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FORUM

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Is habituation a mechanism for neighbor recognition in green frogs?

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Introduction

Acoustically mediated neighbor recognition is prevalent among territorial songbirds (see reviews in Falls 1982; Stoddard 1996), and is also known to occur in several mammals (see Temeles 1994), an anuran amphibian (Davis 1987) and a fish (Myrberg and Riggio 1985). Few studies have attempted to determine how territorial residents learn to discriminate between the acoustic signals of neighbors and strangers. Habituation, defined as a response decrement to repeated stimulation that does not result from sensory adaptation or effector fatigue (Thompson and Spencer 1966), is a form of learning that is commonly suggested as a mechanism of neighbor recognition (e.g., Shettleworth 1998), and some empirical evidence supports this hypothesis (reviewed in Peeke 1984). Peeke (1984) suggested that aggressive responses to repeated or continuous exposure to a neighbor should exhibit three defining characteristics of habituation (response decrement, retention, and recovery to a novel stimulus). Therefore, habituation should produce long-lasting, stimulus-specific decrements in aggression exhibited toward familiar neighbors, while preserving the ability to respond aggressively to unfamiliar animals, thereby satisfying the requirements of a behavioral mechanism of neighbor recognition.

Owen and Perrill (1998) recently examined habituation of the aggressive response of territorial male green frogs (*Rana clamitans*) to the acoustic signals of simulated new neighbors to determine whether aggression directed toward new neighbors exhibits the three characteristics of habituation that would satisfy Peeke's (1984) requirements for a behavioral mechanism. In two field playback experiments, they recorded changes in aggression in response to repeated broadcasts of synthetic advertisement calls during two training sessions separated by a 15-min retention interval and in response to a novel stimulus broadcast immediately after the second training session. They defined three components of an aggressive response: movement, high-intensity advertisement calls (Wells 1978), and encounter calls, which are characterized by lower dominant frequencies compared to advertisement calls (Bee and Perrill 1996). Owen and Perrill concluded that habituation of aggression satisfies Peeke's (1984) requirements, and suggested that habituation allows males to discriminate between the acoustic signals of familiar and unfamiliar males. Here, we argue that the study by Owen and Perrill falls short of demonstrating habituation as a mechanism of neighbor recognition on several conceptual and methodological grounds.

Short-term and long-term habituation

A distinction between short-term habituation (STH) and long-term habituation (LTH) was first suggested over 40 years ago (Sharpless and Jasper 1956). STH refers to the response decrement observed within a single habituation training session. LTH is typically measured as decreased responsiveness across repeated habituation training sessions or on a remote test after some intervening period of no stimulation (e.g., Petrinovich 1984; Wagner 1976). LTH results from the long-term retention or memory of the habituation stimulus and can be retained on the order of weeks (e.g., Carew et al. 1972; Leaton 1974). Although the exact interstimulus interval parameters that distinguish LTH from STH differ across species and procedures, investigators examining LTH often allow retention intervals between 1 and 24 h to transpire after a stimulus exposure to ensure that short-term effects are given sufficient time to dissipate (e.g., Beck and Rankin 1997; Marlin and Miller 1981).

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According to Peeke (1984), a mechanism of neighbor recognition should produce effects that are "long lasting (at least long enough to cover a breeding cycle in cases where territoriality is concerned with protecting a breeding area)" (p. 397). Elsewhere, Peeke argues that habituation "should not be a transient phenomenon, rather it should have some permanence, at least over a period of days" (Peeke et al. 1979, p. 172). In his studies of habituation of territorial aggression in white-crowned sparrows (*Zonotrichia leucophrys*), Petrinovich (1984) assessed long-term effects across days. Changes in responsiveness that occurred during a single day of testing were considered short-term effects. Clearly, Peeke (1984) and Petrinovich (1984) maintain that LTH is required for habituation to function as a mechanism of reduced aggression between established territorial neighbors. Owen and Perrill did not test for the effects of LTH by re-assessing the level of aggressive responsiveness over a behaviorally relevant retention interval.

