

## ORIGINAL PAPER

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**The roles of visual and proprioceptive information during motor program choice in frogs**

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**Abstract** Previous studies have shown that leopard frogs, *Rana pipiens*, use tongue prehension to capture small prey and jaw prehension to capture large prey. After hypoglossal nerve transection, the frogs fail to open their mouths when attempting to feed on small prey, but open their mouths and capture large prey. Here, we investigate how visual information about the prey and proprioceptive information from the tongue interact to influence the motor program choice. Using pieces of earthworm of various sizes, we found that *Rana* exhibits two different behavior patterns based on prey size. The frogs captured the 1.5-cm prey using tongue prehension, whereas 2.0-cm and larger prey were captured using jaw prehension. After hypoglossal transection, the frogs never opened their mouths when they tried to feed on 1.5-cm prey. When feeding on 3.0-cm and larger prey after transection, they always opened their mouths and captured the prey using jaw prehension. When offered 2.0-cm prey, they alternated randomly between opening and not opening the mouth. Therefore, deafferentation changed the pattern of motor program choice at the behavioral border. This implies that afferents from the tongue interact with visual input to influence motor program choice.

**Key words** Feeding behavior · Deafferentation · Afferent modulation · Sensory feedback

**Introduction**

A problem that the brain must solve in planning goal-directed movements is to choose the most appropriate

motor program for the current conditions, both external (e.g., target size, distance, and velocity of movement) and internal (e.g., initial position, inertia, etc.). At some level, information about self and information about the target must interact during movement planning. In the present study, we examine how vision (information about target) and proprioception (information about self) influence motor program choice during prey capture in frogs. First, we present the results of a behavioral experiment that addresses how visual analysis of prey size is used to choose between alternative motor programs for feeding. Second, we investigate the pattern of choice at the sensory border between alternative motor programs. Finally, we present data on how visual information interacts with proprioceptive information to influence the choice of motor programs at the sensory border.

Prey capture in frogs is a goal-directed behavior in which a sensory stimulus (usually visual) concerning the prey item initiates a centrally programmed motor output (Grobstein et al. 1983). Although frogs and toads have been shown to snap in response to tactile stimulation (Comer and Grobstein 1981) and olfaction has been suggested to contribute to prey recognition (Shinn and Dole 1978), visual input appears to be the dominant sensory modality. When vision is intact, anurans seldom lunge or snap at prey items that are not moving (Lettvin et al. 1959; Kaess and Kaess 1960; Ewert 1985; Satou and Shiraishi 1991; personal observation).

Anuran feeding behavior, while usually referred to as rigidly stereotyped, has been shown to vary in response to prey type (Honigmann 1944; Weerasuriya 1989; Nishikawa et al. 1992; Anderson 1993; Anderson and Nishikawa 1993). Several studies have investigated which features of the prey elicit feeding behavior (for review see Ewert 1981), but few studies have approached this question in intact, freely moving animals with natural prey items. Furthermore, previous studies investigated the probability that the frogs and toads will respond to a given stimulus, rather than variations in motor output elicited by different stimuli.

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In previous studies (Anderson 1993; Anderson and Nishikawa 1993), we showed that the feeding motor output of *Rana pipiens* varies depending upon the type of prey that the frogs are offered. When feeding on waxworms (approximately 1.5 cm in length), *R. pipiens* uses tongue prehension to catch prey. When feeding on earthworms (approximately 4–6 cm in length), they capture prey using jaw prehension (Anderson 1993).

During tongue prehension, the head remains horizontal with respect to the substrate, and the lunge distance is shorter than for jaw prehension. The mouth remains open for a longer period. The tongue is protracted farther and through a greater angle relative to the mandible. It is also retracted more slowly than during jaw prehension. We termed this behavior "tongue prehension" because the tongue is protracted onto the prey, adheres to the prey and the prey is returned to the mouth without contacting the jaws.

During jaw prehension, the head is rotated downward, which places the jaws in a position to grasp the prey. The frogs lunge farther than for tongue prehension, the mouth remains open for a shorter period, and the tongue is protracted less far and through a smaller angle than for tongue prehension. We termed this behavior "jaw prehension" because, although the tongue makes initial contact with the prey, the prey is grasped with the jaws and is transported into the oral cavity with the forelimbs. These studies and others (Valdez and Nishikawa 1996) suggest that the choice between tongue prehension and jaw prehension is based on a visual analysis of prey size.

Feedback modulation by tongue afferents differs between these two behavior patterns. When feeding on waxworms, afferent input from the tongue (which travels to the brain in the hypoglossal nerve) is necessary for mouth opening (Anderson and Nishikawa 1993). If the hypoglossal nerve is transected, the mouth fails to open although the feeding behavior appears otherwise normal. Electromyographic studies show that the mouth fails to open after hypoglossal transection because the mouth opening and closing muscles are activated simultaneously (Nishikawa and Gans 1992; Anderson and Nishikawa 1993). In intact frogs, the mouth-closing muscles are inhibited until the mouth-openers have reached maximum contraction. Thus, hypoglossal feedback alters the phase of activity in the jaw muscles.

In contrast, when feeding on earthworms, the mouth opens normally even after transection of the hypoglossal nerves. During jaw prehension, hypoglossal feedback is not necessary for mouth opening, and some other coordination mechanism is used. These studies demonstrate that hypoglossal feedback is necessary for mouth opening when feeding on waxworms, but not when feeding on earthworms. Thus, the influence of hypoglossal afferents on jaw muscle activity is gated by visual information about the prey (Anderson and Nishikawa 1993). This suggests that visual and

proprioceptive information interact in controlling prey capture.

