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Labial-licking for Chemical Sampling by the Leopard Gecko (*Eublepharis macularius*)

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Lizards perform several distinct types of lingual protrusions, including tongue-flicks directed to environmental substrates, tongue-flicks passing through the air, but not contacting a substrate (e.g., Burghardt et al., 1986), and short extensions directed to the individual's own labial scales. Tongue-flicking, i.e., outward protrusion of the tongue directed at a substrate or into the air, is a primary method of sampling environmental chemicals for transfer to the vomeronasal organ for sensory analysis (Gillingham and Clark, 1981; Young, 1990, 1993; reviewed by Halpern, 1992). In labial-licking the tongue contacts only the anterior and lateral labial scales and adjacent surfaces surrounding the mouth. The tongue is neither swept through a volume of air nor brought into contact with a substrate beyond the lizard's body. Instead, the ventral tip and other portions of the tongue are rubbed on the labials.

To examine the possible relationship between labial-licking and chemical cues and to detect possible differences in timing of and relative frequencies of labial-licks and tongue-flicks in response to chemical cues, we studied the lingual responses of the leopard gecko (*Eublepharis macularius*) to prey chemicals and an odorless control substance presented on cotton swabs rubbed on the oral epithelium. When presented prey chemicals on cotton swabs held anterior to their snouts in an earlier study, leopard geckos performed labial-licks and tongue-flicks; after directing tongue-flicks to the swabs, they frequently bit them (Cooper, 1996). Leopard geckos and their relatives use their tongues extensively while foraging (Evans, 1961; Vogel, 1979; pers. obs.) and can discriminate prey chemicals from control stimuli (Dial, 1978; Cooper, 1996). After experimental removal of bitten prey, leopard geckos attempt to relocate prey by strike-induced chemosensory searching (Cooper et al., unpubl. data) Based on earlier findings of elevated lingual protrusions in response to prey chemical stimuli in *E. macularius* and other lizards (Deperno and Cooper, 1993, 1994; Cooper, 1996; Cooper et al., unpubl. data), we predicted that both tongue-flicking and labial-licking would be elevated and that labial-licking would be elevated rapidly, but briefly.

Sixteen adult *E. macularius*, all longterm captives, were housed individually in plastic terraria (41 cm × 28 cm × 23 cm), each having a ventilated plastic cover

and a plastic shelter. The ambient room temperature was maintained at 28–30 C and heat lamps allowed thermoregulation during the lighted portion of a 12:12 LD cycle. Water and food were available *ad libitum* prior to the experiment. The lizards were not fed for three days prior to each experimental condition to ensure equal feeding motivation for each stimulus condition.

Each lizard was tested twice: once in each condition on separate days. To prevent the lizards from hiding from the experimenter, the plastic shelters were removed from the terraria 20 min prior to each experimental condition and replaced immediately at the conclusion of that trial. Trials were conducted on 21 and 24 April 1994 between 1200 and 1500 h Eastern Standard Time.

Lingual responses of each lizard were observed after two stimulus presentations in which the cotton tip of an applicator bearing distilled water or distilled water and prey surface chemicals was inserted into the lizard's mouth. The distilled water stimulus, an odorless control for effects of the experimental procedures on lingual protrusion, was prepared by dipping the cotton tip of a 15 cm wooden applicator into distilled water. The cricket stimulus was prepared by pressing the cotton tip of an applicator wetted as above directly against the body of a live cricket and rolling it repeatedly over the cricket's integument. A fresh swab was prepared prior to each trial.

To start a trial, the investigator slowly approached the terrarium, partially removed the lid of the terrarium, and gently grasped the lizard. The swab was then pressed against the lateral labials and sometimes the anterior labials to induce opening. Then, the swab was rolled across the palate, presumably resulting in transfer to the vomeronasal organs (Graves and Halpern, 1989), and was then removed from view. The lizard was gently released in its cage and immediately the number of tongue-flicks and labial licks were counted in one minute intervals for five consecutive minutes.

