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Effects of movement and eating on chemosensory tongue-flicking and on labial-licking in the leopard gecko (*Eublepharis macularius*)

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Summary. Two forms of lingual protrusion, tongue-flicking and labial-licking, were differentially affected by combinations of movement and eating conditions in a eublepharid gecko (*Eublepharis macularius*). Tongue-flicking, in which the tongue contacts substrates beyond the lizard's body, occurred at increased rates during locomotion and during locomotion was significantly more frequent after eating than in a baseline condition. Labial-licking, in which a protruded portion of the tongue touches the labial, mental or rostral scales that surround the mouth, increased after eating. Unlike tongue-flick rates, by far the highest labial-lick rates were observed in stationary lizards after eating. The elevated tongue-flicking rates during movement after eating may be a manifestation of a postingestive chemosensory search for prey. In addition to grooming, several possible chemosensory functions of labial-licking are discussed, including gustatory sampling, sampling prey chemicals on the labials for transfer to the vomeronasal system, and redistribution of chemicals on the tongue to enhance transfer. It is suggested that labial-licking might help motionless lizards maintain vigilance for visual prey stimuli associated with the specific chemical prey cues. Another possible explanation for the increased labial-lick rate while motionless after eating is that prey chemicals induce tongue-flicking, but that the distance protruded is lessened and the tongue does not contact environmental substrates. Tongue-flicking while stationary is unlikely to lead to detection of additional prey and might incur detection by the lizard's predators or prey.

Key words. tongue-flicking – labial-licking – feeding – foraging – Lacertilia – Eublepharidae – *Eublepharis*

Introduction

Lingual protrusion has been studied primarily and extensively as an indication of chemical sampling of the external environment by squamate reptiles (reviewed by Burghardt 1970; Simon 1983; Halpern 1992; Mason 1992; Cooper 1994a). However, the tongue in various lizards may be protruded in many different ways having different functions including not only chemical sampling, but grooming, prey prehension, swallowing, drinking, and feeding by licking (*e.g.*, Burghardt *et al.* 1986; Bels *et al.* 1994; Cooper 1994b). The type, extent and duration of lingual protrusion differ among social, feeding, exploratory and defensive contexts (Gove & Burghardt 1983). The tendency to touch substrates, objects, or merely to pass the tongue through the air during lingual protrusions also varies markedly in different behavioral settings (Burghardt *et al.* 1986). Because many squamates exhibit two distinct types of lingual protrusions, tongue-flicking and labial-licking, when exposed to prey chemicals before feeding or after feeding, it is important to understand the relationships of these behaviors to chemical sampling.

Tongue-flicking, in which the tongue is protruded from the mouth through a volume of air and often contacts a substrate, serves to sample chemicals that are delivered indirectly to the vomeronasal organs (Gillingham & Clark 1981; Graves & Halpern 1989; Young 1990, 1993). Thus, tongue-flicking serves as a convenient index of chemosensory investigation by vomerolfaction (Burghardt 1967; Cooper & Burghardt 1990a,b). In addition to this vomerolfactory function, tongue-flicking may gather samples for gustation (Schwenk 1985) when environmental substrates are contacted.

Labial-licking is protrusion of the tongue to contact the labial and/or rostral and mental scales and perhaps immediately adjacent surfaces; the tongue is not swept through the air or brought into contact with substrates beyond the body (DePerno & Cooper 1993, 1994; Cooper 1994b). Sometimes the tongue contacts primarily the laterally placed labials, especially after eating (*e.g.*, Cooper 1994b), but also at times after contact with prey chemicals by tongue-flicking or experimental oral contact (DePerno & Cooper 1996).