As evidence of retention, Owen and Perrill reported significantly fewer movements (their Fig. 2) and a significant reduction in the time required to reach their response decrement criteria (their Fig. 4) in the second training session compared to the first session. We agree that habituation was retained between sessions. However, retention over a 15-min interval does not constitute evidence of a long-term effect, as defined by Peeke (1984) and Petrinovich (1984). Consider, for example, the study by Peeke et al. (1979), which examined the retention of habituated territorial aggression in three-spined sticklebacks (*Gasterosteus aculeatus*). In their "short-term retention group," Peeke et al. (1979) re-tested males after a 3- to 5-day retention interval. Their "long-term retention group" was re-tested after a 14-day retention interval. Significant retention was demonstrated after 3–5 days, and some residual effects of habituation were also noticeable after 14 days. Thus, there are clear differences in what Peeke (1984) intended by "long lasting" effects and what the study by Owen and Perrill demonstrated.

An important question is whether territorial male green frogs would be required to remember the calls of nearby neighbors for potentially long periods of time. For example, should males be expected to remember their neighbors' calls between nights of chorusing? During their breeding season, males establish and defend territories that females use as oviposition sites (Wells 1977, 1978). Wells (1977) has shown that males can occupy a single territory for periods lasting up to 7 consecutive weeks. Although males shifted territories frequently, 76% of all males occupied a single territory for a week or more, and 25% did so for at least 4 weeks. Martof (1953) reported similar observations in an earlier study of territoriality in green frogs and noted two additional social phenomena. First, males exhibited a "clustering effect" in which small groups of two to five frogs tended to remain together when changes in territory residency occurred. Second, and more importantly, males within clusters tended to stay in the same relative positions with regard to one another when these changes took place. Moreover, these small groups of males maintained stable spatial relationships for periods as long as 43 days.

This early work by Martof (1953) and Wells (1977) strongly suggests to us that territorial male green frogs would potentially benefit from recognizing their nearby neighbors over intervals of days to weeks, and certainly between nights. We do not consider Owen and Perrill's demonstration of reduced aggression after a 15-min retention interval to be evidence for a long-term effect. Rather, we consider the response decrements they observed to be the result of STH, because stimulus exposures occurred during two closely spaced training sessions on a single day. Because the study by Owen and Perrill did not investigate "long-lasting" effects over a behaviorally relevant time interval, such as between days, it also does not demonstrate that habituation is a mechanism of neighbor recognition in green frogs, since such a claim requires the long-term retention of the effect.

Stimulus specificity of habituation

A common characteristic of habituation is that response decrements to a particular stimulus exhibit recovery to a novel stimulus. Peeke (1984) argued that response decrements to a specific neighbor mediate the low levels of aggression between neighbors, and that a tendency to respond to effective novel stimuli allows residents to behaviorally discriminate between familiar and unfamiliar animals. Owen and Perrill presented males with a novel stimulus that differed from the training stimulus by changes in the frequency spectrum and repetition rate. In experiment 1, the training stimulus had a dominant frequency of 350 Hz and was presented at a rate of 1 per 15 s, while the novel stimulus had a dominant frequency of 450 Hz and was presented at repeated successive intervals of 5, 10, and 15 s. In experiment 2, the dominant frequencies and repetition rates of the training and novel stimuli were reversed.

Owen and Perrill did not provide adequate data to support their claim that aggressive responses recovered upon presentation of the novel stimulus. For example, data for changes that occurred in two of three components of aggression (movements and high-intensity advertisement calls) in response to the novel stimuli are not reported. The only evidence provided to demonstrate recovery of habituated responses is a significant decrease in the dominant frequency of calls given in response to the novel stimulus compared to unsolicited control calls in experiment 2 (their Fig. 3), indicating the recovery of aggressive calling. This comparison suggests that one of three components of the aggressive response recovered in one of two experiments. Given the absence of other relevant data, we consider this result to be rather weak evidence of stimulus specificity. In addition, we note that Owen and Perrill compared responses to the novel stimulus to unsolicited control calls, which were recorded prior to the presentation of any stimulus as a baseline measure of calling behavior. Such a comparison does not "indicate that male green frogs can discriminate familiar from unfamiliar stimuli" (p. 209), as Owen and Perrill suggested, because responses to the unfamiliar stimulus were compared to calls that were not given in response to any stimulus. To test the hypothesis that males actually discriminated between familiar and unfamiliar stimuli, an appropriate comparison in their study would have been to compare calls given at the end of the second training session (End-2) to those given at the beginning of the novel stimulus broadcast.