During the initiation of a behavior, an organism must choose an appropriate motor program that will result in the most successful outcome given current circumstances. The pattern of choice at the sensory border between the two motor programs provides insights into the mechanisms of motor control (Abbs and Cole 1987; Grobstein 1992). There are many examples in which the nervous system chooses the most appropriate motor program for the current circumstances from a set of possible motor programs based on incoming sensory information (Grobstein 1991). In some cases, continuous variation in stimulus parameters (e.g., size of prey) results in a discrete switch between motor programs (e.g., tongue prehension vs jaw prehension). Alternatively, the activity expressed by the CNS may be intermediate between two motor programs, the combination being termed a "blend" (Stein 1989). Behavioral blends at the border of various sensory inputs have been identified in molluscs (Croll and Davis 1987; Huang and Satterlie 1990), locusts (Mohl 1989), cockroaches (Reingold and Camhi 1977), turtles (Stein 1989), and cats (Carter and Smith 1986). For example, turtles may exhibit either a rostral scratch, a pocket scratch or a caudal scratch response depending upon the location of a tactile stimulus relative to the receptive fields of cutaneous sensory neurons. If a stimulus is given at the border between receptive fields, then the resulting motor output may be a blend of two different scratch types (Stein 1989).

The concept of motor equivalence (Abbs and Cole 1987) suggests another set of possibilities. If the success of the outcome is not affected by which motor program is used (i.e., the motor programs are equally efficient), then alternation between the two motor programs may occur at the sensory border. For example, Grobstein et al. (1985) showed that the distance between frog and prey determines whether the frog simply "snaps" at the prey (if it is close enough), or whether it "hops" then "snaps". The behavioral output switches at what is termed the "snap/hop" border (Grobstein 1992). At this border, the frogs alternate randomly between "snap" and "hop/snap".

In this study, we ask how frogs choose between two different motor patterns for prey capture, tongue prehension and jaw prehension. We first test how prey size influences the motor pattern elicited when a frog feeds. If feeding behavior is modulated by prey size, then there should be an intermediate prey size at which one of three possible outcomes may occur. The first possibility is that a behavioral blend will be observed at the sensory border. For example, the frogs would exhibit movements of the head, jaws, and tongue that are intermediate between those expressed during tongue prehension and jaw prehension. Alternatively, there may be a discrete switch from tongue prehension to jaw prehension at the sensory border. If tongue

prehension and jaw prehension are equally efficient at capturing intermediate-sized prey (i.e., motor equivalence), then we hypothesize that there should be a size of prey at which either behavior pattern is elicited, in random order. Secondly, we test whether proprioceptive information from the tongue, which travels to the brain in the hypoglossal nerve, also influences motor program choice. If hypoglossal afferents affect motor program choice, then the pattern of choice at the sensory border should differ between intact and deaf-ferented frogs.

To address these issues, frogs were presented with pieces of earthworms ranging from the size of whole waxworms (i.e., 1.5 cm) to whole earthworms (i.e., 4.5 cm) and the movements of the head, jaws and tongue were compared among the sizes of prey both before and after hypoglossal transection. This allowed us to answer the following questions: (1) How is visual information used by the frogs to choose the appropriate motor program for capturing different-sized prey?; (2) What is the pattern of motor program choice at the border between the alternative motor programs?; (3) How do hypoglossal afferents affect the choice between motor programs at the sensory border?

## Materials and methods

Adult *Rana pipiens* ( $n = 4$ ) were obtained from animal suppliers. The individuals used in this study ranged from 62 to 67 mm in snout-vent length. Feeding attempts were videotaped using a Display Integration Technologies model DIT 660 high-speed video camera. The frogs were filmed at 120 fields per second with synchronized strobe illumination, and were allowed to feed unrestrained on a flat stage covered with a damp paper towel. Filming was done at room temperature (approximately 21–23°C).

In order to investigate the role of prey size in motor program choice, frogs were presented with pieces of earthworms of various sizes. For each frog, one to four feeding sequences were obtained both before and after hypoglossal transection for each prey size. At the beginning of each trial, the prey items were placed at a standard position, approximately 5–6 cm directly in front of the frogs. The frogs then chose to strike at varying distances from the prey by sometimes stepping or hopping towards the prey before initiating the snap. The distance to prey was measured from the tip of the snout to the prey at the onset of forward movement, and it differed among the prey sizes (Table 1).

We used video motion analysis to quantify the kinematics of head, jaw, and tongue movements during feeding on each of the prey sizes. These data were used to compare feeding kinematics among the prey sizes. For the kinematic analysis, we followed the same procedures as in Anderson (1993) and Anderson and Nishikawa (1993). The feeding sequences were analyzed using Peak Performance Technologies 2D motion analysis software on an IBM compatible computer. For each frame of the feeding sequence, the X, Y coordinates of the prey item, a non-moving reference point, and 15 points on the frogs were digitized from the video monitor. The resolution of the video image varied between 32 and 51 pixels/cm. Feeding sequences consisted of 24–32 video fields (200–267 MS). All feeding attempts were analyzed except those in which the frog or the prey item moved out of view of the camera. The earthworm pieces that were presented to the frogs were 1.5, 2.0, 3.0, and 4.5 cm in length. The smallest size earthworm piece (1.5 cm) was approximately the same length as an average waxworm, and the longest was approximately the same length as an average earthworm. To avoid having the frogs habituate to a particular prey size, different sized earthworm pieces were offered on different days and the sizes were offered in random order.

The 17 kinematic variables included in this study were defined by Anderson (1993) and are listed in Table 1. These variables include 5 measurements relating to movements of the head (e.g., time of first forward movement and lunge length), five measurements relating to movements of the jaws (e.g., duration of mouth opening and closing, gape distance and maximum gape angle), and seven measurements relating to movements of the tongue (e.g., duration of tongue protraction and retraction, maximum tongue length and tongue angle).

