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Ontogeny of Suckling Mechanisms in Opossums (*Didelphis virginiana*)

Key Words

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Abstract

Although adult mammals have several different mechanisms for drinking liquids, relatively little is known about the variation in mechanisms used by infants for suckling and how the mechanics of suckling change as a function of ontogeny. We examined suckling in infant opossums, animals that as adults use a lapping mechanism to drink. The youngest infants (45 days old) used a suction, pumping mechanism, based on dorsal/ventral tongue movements, similar to what has been documented in infant primates and pigs. However, slightly older infants (66 days old), when removed from their mother, used a lapping or licking mechanism that is closer to the anterior/posterior tongue movements that adult opossums use during drinking. Littermates remaining with the mother continued to use the pumping mechanism observed in the youngest animals. These older animals lacked teeth and were not physiologically mature enough to live independently of their mothers. We conclude that the neural controls for two distinct feeding mechanisms exist simultaneously in infant opossums and that the change from a suckling, infantile motor pattern to an adult lapping one is not strictly a temporal change.

Introduction

Suckling is the broad term used to describe feeding behavior in infant mammals. The mechanics of suckling include several continuous steps: acquiring milk from the nipple, moving the aliquot through the oral cavity and oropharynx, and finally swallowing, the movement of the milk into the esophagus. Most studies of infant feeding have examined only acquisition and have not considered the mechanics of either intraoral transport or swallowing [Ardran et al., 1958a, b; Colley and Creamer, 1958; Ardran and Kemp, 1959; Bosma, 1967; Wolff, 1968; Gordon and Herring, 1987].

Different mammals appear to use different mechanisms for obtaining milk from the nipple. Early work by Ardran et al. [1958a, b] and Ardran and Kemp [1959] indicated that infant lambs and kids, as well as humans, use an expression mechanism where the tongue and upper jaw put positive pressure on the nipple. This is a different mechanism than one described for infant macaques and pigs [German et al., 1992]. Macaques and pigs use a suction mechanism to obtain milk from the nipple. Dorsal/ventral movement of the tongue increases intraoral volume, reducing pressure and inducing milk to flow from the nipple into the mouth.

Less is known about intraoral transport and swallowing in infant mammals. German et al. [1992] showed two dif-

ferent methods for pigs and macaques. Pigs use a second pump, similar to the mechanism of acquisition, based on dorsal/ventral movement of the posterior tongue. Infant macaques use a squeeze-back mechanism, similar to what has been seen in adult macaques [Franks, et al., 1984; Hiie-mae and Crompton, 1985].

Several researchers have examined the electromyographic patterns (EMG) of muscles involved in suckling and other oral functions in infants [Gordon and Herring, 1987; Westneat and Hall, 1992]. Their papers provide excellent descriptions of the motor patterns for three species. Gordon and Herring [1987] compare genioglossus activity in infant dogs and pigs. Westneat and Hall [1992] compare muscle activity in masseter, digastric, sternohyoid and genioglossus for different behaviors in infant rats: suckling, stretch response, nipple attachment and chewing. Neither of these papers, however, intended to directly address the question of mechanism. Gordon and Herring [1987] assumed the mechanisms suggested by Ardran and co-workers, while Westneat and Hall deferred the issue.

Westneat and Hall [1992], however, did address the relationship between suckling and adult feeding. Specifically, they asked, 'Does adult mastication gradually develop from a previously established suckling neuromuscular network, or does it develop completely separate neural controls and neuromuscular patterns of activity?' [Westneat and Hall, 1992, p. 550]. They summarized prior results based on environmental factors, such as olfactory cues, and physiologic variables, such as nutritional or hydrational state, and concluded from this literature that there is a major difference between infant and adult feeding with respect to the impact of the environment as well as physiology. Some of their experimental EMG results support this view, but others present an alternative picture. They measured several parts of suckling behavior: nipple attachment, stretch response between times of milk letdown, and what they termed 'rhythmic sucking', the rhythmic movement of jaws and tongue while attached to the nipple. The EMG patterns (superficial masseter, anterior digastric, sternohyoideus, and genioglossus) for nipple attachment and the stretch response were reportedly indistinguishable from chewing in slightly older animals. Rhythmic sucking, however, was different from chewing in all seven motor pattern variables they examined. One problem with their measurements of rhythmic sucking is that, at each age (6 days, 9 days, 12 days and 18 days), they observed an average frequency of less than 1 Hz, although all other behaviors had frequencies ranging from approximately 2.5 to 4.5 Hz. This is different from most other published estimates for infant mammals, where frequencies between 3 and 5 Hz have been reported [Wolff,

