

## The call-timing algorithm of the white-lipped frog, *Leptodactylus albilabris*

Steven W. Moore<sup>1</sup>, Edwin R. Lewis<sup>2</sup>, Peter M. Narins<sup>3</sup>, and Pamela T. Lopez<sup>3</sup>

<sup>1</sup> Intercampus Graduate Group in Bioengineering, University of California at San Francisco and University of California at Berkeley, Berkeley, California 94720, USA

<sup>2</sup> Electronics Research Laboratory, University of California, Berkeley, California 94720, USA

<sup>3</sup> Biology Department, University of California, Los Angeles, California 90024, USA

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**Summary.** 1. The call-timing algorithm of the white-lipped frog, *Leptodactylus albilabris*, was studied in the field using playback of conspecific advertisement calls ('chirps') recorded with a digital electronic device developed for these studies.

2. A call refractory period is present. The interval between the onsets of successive chirps from one individual is always greater than this period of time.

3. Call timing is characterized further by a delayed, post-stimulus inhibition period during which a subject does not initiate a chirp. The calling gap produced by this delayed inhibition seems to be necessary for continued chorusing, and thus may be an important component of intraspecific communication in this species.

4. Calling males show spontaneous chirp interval distributions that suggest an underlying periodic call oscillator with noise or random variability.

5. Call timing was investigated also in one individual of *Leptodactylus melanonotus*. Data from this frog suggest that call timing in this species also involves a noisy periodic call oscillator, a refractory period, and delayed, post-stimulus inhibition.

ergetically demanding (Bucher et al. 1982; MacNally 1984) and, for some species, increases the risk of predation (Tuttle and Ryan 1981). Consequently, one expects to find behavioral mechanisms that increase the benefits of calling while reducing its costs.

Male frogs typically congregate and call together from the edges of ponds or other suitable breeding habitat. Whatever the selective advantages might be for these aggregations (Alexander 1975; Emlen and Oring 1976; Ryan et al. 1981), grouping accentuates the problems of vocal competition and acoustic interference. One solution to these problems is to call louder than the competition. The impressive volume of most frog calls suggests that loud voices indeed have a selective advantage. Members of one species, *Leptodactylus albilabris*, have been shown to increase their call intensity in response to increasing intensity of playback calls (Lopez et al., in press).

An alternative, and seemingly less expensive, way of dealing with call interference is for male frogs to adjust the timing of their calls relative to the calls of neighboring males. The fact that such interactive call timing is common in anurans (Wells 1977) as well as many insects (Alexander 1975) implies that it too has selective advantages. Indeed, timing in both anuran and insect choruses is often very precise (Loftus-Hills 1974; Rosen and Lemon 1974; Alexander 1975).

Some interacting frogs synchronize their vocalizations so that calls are simultaneous (Tuttle and Ryan 1981); others avoid call overlap (Lemon 1971; Sullivan 1985). Choruses sometimes comprise duets, trios, quartets, or more complex vocal organizations (Jameson 1954; Hardy 1959; Duellman 1967; Foster 1967). Various algorithms for interaction have been identified or proposed to explain these behaviors in several species. Rosen and

### Introduction

In the anuran amphibians (frogs and toads) vocalizations serve mainly a reproductive function. The male produces an advertisement call that attracts receptive, conspecific females (Martof and Thompson 1958; Littlejohn and Loftus-Hills 1968; Littlejohn and Watson 1974), and also plays a role in male-male interactions such as the establishment and maintenance of territories (Brattstrom and Yarnell 1968; Allan 1973). However, calling is en-

Lemon (1974) suggested an excitation-inhibition model for call timing in *Hyla crucifer*. Loftus-Hills (1971) found that *Limnodynastes dorsalis* calls after a fixed delay following a stimulus call. He later discovered that call timing in *Pseudacris streckeri* can be explained in large part by dividing the calling cycle into three phases: a 'refractory phase' immediately following each call, during which the frog does not call again; an 'excitatory phase' during which a stimulus call would elicit another call from the frog after a fixed delay; and an 'activation phase' during which the frog's call is initiated and produced and during which a stimulus has no effect (Loftus-Hills 1974).

The current paper concerns the call timing algorithm of the White-Lipped Frog (*Leptodactylus albilabris*) from Puerto Rico. This fossorial animal has a presumptive advertisement call consisting of short (30–40 ms) 'chirps' repeated fairly regularly 3 to 4 times per second. While standing next to groups of these animals in the field, we thought we could hear beats or similar sound patterns in their calling. Sometimes it sounded as though many of the frogs were calling in synchrony and at other times as though there were two subgroups producing alternate chirps, 180 degrees out of phase. Occasionally some groups seemed to shift suddenly from one of these modes to the other. Our goal was to use playback experiments to decipher the call-timing algorithm of this animal.

In previous research, when playback experiments have been used to study interactive call timing, the stimuli have been either natural calls recorded on magnetic tapes (e.g., Lemon 1971; Schwartz and Wells 1985) or synthetic calls generated by electronic sound synthesizers (e.g., Loftus-Hills 1971; Narins and Capranica 1978). In the studies reported in this paper, we combined these approaches by employing natural calls from a digital electronic recorder.

## Materials and methods

Our acoustic playback experiments with *L. albilabris* males were conducted alongside quiet roads at elevations between 650 m and 800 m in the Luquillo Mountains of eastern Puerto Rico from June 23 through July 3, 1985, and from July 19 through July 22, 1986. The El Verde field station of the Center for Energy and Environmental Research served as our field laboratory during this time. All recordings were made between 1800 and 0200 h (a period of active calling).

Single chirps from isolated calling males were recorded in the field on magnetic tape, then transferred at the field laboratory to a digital electronic recording/playback device developed by us for these studies. Because playback of the recorded vocalization can be triggered instantaneously (without the delays inherent in current tape and compact disk recorders), we believe

this device has potential value for other researchers in the field of animal communication, and we include a brief description of the device in the appendix to this paper. Once we had recorded a clear chirp positioned close to the beginning of the digital memory and with background noise amplitudes at least 30 or 40 dB below the chirp amplitude, we replayed this call in various temporal patterns by triggering the digital recorder with electrical pulses at the desired times. For most experiments, the pattern was fixed and independent of the response of the frog. In these cases we did not use the digital recorder itself in the field, but instead used it to produce specific chirp patterns that were recorded directly on cassette tapes (with a Sony WM-D6) for use as repeatable stimuli in the field. The timing patterns used to trigger the digital recorder for this purpose were generated by an electronic signal generator (B&K Precision 3020). Two types of stimulus tapes were produced.