The surgical procedures for bilateral hypoglossal transection are described in Nishikawa and Gans (1992) and Anderson and Nishikawa (1993). Briefly, the frogs were anesthetized by immersion

**Table 1** Means  $\pm$  SE for the kinematic variables when the frogs fed on the different sizes of prey. The  $F$ -values and  $P$ -values were calculated from a 2-way ANOVA with the size of the prey and individual (data not shown) as main effects. Asterisks indicate significant differences among prey sizes. Columns that share the same lowercase letter are not statistically different based on post hoc tests

	1.5 cm	2.0 cm	3.0 cm	4.5 cm	$F$ -value	$P$ -value
Time of first forward movement (ms)	$-68.0 \pm 5.5$	$-70.8 \pm 5.1$	$-69.4 \pm 5.2$	$-79.8 \pm 8.0$	0.92	0.442
Time to prey contact	$17.2 \pm 1.3$	$20.1 \pm 1.6$	$22.9 \pm 1.8$	$20.9 \pm 1.3$	2.58	0.071
Time of max. tongue protraction	$19.3 \pm 1.2a$	$21.5 \pm 1.6a$	$25.7 \pm 1.2b$	$22.3 \pm 1.2a$	4.43	0.010*
Time to max. displacement of upper jaw tip	$49.3 \pm 6.4a$	$73.6 \pm 5.4b$	$87.5 \pm 8.4b$	$67.3 \pm 5.1b$	6.77	0.001*
Time of mouth closing	$134.0 \pm 5.8a$	$117.3 \pm 5.0b$	$138.1 \pm 7.7a$	$136.8 \pm 9.1$	4.57	0.009*
Duration of mouth opening (ms)	$60.4 \pm 4.5$	$67.3 \pm 7.1$	$55.5 \pm 4.3$	$53.5 \pm 5.1$	1.85	0.159
Duration of tongue protraction	$19.4 \pm 1.2a$	$21.5 \pm 1.6a$	$26.4 \pm 0.9b$	$22.2 \pm 1.2a$	5.86	0.003*
Duration of tongue at target	$10.4 \pm 1.1$	$9.7 \pm 0.9$	$11.8 \pm 1.2$	$9.7 \pm 0.9$	0.73	0.543
Duration of tongue retraction	$65.9 \pm 3.9$	$63.2 \pm 5.6$	$57.6 \pm 5.0$	$60.47 \pm 5.3$	0.72	0.547
Duration of mouth closing	$75.7 \pm 6.4a$	$58.3 \pm 3.1b$	$82.6 \pm 6.0a$	$83.3 \pm 6.7a$	5.88	0.003*
Maximum gape angle ( $^{\circ}$ )	$59.6 \pm 1.9a$	$57.6 \pm 2.4a$	$66.0 \pm 3.0b$	$58.4 \pm 2.5a$	3.68	0.022*
Tongue angle	$124.8 \pm 5.2a$	$109.6 \pm 4.0b$	$104.6 \pm 6.4b$	$114.7 \pm 6.7b$	3.68	0.022*
Max. downward head rotation	$24.3 \pm 2.8a$	$52.3 \pm 3.6b$	$51.9 \pm 4.6b$	$45.5 \pm 3.2b$	26.07	0.001*
Distance to prey (cm)	$3.3 \pm 0.3$	$3.8 \pm 0.4$	$3.8 \pm 0.3$	$4.3 \pm 0.5$	1.06	0.378
Lunge length	$3.4 \pm 0.4a$	$4.0 \pm 0.3b$	$4.0 \pm 0.4b$	$5.2 \pm 0.6b$	4.57	0.009*
Gape distance	$1.7 \pm 0.5$	$1.7 \pm 0.1$	$1.8 \pm 0.1$	$1.8 \pm 0.1$	1.01	0.403
Maximum tongue reach	$1.6 \pm 0.5a$	$1.1 \pm 0.1b$	$1.1 \pm 0.1b$	$1.2 \pm 0.1b$	5.69	0.003*

in a bath of 0.1% buffered tricaine methanesulfonate salt (MS 222) and were placed on a damp paper towel under a dissecting microscope. A small incision was made on the ventral surface of the lower jaw and the hypoglossal nerve was exposed. A section of the hypoglossal nerve, approximately 1–2 mm in length, was excised distal to the branch that innervates the *m. genioglossus*. This procedure transects afferent fibers that appear to innervate the epithelium of the tongue (Anderson and Nishikawa 1995) and motor fibers that innervate the tongue protractor muscles, *m. genioglossus*. The incision was closed with a cyanoacrylate surgical adhesive. The frogs were allowed to recover from the anesthesia and feeding trials were begun the following day. Following bilateral hypoglossal transection, only three of the four frogs attempted to feed on all of the prey sizes, so the fourth frog was excluded from the post-surgery analysis. Only the first four to ten feeding attempts were videotaped, so presumably motor learning did not affect the outcome (Nishikawa and Innocenti 1994).

To examine how motor output varies with prey size, two-way ANOVAs were performed on the 17 kinematic variables using Statview 512+ and SuperAnova software on a Macintosh IIfx computer with prey size (fixed) and individual (random) as main effects (type III sums of squares). Using individual as a main effect takes any differences among individual frogs (e.g., differences in body size) into account. Student-Neuman-Keuls post hoc tests were used to determine which prey sizes differed significantly from each other on the basis of prey capture kinematics.

