

Kinematic analysis of an appetitive food-handling behavior: the functional morphology of Syrian hamster cheek pouches

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Summary

Prodigious food hoarding in Syrian hamsters *Mesocricetus auratus* Waterhouse is strongly linked to appetite and is made possible by large internal cheek pouches. We provide a functional analysis of the cheek pouch and its associated retractor muscle. Frame-by-frame analysis of videotaped pouch-filling behavior revealed multiple jaw cycles for each food item pouched and the use of more jaw cycles to pouch large food items (~2.5 g chow pellets) than small (corn kernels or sunflower seed with husks). These results stand in contrast to previously reported pouching kinematics in the externally pouched *Dipodomys deserti*, which uses only one jaw cycle per pouching event. Comparison of pouching and mastication in the same individuals also suggests that in Syrian hamsters, feeding jaw cycles are modulated to

accommodate pouch filling primarily by the addition of a pause between fast open and fast close phases, which we call 'gape phase'. Contrary to previous assertions, the retractor muscle does not merely provide structural support for the full pouch during locomotion. Video analysis of ten hamsters with unilaterally denervated retractor muscles and electrophysiological study of an anaesthetized subject confirmed that retractor muscle activity during pouch filling increases pouching efficiency for food items subsequent to the first.

Supplementary material available online at
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Key words: *Mesocricetus*, feeding, hoard, retractor.

Introduction

In 1918, Wallace Craig coined the term 'appetitive' to describe behaviors that are aimed at eliminating a 'state of agitation' (appetite), caused by the lack of a stimulus (food or sex). He distinguished these from 'consummatory' responses, i.e. those that consummate, or resolve the state of agitation through acquisition of the required stimulus, consequently decreasing the appetite. Thus, feeding and copulation are consummatory, while all behaviors directed toward finding and obtaining food or a mate are appetitive. In nature, feeding and copulation generally cannot occur unless preceded by appetitive behaviors, such as foraging or courtship, which must, therefore, be subject to strong and direct selection pressures. This principle has been widely recognized in studies of reproductive behavior, and provides the foundation for morphological research on characters related to sexual selection and mate choice. However, the morphology supporting food-directed appetitive behavior, particularly in mammals, remains largely unexplored, possibly because the structures involved (e.g. forelimbs, jaws, postural head and neck muscles) are also used in behaviors unrelated to feeding, thereby confounding interpretations of their functional morphology with respect to appetite. Rodent cheek pouches, in contrast, are specialized for carrying food to storage sites. They are involved in acquiring (appetitive behavior) but not consuming food. Therefore, pouch structure and function should

reflect selection acting on appetitive behavior independent of consummatory behavior.

There is no question that the rodent cheek pouch is ideally suited for carrying food. It is a subcutaneous, expandable, paraoral sac with its caudal tip extending as far posteriorly as the scapula in some species and its opening either within the mouth (for 'internal' cheek pouches) or lateral to the mouth (for 'external' cheek pouches). Although there have been anecdotal reports of alternative uses for cheek pouches, including one unconfirmed report of a Syrian hamster filling its pouches with air and using them as pontoons (Vorontsov, 1979), by far the most commonly reported function is carrying food (Keyes and Dale, 1944; Van Hoosier and McPherson, 1987). Primary roles in locomotion, displays, fighting or social behavior (unless directly related to food gathering) are unlikely, given cheek pouch anatomy and location. A recent field study of Syrian hamsters (Johnston, personal communication) reported extensive use of cheek pouches in food gathering and stockpiling (collectively termed food hoarding).

Food hoarding in hamsters has been called a 'quintessential' appetitive behavior (Bartness and Day, 2003), because it is strongly influenced by hunger, but is qualitatively and quantitatively dissociable from food intake. The amount of food hoarded increases significantly (up to tenfold) when food is made available to Syrian and Siberian hamsters that were