The functions of labial-licking are uncertain. Because extensive labial-licking often occurs after eating, it has been presumed to be grooming behavior. It has been suggested recently that labial-licking rids the vomeronasal organ of previous chemical samples (Desfilis *et al.* 1993). However, no necessity to do so has been established. That labial-licking increases after exposure to prey chemicals in geckos (*Eublepharis mac-*

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ularius) that have not eaten (Cooper *et al.* in review; DePerno & Cooper in review) is consistent with either the elimination hypothesis or with chemosensory investigation by gustation or vomerolfaction. For the latter, contact with the labials might somehow increase the concentration or availability of chemicals sampled by tongue-flicking or oral contact on portions of the tongue that participate in the indirect transfer of chemicals to the vomeronasal ducts.

To provide further information regarding possible functions and distinctness of the two lingual behaviors, we examine experimentally the effects of eating and movement on rates of tongue-flicking and labial-licking in the leopard gecko *E. macularius*. Eating might induce labial-licking for elimination of chemicals from the vomeronasal organs, chemosensory investigation, or cleaning; it might stimulate tongue-flicking for chemosensory investigation. Because the leopard gecko can discriminate prey chemicals from control substances sampled from cottong swabs by tongue-flicking (Cooper 1995a), post-ingestive tongue-flicking might be a primary component of search for additional prey. Lingual sampling associated with active search for chemical prey cues should increase during movement. If tongue-flicks and labial-licks serve similar functions, their rates should be strongly correlated. Therefore, we examined correlations between tongue-flicks and labial-licks within and between conditions.

Material and methods

Sixteen adult *Eublepharis macularius* were housed individually in translucent plastic terraria (51 × 26 × 32 cm), each containing a water bowl and a plastic shelter. All were long term captives maintained on a 12:12 h LD cycle under fluorescent lighting at an ambient temperature of 26–27°C. They were fed crickets to satiation twice per week. Except during the experiments, crickets often remained in the cages on nonfeeding days. Water was available *ad libitum*.

To ascertain possible effects of movement and eating on lingual behaviors, we conducted a two × two factorial experiment. Each lizard was observed once after eating a cricket and once after not eating and its lingual behaviors were recorded separately during locomotion and while the lizard was still. When only the tip of the tongue was protruded slightly, it was sometimes difficult to assign a lingual protrusion to either tongue-flicking or labial-licking. If the tip was protruded straight forward and withdrawn without pronounced contact with the scales surrounding the mouth, we designated the behavior a tongue-flick. Only when licking was obvious was the protrusion designated a labial-lick.

The experiments were conducted from 28 November–12 December 1991. Lizards were not fed for at least three days before trials to ensure that all lizards were hungry during trials. Although *E. macularius* is nocturnal, trials were conducted under fluorescent lighting in the daytime (1100–1700). Lingual behaviors were qualitatively similar to those observed at night under low intensity red light (Cooper *et al.* in review). Each lizard was tested only once per day.

Twenty minutes before trials began, an experimenter removed the lids and plastic shelters from the cages, being careful not to disturb the lizards. To begin a trial, an experimenter slowly approached the cage and either released a cricket (eating condition), or simulated release (baseline condition), then withdrew to observe. Release of a cricket was simulated by placing a tethered cricket on the floor of the cage and removing the cricket without allowing physical contact between cricket and lizard when the lizard prepared to attack. Each lizard was observed in its home cage for five minutes either immediately after completion of swallowing a cricket or beginning immediately after removal of the cricket in the control baseline condition. The latter condition indicated lingual protrusion rates in the experimental setting not attributable to attack and ingestion of prey. Sequence of eating conditions was counterbalanced.

For both types of lingual protrusion, the data examined were numbers of protrusions per second averaged over the five minute observation period. Because zero values rendered the distributions of tongue-flicking and labial-licking nonnormal, data for both types of lingual protrusion were analyzed separately using nonparametric Friedman two-way analyses of variance. Paired comparisons were conducted using procedures outlined by (Zar 1984). However, the nonparametric analyses did not allow testing for interaction between eating and movement conditions. To test for interaction, data were also analyzed by parametric analysis of variance using a two factor design with repeated measures on both factors (Edwards 1968). Because ANOVA is robust under a wide range of violations of its assumptions, it is assumed that the results give a good approximation of the interactions. Significance levels are two-tailed with alpha = 0.05.