1968; Gordon and Herring, 1987; German et al., 1992]. This leads to the question, what relationship exists between their behavior 'sucking' and the actual feeding measured in the other studies [Gordon and Herring, 1987; German et al., 1992]. In their study, milk was delivered through a cannula, rather than obtained through oral mechanics.

These results led Westneat and Hall to raise a number of significant issues concerning the interactions among different levels of physiological organization through ontogeny. Their measurements of EMG activity of relevant muscles suggest that some aspects of behavior remain relatively constant, while others, such as central control, are changing with the transition from suckling to adult feeding. They further ask about the relationship between the neural basis of these behaviors and the resultant motor pattern at the anatomical periphery. If motor pattern is constant, what is the effect of major changes in neural control on the portions of sucking that are different from adult behavior?

We believe that the examination of a related level of biological organization, the kinematics of jaw and tongue function in infant mammals, will shed some light on these questions. Although the previous work on pigs and primates is relevant, both these groups of animals have adult behaviors, especially the kinematics of drinking, that are similar to those found in infant suckling. Opossums, however, lap as adults, a behavior not normally ascribed to infants. Furthermore, the question still remains as to whether, in suckling infants, other aspects of infant feeding behavior, such as intraoral transport and swallowing, are infantile or adult mechanisms. Although the initial acquisition of milk from a nipple is obviously different from acquisition of liquid for an adult, in neither case is there a significant external constraint on subsequent intraoral transport or swallowing.

Materials and Methods

Two mothers with litters of different ages were obtained from Our Zoo [Neshkoro, WI]. All work reported here was performed under the IACUC guidelines established by Harvard University. The youngest animals were still attached to the teat with an intact perioral membrane and unopened eyes. The older animals had open eyes, were mobile, and lacked a perioral membrane. None were weaned or had erupted teeth, and fur was sparse. Feeding was recorded when the younger animals were 45 days old and when the older ones were 66 days old. They were hand fed every 2–4 hours and housed in modified incubator cages. Each animal was fed infant formula for dogs [Esbilac, Pet Ag, Hampshire, IL]. A special nipple was built out of soft plastic to approximate the size and shape of the maternal opossum teat. These nipples were attached to small bottles designed for puppies or kittens.

Radio-opaque tongue markers were inserted into the body of the tongue in infants from each litter. The opossums were anesthetized IM with a mixture of Ketacet (6.0 mg/kg) and Rompun (1.0 mg/kg), and

several sterilized metal markers (0.38 inch diameter wire, 1–3 mm long) were inserted into the tongue with a hypodermic syringe and plunger. The older animals received three markers along the midline of the tongue, but the youngest animals, with smaller tongues, received only two markers. A fourth marker was attached to the hyoid bone during a subsequent procedure, while the animal was anesthetized with a mixture of halothane and oxygen. Two mandibular and two maxillary reference markers were also inserted in the older animals using a syringe. One maxillary marker was inserted under the mucosa at the posterior end of the tooth row and the other under the mucosa anterior to the incisal gingiva. The mandibular markers were placed below the mucosa at the labial, posterior edge of the tooth row and anterior to the incisal gingiva. The younger animals (about 2 g in weight) were too small to receive these markers.