previously food-deprived or food-restricted (Bartness and Clein, 1994; Buckley and Schneider, 2003; Lea and Tarp, 1986), but the amount of food these hamsters consume is unchanged from pre-fasted levels (Silverman and Zucker, 1976). Hunger is a state of agitation caused by the lack of internal energy reserves, and food hoarding (including pouch filling) by itself can do nothing to resolve this internally driven state of agitation. There are no secretory cells within the cheek pouch walls to aid in digestion (Hardy et al., 1986; Ryan, 1986), and adult Syrian hamsters do not consume food directly from the pouch, but place it into a food pile (hoard), from which meals are taken (Etienne et al., 1982). Pouch-filling is therefore not consummatory with respect to hunger, although in internally pouched rodents, it might be termed 'incipient consummatory behavior' (Craig, 1918), in that the food must enter the oral cavity before it can enter the cheek pouch. However, such behavior is, in truth, appetitive, because it does nothing to decrease the appetite, and will presumably continue until complete consummatory behavior is elicited (chewing and swallowing), and the condition of hunger is thereby resolved [additional references similarly define appetitive and consummatory ingestive behaviors (Ammar et al., 2000; Taylor et al., 2007)]. Thus, rodent cheek pouches present a unique opportunity to study the morphology of a functionally isolated, appetitive specialization.

Internal cheek pouch use in Syrian hamsters has been casually observed by many, but the functional morphology of the internal cheek pouch and its associated musculature remains largely unexplored. Our goal here was to describe pouch-filling behavior in the Syrian hamster. Specifically, we studied the kinematics of jaw cycles during pouch filling and the role of the pouch retractor muscle. For clarity, this behavior can best be described in three steps. First, food must be moved in a primarily posterior direction in order to enter the oral cavity. Second, the food must be diverted laterally, through the pouch aperture. Third, if multiple food items are added to the cheek pouch, then food within the pouch must be moved caudally in order to make room for subsequently pouched items. Videotapes of chewing and pouch-filling behavior in a mirrored filming chamber were used to elucidate the behavior patterns associated with each step of pouch filling.

The anatomy of internal and external cheek pouches has been described in detail elsewhere (Priddy and Brodie, 1948; Ryan, 1986; Ryan, 1989), and other authors have speculated on the relationship between cheek pouch form and function in rodents (Keyes and Dale, 1944, Long, 1976). In Syrian hamsters, cheek pouch function has been examined using electromyographic stimulation (Priddy and Brodie, 1948) and videotaping (Gorniak, 1977), but jaw activity and the role of the retractor muscle in pouch filling have not been described. One externally pouched rodent, *Dipodomys deserti*, has been shown to coordinate forelimb activity with one jaw cycle per pouching event, defined as the insertion of one load of food from paws into cheek pouches (Nikolai and Bramble, 1983). Due to the location of the internal cheek pouch opening (on the medial cheek wall, lateral to the diastema), food must pass through the oral cavity in order to enter the pouch, thereby necessitating jaw abduction and some means of manipulating food items laterally, through the diastema. Given the reported

coordination of jaw activity with forelimbs during external cheek pouch use in *Dipodomys*, it seemed reasonable to hypothesize, first, that forelimb and jaw activity would be coordinated to move food into the oral cavity in an internally pouched rodent, and second, that moving food items laterally through the pouch opening would require multiple jaw cycles, particularly for large items as they pass by increments from the oral cavity through the diastema and pouch aperture. Further, we wished to compare pouching jaw cycles to masticatory jaw cycles in the same individuals.

Mammalian mastication is a conserved cyclic motor pattern with four phases, varying in length based on food consistency (Hiimae, 2000). (i) Slow open (SO) is the initial widening of gape, and is followed by (ii) Fast open (FO), which involves a large, quick abduction of the mandible. (iii) Fast close (FC) is the equally quick adduction of the mandible that immediately follows FO, and ends when the closing jaw makes contact with the food item, at which point (iv) Slow close (SC), also called the power stroke, begins. SC may be followed by an intercusp phase, then SO, as the jaw relaxes from the power stroke and begins the next cycle. Lateral movements of the mandible, particularly during occlusion, are another important aspect of mammalian mastication, but one that we were unable to quantify in this study. Instead, pouching and mastication were compared by measuring gape distance and cyclic patterns of jaw activity.