Spearman rank correlations were conducted between the tongue-flick and labial-lick rates within each of the movement-eating conditions, between movement conditions within eating conditions, and between eating conditions within movement conditions. Because tied ranks were numerous, all correlations were corrected for ties (Siegel 1956). Alpha was set at 0.01 to compensate for the number (8) of correlations conducted.

Results

Lizards tongue-flicked at low rates in all four conditions, but the mean rate was substantially higher while moving after eating than in the remaining conditions (Fig. 1). The main condition effect was significant ($X_r^2 = 12.65$, df = 3, $P < 0.001$). Paired comparisons showed that the number of tongue-flicks per second was significantly greater while moving after eating than while moving after not eating ($P < 0.05$), while still after eating ($P < 0.05$), and while still after not eating ($P < 0.005$). Although substantially more total tongue-flicks were performed while moving by lizards that had not eaten than in either condition while not moving, no other differences were significant. Parametric analysis of variance revealed a significant effect of movement ($F = 12.62$; df = 1, 45; $P < 0.001$), but no effect of eating ($F = 2.22$; df = 1, 45; $P > 0.05$) and no interaction between movement and eating ($F = 1.82$; df = 1, 45; $P > 0.05$).

Lizards labial-licked frequently while remaining still after eating, infrequently while moving after eating or while at rest without having eaten, and not at all while moving without having eaten (Fig. 2). The main condition effect was significant ($X_r^2 = 22.86$, df = 3, $P < 0.001$). Paired comparisons showed that the number of labial-licks per second was significantly greater while not moving after eating than in each of the other conditions ($P < 0.001$ each). No other differences were significant according to the Friedman test, but six individuals performed at least one labial-lick while moving after eating whereas no lizard labial-licked while moving without having eaten (sign test, $P = 0.03$, two-tailed). Parametric analysis of variance showed significant effects of eating ($F = 177.86$; df = 1, 45; $P < 0.001$) and movement ($F = 133.93$; df = 1, 45; $P < 0.001$). However, the interaction between eating and

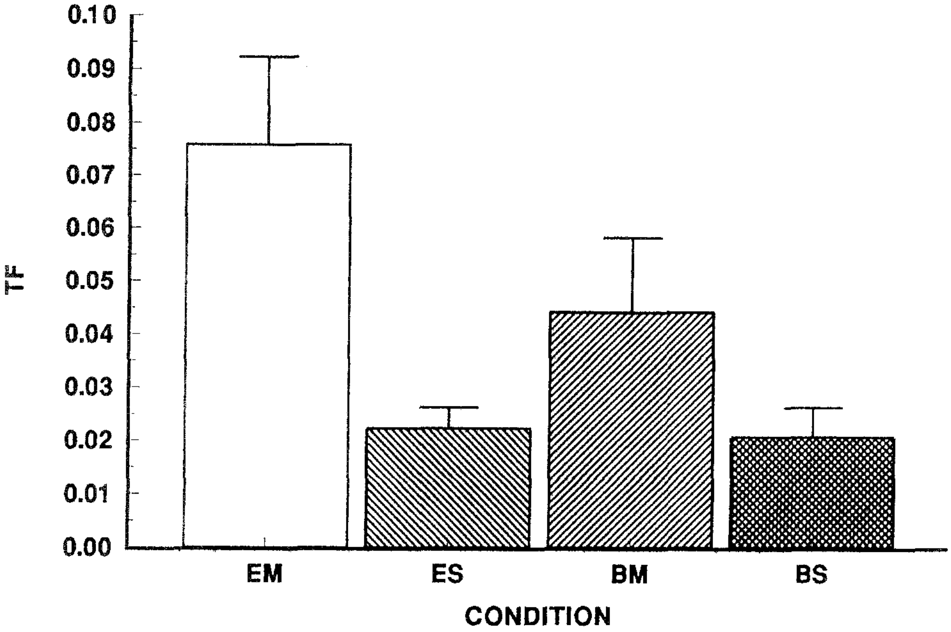

Fig. 1 Mean tongue-flicks (TF) per second (+SE) by moving (M) and stationary (S) leopard geckos (*Eublepharis macularius*) after eating (E) and in baseline (B) condition without exposure to prey chemicals

movement was significant (F = 36.87; df = 1, 45; $P < 0.001$).