Movement was filmed with an IPL recording camera (Photo-Sonics) mounted on a Siemens cineradiographic apparatus at 100 or 150 frames per second (fps) using 16 mm Kodak plus-X Reversal Film. Judged by their reluctance to feed, the animals did not like to feed on formula containing barium. This was noticeably different from other infant mammals previously studied [German et al., 1992]. Milk therefore contained only small amounts of barium.

The recording procedures were slightly different for the two ages of individuals, although both included a behavioral control. Older animals were taken from their mothers and trained to feed from the bottle in front of the x-ray apparatus. They were then filmed prior to any surgery. For the older animals this was several days after being removed from their mothers. These animals were then filmed again 24 h after marker insertion to ensure that the markers did not interfere with normal feeding. The younger animals were taken from their mothers, marked (tail colored) and filmed within 2 h. Food was withheld for 2 h, and the markers were implanted. After waking, they were returned to the mothers for at least 24 h. They were taken from the mothers a second time and filmed with markers in place. There were several reasons for the difference between the two age groups. Most importantly, behavior appeared to change with the time animals were separated from their mothers. Further, the perioral membrane began to disappear soon after even the youngest animals were separated from their mothers. Finally, it was possible to return the youngest animals to the mothers without difficulty. However, attempts to return older animals, even when separated from the mothers for a short period of time, were not successful because the mothers failed to recognize the infants and attacked them.

Observations were made on the littermates (8–10 individuals) remaining with the mothers of both ages. Although only the oral floor, cheeks and, occasionally, the tongue were visible, it was possible to observe many infants of both ages several times a day for two weeks. No quantitative analysis was possible, but qualitative assessments of suckling (movement of the oral floor and cheeks and visibility of the tongue) were made. Attempts to film natural suckling were unsuccessful due to maternal behavior.

The positions of the radio-opaque markers and of the liquid were analyzed in each frame using a digital imaging system consisting of L&W 4500 projector and cinechain, a Coho video camera and a Data Cube MaxVision image analysis system attached to a Zenith 386 microcomputer. For each animal, several sequences of suckling and crinkling were selected in which the animal did not turn its head out of the lateral plane. These clear X-ray images were digitized and then analyzed.

For graphical analyses in both ages of animals, the movement of a radio-opaque marker was examined as a function of time. In figure

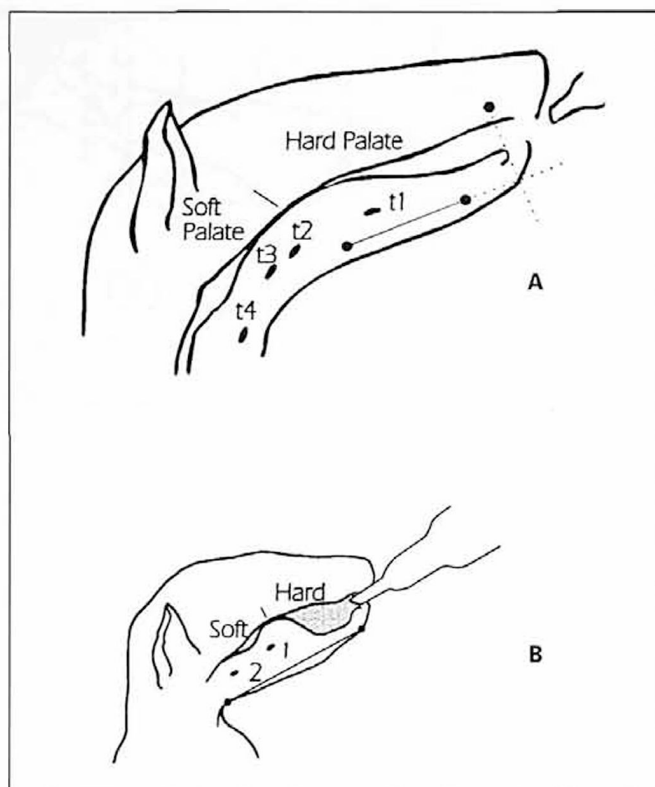


Fig. 1. Single frames traced from cine-x-ray film of **A** a 66 day old individual and **B** a 45 day old individual. Anterior is to the right and dorsal is up. Tongue and hyoid markers are solid ovals (t1–t4 in 66 days, and 1–2 in 45 days), and reference markers are solid circles. The solid line between the lower reference markers indicates the horizontal axis for determining movement of the markers. The vertical dotted line is the vertical axis.