The purpose of the Type-I tapes was to determine whether or not the frogs could phase-lock to perfectly periodic stimulus chirps. Each of these tapes contained a series of 30- or 60-s segments, each with a different stimulus chirp rate. As the experiments proceeded, three versions of the Type-I tape were used. The first began with a rate of 1 chirp per second, increased to 10.5/s, then decreased again to 1/s. When our first subject showed evidence of being able to phase-lock at all these rates, we made a second tape that went from 1/s all the way to up to 17.6/s and then back to 1/s. Again, the frog apparently phase-locked at the highest rate, so we generated a third tape with a maximum rate of 22.3/s, which represents the highest stimulus rate we could produce without overlapping successive chirps (each of which was approximately 40 ms in duration). With the equipment available to us in the field laboratory, we could detect no difference in the responses to the ascending and descending portions of these stimulus tapes, so we made the third tape with an ascending series only. On return to our laboratories in California, we verified that responses on the ascending and descending series were indeed the same. In all three of these tapes, the change in rate between adjacent steps was approximately half an octave; thus the rate roughly doubled (or halved) every two steps.

The Type-II tape was designed to test the hypothesis that the strategy employed by these frogs might be to place their chirps in gaps between the chirps of several neighboring frogs. For example, one 2-min segment of the tape had chirps presented with fixed period at a rate four times the normal calling rate for the species except that every fourth chirp was missing; thus a gap occurred periodically with a rate equal to the normal calling rate.

In another set of experiments (echo experiments), we used a protocol similar to one used by Narins (1982) with *Eleutherodactylus coqui*. In the field, a stimulus call was triggered from the digital recorder after a certain preset delay time following each of the subject's calls.

We began each experiment by locating an isolated, calling *L. albilabris* male. Then we placed a directional microphone (Sennheiser ME-88 or AKG CE-8) and a loudspeaker (Calrad 20-257 or Realistic Minimus-0.6) approximately 1 m from the frog. The microphone and speaker were oriented to minimize microphone pickup of the speaker output, and connected by coaxial cables to an observation point 10 m distant. The prerecorded conspecific calls were played to the frog from either a cassette tape player (Sony WM-D6) or our digital recording device. Stimulus volume (approx. 70 dB SPL) was selected at a level found earlier to be sufficient to elicit clear vocal responses in this species but not intense enough to elicit the presumably aggressive chuckle call (Lopez et al., in press). The stimulus volume settings had been calibrated earlier in the open at a distance of 1 m from the speaker using a sound-level meter

(GenRad 1982). A cassette recorder (TEAC R-61) was used to record simultaneously both the stimulus calls and the subject's calls on separate channels. We used a field oscilloscope (Tektronix 305 DMM) to monitor the signals during recording and confirm that the frog and stimulus calls were not being distorted before recording and that the stimulus calls from the speaker were not being picked up significantly by the microphone.

The subject's spontaneous calling was recorded for 1 min. Then the subject was presented with one or more of the stimulus protocols. During 1985, the Type-I (periodic) tapes were presented to 11 subjects. Echo experiments were done the same year with two of the animals that had been presented with the Type-I tapes and with three that had not. During 1986, four subjects were presented with the Type-II (gap) tapes. Typically one or two animals were tested per night.

The recorded calls were analyzed as follows: The data tapes were played through two lab-built threshold detectors (one for the stimulus chirp channel, one for the response channel), each adjusted so that a narrow electrical pulse was generated by the detector whenever a chirp occurred on its channel. The pulse output from each detector was accompanied by a light flash. Observing the flashes while listening to the recording allowed us continually to confirm one-to-one correspondence between chirps and pulses. To eliminate spurious pulses that might have resulted from the low-frequency wind noise present in some recordings, the amplitude of that noise was strongly reduced with a spectral filter. However, the spectrum for calls emitted by *E. coqui* overlaps extensively with that of *L. albilabris* and could not effectively be separated by spectral filtering. Therefore, occasional outbreaks of loud calling by one or more nearby *E. coqui* made a few segments of our data tapes unusable. Two-channel oscilloscope presentations of chirps and pulses allowed us to verify that the time of each pulse accurately matched the time of onset of each chirp. Minor variations in the shape of chirp envelopes introduced some variability in pulse time relative to actual chirp onset time, but the steep onset of the chirps minimized this problem, and pulse intervals consistently matched the corresponding chirp intervals to within approximately 5 ms. A systematic error of about 3–4 ms was introduced by sound propagation delays from speaker to frog and frog to microphone; however, the discussions and conclusions contained in this paper are based on features of the data that are not appreciably altered by timing errors of this magnitude, and we did not attempt to correct the data for those errors.

The train of pulses from each detector was fed into a digital computer that recorded the time (to the nearest  $\mu$ s) and the channel for each pulse. In this way a computer data file containing the source (stimulus or frog) and time of every call was stored for each subject in each experiment. These data files were used by laboratory-written software to generate the histograms and other graphs presented in this paper.

## Results and preliminary discussions

For the sake of clarity in the text that follows, a chirp produced by a subject during the experiments will be referred to simply as a 'chirp'. A recorded chirp, played to a subject as an audio stimulus in the field, will be referred to as a 'stimulus'.

The responses of subjects to the Type-I (periodic) stimulus tapes form the basis for most of the

analysis in this paper. Nine of the eleven subjects presented with the Type-I tapes interacted strongly with those stimuli. For these nine animals, the data demonstrate that call timing behavior in response to each of our stimulus rates was repeatable for each individual and remarkably consistent among all individuals. Though weaker and less precise, the calling patterns in the two remaining subjects paralleled those of the strongly responding frogs for each stimulus rate. Most of the subjects were tested either with the second or third versions of the Type-I tapes. Only one animal (frog 7) was tested with both versions, and the data from that animal are used to illustrate our analytical approach. Subsequently, by pooling available data from the entire group of strongly-responding subjects from 1985, we show that the conclusions drawn for frog 7 are consistent with data obtained from all of those subjects.

