Olfaction: Responses of a Decapod Crustacean Are Enhanced by Flicking

Abstract. *Periodic movements of the olfactory organs, known as "flicking," temporally enhance the response of the olfactory receptors of the spiny lobster to changes in stimulus concentration. This reflex provides the lobster with a physiological mechanism to compensate for the indiscrete temporal nature of chemical stimuli.*

In diverse organisms, the sense of smell is associated with intermittent interaction between the odor environment and the chemosensory organ. Roaches and moths vibrate their antennae in the presence of pheromone odors (*1*). Cyclosmate fish pass water over the olfactory lamellae in concert with mouth movements and the inhalation cycle (*2*). Salamanders ventilate over the olfactory and vomeronasal surfaces at a basal rate of one to two per second (*3*). Snakes, which rely on the vomeronasal system more than other vertebrates, flick their tongues, thereby delivering odors into the vomeronasal pouches in phases (*4*). Mammals tidally flow air over the olfactory mucosa "sniffing" odors (*5*). These phenomena increase in rate at the onset of novel odors, denoting their active role in odor perception. Although one might assume that this intermittent interaction has some fundamental significance for the underlying chemoreceptive process, physiological studies of the effects of periodic sampling on the sense of smell have been few. In mammals, electrophysiological investigation of the olfactory bulb and lateral hypothalamus shows that responses to different odorants are patterned as a result of the superimposed inhalation cycle (*6*). The various degrees of central neural organization in animals that exhibit intermittent olfactory sampling argue that the physiological effect of this phenomenon is at a lower level in the chemosensory sequence, likely at the receptors themselves. We have studied the olfactory system of a decapod crustacean to demonstrate intermittent sampling and describe its effect on the unitary discharge of primary olfactory receptors.

In decapod crustaceans, the olfactory sense is concentrated in sensilla borne on the paired bifurcate antennules (first antennae) and can respond to nanomolar quantities of L-amino acids and related compounds (*7*). Although both filaments are chemosensory (*8*), behavioral evidence (*9, 10*) shows that only the lateral filament is necessary for orientation to distant odor sources. Lateral-filament chemosensitivity is commonly attributed to the exclusive presence of aesthetasc setae on that filament. These setae, each innervated by as many as 400 bipolar receptor cells (*11*), are arranged in rows in a densely packed tuft on the ventral face of the distal third of the filament, in a position to be maximally affected by periodic sharp downward movements of the filament known as flicking. High-speed cinematography shows that flicking splays out the otherwise tightly packed aesthetasc hairs, presumably allowing increased exposure to the surrounding chemical environment (*12*). Flicking occurs spontaneously at about 1 Hz, depending on the species, and reflexively increases in rate in the presence of novel stimuli of diverse modalities. In the spiny lobster, *Panulirus argus*, an organism with long filamentous antennules, flicking can double in rate with the onset of amino acid stimuli (*13*). Since flicking is maximal at the onset of chemical stimulation, and since a preliminary analysis of antennular chemoreceptors showed that most were unaffected by flicking during and after adaptation (*13*), we hypothesized that flicking modulates the response onset. We report here that flicking temporally enhances the discharge of the primary receptors by interrupting a chemical diffusion barrier, presumably created by the dense packing of the receptor hairs.

We mounted excised antennules in a Lucite recording chamber with the aesthetasc hair tuft extending into an olfactometer. Two linearly increasing concentration profiles of the amino acids taurine or glutamic acid served as stimulants (*14*). Densitometry studies showed that chemical stimuli flowed through the olfactometer with a homogeneous front in the vertical plane to assure that receptors, moved through the vertical plane by flicking, would not be exposed to different concentrations during any one flick. The stimulus exited the olfactometer to prevent possible stimulation of other parts of the preparation. Perfusion of the antennular artery with *Panulirus* saline (*15*) maintained preparation viability. Chemoreceptor discharge was recorded extracellularly, amplified with conventional capacity-coupled electronics, and stored on magnetic tape for subsequent photography and analysis. Flicking was elicited in one of two ways: (i) actively, by electrically stimulating the motoneuron of musculus reductor$_4$, the muscle that naturally produces flicking in *P. argus* (*9, 16*) or (ii) passively, by mechanically deflecting the filament with a Lucite rod attached to a pen-driver motor, in turn driven by a ramp voltage to approximate the natural flicking movement. A photoresistor placed across the recording chamber monitored movement of the filament and allowed the two modes of flicking to be compared; passively elicited flicks resembled active flicks in velocity, maximum displacement, and duration. This conclusion was supported by the identical responses of antennular mechanoreceptors, which respond to actively and passively elicted flick movements with phasic discharge. We compared the discharge of single chemoreceptor neurons in response to chemical stimulation with and without concomitant flicking at 1 Hz. Stimulation with flicking preceded and followed stimulation without flicking to avoid bias.

