Serinus canaria*) are capable of determining relative male status by eavesdropping on audio interactions: Leboucher and Pallot (2004) likewise created winners and losers of audio interactions in playbacks to females, who subsequently gave more copulation solicitation displays in response to the winner's songs.

Visual eavesdropping has been documented as well, and a frequent subject of such studies is the Siamese fighting fish, *Betta splendens*. In one experiment, subject males were held in the center portion of a tank; on either side were placed a pair of males that could engage in visual aggressive interactions (Oliveira et al. 1998). Subjects were found to spend more time on the side of the tank facing interacting males versus non-interacting males. Moreover, when faced with males that had won encounters, subjects were more hesitant to approach and took longer to display than when they were faced with losers; thus, they clearly were able to utilize information gathered through observation. Likewise, male green swordtails (*Xiphophorus helleri*) visually eavesdropped on aggressive contests between two other swordtails, and behaved much less aggressively when subsequently presented with the winner of the observed bout (Earley and Dugatkin

2002). Although individuals of these two fish species behaved oppositely toward winners and losers than did male great tits, it is obvious that animals are capable of making informed decisions based on what they have gleaned from eavesdropping.

As these examples demonstrate, it is possible to present individuals with known stimuli and subsequently determine what they have perceived. And, as the great tit and canary examples illustrate, such experiments can involve communicative signals. However, communication research rarely considers the behavioral potential present in animals that have perceived signals but are not actively demonstrating behavioral responses. Hence, an example of such an experiment will be described below.

Latent Systems in California Ground Squirrel Responses to Alarm Calls

California ground squirrels (*Spermophilus beecheyi*) produce a variety of anti-predator vocalizations, one of which is a repetitive call produced in response to mammalian predators; Owings et al. (1986) speculated that this call might be a form of tonic communication, with a cumulative effect over an extended time scale that could serve to increase or maintain vigilance in perceivers. Repetitive calling typically is composed of a "chatter" call (a multiple-note vocalization), followed by a series of "chat" (single-note components of a chatter call; Owings and Virginia 1978). Squirrels exhibit higher vigilance levels during than after repetitive calling, but this pattern is only significant for the first five minutes of calling, a finding that provides limited support for the tonic communication hypothesis (Loughry and McDonough 1988). However, probes were not used in this study.

We (L.E.M and D.H.O.) conducted an experiment designed to test whether repetitive calls exert a lingering effect on squirrels perceiving the call. (Since this experiment is reported in more depth elsewhere (Miyasato and Owings *in prep*), only a brief summary will be presented here.) Moreover, we tested whether visual stimuli (taxidermic mounts of squirrels in either an upright bipedal posture or a quadrupedal posture) modified the effects of repetitive call playbacks. The mounts were meant to mimic vocalizing squirrels. Leger et al. (1979) previously had shown that California ground squirrels use the quadrupedal posture more often than the bipedal stance during high-urgency situations, so we predicted that the pairing of the quadrupedal squirrel model with the alarm call playback would be the most evocative condition.

Playbacks were conducted during the spring and summer of 2003 and 2004 on free-living, marked adult squirrels living along the Alameda watershed in Alameda County, California. The repetitive calls were recorded from adult squirrels from a nearby population by L.A. Rabin; calls were elicited in response to domestic dog presentations. Prior to the start of the trials, squirrels were drawn into filming range by scattering black oiled sunflower seeds throughout the testing area. Once the focal animals were in range and had begun to forage, the initial playback was initiated. Playback involved presentation of the combined visual and audio stimuli (in four possible combinations: quadrupedal model/anti-predator call, bipedal model/anti-predator call, quadrupedal model/control call (mallard *Anas platyrhynchos* "hail" calls), and bipedal model/control call); this continued for three minutes. Initial playback was followed by an average delay of nine minutes during which no visual or audio stimuli were presented. A probe test then was conducted; this consisted of playback of only the brief, initial chatter portion of a repetitive alarm call. This probe stimulus always consisted of the brief chatter call; there was no control call, nor were visual stimuli presented. Squirrel behavior then was observed for two more minutes following probe playback. Focal squirrels were videotaped for subsequent quantitative behavioral analysis, and relevant details regarding the subjects' responses were narrated onto the audio track of the videotapes.