Owen and Perrill also did not carefully consider possible explanations for the presumed response recovery. For example, Owen and Perrill "altered the delivery rate as well as the dominant frequency [of the novel stimuli] to add enough variation to simulate two different frogs" (p. 210). Because the training and novel stimuli differed along two dimensions, frequency and repetition rate, their experiments were not designed to determine the precise perceptual basis of the presumed renewed aggression to the novel stimulus. Nevertheless, the authors clearly imply in their Discussion that the recovery observed in experiment 2 can be attributed to the differences in spectral frequency between the training and novel stimuli. In fact, it is equally plausible that differences in stimulus repetition rate were solely responsible for eliciting recovery.

We also note the absence of recovery in experiment 1, in which the dominant frequency of the novel stimulus was 100 Hz higher than the training stimulus (instead of lower, as in experiment 2; see their Fig. 3A). No explanation for this asymmetry in responses to the novel stimuli is provided, although a consideration of one hypothesis is warranted. In response to real and simulated intruders, the defining characteristic of aggressive vocalizations by male green frogs is a decrease in the dominant frequency of the call (Bee and Perrill 1996; Bee et al. 1999). A possible explanation for the recovery of the response to a 100-Hz decrease in dominant frequency (their experiment 2) is that males responded to a perceived territorial challenge in the form of a sudden switch to aggressive calling by the simulated neighbor. Although a 100-Hz decrease is larger than the decreases typically observed during playback tests (M.A. Bee, S.A. Perrill, P.C. Owen, unpublished data), the hypothesis that the 350-Hz novel stimulus was perceived not as a new male, but as an aggressive one, is worth considering.

Conclusions and recommendations

The contributions of the study by Owen and Perrill (1998) to our current understanding of habituation as a potential mechanism of neighbor recognition are limited for three main reasons. First, Owen and Perrill did not demonstrate that repeated broadcasts of conspecific calls can produce long-lasting effects on aggressive responsiveness in the form of LTH. Consequently, they have not demonstrated that habituation can be a mechanism underlying neighbor recognition, since neighbor recognition in the field requires long-term retention (Peeke 1984). Second, Owen and Perrill concluded that they have demonstrated recovery of aggressive responses to a novel stimulus, that this recovery was likely due to a difference in the frequency spectrum of the novel stimulus, and that this ability probably allows males to discriminate between familiar and unfamiliar animals based on size-related frequency differences in their calls. We believe that these conclusions are unjustified due to the absence of relevant data (e.g., the extent of recovery for movements and high-intensity advertisement calls to the novel stimulus), inappropriate statistical comparisons (e.g., comparing responses to the novel stimuli to unsolicited control calls), limitations of experimental design (e.g., changing the novel stimulus along two dimensions), and the failure to consider a legitimate hypothesis for the asymmetric recovery of aggressive calling. Third, Owen and Perrill examined a mechanism of neighbor recognition in a species that is not currently known to discriminate between neighbors and strangers. Because green frogs are not known to exhibit this behavior, we find their results difficult to place within a relevant context.

We offer the following recommendations to future investigators who may examine habituation as a mechanism of neighbor recognition. First, it is critically important to choose species for study that are known to discriminate behaviorally between neighbors and strangers in their natural environment. Second, we generally agree with Peeke's (1984) treatment of this subject and emphasize here that demonstrations of response decrement, recovery to a novel stimulus, and long-term retention over behaviorally relevant time intervals be considered minimal criteria for assessing the role of habituation as a mechanism of neighbor recognition. Researchers should also be aware of studies that suggest a role for more complex cognitive processes (e.g., Beecher et al. 1996; Godard 1991; Richards 1979). Third, it is important to report how each component of aggression changes over the course of habituation training and during tests of recovery and long-term retention. Fourth, to test the hypothesis that habituation allows residents to distinguish between familiar and unfamiliar stimuli, the appropriate comparisons must be made (as discussed earlier). Finally, if the perceptual basis of neighbor recognition is investigated using an habituation paradigm, future investigators should strive to alter novel stimuli along single, independent dimensions where possible, especially when using computer-generated sounds or video images. This procedure would avoid complicating the interpretation of recovery to the novel stimulus and would allow researchers to draw firmer conclusions about the cues that mediate recognition. By considering these recommendations, future investigators may avoid several potential problems associated with tests of habituation as a mechanism of neighbor recognition.

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