1A, the location of the tongue markers (solid ovals labeled T1–T3) in Cartesian coordinates was relative to a horizontal axis drawn between the two lower reference markers (solid circles) and a perpendicular vertical axis through the lower anterior marker, which was designated as the origin. The variable gape was measured as the linear distance between the lower and upper anterior markers. A short line in figure 1A and B indicates the junction between the hard and soft palates. For the younger animals lacking reference markers, recognizable anatomical points were used (fig. 1B). These are not as accurate or repeatable as inserted markers, resulting in more variable data.

Results

The two different ages used distinct mechanisms for acquisition of milk from the nipple and subsequent transport through the oral cavity. The youngest animals, 45 days of age, relied on dorsal/ventral or circular tongue movements,

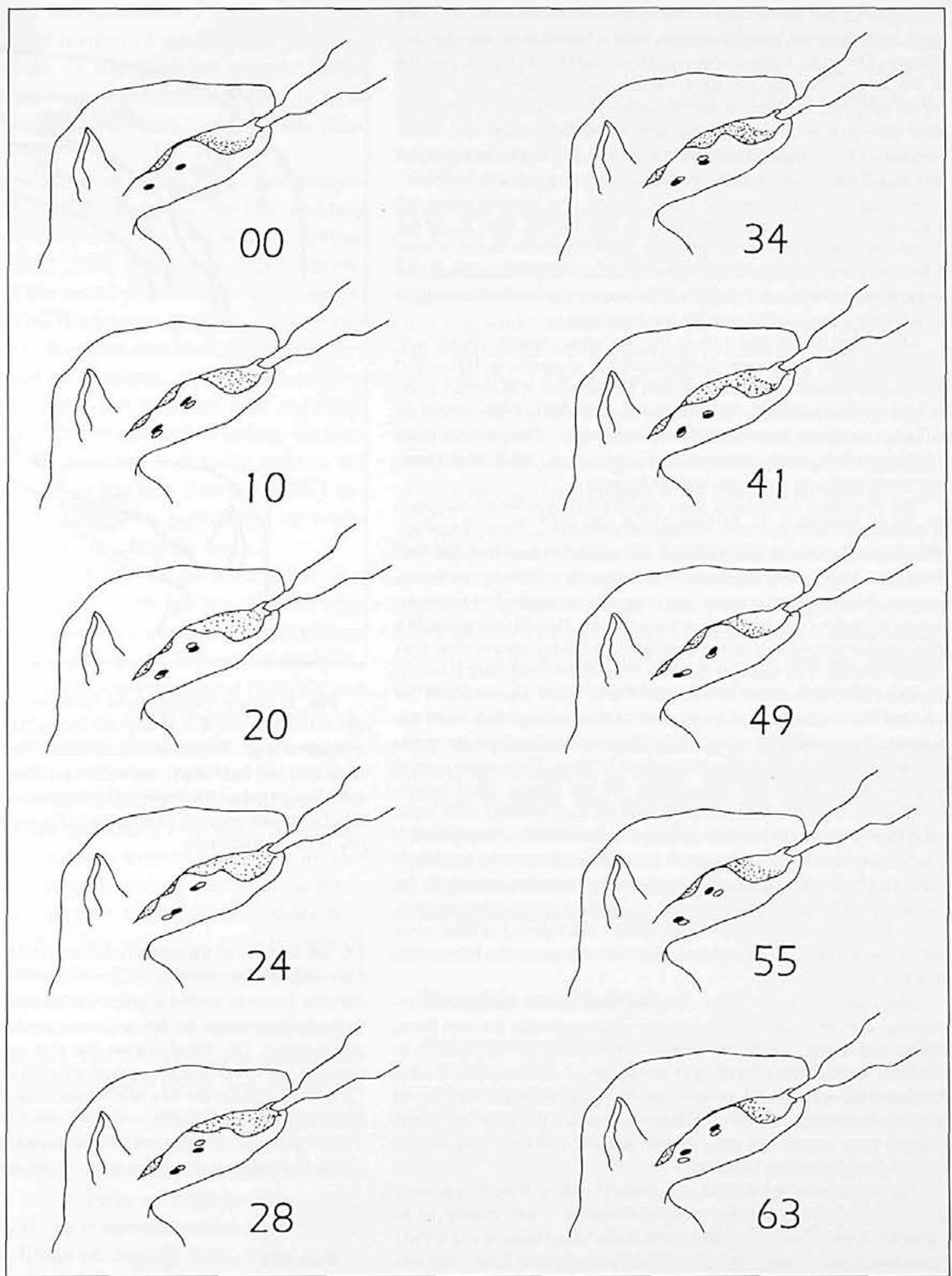


Fig. 2. Suckling in infant opossums of 45 days of age. These infants have been separated from their mothers for only a brief period of time. Numbers are frame numbers, each frame is 10 msec. Solids dots are the location in each frame of radio-opaque markers in the tongue body. The open circles indicate the position of the tongue markers in the previously illustrated frame. The stippled area is the location of milk containing a small amount of barium.

One representative sequence is shown in figure 2. Jaw movement in these individuals was minimal, and no repetitive cyclic patterns were discernable. The tongue tip was not clearly visible in every frame, but in those frames where it was visible it did not move between frames.

Initially the middle of the tongue was pressed against the hard palate, near the location of the first tongue marker, just anterior to the hard palate – soft palate junction (fig. 2, frames 00–10). In front of this seal, the tongue moved downwards, as milk flowed from the nipple into the oral cavity (fig. 2, frames 00–20). Between frames 00 and 10, the first marker moved upwards, maintaining the tongue to palate seal. The seal moved posteriorly