We instantaneously sampled, at 6-s intervals, the following seven behavioral measures for each focal squirrel: posture (bipedal, sitting upright, quadrupedal), whether the head was elevated or not, whether the animal was feeding, and any locomotion (walking or running). Principal components analysis (PCA) was performed on these seven measures for the two minutes following probe playback, and components with eigenvalues greater than 1.0 were subjected to analysis by general linear model, using a mixed repeated measures/between-subjects analysis of variance (ANOVA).

Because interpretation of the results can be somewhat confusing, the main phases of the experiment are reprised here: (1) an initial playback consisting of a combination of visual stimulus (quadrupedal or bipedal model) and audio stimulus (repetitive call or control call); (2) a delay during which no stimuli were presented; and (3) a probe test (brief alarm call without visual stimulus). The focal squirrel's response to the probe playback was videotaped for two minutes and later analyzed. The behavior quantified thus was produced in the immediate aftermath of the brief probe stimulus, and therefore provided an index of the delayed effects of the initial visual or audio stimuli on reactions to the subsequent probe playback. Since the probe stimulus was always a short alarm call, squirrels were expected to take precautionary measures after hearing it. Hence, in order for any lingering effects from visual or audio conditions of the initial playback to be detected, they would have to differentially affect the squirrels' responses to the probe stimulus.

And this differential effect is exactly what we found. The results of this experiment showed that latent systems indeed were operating with respect to the squirrels' responses to the playbacks, although not in a manner that we originally had anticipated. Sex differences played a significant role in squirrel responses to probe tests: females differentiated more strongly on the basis of the earlier auditory stimulus (anti-predator call versus control call) they had heard during the initial playback, whereas males discriminated more on the basis of the earlier visual stimulus (quadrupedal versus bipedal model). Female audio discrimination was most pronounced in their initiation of movement during the first minute following broadcast of the probe stimulus; they moved about more often if they previously had heard the control call than if they had heard the repetitive alarm (Figure 3.1). This suggests that repetitive calls had a suppressive effect on subsequent mobility in the females; it might be surmised that movement would call a predator's attention toward an individual and therefore that it should be minimized in certain high-risk situations.

On the other hand, during the second minute following probe playback, males showed less relaxation if they previously had seen the quadrupedal model than if they had seen the bipedal model during the initial playback (Figure 3.2). Although it may seem counterintuitive for animals to act more cautiously after seeing an animal vocalizing "on all fours" as opposed to a caller that is sitting upright, Leger *et al.* (1979) played back alarm whistles to California ground squirrels and found that, as they increased the number of whistles, quadrupedal freezing increased as upright postures declined. This appeared to be due to a trade-off between "safety" and "information-obtaining" behavior: bipedal animals are better able to survey their surroundings, but are more conspicuous to predators, whereas the converse is true for quadrupedal animals, which are less noticeable and also are poised for a quick escape.

Thus, employing a probe stimulus revealed that previously experienced audio and visual stimuli had lingering effects on squirrels, and that males and females were differentially affected. This is a clear demonstration of latent systems in California ground squirrels, and illustrates the following points: (1) the need to be aware of the potential for such behaviorally "invisible" effects of communicative signals and their associated contextual cues on perceivers; and (2) the importance of designing appropriate probe tests that will elicit the heretofore latent behavior. This second point may be difficult to fulfill in many situations; nonetheless, if possible cases of latent systems are simply ignored because of this difficulty, a great many instances could remain "latent" themselves.