Chemoreceptors of the aesthetasc responded to a shallow gradient of taurine stimulation by gradually increasing their discharge frequency (Fig. 1A, middle trace). This discharge could continue for more than 3 minutes without adapting. With flicking, the response began sooner and with a higher initial frequency (Fig. 1A, upper trace) (Table 1). The initial response consisted of a series of phasic bursts, each beginning at the end of the downstroke of the flicks. These bursts were superimposed on an ever-increasing rate of discharge that followed the overall increase in stimulus concentration (Fig. 1B). Passively flicking the antennule elicited a discharge pattern similar to that observed with active flicks (Fig. 1A, lower trace), which demonstrates that the observed effects were not due to ephaptic excitation of the chemoreceptors. In addition, flicking increased the maximum frequency of discharge to a given stimulus and decreased the time by which the maximum frequency was reached (Table 1). However, the mean frequency of discharge throughout the first 40 seconds of the response was not significantly different between flicked and unflicked trials (Table 1), which indicates that the chemoreceptor responses were not potentiated by flicking but only altered in their discharge pattern. Thus, with flicking, the overall response was not only advanced in time, but also reached a greater maximum discharge earlier (the response profile is skewed to the right) than it did in the absence of flicking.

The discharge of antennular chemoreceptors followed the rate of rise of the stimulus. Stimulating with a greater rate

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of increase of stimulus concentration, one rising 24 times more rapidly than the shallow gradient, elicited a more robust response, but the relative effect of flicking on this response was similar to that observed with the more shallow profile (Table 1). With either stimulus profile, the response with flicking started approximately 5 seconds sooner and reached a maximum frequency 25 to 38 percent higher and 11 to 13 seconds sooner than did the response without flicking. These results indicate that the observed phenomena are independent of the rate of stimulus onset. Flicking produced the same qualitative effects on the responses of other receptors to glutamic acid stimulation as those described for taurine-sensitive receptors. This observation suggests an independence of stimulus quality on the effects induced by flicking.

To test whether the effect of flicking persists in steady-state stimulation, we stimulated the preparation with the steep taurine gradient and waited 6 seconds after the limiting concentration had been reached before flicking the antennule. The discharge frequency doubled on the first flick in a phase-locked burst, but increased only slightly on subsequent flicks; the overall response frequency continued to decline to preflick levels (Fig. 1C). The lack of a sustained effect of flicking in steady-state stimulus conditions supports the hypothesis that the primary function of flicking is to resolve changes in the odor environment.

The observed phenomena can be explained if we assume that the receptors in the densely packed aesthetasc hair tuft are not freely in contact with the surrounding chemical environment and that flicking serves to increase water circulation into the hair tuft, as proposed by Snow (*12*) on morphological grounds. To test this theory, we devised a simple assay to determine whether an antennule immersed in a solution of tryptophan and flicked would pick up more amino acid than the same antennule immersed without flicking (*17*). In such trials, the mean amount of amino acid loaded with flicking was twice as great as that without flicking (*18, 19*). Further, immersion of an antennule in the tryptophan solution for upward of 30 seconds without flicking loaded the hair tuft less than a 1-second immersion with one flick. The hair tuft is apparently a barrier to diffusion of dissolved substances and the splaying effect of the flicking movement removes this barrier, allowing stimuli to pervade the tuft's interior spaces. This conclusion is supported by the ability of one flick to enhance receptor discharge after 6 seconds of exposure to $10^{-4}M$ taurine (Fig. 1C). Intermittently increased rates of stimulus-receptor interaction (*20*), reflecting superimposed increases in stimulus concentration at the receptor sites because of flicking-modulated accessibility of the aesthetasc hair tuft, would account for the observed pattern of receptor discharge with flicking.

Temporal enhancement of the receptor discharge summed over repetitive flicks enhanced the detection of changes in the lobster's odor environment by creating a greater peristimulus discharge to a given increment of odor change than would have occurred in the absence of flicking. The basis of the demonstrated temporal enhancement was the individual flick. That behavioral responses of *P. argus* to dilute taurine stimulation applied directly to the aesthetasc tuft occurred within 1 second of stimulation (*21*) indicates that spiny lobsters, like other organisms (*22*), can at least partial-

Table 1. Effects of flicking on responses of *P. argus* antennular chemoreceptors to taurine stimulation. Data were subjected to a two-way analysis of variance and subsequent a posteriori testing of the means (*19*). The tests showed that differences between flicked and nonflicked trials accounted for the significantly different variances. Onset frequency refers to spikes per second during the first 2 seconds of a response. Onset time is the start of the response relative to the start of stimulus flow. Maximum frequency is the maximum discharge rate sampled according to 100-msec time bins. Time to maximum is the time of maximum frequency relative to the start of stimulus flow. Mean frequency is the spikes per second over the first 40 seconds of the response to the shallow gradient or 18 seconds to the steep gradient. Abbreviation: N.S., not significant.