Tongue-flicks/sec while moving were not significantly correlated with labial-licks/sec while still after eating ($r_s = -0.11$, n = 16, $P > 0.05$), and were marginally, but not significantly, correlated in the baseline condition ($r_s = 0.53$, n = 16, $P < 0.05$). Tongue-flicks/sec while still were not significantly correlated with labial-licks/sec after eating ($r_s = -0.02$, n = 16, $P > 0.05$) or not eating ($r_s = 0.00$, n = 16, $P > 0.05$). Tongue-flicks/sec after eating were not significantly correlated with labial-licks/sec after not eating either while moving ($r_s = 0.00$, n = 16, $P > 0.05$) or still ($r_s = 0.06$, n = 16, $P > 0.05$). Tongue-flicks/sec after not eating were not significantly correlated with labial-licks/sec after eating either while moving ($r_s = -0.16$, n = 16, $P > 0.05$) or still ($r_s = 0.14$, n = 16, $P > 0.05$).

Discussion

Tongue-flicking rates increased during locomotion. In a wide taxonomic range of lizards, tongue-flicking rates are believed to increase in association with locomotion (Evans 1961), especially in active foragers that can identify prey chemicals (Cooper 1994b,c, 1995b) sampled by tongue-flicking. The eublepharid *E. macularius* forages actively, but moves more slowly and deliberately then typical active foragers, such as teiids and most lacertids, and tongue-flicks at lower rates (Cooper, qualitative observations).

Although the interaction between movement and eating was not significant according to the parametric test, the nonparametric analysis of variance showed that the tongue-flicking rate while moving was higher after eating then after not eating. In a previous study we (Cooper *et al.* in review) found only a suggestion that tongue-flicking rates by leopard geckos increased after prey that had been bitten was removed from the lizards' mouths and movement rates increased only after a delay of about five minutes (possibly due to experimental handling of lizards). Those results hinted that leopard geckos may perform strike-induced chemosensory searching, but did not firmly demonstrate its presence.

The present finding that the tongue-flicking rate increases more during movement after eating than during movement when the lizards had not eaten suggests the presence of a postingestive chemosensory search for additional prey. The low, nearly identical rates of tongue-flicking while not moving in geckos that had and had not eaten is also consistent with this interpretation because additional chemical cues to location of prey are unlikely to be obtained at the feeding site. Contact with the substrate at that site would gather only redundant information. Other prey items at that spot would very likely be detected and attacked based on visual or auditory stimuli. On the other hand, while a lizard is moving, it may encounter chemical information regarding the location of additional prey.

Labial-licking rates increased after eating, but only while the lizards remained motionless. The absence of any increase in labial-licking rate during movement accounts for the significant interaction between eating and movement. Nevertheless, the greater number of individuals that labial-licked while moving after eating than while motionless in the baseline condition suggests that eating does produce a minor general increase in labial-licking, but that the increase is much greater while motionless.

Although some labial-licking appears to be grooming (*e.g.*, Bels *et al.* 1994; Cooper 1994b), most labial-licks observed during this study lacked the hallmark of such grooming: relatively great protrusion of the tongue combined with broad wiping of the labials along most of one side. Instead the lizards often protruded only the tips of their tongues and contacted only the rostral, mental, and anteriormost labial scales. These