Responses of lizards were tested in a randomized block design with sequence of stimulus presentation counterbalanced. Parametric analysis was precluded by nonnormality of the distributions of lingual protrusions. Nonparametric techniques of analysis of variance are inappropriate for a condition × time design. As a compromise, Wilcoxon signed-ranks matched-pairs tests were used to analyze differences between control and experimental conditions and between behaviors (Hollander and Wolfe, 1973). Because tests were conducted separately for each of five minutes, significance levels were adjusted downward from the usual $P < 0.05$. Using the Bonferroni adjustment, $P < 0.01$ (two-tailed) was required for significance (Wright, 1992).

Many labial-licks occurred immediately in the cricket condition after the prey chemicals had contacted the labials and the oral epithelium, but rates of labial-licking decreased rapidly to a minimum in the third minute and remained at low levels during the final two minutes (Fig. 1). The difference in labial-licks (Fig. 1) between the cricket and the control conditions was significant during minute 1 ($T = 7.5$, $N = 14$, $P < 0.005$). Differences in the remaining minutes were not significant (minute 2, $T = 38.5$, $N = 15$, $P > 0.10$; minute 3, $T = 13.5$, $N = 9$, $P > 0.10$; minute 4,

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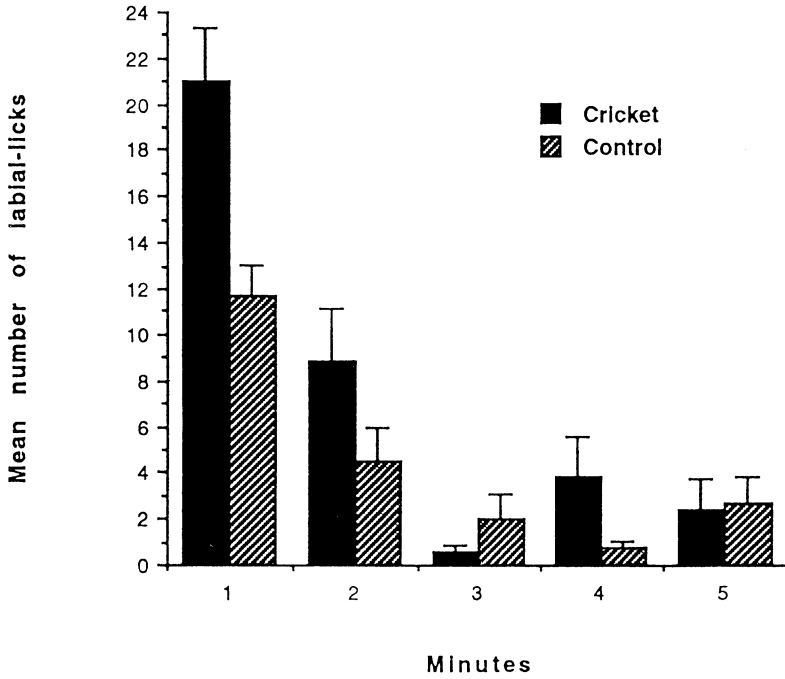


FIG. 1. Mean numbers (bars) and standard errors (vertical lines) of labial-licks performed in five consecutive minutes by 16 adult *Eublepharis macularius*.