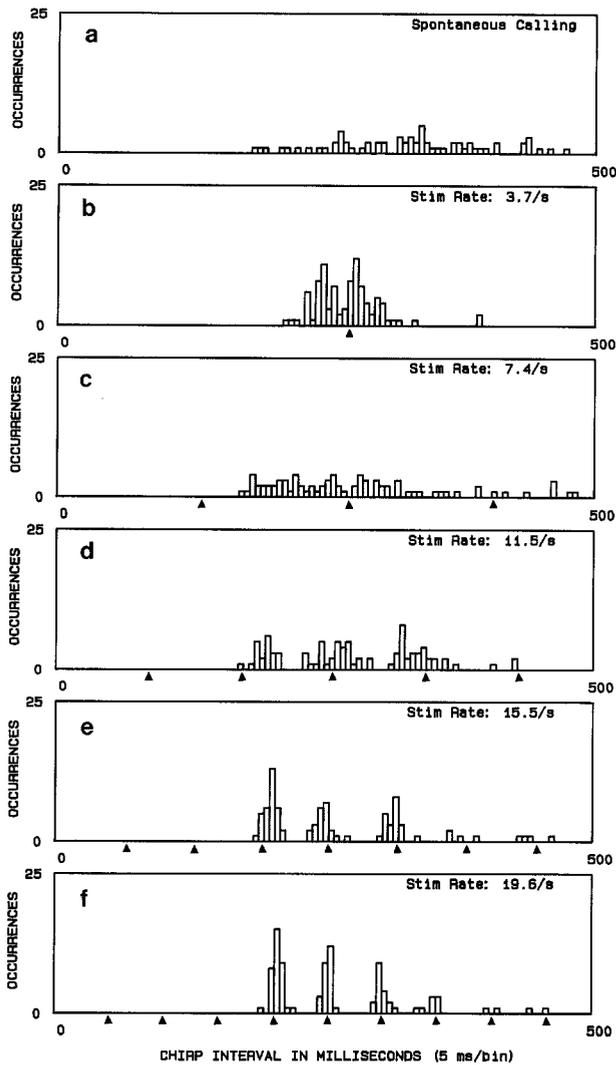
### *Chirp refractory period*

In Fig. 1, the differences in chirp distribution seen for the various stimulus rates, and the differences between those distributions and the spontaneous distribution, clearly demonstrate that this animal was actively responding to our stimuli. Regardless of stimulus rate, however, this subject never produced intervals of less than about 170 ms between the onsets of adjacent chirps. A minimum interval of from 170 to 210 ms was observed for all subjects, including the weakly responding ones, independent of stimulus protocol. Thus, this minimum interval apparently represents some sort of refractory period.

### *Hypothesis 1: constant phase algorithm*

The distributions of chirp intervals for the three highest stimulus rates in Fig. 1 show several distinct peaks, with each peak occurring at an integral multiple of the stimulus interval. The fact that the peaks are centered on integral multiples of the stimulus interval indicates that the phase of each chirp – that is, its relative placement in the interval between two adjacent stimuli – remains fairly constant for any given stimulus rate. This suggests the hypothesis that the frog's call-timing algorithm is simply to place its chirp at the same phase relative to periodic stimuli independent of stimulus rate.

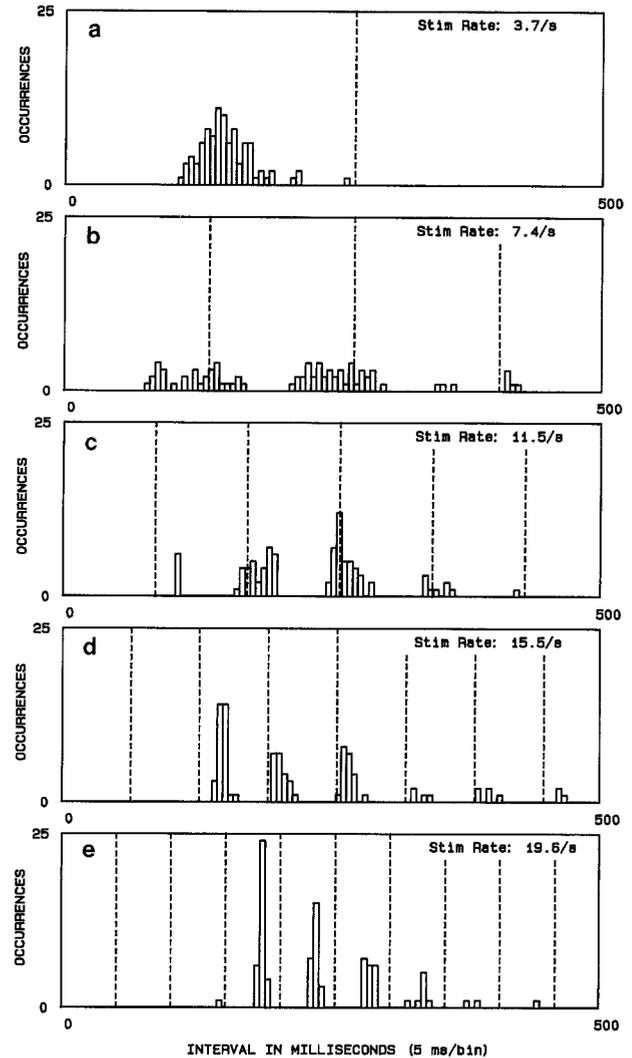
The phase of the response chirps relative to the stimuli could, in principle, range from perfect synchrony, in which stimulus and response occur at the same time, to perfect alternation, in which



**Fig. 1a–f.** Chirp interval histograms for *L. albilabris* under various stimulus conditions. These histograms show the distribution of inter-chirp intervals observed during 25 s of recorded calling with the stimulus rates indicated in the upper right corner of each graph (spontaneous calling = no stimulus presented). The small triangles are positioned at integral multiples of the stimulus period. Occasional intervals greater than 500 ms (corresponding to pauses in calling) are omitted

each chirp occurs exactly half way between two adjacent stimuli. To test the constant-phase hypothesis, we plotted the periodic-stimulus data in a slightly different way (Fig. 2). In these multicycle histograms we are able to see the phase relationships between chirps and stimuli and the distributions of chirps over the various stimulus periods following the previous chirp.