A discriminant function analysis was used to determine objectively how movement patterns differed among prey sizes. The analysis was performed using SPSS release 4.1 on a VMS/VAX system mainframe. The purpose of the analysis was to test whether prey size could be identified accurately on the basis of kinematic variables, and to determine which variables were most important in identifying prey size. The data included 25 waxworm and 25 earthworm feedings from previously published studies (Anderson 1993). These data were combined with pre-surgery feeding sequences from the present study (12 feeding sequences for each prey size). The independent variables were "waxworm-like" stimuli and "earthworm-like" stimuli. We coded prey capture sequences of 1.5 cm earthworm pieces and intact waxworms as "waxworm-like" and the 3.0- and 4.5-cm pieces of earthworm and intact earthworms as "earthworm-like". The dependent variables were the 17 kinematic variables listed in Table 1. The computer then assigned the 2.0-cm pieces into either the "waxworm-like" group or the "earthworm-like" group on the basis of the kinematic variables. If discrete switching occurs between tongue prehension and jaw prehension, we would expect the 2.0-cm pieces to be grouped unambiguously as either "waxworm-like" or "earthworm-like". If a behavioral blend or motor equivalence occurs, then we would expect the grouping to be more ambiguous than if there is a discrete switch. This analysis also determines which variables are most important in classifying the feeding attempts into the different groups.

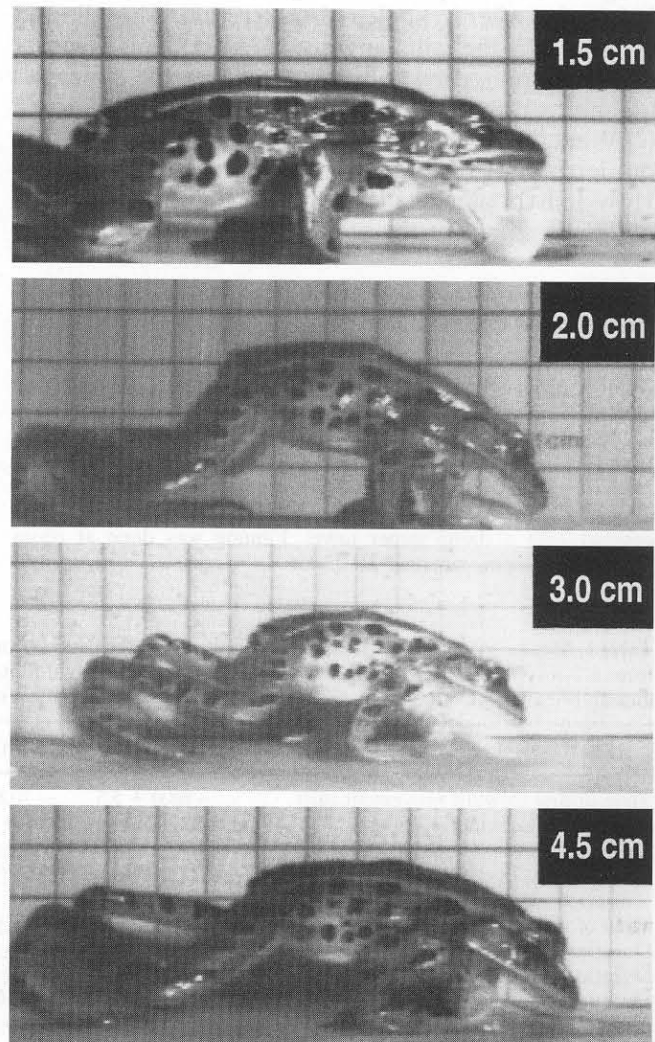
## Results

In this study, we varied the length of the prey systematically in order to determine: (1) whether prey size is used to choose between alternative motor programs; (2) to what extent *Rana* can discriminate differences in prey size on the basis of vision; (3) what movement pattern is observed at the sensory border between the two motor programs; and (4) whether hypoglossal afferents also influence motor program choice. We first describe the qualitative changes in feeding behavior across different prey sizes, and then present a quantitative analysis of variation in prey capture kinematics.

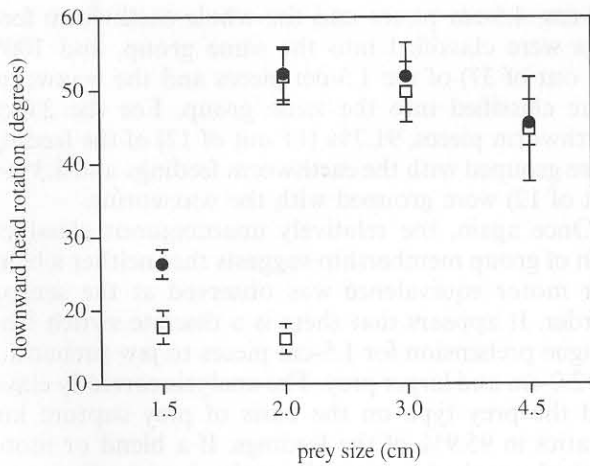
## Overview of behavior patterns

When the frogs were offered the smallest prey size (1.5 cm), they always performed tongue prehension (Fig. 1). When feeding on the 1.5-cm prey, they lunged forward and kept the head and body level with the substrate. Downward rotation of the head was  $24.3^\circ$  (Table 1; Fig. 2). The tongue was protruded, made contact with the prey, and returned the prey to the mouth without contacting the jaws (Fig. 1).