between frames 10–20, as a slightly more posterior portion of the tongue contacted the soft palate. The tongue near the first marker moved downwards, increasing the space inside the oral cavity. Between frames 20 and 34 the tongue near the first marker continued to move downwards and slightly backwards. There was considerable movement in the tongue that was not reflected in marker movement. The seal between palate and tongue shifted further in a posterior direction along the soft palate (fig. 2, frames 24–34), and a portion of the tongue anterior to the first marker moved dorsally, upwards towards the hard palate. These movements created a second space within the oral cavity, and milk flowed backwards into this space. The rising portion of the tongue continued to rise, and by frame 49 it again contacted the hard palate, sealing this bolus of milk inside the posterior region of the oral cavity. Finally, the tongue posterior to the seal, near marker two, moved upwards to reach first the posterior most edge of the hard palate and a large portion of the soft palate, forcing the milk in the anterior oral cavity into the oropharynx (fig. 2, frames 49–63).

The main movement of the tongue was in a dorsal/ventral direction. Figure 3 traces the dorsal/ventral movement of the first tongue marker as a function of time.

Downwards in this graph is dorsal, and a dotted lines indicate the frames illustrated in figure 2. There was circular movement, i.e., both dorsal/ventral and anterior/posterior in the more posterior, second tongue marker. Figure 4A is a trace of marker movement in the plane of the film, showing movements in two directions. The location of a marker in space at each point in time is connected to produce a graph of the marker movement for one cycle. The circular movements were anterior and downwards, followed by posterior and upwards. This tongue movement produced a pumping motion that was externally visible while the animal was feeding. A similar pumping movement of the oral floor could also be seen in littermates still attached to maternal teats. There was no observed qualitative difference between