Juvenile Anti-Predator Responses, Revisited

So, what about the development of anti-predator behavior in those juvenile animals that were discussed earlier? There is not as yet enough known about the mechanisms by which this type of behavior develops to state definitively that latent systems play a major role. However, as previously mentioned, vervet monkeys appear to habituate to alarm calls produced by an "unreliable" caller, and this generalizes to other calls with the same referent that were produced by that individual (Cheney and Seyfarth 1988). The study also showed that vervets still responded to similar calls from different individuals, as well as calls with different referents produced by the unreliable caller. These results lend support to the hypothesis that, at least over the short term, vervets actively suppress responses toward signals that they deem unworthy of further attention. Moreover, since vervet monkeys are one of the many species in which juveniles show more generalized anti-predator behavior than do adults (in this case, alarm calling to a wider range of stimuli; Seyfarth and Cheney 1980), it seems reasonable to predict that such a mechanism could work to winnow down the set of stimuli to which these infants respond, until their behavioral repertoire is more similar to their elders'.

This is just one example of a mechanism by which behavior systems may undergo change through the transformation of existing systems. Although the ability to expand a behavioral repertoire is very important in adapting to an animal's changing needs, so too is the capacity to modify or even suppress behavior if and when it becomes inappropriate; latent systems arise as a means of implementing these modifications. Hence, latent systems may play a crucial role in maintaining behavioral flexibility; investigating the mechanisms by which these systems operate will contribute much to our understanding of how animals adjust to an ever-changing environment.