When offered the longer pieces of earthworm (2.0, 3.0, and 4.5 cm), the frogs always fed using jaw prehension. They lunged forward, but rotated their heads



**Fig. 1** Video images from intact *Rana pipiens* showing behavior when feeding on four sizes of earthworm pieces. The frogs use tongue prehension when feeding on 1.5-cm prey and jaw prehension when feeding on 2.0-, 3.0-, and 4.5-cm prey. During tongue prehension, the head remains level, the frogs lunge for a short distance and the tongue is protracted fully. During jaw prehension, the head is rotated downward, the frogs lunge farther and tongue protraction is reduced



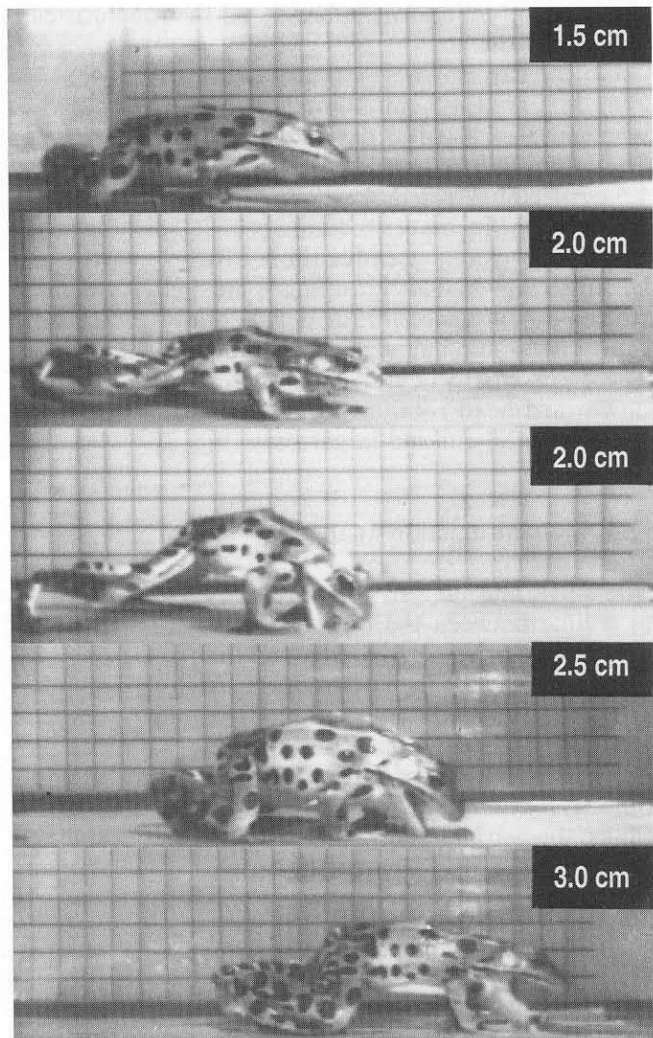
**Fig. 2** This scatter plot illustrates differences in the maximum degree of downward head rotation between tongue prehension and jaw prehension before and after bilateral transection of the hypoglossal nerves. The discriminant function analysis showed that this variable was the most important in distinguishing between these motor programs. Here, we plotted maximum downward head rotation (mean  $\pm$  SE) for each of the four earthworm sizes before (*filled circles*) and after (*unfilled squares*) hypoglossal transection. For 2.0 cm after surgery, the values were plotted separately for sequences in which the mouth opened or failed to open. The *upper square* overlaps nearly completely with the *filled circle*. In intact frogs, the head was rotated downward to an average angle of 24.3° when feeding on the 1.5-cm earthworm pieces, whereas downward head rotation averaged 45.5–52.3° when feeding on the 2.0-, 3.0-, and 4.5-cm pieces. After hypoglossal transection, when offered 2.0 cm earthworm pieces, either tongue prehension or jaw prehension was elicited. The *upper square* corresponds with the degree of downward head rotation observed during jaw prehension (mouth opens) and the *lower square* corresponds with the decreased downward head rotation observed during tongue prehension (mouth fails to open). No intermediate behavior patterns were observed

downward to a significantly larger degree (45–52°) than for the 1.5-cm earthworm piece (Table 1, Fig. 2). The tongue made contact with the prey, although the tongue was not used to return the prey to the mouth. Instead, as the head rotated downward, the jaws made contact with the substrate and closed on the prey, which resulted in prey capture (Fig. 1).

#### Univariate ANOVAs

In order to determine how movement patterns change across prey size, we compared the kinematic variables during feedings on 1.5-, 2.0-, 3.0-, and 4.5-cm pieces of earthworm (Table 1). Of the 17 variables that were measured, 10 differed significantly among prey sizes (Table 1). Of the ten significant variables, five variables distinguished the 1.5-cm prey from the other prey sizes.

The head was rotated downward to a significantly smaller degree when feeding on the 1.5-cm earthworm pieces (Table 1;  $F = 26.07$ ,  $P < 0.001$ ) than when feeding on 2.0-cm and larger pieces of earthworm (Fig. 1; Fig. 3). Lunge length was significantly shorter when



**Fig. 3** Video images from *Rana pipiens* showing feeding behavior for four sizes of earthworm pieces following transection of the hypoglossal nerves. When feeding on the 1.5-cm pieces following transection, the mouth never opened. When feeding on 2.5-, 3.0- and 4.5-cm pieces following surgery, the frogs always opened their mouths and fed using jaw prehension, although the tongue was not protracted. Of the 21 feeding attempts filmed following surgery when the frog was offered the 2.0-cm prey, 11 attempts resulted in failure of the mouth to open, while the mouth opened in 10 of the trials

feeding on the 1.5-cm earthworm piece than when feeding on larger pieces (Table 1;  $F = 4.57$ ,  $P = 0.009$ ), and the time to maximum forward displacement of the upper jaw tip was significantly shorter than for the larger prey (Table 1;  $F = 6.77$ ,  $P = 0.001$ ). Maximum tongue reach was significantly greater for 1.5-cm pieces than for the 2.0-, 3.0- and 4.5-cm pieces of earthworm (Table 1;  $F = 5.69$ ,  $P = 0.003$ ). In addition, the angle through which the tongue was protracted was significantly larger for the 1.5-cm pieces than for the 2.0-, 3.0-, and 4.5-cm pieces (Table 1;  $F = 3.68$ ,  $P = 0.022$ ). When feeding on earthworm pieces larger than 1.5-cm, downward head rotation increased, lunge length increased,

tongue protraction was reduced and the prey was captured with the jaws.