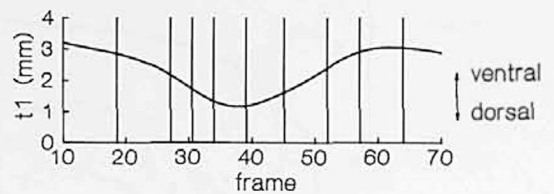


Fig. 3. Dorsal/ventral movement of the first (t1) radio-opaque marker vs time in 45-day-old infants during suckling. The location of the marker relative to the lower jaw is plotted against frame number, where each frame is 10 msec. The vertical dotted lines indicate frames illustrated in figure 2. As the curve moves downward, with increasing frame number, the tongue marker is moving ventrally.

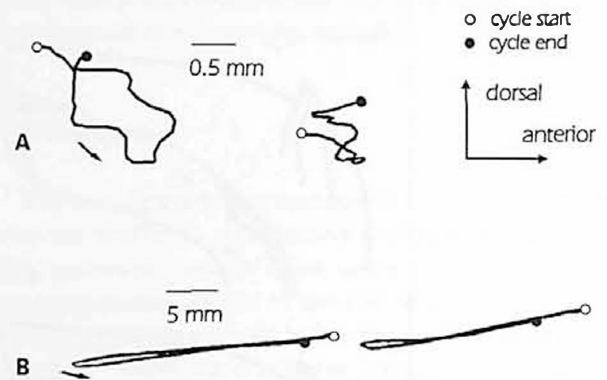


Fig. 4. Observed movement of first two tongue markers from films taken in the lateral plane of **A** a 45-day-old infant and **B** a 66-day-old infant. Up is dorsal and left is anterior. These curves trace the movement of each marker in the lateral plane over time. The younger infant has circular movements, with both dorsal/ventral and anterior/posterior movement. The older individual has primarily anterior/posterior movements.

the experimental infants suckling on a bottle and the controls suckling on their mothers. In neither case was the tongue visible during feeding.

The kinematics seen in the older individuals was different from that in the younger ones. These animals had been separated from their mothers for several days and hand fed with bottles and formula. Initially, prior to filming, the infants attempted to suckle with what appeared to be a similar pumping mechanism. This activity could be elicited for less than one day, often for only a few hours, after separa-