Condition	Onset frequency (spike/sec)	Onset time (sec)	Maximum frequency (spike/sec)	Time to maximum (sec)	Mean frequency (spike/sec)
Shallow stimulus gradient (N = *10 units*)					
Flicking, 1 Hz	14 ± 2	26 ± 2	72 ± 8	34 ± 3	25 ± 3
No flicking	10 ± 2	29 ± 3	50 ± 8	44 ± 4	20 ± 3
Flicking, 1 Hz	16 ± 2	24 ± 2	66 ± 8	31 ± 3	22 ± 3
Mean difference*	5 ± 1	(−)5 ± 1	19 ± 7	(−)11 ± 2	N.S.
Steep stimulus gradient (N = *5 units*)					
Flicking, 1 Hz	47 ± 19	13 ± 1	126 ± 24	16 ± 1	47 ± 15
No flicking	23 ± 8	18 ± 2	78 ± 14	29 ± 4	39 ± 11
Flicking, 1 Hz	49 ± 22	12 ± 0.5	104 ± 16	16 ± 0.5	44 ± 12
Mean difference*	N.S.	(−)6 ± 2	37 ± 9	(−)13 ± 4	N.S.

*Average value of the two flicking trials minus the no-flicking value.

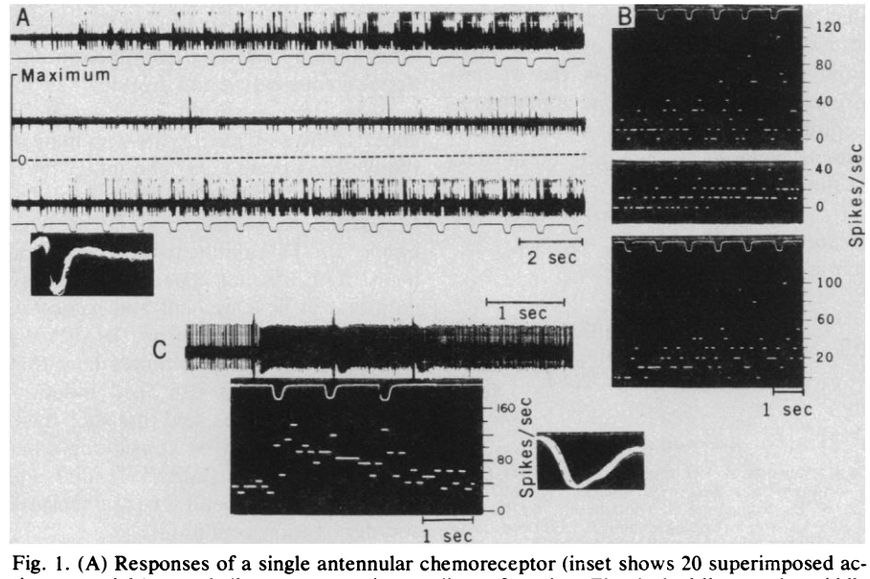

Fig. 1. (A) Responses of a single antennular chemoreceptor (inset shows 20 superimposed action potentials) to a shallow concentration gradient of taurine. The dashed line on the middle trace depicts the stimulus profile relative to the concentration reference points at the left margin; the dashed line rises from 0 to about 4 percent of maximum ($10^{-4}M$) concentration over 18 seconds. (Upper trace) Response of the cell with active flicking at 1 Hz. (Middle trace) Response without flicking. (Lower trace) Response with passively elicited flicking at 1 Hz. The photoresistor output shown below the upper and lower traces marks flicks, a downward movement indicating antennular depression. (B) Frequency plots depicting the first 6 seconds of the three responses in (A). (C) Response of a single antennular chemoreceptor (inset shows 20 superimposed action potentials) to a steady-state concentration of taurine ($10^{-4}M$) with active flicking at 1 Hz. Flicks are marked by artifacts of large amplitude. The associated frequency plot details the response.

ly discriminate chemical stimuli with information contained in spike trains shorter than 1 second, such as those following individual flicks. Repetitive flicking could therefore provide multiple points for discriminating gradual odor changes. The increased flicking rates accompanying initial detection of a novel odorant would further the lobster's temporal resolution of its odor environment.