On the basis of these variables, it is evident that the 2.0-, 3.0-, and 4.5-cm feedings represent one group, whereas the 1.5-cm feedings represent another group. The quantitative analysis shows that kinematics of 1.5-cm feedings resemble those of waxworm feedings, whereas the kinematics of feedings on 2.0-cm and larger earthworm pieces resemble those of whole earthworm feedings (Fig. 3). This grouping is most clearly demonstrated by differences in the degree of downward head rotation for 1.5-cm versus 2.0-cm and larger prey. There appears to be a discrete switch in the degree of downward head rotation from 24° for 1.5-cm prey to 45–50° for 2.0-cm and larger prey (Fig. 3).

#### Discriminant function analyses

The univariate analysis compares only a single variable at a time between the different treatments. However, movements of the head, body, and tongue are obviously associated with each other during feeding. To understand this interaction, we used a discriminant function analysis, which combines the multiple variables into one common analysis that groups the feeding attempts objectively on the basis of all kinematic variables and ranks the variables in order of their importance in determining group membership.

We first re-analyzed the waxworm and earthworm data from Anderson (1993) to see if the prey type that was offered could be identified on the basis of prey capture kinematics. For whole earthworms and waxworms, the combination of kinematic variables produced a 100% correct identification of prey type. All 25 of the waxworm feedings were placed into one group, and all 25 of the earthworm feedings into another. The kinematic variables that best distinguished between prey types were, in order of importance, downward rotation of the head, duration of tongue retraction, tongue angle, time of mouth closing, and maximum tongue reach. The unambiguous identification of prey type on the basis of prey capture kinematics suggests that the motor programs produced when feeding on waxworms and earthworms are discrete, with little overlap between them, even when multiple kinematic variables are considered.

We next examined the behavior patterns produced during feeding sequences on the four different sizes of prey. We tested the hypotheses that either two discrete behavior patterns would be observed, or that a behavioral "blend" would be found at the border between the two behavior patterns. In this analysis, we coded the feeding sequences for the 1.5-cm earthworm pieces as "waxworm-like", and the feeding sequences for the 3.0- and 4.5-cm pieces as "earthworm-like". The computer then classified the 2.0-cm feedings into one of the two groups. Based on this analysis, 93.9% (46 of 49) of the

3.0-cm, 4.5-cm pieces and the whole earthworm feedings were classified into the same group, and 100% (37 out of 37) of the 1.5-cm pieces and the waxworms were classified into the same group. For the 2.0-cm earthworm pieces, 91.7% (11 out of 12) of the feedings were grouped with the earthworm feedings and 8.3% (1 out of 12) were grouped with the waxworms.

Once again, the relatively unambiguous classification of group membership suggests that neither a blend nor motor equivalence was observed at the sensory border. It appears that there is a discrete switch from tongue prehension for 1.5-cm pieces to jaw prehension for 2.0-cm and larger prey. The analysis correctly classified the prey type on the basis of prey capture kinematics in 95.9% of the feedings. If a blend or motor equivalence had occurred at the intermediate prey sizes, then the accuracy of group assignment should have been reduced.

Both the discriminant function analyses and the univariate analyses showed that several kinematic variables are important in discriminating between jaw prehension and tongue prehension. In the univariate analyses, these were downward head rotation, lunge length, time to maximum displacement of upper jaw tip, maximum tongue reach, and tongue angle. In the discriminant function analyses, these were downward head rotation, duration of tongue retraction, tongue angle, time of mouth closing, and maximum tongue reach. Thus, these analyses show that differences between jaw prehension and tongue prehension involve movements of the head, jaws and tongue, although head movements (particularly downward head rotation) were the most important.

A detailed analysis of downward head movement shows that the decision to exhibit jaw prehension or tongue prehension takes place very early in the feeding sequence. At the onset of mouth opening, there was already a significant difference between the 1.5- and 4.5-cm prey in the degree to which the head was rotated downward (paired *t*-test;  $t = -5.11$ ;  $P < 0.001$ ).

#### Effects of hypoglossal transection on motor program choice

The next experiment investigated the role of hypoglossal afferents in motor program choice across the prey sizes. The hypoglossal nerve was transected in three frogs and the frogs were offered the same range of prey sizes. For all three frogs, the results were identical. When feeding on 1.5-cm pieces of earthworm after transection, the mouth never opened in any of the feeding attempts (Fig. 2). The frogs always exhibited tongue prehension, as demonstrated by the small extent of downward head rotation that occurred whenever the mouth failed to open (Fig. 2). As previously shown by Anderson and Nishikawa (1993), this is typical of tongue prehension. When offered the 3.0- and 4.5-cm

earthworm pieces, the frogs always lunged forward, exhibited a large degree of downward head rotation and opened their mouths in an attempt to capture the prey, although their tongues were not protracted as expected due to denervation of the *m. genioglossus*. The frogs always exhibited jaw prehension, as demonstrated by the large extent of downward head rotation that occurred whenever the mouth opened (Figs. 2, 3).

At the intermediate prey length (2.0 cm) that represents the border between the two behaviors, the deafferented frogs alternated randomly between tongue and jaw prehension. Of the 21 feeding attempts filmed when the frogs were offered the 2.0-cm prey, 11 attempts resulted in the failure of the mouth to open, while the mouth opened in 10 of the trials (Fig. 2). The probability of mouth opening (47.6%) did not differ significantly from the random expectation of 50%. In one frog, its first attempt resulted in no mouth opening. On the next attempt, the frog opened its mouth and captured the prey. This was followed immediately by three failures to open the mouth and it opened its mouth on the last attempt. The second frog did not open its mouth on the first attempt, opened the mouth on the second, the third attempt resulted in no mouth opening, followed by a successful mouth opening in which the prey was captured. The last frog did not open its mouth on the first attempt, opened it on the second, followed by a failure and then a mouth opening. This was followed by two consecutive mouth openings, a failure, two more mouth openings and finally a failure to open the mouth.