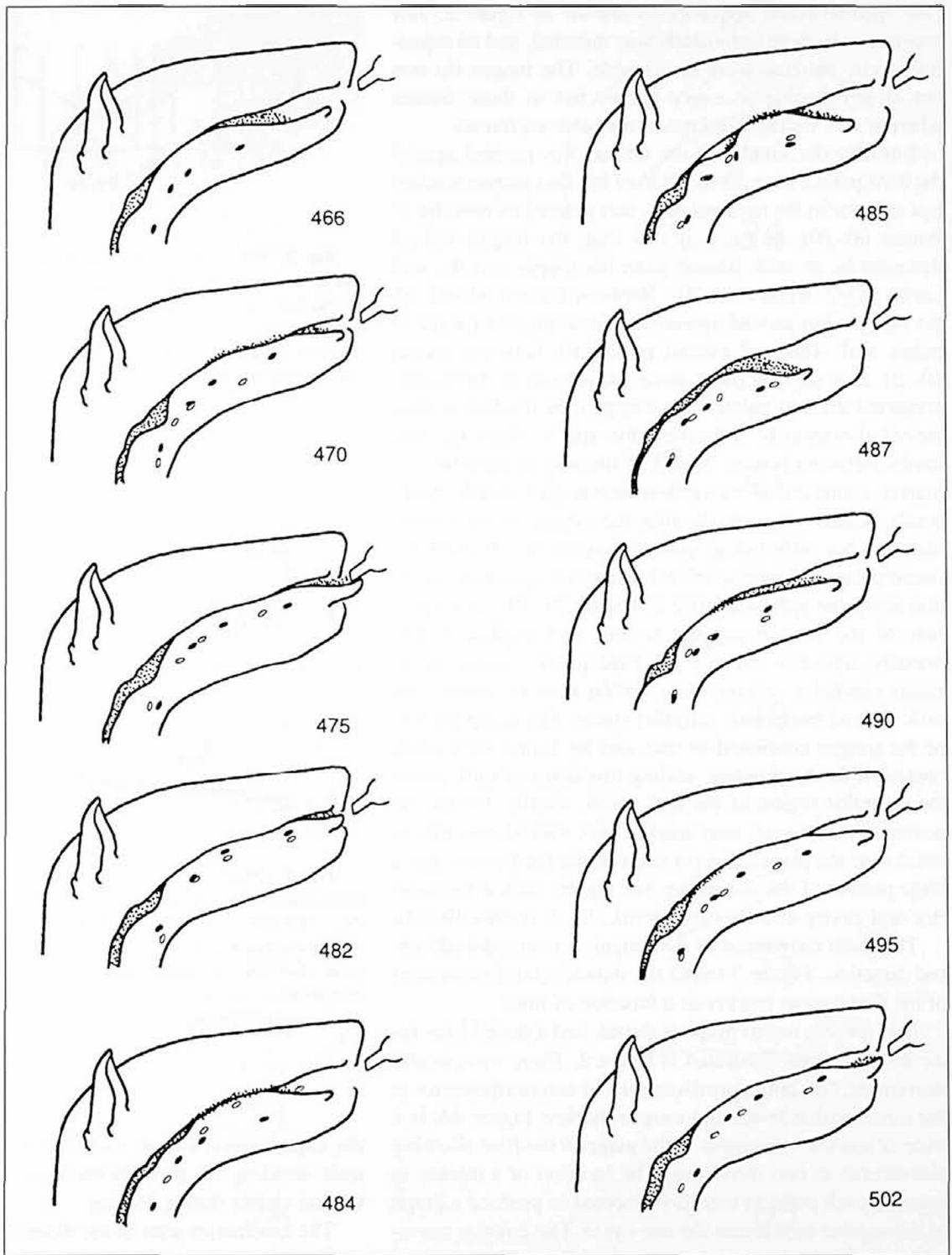


Fig. 5. Lapping on a bottle by infant opossums of 66 days of age. The infants have been separated from their mothers and bottle fed for at least 12 h. Numbers are frame numbers, each frame is 10 msec. Solid dots are the location in each frame of radio-opaque markers in the tongue body. The open circles indicate the position of the tongue markers in the previously illustrated frame. The stippled area is the location of milk containing a small amount of barium.

tion from the mother. This activity was superseded by attempts at lapping, but few animals survived for more than 36 hours. Animals that did survive preferred to lick or lap at the nipple with significant tongue protrusion.

X-ray images of feeding in the older animals indicated that the seal between hard palate and tongue was longer, flatter, and less mounded in appearance than in the younger animals. Milk transported in the previous cycle was visible in the valliculae and oropharynx (fig. 5, frame 466). The seal, and contact between tongue and palate, lengthened considerably as the tongue moved forward (fig. 5, frame 466), and the tip of the tongue protruded to touch the nipple (frame 470). Tongue protrusion involved forward movement of the body of the tongue coupled with intrinsic expansion. The tip of the tongue contracted during frames 464–475 as compared to the rest of the tongue which expanded. These animals would not take the nipple into their mouths, instead reaching with their tongue tip to lick milk from the end of the nipple. The tongue tip extended to form a characteristic curl to collect the milk (fig. 5, frame 475).

Tongue retraction began near frame 482, and at this point milk was visible between the tongue and hard palate through most of the oral cavity (frame 484). The tongue continued to retract very quickly (frames 484–487), dropping slightly from contact with the hard palate. The posterior parts of the tongue moved posteriorly and with some intrinsic contraction between pairs of markers. Slight dorsal movement occurred at this time in the anterior parts of the tongue. Finally, forward movement of the tongue squeezed this milk into the esophagus for a swallow (frames 490–502). During this time, a new bolus was being collected from the nipple.