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FIGURES

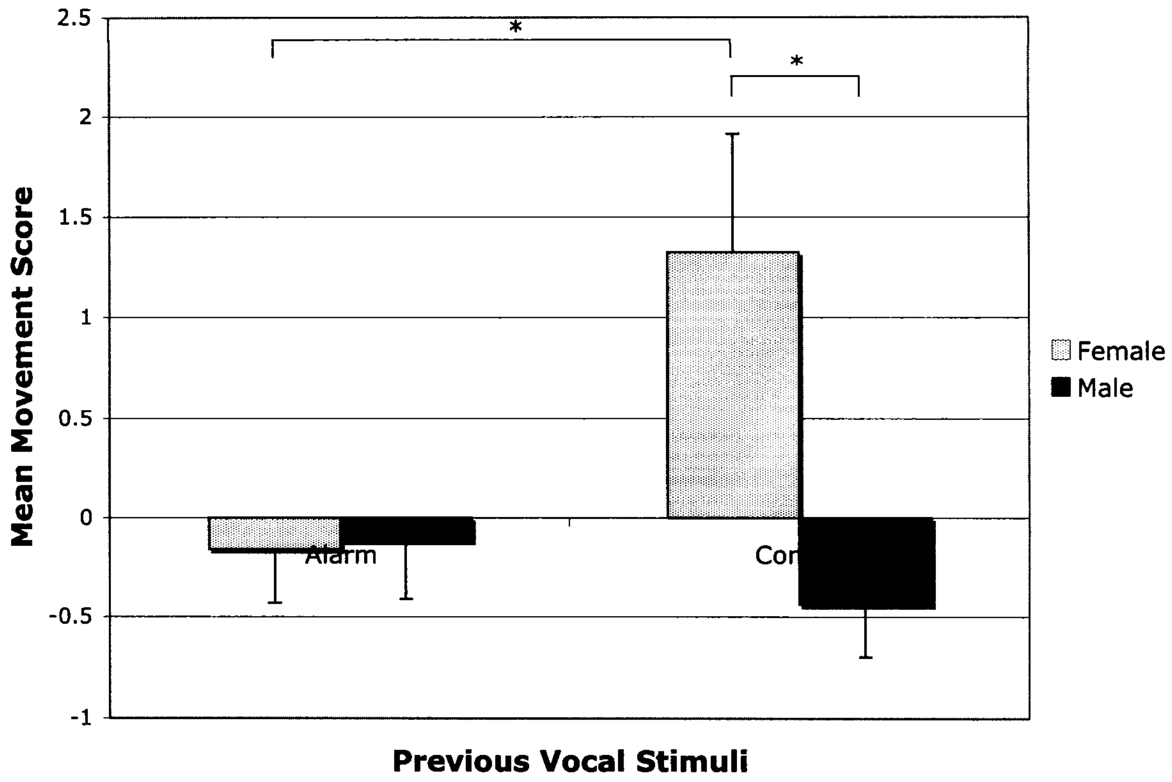

Figure 3.1. Differential effect on initiation of movement evoked by interaction between past vocal stimuli and subjects' sex during the initial minute following probe playback (vocal*sex interaction: $F_{1,8}=15.816$, $p=0.004$; $N=12$: 6 females, 6 males). Shown are mean (±s.e.) principal components analysis scores for factor 2, which was associated with the following changes in behavior: increased running, increased walking, and increased time in the quadrupedal stance. Females showed a greater degree of movement if they heard the control stimulus in the period preceding the probe than if they heard the alarm ($t_{10}=-2.290$, $p=0.045$); females also showed more movement in response to the control than did males ($t_{10}=2.749$, $p=0.021$).