For 2.0-cm prey, two discrete groups were observed following surgery. In 6 of 11 digitized trials after hypoglossal transection, the mouth opened, whereas in the other 5 the mouth failed to open. Kinematic analysis showed that when the mouth opened, the frogs also exhibited a large degree of downward head rotation ( $52^\circ$ ), whereas when the mouth failed to open, downward head rotation was reduced (Figs. 2, 3). This result demonstrates that, at the intermediate prey size, the deafferented frogs alternate randomly between two motor programs, one being tongue prehension (in which the mouth does not open following hypoglossal transection) and the other jaw prehension (in which the mouth opens normally). Downward head rotation was significantly greater in trials when the mouth opened than in trials when the mouth failed to open (ANOVA,  $F = 66.24$ ,  $P = 0.0001$ ). There was no difference in downward head rotation between feedings before surgery in which jaw prehension was used and feedings after surgery when the mouth opened (ANOVA,  $F = 0.005$ ,  $P = 0.9418$ ).

At the intermediate prey size (2.0 cm), no intermediate behavior was observed (Figs. 2, 3). For example, if a behavioral blend occurred at the sensory border, then we would have expected to observe sequences in which the degree of downward head rotation was intermediate (e.g.,  $33^\circ$ ) between jaw prehension ( $50^\circ$ ) and tongue

prehension ( $25^\circ$ ), but no such intermediate behavior was observed (Fig. 3).

No behavioral blends were observed between the two motor patterns either before or after surgery (Fig. 2). For 1.5-cm prey, the frogs exhibited a small degree of downward head rotation ( $18\text{--}24^\circ$ ), which is characteristic of tongue prehension. There was no difference in downward head rotation before versus after surgery (ANOVA,  $F = 3.59$ ,  $P = 0.0734$ ). For 3.0-cm and larger prey, the frogs exhibited a large degree of downward head rotation ( $45\text{--}50^\circ$ ), which is characteristic of jaw prehension. At these sizes, there was also no difference in downward head rotation before versus after surgery (ANOVA, all  $P > 0.70$ ).

Hypoglossal transection changed the probability with which jaw prehension was elicited by 2.0-cm prey. Before transection, 11 of 12 (91.7%) prey capture attempts were classified as jaw prehension on the basis of the discriminant function analysis, whereas after hypoglossal transection only 10 of 21 (47.6%) were classified as jaw prehension on the basis of mouth opening. The difference is statistically significant (Fisher's exact probability test, two-tailed,  $P = 0.02$ ).

These results demonstrate that following bilateral transection of the hypoglossal nerves in the tongue, the choice of motor programs changed at the intermediate prey size. Before hypoglossal transection, the frogs nearly always used jaw prehension for the 2.0-cm and larger prey and tongue prehension for the 1.5-cm prey. After hypoglossal transection, the frogs always exhibited jaw prehension and the mouth opened when feeding on 3.0-cm and larger prey. They always exhibited tongue prehension and the mouth failed to open when feeding on 1.5-cm prey. However, at the intermediate prey size, the frogs alternated randomly between tongue prehension (mouth failed to open) and jaw prehension (mouth opened). In some feeding attempts, the frogs lunged forward and exhibited the typical tongue prehension behavior. In others, they rotated the head downward and opened the mouth to capture the prey. Thus, hypoglossal transection altered the choice of motor programs at the sensory border between jaw and tongue prehension.

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## Discussion

The observations reported here have several important implications for understanding the control of feeding behavior in frogs. First, vision is the sensory modality used to choose between alternative motor programs (i.e., tongue prehension and jaw prehension) and size is the characteristic of the prey upon which the choice is made. Second, tongue prehension and jaw prehension are discretely different behavior patterns. No behavioral "blend" is present at the sensory border between the two behaviors. Finally, sensory information from

the tongue, returning to the brain in the hypoglossal nerve, influences the choice of motor program used for prey capture.

### Visual analysis of prey size

Previously, we identified two feeding motor programs in *Rana pipiens*, tongue prehension and jaw prehension (Anderson 1993). Which of these behavior patterns will be elicited depends on whether the frog is offered small prey (tongue prehension) or large prey (jaw prehension). In the present study, we tested whether this decision is in fact based on a visual analysis of prey size. The results showed that when *Rana* are offered 1.5-cm earthworm pieces, they feed as when feeding on waxworms. The prey is captured using tongue prehension. When the frogs are offered 2.0-, 3.0- or 4.5-cm pieces of earthworm, they feed using jaw prehension, as when feeding on intact earthworms. These observations demonstrate that: (1) motor program choice is based on a visual assessment of prey size; (2) the border between the alternative motor programs occurs between 1.5 and 2.0 cm; and (3) the frogs are able to distinguish the length of prey to within 0.5 cm.

Kinematic analyses demonstrated that tongue prehension and jaw prehension differ primarily in movements of the head and tongue. Tongue prehension is characterized by reduced downward rotation of the head and reduced lunging, and increased tongue protraction. These differences are highly correlated, and downward rotation of the head is the most important variable in distinguishing between tongue prehension (less than 30°) and jaw prehension (more than 30°).

By the time the mouth begins to open and the tongue is first visible, the degree to which the head is rotated downward already differs significantly between the 1.5-cm feedings and the larger pieces of earthworm. The behavioral choice is made before the mouth opens, long before contact with the prey or the substrate.

Based on univariate statistics and discriminant function analysis, the border between tongue prehension and jaw prehension appears to be relatively discrete. In intact frogs, neither a behavioral blend nor motor equivalence was observed at intermediate prey sizes (Fig. 2). For 2.0-cm prey, the frogs exhibited jaw prehension in 91.7% of the trials and tongue prehension in 8.3% of the trials.