In Fig. 2 it is clear that the actual phase of the chirps relative to the stimuli varies from one stimulus rate to another; some stimulus rates result in chirps that fall between stimuli (e.g., Fig. 2a



**Fig. 2a–e.** Multicycle histograms for *L. albilabris* showing the distribution of chirps relative to stimuli at various, periodic stimulus rates. Intervals displayed in the distributions are the intervals between the onset of each of the subject's chirps and the onset of the first stimulus following the subject's previous chirp. Each graph represents 25 s of calling. Stimulus rate is given in the upper right corner of each graph. Dashed vertical lines indicate the onset times of stimuli occurring subsequent to the first stimulus following one of the subject's chirps

and 2e), and others result in chirps that are synchronized with the stimuli (e.g., Fig. 2c). Occasionally the distribution of chirp phases depended conspicuously on the stimulus cycle in which the chirps occurred (compare the third cycle with the others in Fig. 2d).

All of this demonstrates that the call-timing mechanism, whatever its nature, does not specifically evaluate the phase between the subject's chirps and the stimulus cycle and maintain it constant over all stimulus rates. Thus hypothesis 1 is eliminated.

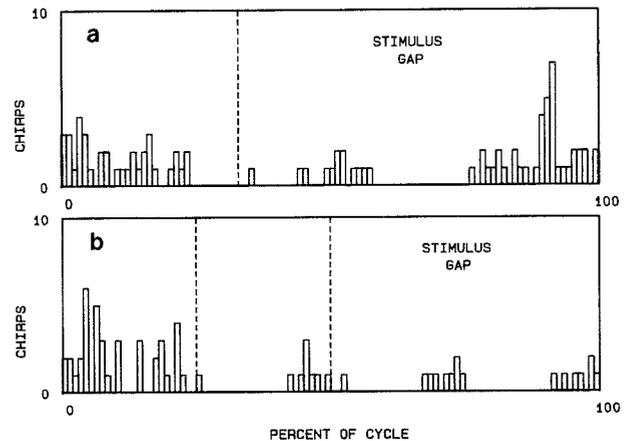
### Hypothesis 2: constant time-delay algorithm

Note that the elimination of hypothesis 1 does not eliminate the possibility that maintaining a particular phase relationship between calling neighbors is selectively advantageous. At the natural calling rate of about 3.7 chirps/s, call alternation was the observed behavior in this animal (Fig. 2a; all chirps during the 25-s sample period used are accounted for in the histogram). The presence of different phase relationships at higher (possibly unnatural) stimulus rates does not necessarily eliminate the potential importance of call alternation during normal vocal interaction. Similar call alternation with stimuli near the natural calling rate was observed in all of the other strongly responding frogs, and this suggests that avoidance of call overlap might be a normal feature of *L. albilabris* calling. If avoiding call overlap increases the fitness of these frogs, then any call timing algorithm that results in non-overlapping calls at normal calling rates would be favored by natural selection and be preserved, even if the lack of overlap occurs as a coincidental side effect of that algorithm.

One algorithm that would avoid overlap at normal rates without directly controlling phase would simply place a constant time delay between the chirp of another frog and the chirp of the responding frog. Such delays have been proposed as components of call timing algorithms in a number of other species (Lemon 1971; Loftus-Hills 1974; Zelick and Narins 1985). If a frog were to follow another frog's chirps (or our stimuli) with a chirp delayed in time by an interval equal to half of the normal chirp interval, this would lead to call alternation at normal calling rates, yet such call alternation would not be preserved at higher stimulus rates. For *L. albilabris*, this particular hypothesis can be eliminated quickly by examination of the positions of peaks in the subject's chirp distributions relative to stimulus onset times in Fig. 2. There is no consistent delay between stimulus onset times and chirp peaks, so the mechanism controlling call timing in this species is not a simple post-stimulus delay.

### Hypothesis 3: gap seeking algorithm

If several neighboring frogs were calling at once, each attempting to avoid call overlap with all of the others, then the effective chirp rate heard by any one animal could be very high. For situations with a variable number of calling neighbors, a fixed time-delay would be an inappropriate algorithm for avoiding call overlap. The remarkable ability of male *L. albilabris* frogs to phase-lock to very



**Fig. 3a, b.** Cycle histograms for *L. albilabris* in response to the Type-II gap tape stimuli described in the text. Each cycle begins and ends on the onset of a stimulus immediately following the stimulus gap. The graphs show the distribution of chirp onset times over this cycle during a 25 s sample. Dashed vertical lines indicate the onset times of stimuli that occur within the cycle. In both graphs one full cycle is 250 ms. **a** Stimulus period: 83 ms, every third stimulus missing. **b** Stimulus period: 63 ms, every fourth stimulus missing

high stimulus rates, combined with their tendency to call between stimuli presented at normal rates, suggested to us that these animals may have evolved a mechanism for placing their chirps into conspicuous gaps among overlapping chirp trains produced by several calling neighbors. It has been suggested that this type of behavior may exist in other frog species (e.g., Zelick and Narins 1985). To test this hypothesis in *L. albilabris*, we presented four new frogs with our gap tapes (Type-II). If these frogs had evolved a mechanism for avoiding call overlap with multiple calling neighbors, then we would expect an individual frog to place its chirps into the gaps in our stimulus trains. Fig. 3 shows cycle histograms for the two different stimulus rates we used for one of the frogs presented with this stimulus tape. The left edge of each graph and the vertical dashed lines correspond to the onset times of the stimuli. The chirps were not concentrated in the gaps for this animal nor for the other three frogs. Thus our gap-seeking hypothesis is eliminated.