The movements of the markers in these individuals were almost entirely anterior/posterior. Figure 6 plots the consistent and regular anterior/posterior movement of the first tongue marker as a function of frame number. There was little, if any dorsal/ventral movement of the tongue. Figure 5b shows movement of the first two tongue markers in both anterior/posterior and dorsal/ventral directions. The location of each point in space is connected with a line to show the trace of movement in a lateral plane over time. The slight dorsal/ventral movements seen were associated with the formation of seals between tongue and hard or soft palate. The only exception is a small dorsal/ventral excursion seen in the tongue tip when milk is collected from the nipple. Even the squeezing of milk for the swallow at the end is an anterior/posterior movement.

The littermates of these animals that remained with their mother did not show any indication of this behavior. Their tongues were never visible, and their mouths were strongly

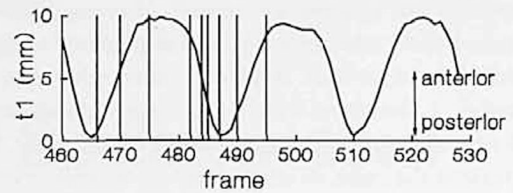


Fig. 6. Anterior/posterior movement of the first radio-opaque marker in 66-day-old infants during feeding. Each frame is 10 msec. The vertical dotted lines indicate frames illustrated in figure 5. As the curve moves upwards with increasing frame number, the tongue marker is moving in an anterior direction.

attached to the maternal teats. They always showed a strong dorsal/ventral movement of the oral floor, identical to what was observed in the younger animals.

Discussion

The two different mechanisms observed in these opossums are similar to mechanisms described for drinking in other mammals. Several other infant mammals use a suction mechanism, similar to the one described here for the youngest opossums. Both infant pigs and macaques [German et al., 1992] use a negative pressure mechanism, primarily driven by dorsal/ventral pumping of the tongue to acquire milk from the nipple. Infant pigs use a second pumping mechanism to move milk through the oral cavity and oropharynx prior to swallowing, whereas infant macaques use a squeeze-back transport mechanism, similar to that used by adult macaques in feeding and drinking [Franks et al., 1984]. Adult macaques [K.M. Hiiemae and A.W. Crompton, unpubl. observ.] and pigs [Herring and Scapino, 1973] both use a modified sucking, negative pressure mechanism for drinking.

The lapping mechanism used by the older, 66-day-old opossums, however, resembles the mechanisms used in drinking by adult opossums and other mammals that lap as adults [Hiiemae and Crompton, 1985; Thexton and Crompton, 1989]. Lapping, in adults, is primarily an anterior/posterior movement, with no or very small dorsal/ventral components. The backwards and forwards excursion is greater in lapping than in other forms of feeding behaviors, such as chewing or intraoral transport.

Although we observed lapping in infant opossums that was indistinguishable from adult behavior, in both general

mechanism and specific tongue movements, these infants were certainly not weaned nor capable of eating solid food. They lacked teeth and were at a developmental stage that indicated at least several weeks of time remaining with their mother. Littermates who did remain with the mother showed no indication of lapping, either at the teats, at the water bottle in the cage, or at small bowls of milk offered to them.

These results strongly suggest that two distinct mechanisms for acquisition of liquid exist simultaneously in infant opossums. The pumping, negative pressure mechanism seems to be common to several species of infant mammals, all of which have different adult mechanisms for drinking. It seems that the adult lapping mechanism exists in infants who are too young to survive independently of maternal care. There are several potential cues that could have

caused these infants to turn on the adult mechanism. Temperature and odor were most likely different between the experimental animals and their littermates who remained with the mother. Thus, the developmental change from infant to adult drinking patterns is not a strictly temporal change. The neural basis for both mechanisms exists from early in development, far sooner than could be realistically utilized by an infant opossum.

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