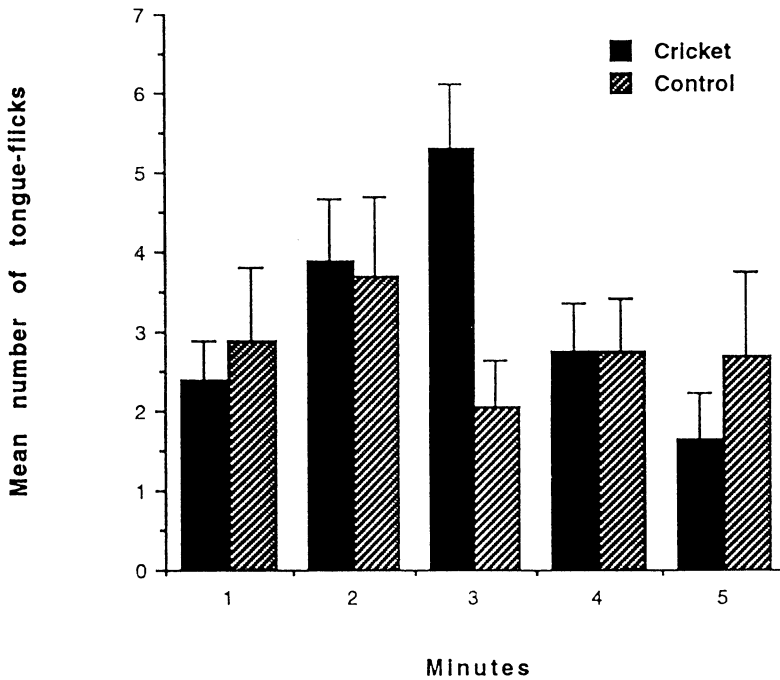


FIG. 2. Mean numbers (bars) and standard errors (vertical lines) of tongue-flicks performed in five consecutive minutes by 16 adult *Eublepharis macularius*.

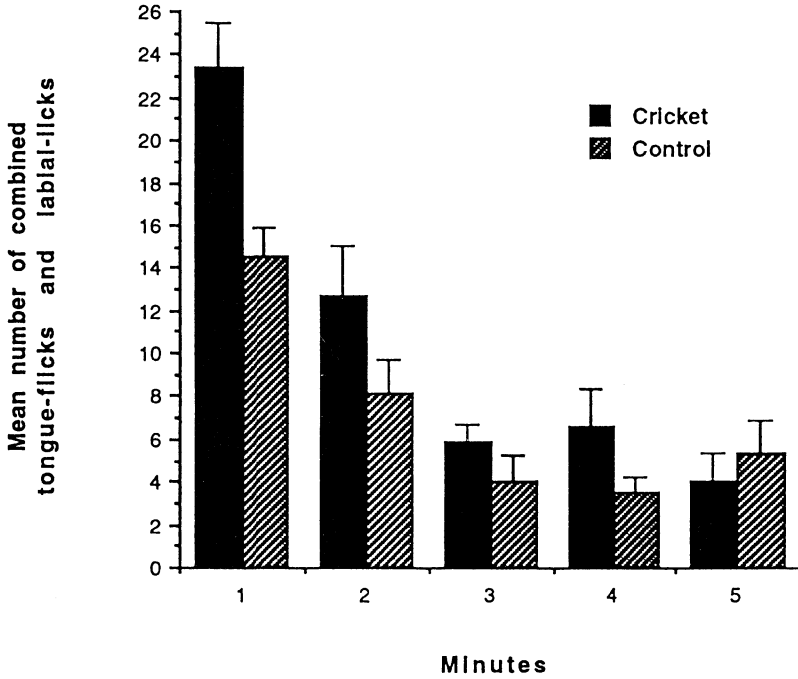


FIG. 3. Mean numbers (bars) and standard errors (vertical lines) of pooled labial-licks and tongue-flicks performed in five consecutive minutes by 16 adult *Eublepharis macularius*.

T = 8, N = 8, $P > 0.10$; minute 5, T = 20, N = 9, $P > 0.10$).

Relatively few tongue-flicks occurred in the first two minutes of the cricket condition, but tongue-flicks increased to a peak in the cricket condition during minute three and decreased thereafter (Fig. 2). The difference in tongue-flicks (Fig. 2) between the cricket and the control condition was significant during minute 3 (T = 12, N = 14, $P < 0.01$), but the differences were not significant in the remaining minutes (minute 1, T = 50, N = 13, $P > 0.10$; minute 2, T = 44.5, N = 13, $P > 0.10$; minute 4, T = 63, N = 16, $P > 0.10$; minute 5, T = 24, N = 12, $P > 0.10$).

The difference in combined tongue-flicks and labial-licks (Fig. 3) between the cricket and the control conditions was significant only during minute 1 (T = 9.5, N = 14, $P < 0.01$). Differences in the remaining minutes were not significant (minute 2, T = 44.5, N = 16, $P > 0.10$; minute 3, T = 33, N = 15, $P > 0.10$; minute 4, T = 49, N = 16, $P > 0.10$; minute 5, T = 52.5, N = 15, $P > 0.10$).