### Hypothesis 4: delayed post-stimulus inhibition

What, then, accounts for the presence of multiple peaks and the observed phases of those peaks in the histograms? The cycle histograms of Fig. 3 and the multicycle histograms of Fig. 2 suggest that the answer, in part, might be some sort of delayed, post-stimulus inhibition. For example, the chirp

distributions in Fig. 3 seem to show a separate silent period for each stimulus in the complete stimulus cycle; and if the appropriate stimulus is assigned to each silent period, then the times from the onsets of the stimuli to the beginnings of the silent periods appear to be the same for all stimuli (approximately 55 to 60 ms). Note that the missing stimulus is not followed by one of these delayed periods of silence. Looking back to Fig. 2, we see the same phenomenon, but with the silent period beginning approximately 35 to 40 ms after the stimulus onsets. In both histograms, the silent periods lasted for approximately 40 ms. This same phenomenon was found in all strongly responding frogs we tested (with the 35–40 ms delay and 40 ms duration of Fig. 2 being typical).

The echo experiments, in which each chirp from the subject triggered (after a preset delay) a stimulus call from the digital recorder, yielded data directly bearing on this notion of delayed inhibition. Two subjects from our group of eleven and three subjects not tested with periodic stimuli were tested with echo stimuli with delays starting at about 20 ms and increasing gradually in steps of a few ms at a time. At the shorter delays, the subjects continued to chirp, and we were unable to detect any unusual calling patterns that might have resulted from the echo. As we increased the delay, we eventually reached a point (between about 30 ms and 90 ms depending on the subject) at which the animal's calling either became intermittent (1 frog) or ceased altogether (4 frogs). The intermittent caller would stop calling for periods of many tens of seconds, then emit one or two chirps (each eliciting an echo), and stop again. When the echo delay was just below the value that finally halted calling, two of the subjects emitted the presumptively aggressive chuckle-like calls.

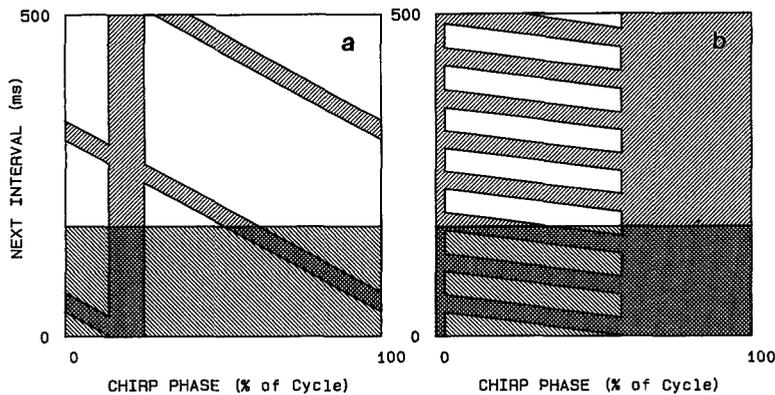
None of the four silent subjects resumed calling at all during the hour or more that we waited for each of them. Even playing recordings of naturally timed conspecific calls failed to restore calling; in other situations, such playback almost always elicited vocalizations from frogs that had recently stopped calling. It is interesting that the delays that silenced the frogs all placed stimuli roughly within the period of post-stimulus inhibition that would have followed the frog's call. Subjects thus appear to be intolerant of chirps initiated by a neighbor during the span of time when that neighbor's calling should have been suppressed by delayed inhibition.

It is not clear, however, that just one such chirp is sufficient to silence a subject. In every echo experiment, after the time delay had been switched

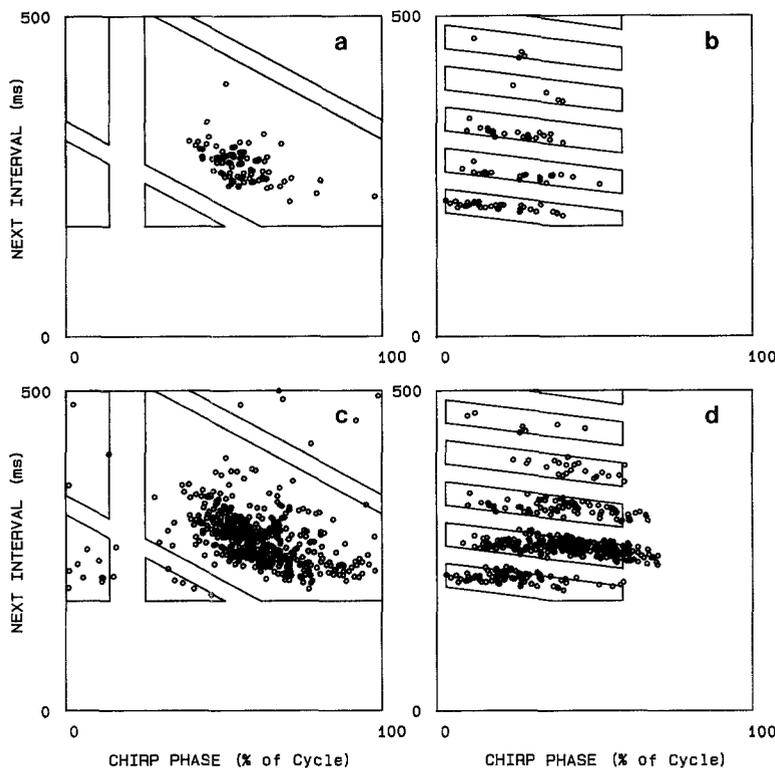
to the value that eventually silenced the subject, the animal produced several chirps and received several apparently offending echoes before ceasing its calling. Typically, after remaining silent for several seconds, the subject would emit two or three more chirps, each eliciting an echo. Then the animal would remain quiet. These results suggest that the calling gap resulting from delayed inhibition is an essential component of the interaction between two calling male *L. albilabris*.