Another goal was to examine the role of the trapezius-derived retractor muscle in cheek pouch use, particularly during caudal movement of items already within the pouch as more food is added. Videotapes of hamsters pouching both large and small food items showed muscle activity in the region of the retractor toward the end of each pouching event. This observation was perplexing in that the retractor is not ideally positioned to play a role in pouch filling, nor has it ever been proposed to do so. Generally, it has been construed as structural support, acting as an 'antagonist to the longitudinal musculature of the pouch wall' (Priddy and Brodie, 1948). When Priddy and Brodie applied electrical stimulation to the facial nerve innervating the pouch wall muscles while observing the location of a pea placed inside the pouch, the pea moved toward the pouch opening and in some cases, into, but not through the opening. Forceps clamped to the everted pouch wall of an anaesthetized hamster were pulled into the cheek pouch by electrical stimulation of the retractor muscle, but there was no reported attempt to observe the effects of such stimulation on food within the pouch.

Given preliminary observations of retractor activity during pouching and the reported anterior movement of food within the pouch by stimulation of muscles that are antagonistic to the retractor, we hypothesized that retractor activity serves to pull food into the pouch, moving food caudally, perhaps by a ratchet-like mechanism. We tested this hypothesis by unilateral denervation of the retractor muscle, and predicted decreased pouching efficiency to the denervated side, compared to the intact (sham-operated) side. We also used electrophysiological stimulation of retractor contraction to observe its effect on food position within the pouch of an anaesthetized hamster.

Materials and methods

In preparation for surgical manipulations and to confirm literature descriptions of pouch anatomy (Van Hoosier and

McPherson, 1987), particularly with respect to functional and morphological relationships between retractor and pouch (Priddy and Brodie, 1948), we began with dissections of male and female specimens of *Mesocricetus auratus* Waterhouse with full and empty pouches. We used the video apparatus described below to observe chewing and pouching behavior in intact and then unilaterally retractor-denervated (cranial nerve XI) hamsters. Electrophysiological observations were obtained from one intact, anaesthetized male hamster, and the second set of pouching efficiency data were collected from previous videotapes of denervated hamsters. All procedures were conducted according to the guiding principles for research, formulated by the American Physiological Society and the National Institutes of Health, and were approved by the Lehigh University Institutional Animal Care and Use Committee.

Dissections (specimen preparation)

Two male and two female hamsters (approximately 6 months old, body mass 115–125 g) were given nebutol anaesthesia (9 mg 100 g⁻¹ body mass) then sacrificed by CO₂ asphyxiation. One male and one female were injected subcutaneously, intramuscularly, pericardially and intraperitoneally with approximately 150 ml of paraformaldehyde solution (4%) and soaked in this solution for 24–48 h, after which they were stored in 70% ethanol. Several additional hamsters were given eight chow pellets (~2 g each) inside their cages, and of those that filled their cheek pouches, one male and one female were immediately sacrificed by CO₂ asphyxiation, fixed and stored as previously described.

Subjects and housing (behavioral studies)

For the study of jaw cycle kinematics during pouching and chewing, adult male and female hamsters *M. auratus* (Charles River and Harlan/Sprague Dawley) were housed on a long-day (16 h:8 h reversed-phase dark:light) photoperiod at 22±2°C, and given food and water *ad libitum* (Purina Laboratory Chow # 2016 and Harlan Global Diet, Frederick, MD, USA), except as noted below in 'Apparatus and Videotaping'. Body mass ranged from 115 to 130 g, and ages from approximately 6 months to 1 year, females being slightly older and larger than males. They were initially housed in 33 cm×20 cm×18 cm opaque Nalgene™ cages with wire tops, and were transferred to 25 cm×20 cm×24 cm clear plastic cages (CrittterTrail® by SuperPet International, Elk Grove Village, IL, USA), hereafter referred to as 'home cages,' several days prior to videotaping (sufficient time to acclimate and establish a nesting site).

To determine the effects of denervated retractor muscle on number of items pouched, 20 additional animals were obtained and housed under conditions identical to the jaw cycle study, but were acclimated for several weeks to home cages connecting them to a food source area of similar dimensions to the filming chamber, and only those hamsters that pouched regularly during the acclimation period were used (N=16). Fourteen of these hamsters pouched exactly two pellets to each side on at least four visits to the filming chamber, and ten video segments from these animals were randomly selected to provide additional data on the effects of denervation on pouching efficiency based on pouching chronology. The subject used for electrophysiological

observations was obtained similarly and housed in the standard Nalgene™ cages described above.