### Hypoglossal afferent input

Hypoglossal sensory information has been shown to coordinate mouth opening and tongue protraction during feeding (Nishikawa and Gans 1992; Anderson and Nishikawa 1993). This afferent information influences the temporal sequence of jaw muscle activity so that the mouth-closing muscles are inhibited from

firing long enough for the mouth to open (Nishikawa and Gans 1992).

Following hypoglossal nerve transection, *Rana* does not open its mouth when attempting to feed on waxworms (Anderson and Nishikawa 1993) or 1.5-cm pieces of earthworm. This suggests that proprioceptive information from the tongue affects the feeding behavior in a similar manner when feeding on 1.5-cm pieces of earthworm as during waxworm feedings. When feeding on 3.0- and 4.5-cm pieces of earthworm, the mouth always opens following hypoglossal transection, as observed in the feedings on whole earthworms. These observations demonstrate that the effect of hypoglossal afferents on motor output depends upon simultaneous visual information that the frog receives about the prey.

### Interactions between visual and proprioceptive information

The results of the present study show that visual analysis of prey size is important for choosing between alternative motor programs, and that proprioceptive information from the hypoglossal nerve is necessary for proper sequencing of jaw muscle activity when feeding on small prey. An interesting observation emerged from this study, which suggests that these two sensory modalities interact in controlling motor program choice. In contrast to intact frogs (which switch discretely from tongue prehension at 1.5 cm to jaw prehension at 2.0 cm), deafferented frogs alternated, in a random fashion, between opening and not opening the mouth when offered a series of 2.0-cm prey after hypoglossal transection. When feeding on intermediate prey sizes following hypoglossal transection, the frogs exhibited motor equivalence. This demonstrates that hypoglossal sensory information interacts with visual information to influence motor program choice, and also suggests that both visual and proprioceptive information are used in a feedforward manner to plan the ensuing movement.

The anatomical location where peripheral sensory information from the tongue and visual information about the prey converge should be the location at which behavioral decision making occurs. In frogs, this is most probably the reticular formation, although higher brain centers, such as the cerebellum, may also be involved. Neuroanatomical studies demonstrate that hypoglossal afferents project to both the medial reticular formation and the cerebellum in *Rana pipiens* (Anderson 1996).

It is important to rule out motor learning as a possible explanation for the pattern of motor program choice that was observed at the sensory border. When *Rana* was offered 2.0-cm pieces of earthworm following hypoglossal transection, the mouth did not open at the first attempt for two of the individuals. The fact that they could later open their mouths and capture the



2.0-cm prey might suggest that they had learned to associate this prey size with a new motor pattern. However, toads require 12–15 trials to learn to open the mouth following hypoglossal transection (Nishikawa and Innocenti 1994), whereas we only included the first 4–10 trials for each frog in this analysis in order to eliminate any learning effects. Thus, we believe that learning had not yet occurred. Ongoing studies are addressing how learning affects motor program choice over a longer time period.

Other studies have also suggested that motor program choice depends upon incoming sensory information. For example, this has been observed in the frog's orienting response toward prey. Grobstein et al. (1985) showed that the distance between frog and prey determines whether it simply "snaps" at the prey (if it is close enough), or whether it "hops" then "snaps". The behavioral output switches at what is termed the "snap/hop" border (Grobstein 1992). At this border, the frogs alternate randomly between "snap" and "hop/snap". In this case, the frog may capture a prey item successfully, regardless of which motor program it chooses to execute. An important question that remains to be answered is whether the switch from tongue prehension to jaw prehension affects capture success for different types of prey. We believe that the reason why frogs switch from tongue prehension for small prey to jaw prehension for large prey is because tongue prehension is only effective when the weight of the prey is less than or equal to the force of adhesion between prey and tongue. Likewise, tongue prehension is more effective for capturing small prey because the tongue can be placed more precisely than the jaws. In addition, prey are transported to the esophagus in a single movement during tongue prehension, which reduces the chance for escape.

It appears that hypoglossal afferents bias motor program choice toward jaw prehension at the sensory border. When hypoglossal sensory information is present, the frogs always use jaw prehension to capture 2.0-cm prey, whereas deafferented frogs alternate randomly between tongue and jaw prehension. It appears that this bias may also enhance capture success. At intermediate prey sizes, jaw prehension is a safer bet than tongue prehension because if the prey is actually too heavy, then the frog is more likely to be successful using jaw prehension than using tongue prehension.

The presence of hypoglossal afferents is a derived feature that has evolved independently numerous times among frogs (Nishikawa et al. 1993). For example, the toad, *Bufo marinus*, has been shown to possess hypoglossal afferents that function similarly in feeding behavior (Nishikawa and Gans 1992) to those of *Rana pipiens*. The hypoglossal afferents of *Bufo* are derived from a different source and have differing central projections than those of *Rana*. In *Bufo*, hypoglossal afferents are re-routed from the glossopharyngeal nerve. They enter the brainstem through the root of the

glossopharyngeal nerve and have their cell bodies in the Gasserian ganglion (Nishikawa et al. 1995). Ongoing studies in *R. pipiens* have shown that the hypoglossal afferents are re-routed cervical spinal afferents. They enter the brainstem through the third dorsal root, have their cell bodies in the dorsal root ganglion of the third spinal nerve, and ascend in the dorsomedial funiculus to the cerebellum and medial reticular formation (Anderson and Nishikawa 1995; Anderson 1996).

It appears that the historical origin of hypoglossal afferents from different sources may have constrained their subsequent use. In both *Bufo* and *Rana*, the hypoglossal afferents play a role in determining the phase relationships of the jaw muscles. This is a typical role for sensory feedback to play in motor control (Pearson 1993). However, in *Rana* alone, these afferents also contribute to functions such as motor program choice.

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