#### *Hypothesis 5: sufficiency of refractoriness and delayed inhibition*

So far, the data have revealed the existence of two important timing elements that lead to absences of chirps. One is the refractoriness following a chirp; the other is the delayed inhibition following a stimulus. These effects can be combined on a single graph if we have a reference point for the previous chirp as well as a reference point for the preceding stimulus (i.e., a combination of interval and cycle histograms). This concise presentation is achieved if one plots an 'interval versus phase' graph, in which the interval following a chirp is plotted against the phase of that chirp (relative to the stimulus cycle). Such graphs are displayed in Fig. 4 for two stimulus rates. The shaded areas are 'forbidden regions', which correspond to periods of inhibition or refractoriness or both. The unshaded boxes enclose 'allowed regions', in which chirps are not eliminated by either of these constraints. The value (171 ms) for refractory period and that (30 ms) for post-stimulus inhibition period each was the minimum observed over all stimulus rates (not just those presented in the figures) for frog 7. The time of onset (38 ms) of post-stimulus inhibition was the maximum observed for frog 7. In this way, we have made conservative estimates (consistent with the data) of the boundaries of the forbidden regions. It is important to recognize that these boundaries are not predictions, nor do they provide any new data; they merely summarize the limitations on call timing that we already have established from the data. It should be noted that the chirp distributions of Figs. 2 and 3 very likely are discrete approximations of continuous probability density functions. Therefore, the gaps that we see in these histograms may simply correspond to regions of very low probability of chirp production. In that case, our forbidden region boundaries should be regarded as contours along continuous probability density surfaces (i.e., probability density functions of two variables: chirp phase and interval). If our data



**Fig. 4a, b.** Interval versus phase diagrams for two stimulus rates: **a** 3.7/s (one cycle = 270 ms), and **b** 15.5/s (one cycle = 64 ms). The crosshatched areas indicate regions in each diagram that correspond to the refractory period (hatching running upper left to lower right) and/or the period of delayed inhibition (hatching running lower left to upper right). Because the axes are interdependent (e.g., any movement in phase along the horizontal axis produces a corresponding movement in time along the vertical axis) the period of delayed inhibition appears not only as a vertical band, but also as a series of diagonal bands. The boundaries of the regions were determined from data for *L. albilabris* (frog 7) as described in the text. Allowed calling regions remain unshaded



**Fig. 5a–d.** Interval versus phase scatter diagrams for *L. albilabris* showing each interchirp interval plotted against the phase (relative to the stimulus cycle) of the chirp beginning that interval. The boxes outline the allowed chirp regions and are the same as those defined in Fig. 4.  
**a** Response of frog 7 to a periodic stimulus rate of 3.7/s (one cycle = 270 ms).  
**b** Response of frog 7 to a periodic stimulus rate of 15.5/s (one cycle = 64 ms).  
**c** Responses of 7 different *L. albilabris* (including frog 7) to a stimulus rate of 3.7/s.  
**d** Responses of 5 different *L. albilabris* (including frog 7) to a periodic stimulus rate of 15.5/s. 25 s of data are displayed for each animal in each of these diagrams

sets had been larger, we might well have had occurrences of chirps in the forbidden regions of Fig. 4.

By viewing the distributions of chirps over the allowed regions of Fig. 4, we can determine whether refractoriness and delayed inhibition together are sufficient to account for the observed call-timing behavior in these frogs. This is accomplished in the scatter diagrams of Fig. 5.

At high stimulus rates, such as the 15.5/s rate of Fig. 5b, chirps were distributed over much of

the area in the allowed regions, though they became less frequent in regions corresponding to longer interchirp intervals. At the lower stimulus rate of 3.7/s, near the natural calling rate, the forbidden regions occupy a much smaller proportion of the total chirp space (Fig. 5a). In spite of the relaxed restrictions, however, the chirps are distributed in only a small area near the center of one of the large allowed regions. Evidently, other constraints are imposed on call timing, but those constraints

are less conspicuous at high stimulus rates where the allowed regions are smaller. Indeed, it was the apparent dominance of refractoriness and delayed inhibition at higher stimulus rates that first brought them to our attention and allowed us to estimate their extent.

#### *Hypothesis 6: history dependence*

The variability in chirp intervals seen in the spontaneous and driven responses shown in Figs. 1 and 2 seem to suggest a partly probabilistic or random algorithm. On the other hand, in light of the recent revelations about 'chaos' (e.g., Chua 1987), one must consider the possibility that apparently random phenomena might arise from deterministic algorithms. For example, the number of stimuli skipped before a chirp might depend on the recent history of chirp intervals and/or the recent history of chirp times relative to stimulus times. We have made a superficial search for such deterministic interactions by means of the following types of scatter diagrams (not shown): (a) chirp interval vs chirp phase (as in Fig. 5), (b) chirp phase vs preceding chirp phase, and (c) chirp interval vs preceding chirp interval. None of these scatter diagrams showed any conspicuous deterministic relationships between the plotted variables. We also checked to see if the present chirp interval depended on the two preceding intervals. Again, no conspicuous deterministic relationship was found.

#### *A periodic process with noise and inhibition*

Most of the spontaneous inter-chirp intervals for frog 7 tended to cluster loosely around a mean value of about 320 ms (Fig. 1a). This type of distribution suggests a fundamentally periodic process with random fluctuations in its output. Although our superficial search for a deterministic but chaotic pattern in the sequence of chirp intervals was not exhaustive, it eliminated the most obvious deterministic possibilities, and we tentatively conclude that calling in *L. albilabris* is driven by a noisy (i.e., partly randomized) periodic oscillator, the output of which is modulated by refractoriness and delayed inhibition.