Apparatus and videotaping

The apparatus design, based on previously published research (Bartness and Clein, 1994), simulated as closely as possible the natural conditions under which hamsters climb from their burrows and pouch food (Siegel, 1985), including a vertical climb to reach a food source area some distance away from the nest site in the home cage. Just prior to videotaping, the home cage was connected to a filming chamber *via* approximately 110 cm of translucent plastic tubing (minimum internal diameter of 4 cm, Habitrail® by SuperPet). The filming chamber (Fig. 1) provided simultaneous lateral, anterior and ventral perspectives from a single camera angle. To study jaw cycle kinematics, four large food items (lab chow pellets cut to 2.5±0.5 g) or 40–60 small food items (whole corn kernels or sunflower seeds with hulls) were placed in the filming chamber before connecting it to the home cage, and new food was added as needed. Hamsters that did not pouch under *ad libitum* feeding conditions were disconnected from tubes and retested several days later, after reacclimation to the photoperiod and 12–16 h of food deprivation. Subjects that failed to pouch after minor food deprivation were removed from the study.

For retractor denervation studies, videotaping occurred between 10 and 25 days post-surgery, and was conducted as described above, except that each hamster was presented with 24 large (2.5±0.5 g) pellets (four at a time) and 130 corn kernels (in four sets of 30 and one set of 10).

All videotaping was completed during the first half of the dark phase of the photoperiod, which is the peak activity period for hamsters (Refinetti, 1999). The filming chamber was directly illuminated with a photo lamp and incandescent bulb (total 850 W), and behavior was videotaped with a Panasonic AG-456 video camera (Matsushita Electronic Corporation of America, Secaucus, NJ, USA) at 60 frames s⁻¹, with frame exposure times of 1/500 s.

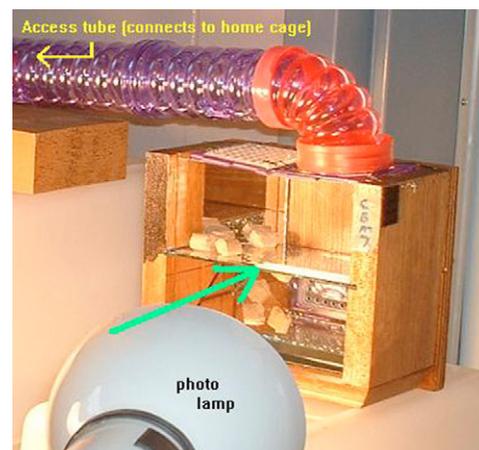


Fig. 1. Mirrored filming chamber containing four large food items (lab chow pellets cut to 2.5±0.5 g). Green arrow shows camera angle. Photo lamp (to decrease shadows) is a 100-W bulb approximately 4.5 m from the filming chamber. Another lamp (750 W) was set up about 1.5 m from the filming chamber.

Retractor denervation surgery

Under nembutol anesthesia (9 mg 100 g⁻¹ body mass), a 2 cm incision was made on the dorsal surface at the third thoracic vertebra, approximately 1 cm lateral to the vertebral column. Under a dissecting microscope, the medial side of the retractor muscle was exposed by cutting the connective tissue between the retractor and the underlying spinotrapezius, latissimus dorsi and platysma cervicale. Branches of the spinal accessory nerve were identified by their entry into the dorsal and ventral slips of the retractor muscle, traced back to their origin as a single nerve emerging from beneath the spinotrapezius, teased apart from accompanying blood vessels and cut, resulting in immediately visible loss of retractor muscle tone. Because individual hamsters varied in the topology of spinal accessory nerve branches, it was not always possible to access the main nerve, central to its branches, without damage to the surrounding muscle tissue, and in such cases each branch was cut until the retractor lost muscle tone. Muscles were returned to natural positions and the wound was closed with suture. Each animal also received sham surgery to the contralateral retractor muscle, in which the spinal accessory nerve was teased apart from blood vessels, but not cut. Thus, each animal served as its own control, and the order of sham and denervation procedures was counterbalanced. Analgesia (buprenorphine, 0.02 mg kg⁻¹) was given subcutaneously before surgery and 12 h postoperatively. Food intake was measured daily for 1 week before and 1 week after surgery and did not differ.