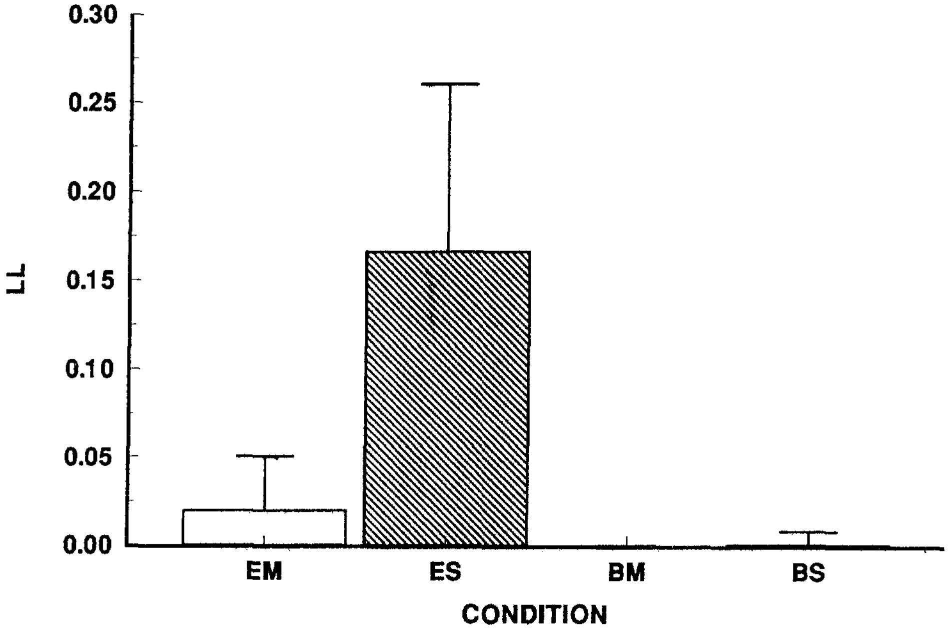

Fig. 2 Mean labial-licks (**LL**) per second (+SE) by moving (**M**) and stationary (**S**) leopard geckos (*Eublepharis macularius*) after eating (**E**) and in baseline (**B**) condition without exposure to prey chemicals

labial-licks appeared to be investigatory. Even lingual protrusions involving broad wiping may have some investigative function. A previous study showed that labial-licking increased in response to the presence of prey chemicals in the mouth (DePerno & Cooper 1996). Possible chemosensory functions of labial-licking include gustatory sampling, sampling of prey chemicals for transfer to the vomeronasal system, and redistribution of chemicals on the tongue to enhance transfer to the vomeronasal system.

That a vast majority of labial-licks were performed by motionless lizards after eating whereas tongue-flicks occur largely during locomotion suggests that labial-licks and tongue-flicks may have different functions. If labial-licking has any investigatory function, it appears to involve extraction of information from chemicals already sampled during biting, ingestion, or tongue-flicking. We have suggested that labial-licks might serve to sharpen or maintain vigilance for visual prey stimuli associated with the chemical cues (Cooper *et al.* 1996; DePerno & Cooper 1996). Their association with stillness is consistent with visual search and suggests a role for additional chemical sampling or resampling to maintain stimulation levels.

Differences in timing also hint that labial-licks and tongue-flicks may have different functions. Tongue-flick rates by leopard geckos exposed to prey chemicals did not increase until the third minute after exposure (Cooper *et al.* 1996; DePerno & Cooper 1996), but labial-lick rates increased immediately. In previous studies of two species of iguanian lizards (DePerno & Cooper 1993, 1994) and leopard geckos (Cooper *et al.* 1996; DePerno & Cooper 1996), labial-licking increased rapidly after exposure to prey chemicals by biting or introduction into the mouth on cotton swabs.

In the latter study labial-licking returned to control levels in the second minute, but in a study of strike-induced chemosensory searching in *E. macularius* (Cooper *et al.* 1996), labial-licking rates remained elevated for several minutes after exposure to prey chemicals. This might be interpreted as indicating that resampling by lizards while motionless helped maintain vigilance longer after visual, chemical, and tactile exposure to the prey than after exposure to chemical cues alone. An alternative hypothesis is that chemical stimulation induces tongue-flicking, but that this is partially suppressed while lizards are stationary in the absence of visual prey cues, resulting in labial-licks.

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