The timing and rates of tongue-flicking and labial-licking differed greatly. Relative numbers of labial-licks and tongue-flicks in the cricket condition changed substantially during the experimental interval. During the first minute, there were significantly more labial-licks than tongue-flicks (T = 0, N = 16, $P < 0.001$). In the second minute the difference was not significant (T = 29, N = 15, $0.05 < P < 0.10$). Relative numbers reversed in the third minute when there were significantly more tongue-flicks than labial-licks (T = 1, N = 14, $P < 0.001$). Frequencies of the two types of lingual protrusion did not differ significantly

in the final two minutes (minute 4, T = 44.5, N = 13, $P > 0.10$; minute 5, T = 25, N = 13, $P > 0.10$).

In the control condition, significantly more labial-licks than tongue-flicks occurred in minute 1 (T = 9, N = 15, $P < 0.005$). In minutes three and four more tongue-flicks than labial-licks were performed, but the difference was significant only in minute four (minute 3, T = 14, N = 12, $0.05 < P < 0.10$; minute 4, T = 6, N = 10, $P < 0.05$). Numbers of the two types were similar in minutes two (T = 43, N = 14, $P > 0.10$) and five (T = 23, N = 10, $P > 0.10$).

Both tongue-flicking and labial-licking were elevated over control rates, indicating that these behaviors are responses to prey chemicals. Labial-licking may be a response to vomeronasal and/or gustatory stimulation as a result of biting prey or tongue-flicking prey chemicals. If prey chemicals are present on the labials, labial-licking could effect further delivery of prey chemicals to the vomeronasal organs (Graves and Halpern, 1989; Cooper, 1994).

Labial-licking has several possible functions. It may occur in contexts unrelated to chemosensory sampling or response. The squamate tongue participates in drinking, grooming, displaying, and food capture and manipulation (e.g., Schwenk and Throckmorton, 1989; Bels, 1994; Cooper, 1994). Labial-licking often immediately follows swallowing (Cooper, 1994), presumably serving a grooming function. Possible chemosensory functions include sampling chemicals on the labials for vomerolfactory or gustatory analysis and redistributing chemicals on the tongue to enhance transfer to the vomeronasal organs. The possible importance of labial-licking for vomerolfaction

might be assessed by observing effects of experimentally blocking the vomeronasal ducts (Graves and Halpern, 1989) on labial-licking rates.

Relative numbers of both labial licks and tongue-flicks in each of the two conditions followed similar time courses, accounting for the similar temporal pattern of significance of differences between the two lingual behaviors in the two conditions. Why labial-licking increases and declines before tongue-flicking increases in response to prey chemicals is unknown. Any explanation must take into account that the two types of lingual protrusion are mutually exclusive: A lizard cannot tongue-flick and labial-lick simultaneously. The rapid decline of labial-licking in response to prey chemicals is consistent with previous data from studies of strike-induced chemosensory searching (Deperno and Cooper, 1993, 1994). Therefore, although handling might have affected rates of labial-licking, it did not greatly modify the time course.

Although both tongue-flicking and labial-licking may reflect vomerolfaction (Cooper and Burghardt, 1990) investigation of prey cues, pooling data for these behaviors might occasionally obscure important effects. This would have occurred in minute 3 in this experiment had the data not been analyzed separately. For species that frequently engage in labial-licking, it may be important to record and analyze tongue-flicks and labial-licks separately.

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Bite Scar Patterns in the Black-bellied Salamander, *Desmognathus quadramaculatus*

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Aggression is an important component of intra-specific social behavior in many plethodontid salamanders (Jaeger and Forester, 1993). Research into the combat tactics of these salamanders may facilitate a better understanding of social behaviors and their evolution. Specific combat tactics have been elucidated by the analysis of bite scar patterns in animals used in staged encounters, in recently collected individuals, and in museum specimens. Bite scars have been reported to accurately reflect observed aggressive patterns in *Aneides* (Staub, 1993), *Desmognathus* (Keen and Sharp, 1984), and *Plethodon* (Jaeger, 1981). In his analysis of combat tactics in *P. cinereus*, Jaeger (1981) showed that attacks on conspecifics are disproportionately directed toward the snout and tail. Those on the snout commonly result in damage to