Electrophysiology

One male hamster was given Metacam analgesic orally (meloxicam, 0.2 mg kg⁻¹). 15 min later, sodium pentobarbital was administered by intraperitoneal injection (0.9 mg kg⁻¹). Anesthesia was maintained throughout the procedure with 0.05 mg kg⁻¹ injections delivered subcutaneously every 30–40 min during the procedure, and a lethal dose was combined with isoflurane inhalant to expedite euthanasia when recordings were complete.

Approximately 15 min after the first anesthesia injection, the anterior 2/3 of one side was shaved from the lumbar vertebrae to the vibrissae. An incision was made exposing the full length of the cheek pouch and retractor muscle. The animal was placed under a dissecting microscope and the same procedure described for retractor denervation was used to isolate and identify the two branches of the spinal accessory nerve that innervate the ventral and dorsal slips of the retractor muscle. These were gently lifted away from the body along with associated blood vessels and connective tissue. Nerves and associated tissue were draped over the leads of an electrode mounted on a micromanipulator, which allowed stable positioning of the tissue and electrode tips approximately 2–3 mm away from the underlying muscle tissue. The electrode was connected to a Grass stimulator (West Warwick, RI, USA) and current was applied at voltages and frequencies sufficient to mimic rhythmic contractions and tetanus that had been observed in pouching videos (voltage ranged from 500 mV to 700 mV, increasing as the retractor muscle became fatigued). Activity was limited to the retractor; no contractions were seen in adjacent muscles. Ringer's solution (0.9% saline) was applied as needed to maintain moisture of all exposed tissue, except the oral cavity and pouch aperture.

A surgical spring-type tissue retractor held the mouth open at full gape, and a single corn kernel was placed within the pouch opening, with the pointed end extending into the pouch and the flat end visible within the oral cavity (matching the orientation most often seen on video tapes). Through the pouch opening, gentle pressure was applied with a blunt probe at the flat end of the corn kernel, pushing it into the pouch by small increments, and alternating retractor tetanus with mechanical pressure, until the kernel was entirely visible through the lateral pouch wall. While observing effects on kernel position through the pouch wall, we continued to alternate repeated retractor tetanus with probe pressure. Lastly, tetanus and mechanical pressure were applied simultaneously.

Behavioral data collection

To maintain consistency in behavioral measurements, all video data were collected by a single observer, using a Panasonic AG-1960 ProLine S-VHS VCR (Knoxville, TN, USA). For jaw cycle kinematic data, frames were counted as SO or SC if the mandible showed only minor or non-detectable changes in position from the previous frame (relative to the maxilla, with jaw closed). Large shifts in position were counted as FO or FC. To compare patterns of jaw movement, gape distance was measured for one male hamster during typical bouts of mastication (lateral view, grinding lab chow pellets) and large- and small-item pouching (ventral view). The different perspectives provided optimal views of mandibular motion during each of these behaviors. To measure pouching efficiency, three pouching transition points were identified from video records: (1) Lateral shift: food moved laterally through the diastema to the extent that a bulge was detectable on the outer cheek in the ventral view; (2) Mouth closing: the mouth closed completely, indicating that the food item was completely within the pouch and no longer within the oral cavity; and (3) End: the hamster ceased neck-stretching (repeated protraction and retraction of the head and lower jaw that sometimes followed insertion of a food item into the pouch) or reached for another food pellet, indicating the end of the pouching event. Interval A was defined as the number of frames from Lateral shift to Mouth closing, and Interval B, from Mouth closing to End. For each hamster, the order of each pellet (whether it was first or second to be pouching to that side) was recorded, and the length of each interval was counted in frames (1 frame=1/60 s) for all visits to the filming chamber where exactly two pellets were pouching to each side (this varied from 4 to 6 visits).