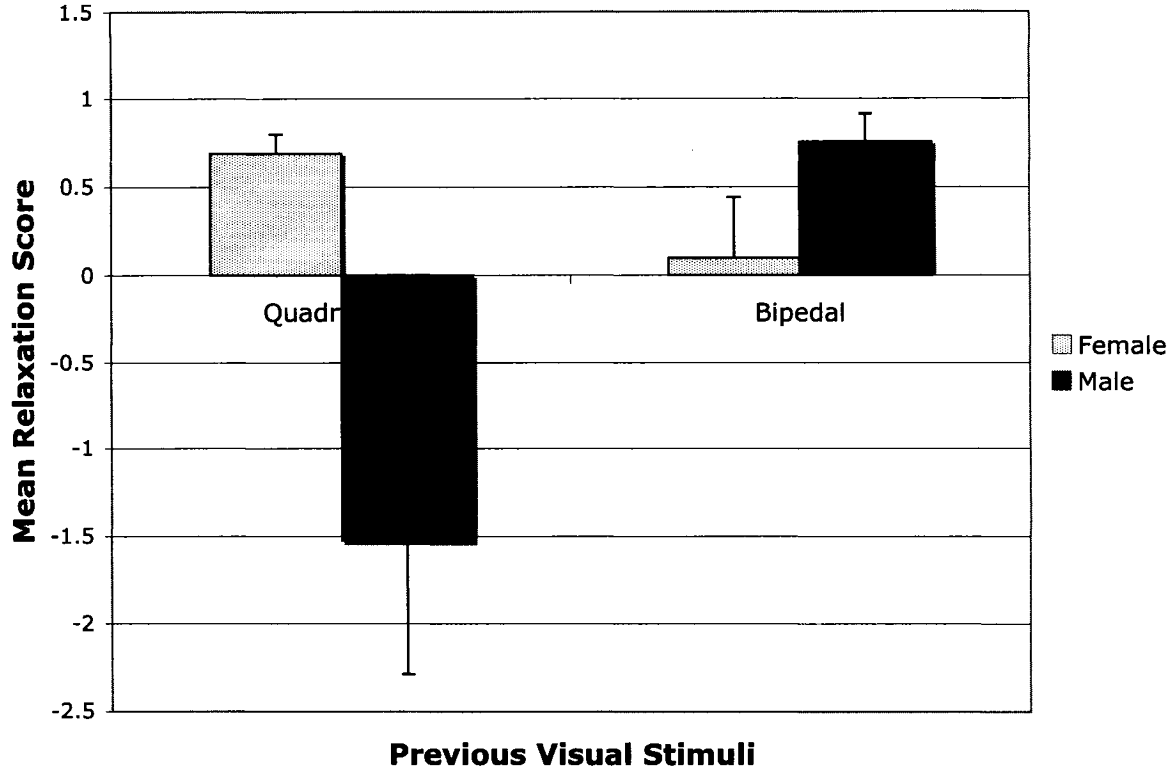

Figure 3.2. Differential effect on relaxation of alarm evoked by interaction between past visual stimuli and subjects' sex during the second minute following probe playback (visual*sex interaction: $F_{1,6}=10.784$, $p=0.017$; $N=10$: 6 females, 4 males). Shown are mean (±s.e.) principal components analysis scores for factor 3, which was associated with increased feeding and increased time in the quadrupedal stance. Males showed much lower levels of relaxation if they had seen the quadrupedal model during period preceding probe playback than if they had seen the bipedal model; conversely, females displayed more relaxation after seeing the quadrupedal model.

CONCLUSION

This playback study demonstrated that California ground squirrels use visual cues associated with ritualized vocal signals to determine their responses to vocalizations, and that males and females do so in different ways. Furthermore, visual and vocal stimuli had different effects in an immediate versus a temporally extended (tonic) time frame. Finally, it was shown that the effects of visual and vocal stimuli could remain behaviorally quiescent for extended periods of time. These "silent" effects were shown to be part of a larger phenomenon of latent behavioral systems that are widespread sources of influence on animal behavior.

The role of cues associated with signal production seldom has been investigated; hence, postural cues from a "vocalizing" squirrel were paired with playback of the repetitive chatter-chat alarm call. Unexpectedly, I observed no differential effects of vocal stimuli in an immediate time frame (during playback). In contrast, visual cues significantly affected perceivers' behavior: subjects showed higher surveillance levels during presentation of the quadrupedal than the bipedal model. This effect was driven primarily by females, who showed a strong surge in surveillance during the first minute of playback; males, on the other hand, exhibited little change in this behavior throughout the observation period. With respect to vigilance, females responded more strongly to the bipedal than the quadrupedal model, whereas males showed the opposite pattern of behavior. Females appeared to remain at an elevated state of alarm throughout the trial;

males, conversely, showed more relaxation of alarm. These visual effects were not dependent on the type of vocal stimulus.

Even though no differential effects of vocal stimuli were observed during playback, the "probe" experiment in Chapter 2 demonstrated delayed effects of both prior vocal and visual stimuli. This use of probe methodology provided a sensitive assay that revealed latent tonic effects that had been evident in only a limited way in prior research. Females displayed less initiation of movement if they previously had heard the alarm than the control calls, a result that implies that repetitive chatter-chat calling exerted a lingering suppressive effect on mobility. Males, on the other hand, differentiated more strongly on the basis of prior visual cues, exhibiting less relaxation of alarm if they previously had been exposed to the quadrupedal model than if they had seen the bipedal model. Although these lingering effects must have persisted throughout the period between the initial repetitive call playback and the onset of the probe stimulus, they remained behaviorally latent until they were evoked by the probe.

This discovery of lingering but latent effects of tonic signals raised the possibility that such effects might be more widespread throughout the field of animal behavior. This was indeed found to be the case. A survey of the literature revealed numerous examples of such latent behavioral systems, in which the underlying cognitive or motivational state brought about by exposure to a given stimulus are sustained even when the behavioral responses they influence are no longer observable. Many of these examples involved systems in which behavior was actively inhibited, a process all too often overlooked in the study of animal behavior. The importance of using probe stimuli to detect the presence of latent systems was emphasized. Until the use of such techniques becomes more commonplace, we will not know the true extent to which latent systems affect animal behavioral processes.

These findings help to illustrate the complexity of animals' assessment systems and the different levels at which transactions between these systems and environmental processes may act to bring about changes in overt behavior. Delving further into such processes as the integration of stimuli across multiple sensory modalities, the patterning of the effects of signals and cues across multiple time frames, and the activation of latent behavioral systems will help increase our understanding of the intricacies of animal behavior and the ways in which individuals adapt to their complex, ever-changing environments.