#### *Generality of results*

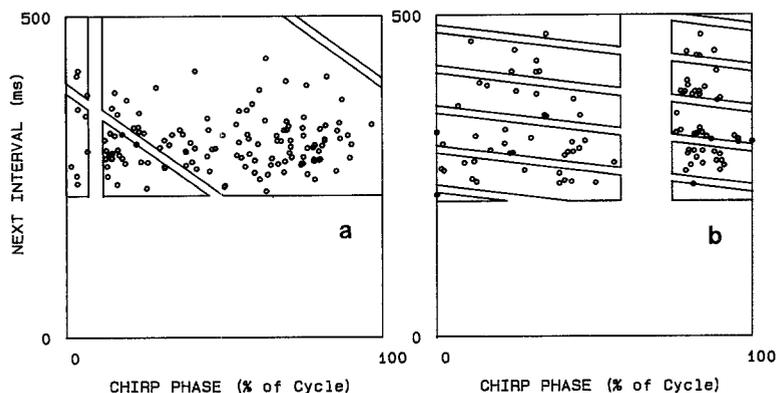
The allowed and forbidden regions indicated in Fig. 4 summarize our conclusions concerning the refractory period and delayed inhibition for a single animal, frog 7. To test the generality of these conclusions, we have pooled the data from all of the animals for which analyzable tapes were available for stimulus rates of 3.7/s (7 frogs) and 15.5/s (5 frogs) and plotted these data on top of the al-

lowed and forbidden regions deduced for frog 7 (Fig. 5c and d). The data for each subject in each graph were accumulated over a 25-s period. There are 511 data points in Fig. 5c, 547 in Fig. 5d. Although the delay between stimulus and onset of inhibition was slightly longer for some of the subjects than it was for frog 7, it is clear that the overall chirp patterns are consistent among all of these animals, and we can conclude that the call-timing algorithm in each of these subjects is essentially the same as that for frog 7. In particular, the clustering of the vast majority of the data points in Fig. 5c and d into allowed regions and the absence of points throughout most of the forbidden regions clearly demonstrate that all of these animals have both a refractory period and a period of delayed inhibition, each of which is qualitatively and (to a lesser extent) quantitatively similar to that of frog 7.

#### *Leptodactylus melanonotus*

Because *L. melanonotus* (found in Central and South America) has a chirp call very similar to that of *L. albilabris*, we briefly investigated the calling behavior of this animal for comparison with that of *L. albilabris*. We obtained call-timing data for only one individual of this species and used only periodic stimuli. The experiment was carried out in a cattle pasture at Finca La Pacifica near Cañas in the Guanacaste province of Costa Rica on July 9, 1985. The laboratory phases there also were carried out at Finca La Pacifica. A Type-I stimulus tape was generated in the manner previously described, but with a chirp recorded in the field from *L. melanonotus* (not the same individual as the subject of the playback experiment). The stimulus chirps were presented at rates ascending from 1.6/s to 22.5/s. The step size was approximately 1/2 octave. The stimulus calls were played from an Aiwa HS-P05 through a Realistic Minimus-0.6 speaker, and recordings were made with a Sennheiser ME-88 directional microphone and a Sony WM-D6 tape recorder. Playback intensity was adjusted to approximate that of a calling frog at the same distance (as estimated with the VU meter on the Sony WM-D6), but was not measured with a sound level meter.

The calling behavior of this particular individual was similar to that of *L. albilabris* in the following ways: (a) there was in fact a conspicuous call timing response to our stimuli; (b) the distributions of spontaneous and stimulus-driven chirp intervals suggested an underlying periodic process with noise; (c) the sharp lower bound on the chirp interval length seemed to imply the presence of a refrac-



**Fig. 6a, b.** Interval versus phase scatter diagrams for *L. melanonotus* at two stimulus rates:

**a** 2.8/s (one cycle = 357 ms), and **b** 16.0/s (one cycle = 63 ms). Each graph represents 50 s of data. The boxes enclose allowed regions as defined in a manner identical to that described in Fig. 4, but in this case the values for the refractory period and delayed inhibition period were measured from interval histograms and multicycle histograms of this *L. melanonotus* individual

tory period; (d) there was apparently some delayed post-stimulus inhibition; and (e) there was no constant, post-stimulus delay and no phase relationship between chirps and stimuli that was consistent across the various stimulus rates.

The presence of a refractory period and delayed inhibition can be seen in the two panels of Fig. 6, which are interval versus phase plots, like those of Figs. 4 and 5. The allowed and forbidden regions shown were determined in the manner described for Fig. 4, but with data from this *L. melanonotus* individual. The chirp refractory period (approximately 220 ms) of this animal was comparable to that (approximately 170–210 ms) of *L. albilabris*. The post-stimulus inhibition period (approximately 10 ms in duration) was conspicuously shorter than that (30 ms or more) of *L. albilabris*. The delay (approximately 40 ms) between onset of stimulus and onset of the inhibition period in *L. melanonotus* was comparable to the typical values (35–40 ms) in *L. albilabris*. At normal calling rates (approximately 2.5/s) for *L. melanonotus*, the delayed inhibition period occupied only about 2–3% of the calling period compared with about 11–13% for *L. albilabris*.

### General discussion

Several authors have noticed that frogs of some species can be entrained to stimuli (Foster 1967; Lemon 1971; Loftus-Hills 1971; Zelick and Narins 1985), but two features of entrainment in *L. albilabris* make it different from that reported for the other species. First, phase-locking extends to stimulus rates that are six to eight times the natural calling rate of these frogs, and five times their theoretical maximum calling rate (given the observed refractory period). To the best of our knowledge, entrainment to stimuli at such extreme rates has not been reported for any other frog species. The second feature is the change in behavior observed at stimulus rates beyond those at which the frog

can produce one chirp for each stimulus. At stimulus rates comparable to their natural calling rate, frogs of this species and others apparently entrain so that each stimulus is followed after a certain delay by exactly one response call. As the stimulus rate is increased, however, a threshold is reached at which the frog no longer maintains this one-to-one call matching. In previously reported cases one of two things happens at this threshold: either the frog falls out of beat with the stimulus and reverts to its spontaneous, somewhat irregular, calling patterns (e.g., Loftus-Hills 1971, 1974), or it shifts suddenly to a single new call interval that skips a fixed integral number of stimuli (2, 3, and possibly more, depending on the stimulus rate) between each call (e.g., Foster 1967; Zelick and Narins 1985). In contrast, the multimodal histograms in Fig. 1d, e, and f indicate that *L. albilabris* is not restricted to a single chirp interval for a given high stimulus rate, but instead can skip any of a range of stimulus intervals before calling again.

The chirp refractory period implied by all of the histograms and scatter diagrams in this paper might be imposed by the peripheral mechanics of calling (e.g., lung filling) or might represent a minimum recycling time for a neural oscillator, such as that suggested for the control of call timing in *Pseudacris streckeri* by Loftus-Hills (1974).