Olfaction usually affects behavior at a great enough distance from the source of an odor that the initial onset and subsequent changes in odor concentration are likely to be gradual and haphazard rather than continuously incrementing, like the changes studied here. Flicking in near-threshold, gradually changing stimulus conditions elicits phasic bursts of spikes in otherwise silent receptors (Fig. 1, first several flicks of the upper trace). The resulting ability to better detect near-threshold changes in stimulus concentration may account for the spontaneity of flicking in the unstimulated animal.

The finding that flicking temporally enhances the detection of, and emphasizes any change in, the lobster's chemical milieu is in harmony with the hypothesis (*23*) that chemical cues are the weakest of the stimulus modalities in the temporal and spatial domain. We propose that flicking represents a physiological mechanism in the lobster to compensate for the inherent temporal weakness of olfactory stimuli. Since all organisms must deal with this characteristic of olfactory stimuli, it is likely that the responses of primary chemoreceptors in the diverse organisms exhibiting phasic stimulus-receptor interaction (such as sniffing and tongue flicking) are similarly modulated in time.

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16. Excitation of the muscle produces natural deflections between 0.3 and 1.5 cm vertical displacement measured at the proximal end of the hair tuft (*13*).
17. Tryptophan was selected for its solubility in water and strong absorption spectrum concentrated in the ultraviolet range [A. White, P. Handler, E. E. Smith, *Principles of Biochemistry* (McGraw-Hill, New York, 1964), p. 94]. Freshly excised antennules were washed by repetitive flicking in seawater for 1 minute. The hair tuft was then gently immersed in $10^{-3}M$ tryptophan for 2 seconds, during which it was either flicked twice (1 Hz) or held steady. The tuft was withdrawn, rinsed by repetitive flicking in 1 ml seawater for 10 seconds, and the absorbance of the rinse read at 280 nm.
18. Spectrophotometric measurements of the amount of tryptophan loaded by five antennules in a total of 15 flick-no-flick-flick sequences varied significantly between flicked and nonflicked trials determined by a two-way analysis of variance ($P < .001$) and a posteriori testing of the means (sum of squares simultaneous test procedure, $P < .001$) (*19*).
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Feeding and Behavioral Activation in Infant Rats

Abstract. *Three-day-old rats that were separated from their mothers and deprived of food were found to be capable of feeding either from small puddles of milk or when milk was infused into the front of their mouths. Such feeding was accompanied by a dramatic increase in behavioral activity and only occurred in a warm environment. These data demonstrate that neural systems for ingestive behavior are present at birth and suggest the existence of feeding-related arousal or motivational systems.*

It has usually been assumed that neonatal rats are not able to feed in any way other than by suckling from the mother. Spontaneous eating and drinking has only been observed in rat pups starting at about 15 days of age (*1*), the beginning of the weaning period (*2*). The infant rat is notoriously resistant to being hand-fed or coaxed to suckle from an artificial nipple (*3*). The ability to recognize and ingest food has not appeared to mature until late in development and has often been presumed to arise from the suckling behaviors of infancy. I report here that even neonatal rats can feed independently of the mother, and that such feeding is accompanied by considerable behavioral activation. Both the feeding and the activation depend on temperature and deprivation conditions.

The altricial status of the newborn rat makes it, in general, an ideal subject for studies of neural and behavioral development (*4*). In this study of the development of the feeding system in rats, 3-day-old rat pups were placed near a large puddle of milk in a clear plastic observation container (*5*). The container was housed inside a moist incubator maintained at 33° ± 1°C. Pups were tested after 1/2, 7, or 22 hours of food deprivation ($N = 5$) (*6*). Food deprivation is a primary determinant of adult ingestive behavior but has little effect on the suckling behavior of young pups (*7, 8*).

The pups ingested the diet. They probed the floor with their snouts and mouthed the milk. Moreover, they increased their intake with longer periods of deprivation. Pups deprived for 1/2, 7, or 22 hours consumed, respectively, 1.5, 3.0, and 5.3 percent of their body weight in a 1/2-hour test (*9*). The pups also exhibited a marked behavioral activation in conjunction with ingestion of the diet.

In order to study the pups' ingestion more closely a procedure was developed that allowed the experimenter to program a pup's exposure to food and then observe its behavior in a structured situation (*10*). A fine polyethylene cannula was installed under the tip of the tongue in the front of the pup's mouth (*11*). This intraoral cannula could be implanted rapidly, without trauma, and did not seem to interfere with mouthing and swallowing. Pups quickly habituated to its presence (*12*). When diet was infused through the cannula a pup could eat by licking and swallowing. Or, if the pup did not active-

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