Post-stimulus inhibition periods have been found in other anurans and regulate vocal interactions both within species (Lemon 1971; Rosen and Lemon 1974; Loftus-Hills 1974) and between species (Littlejohn and Martin 1969). However, the post-stimulus inhibition seen in *L. albilabris* differs from these others by being delayed. Thus, in this animal, the inhibitory response to one stimulus can occur after an intervening stimulus. For example, in Fig. 3b, two silent periods occur in the stimulus gap.

The data for stimulus rates near the natural calling rate (Fig. 2a) imply that the normal interaction between two calling neighbors is call alterna-

tion. Perhaps the changes in calling patterns that we perceived and that led us originally to investigate the dynamics of chorusing in *L. albilabris* are simple consequences of such alternation. Specifically, if we were listening to two frogs that were both alternating with a third individual slightly farther away, we might have heard mainly synchronized calling. If these individuals suddenly started interacting with each other instead of the third frog, we would have heard the pattern suddenly switch to call alternation. As mentioned, we often heard groups of *L. albilabris* males suddenly switch from calling in apparent synchrony to apparent alternation, and vice versa. Thus, large choruses of *L. albilabris* males might comprise large numbers of interacting duets.

The tight clustering of points in the scatter diagrams of Fig. 5a and c and the reduced spread in the chirp distribution for stimuli near the natural calling rate (Fig. 1b) relative to that for spontaneous calling (Fig. 1a) indicate that the call-timing algorithm of *L. albilabris* involves at least one, as yet uncharacterized, element beyond those identified in this paper. On the other hand, for the one *L. melanonotus* subject studied, a noisy periodic oscillator modulated by refractoriness and delayed inhibition alone seems sufficient to explain the uniform distribution of points seen in Fig. 6.

Thus, if the single *L. melanonotus* individual studied is typical of his species, then it appears that there is one qualitative difference between the call-timing algorithms of *L. albilabris* and *L. melanonotus*. The other differences, however, appear to be minor quantitative ones, and the fundamental algorithms probably are very similar.

In general, the degree of similarity between the call-timing algorithms of two species of anurans probably reflects the phylogenetic proximity of those species. Since two or more different call-timing algorithms may yield the same calling pattern, such algorithms are somewhat removed from the direct selective pressures sculpting vocal patterns or any other overt phenotypic characteristic (including morphology). Thus, such algorithms are partly shielded from convergence and other evolutionary processes that can obscure phylogenetic relationships. Indeed, future comparisons of call-timing algorithms among the Anura may provide more reliable estimates of phylogenetic proximity between species than the morphological features traditionally employed.

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## Appendix

Here we provide a brief description of the circuitry and operation of the digital electronic recording device so that interested researchers may construct a similar device for their own purposes.

Unlike conventional tape recorders or synthetic call generators, this device allows for the possibility of interactive playback using actual recorded calls, whereby a call from the subject is used to trigger playback of a prerecorded call, possibly with some arbitrary predetermined delay or other experimental timing modification.

The essential components of the device are a 10 kHz clock, an 8 kilobyte random access memory (RAM), an address counter, an analog to digital (A/D) converter, a digital to analog (D/A) converter, and a 4 kHz low pass filter, all interconnected as diagramed in Fig. 7. The filter cutoff frequency, the clock rate, and memory size were chosen using the Nyquist Sampling Theorem and our knowledge of the duration and energy distribution of the calls of *L. albilabris* (Lewis and Narins 1985) to ensure accurate reproduction of those calls. The duration and energy distribution of the calls of *L. melanonotus* are within the limits imposed by these components and, therefore, also were recorded and reproduced without distortion by this device.

To record a frog call, the RAM is 'write-enabled' so that it can record data, and an electronic pulse generated by a push button or external device such as a signal generator is used to start the recording by resetting the address counter to zero. With each subsequent clock pulse (i.e. 10 000 times/s), the amplitude of the incoming signal at that moment is sampled and digitized by the A/D converter, then stored at the memory address specified by the address counter. Each clock pulse also

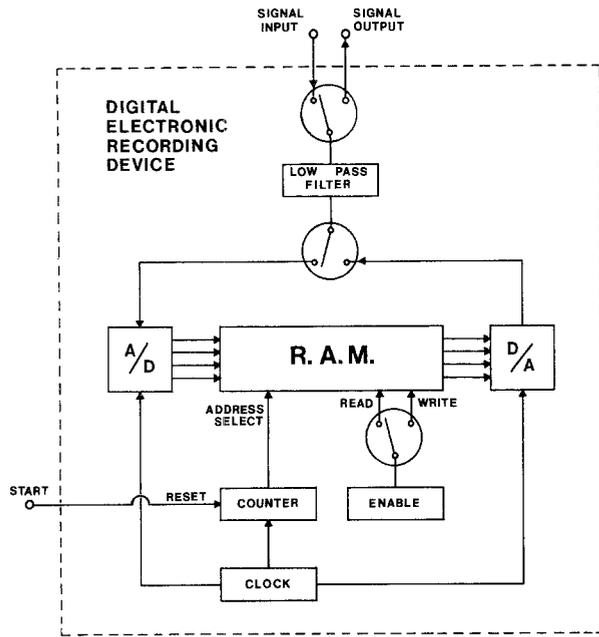


Fig. 7. Simplified schematic drawing of our digital electronic device for recording and playing back frog calls. The three switches shown are all moved to the left for recording a call and to the right for playing back a call. Arrows indicate the direction of information flow. *A/D* = Analog to digital converter; *D/A* = digital to analog converter; *R.A.M.* = random access memory

causes the address counter to step once so that the digitized values of the incoming signal are stored sequentially in the memory. To play back a recorded call, the RAM is 'read-enabled' and the address counter is again reset to zero. The address counter starts counting again, and each time it does so the digital value stored at the specified address in memory is read and passed through the D/A converter, which transforms it back into an analog signal. The low pass filter is used on the input during recording to ensure that extraneous high frequencies in the signal will not cause distortion of the frog call during playback, and the filter is used on the output during playback to smooth the signal from the D/A converter and recover the original frog call. We recommend that anyone building a device of this type for battery-powered field work use components with low power consumption such as the complimentary metal oxide silicon (CMOS) integrated circuits.

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