Data analysis

Jaw cycle kinematic data were based on three females and four males that provided multiple unobstructed views of mastication (grinding bites of dry lab chow), pouching of large food items and pouching of small food items. The number of masticatory and pouching events providing sufficiently clear views for analysis varied between hamsters, as did the number of cycles per event (Table 1). Thus, for each animal, the length of time in each phase of the jaw cycle was averaged across multiple occurrences of each type of event (mastication, pouching large items, and pouching small items), and the resulting means were used in all test statistics. Occasionally, hamsters 'froze' for extended periods of time during jaw cycles,

Table 1. Number of unobstructed events and jaw cycles per event contributing to means used in statistical analyses

Gender	Number of events		
	Mastication	Pouching pellets	Pouching seeds
F	3 (7,5,6)	2 (6,12)	11 (8,2,7,6,3,3,2,6,4,2,5)
F	1 (12)	5 (6,6,3,6,4)	6 (2,3,3,4,5,3)
F	3 (7,7,5)	4 (9,6,8,3)	9 (4,5,3,2,4,4,4,3,3)
M	6 (14,6,9,13,12,7)	7 (6,6,5,7,6,5,5)	6 (8,8,5,4,3,5)
M	2 (5,5)	4 (9,7,6,6)	14 (2,3,3,2,3,2,2,2,3,2,2,2,2,2)
M	3 (7,14,13)	4 (8,7,6,5)	9 (10,4,4,7,4,3,2,3,2)
M	6 (9,7,7,12,12,14)	7 (11,9,7,4,8,8,9)	16 (3,4,4,2,2,3,4,3,2,2,2,2,3,2,2)

N=7 (3 females and 4 males). Values in parentheses are the number of jaw cycles per event.

resulting in total cycle lengths that far exceeded all other cycle lengths. Thus, for each event, means \pm s.d. were calculated for each phase, and any data outside 98% confidence limits were trimmed by removing the entire cycle containing the outlier. Data for males and females were combined, and a 3 \times 3 factorial repeated-measures ANOVA was used to compare the mean lengths of three phases of the masticatory cycle during each behavior (FO, FC, and the combined SC and SO phases, which could not be distinguished during pouching), with *post-hoc* comparisons only if the main effects were significant. Average number of frames to complete a jaw cycle during each behavior was compared with a one-way repeated-measures ANOVA, with *post-hoc* comparisons only if the main effects were significant. The length of the observed pause between fast open and fast close phases while pouching large vs small food items was compared with a paired *t*-test. All statistical analyses were considered significant at $P<0.05$.

To determine the effects of denervation on the number of food items pouching, the percentage of items pouching to the denervated side (out of the total number of items pouching to both sides) was averaged for each animal across visits to the filming chamber where pouching occurred. These were compared to the null hypothesis mean of 0.50, using one-tailed, one-sample *t*-tests, with $P=0.05$. All 16 hamsters pouching large items during at least six visits and small items (whole corn kernels) during at least five visits, for a total of 88 visits where large items were pouching and 100 visits where small items were pouching. The total number of items pouching in a single visit ranged from 1 to 4 for large items, and 2 to 30 for small items.

To quantify the effects of denervation on pouching efficiency based on item sequence, the mean length of each pouching interval (see Results, 'Behavioral Data Collection') was averaged across visits and similar pouching events, and these means were compared in three repeated measures 2 \times 2 ANOVAs with $N=10$ [condition of retractor (intact vs denervated) \times order of pellet (first vs second)] and $P=0.05$. *Post-hoc* comparisons were made only if the main effects were significant, with $P=0.05$.

Results

Anatomy

The retractor muscle originates from the lumbodorsal fascia and the spinous processes of the last three thoracic vertebrae, proceeds rostroventrally, and bifurcates approximately 1 cm before reaching the caudal end of the cheek pouch. A dorsal slip

inserts on the dorsomedial surface of the cheek pouch about 1–1.5 cm anterior to its caudal end, and a ventral slip inserts on the ventrolateral surface in the same region (Fig. 2A). In a fixed specimen with empty pouches, longitudinal folds (Fig. 2B) run the full length of the cheek pouch up to the points of insertion of the retractor, where the walls become convoluted in the transverse plane, forming a distinct, rigid apex pocket that extends caudad, between the two slips of the retractor muscle (Fig. 2C). In an unfixed specimen, the cheek pouch walls are semi-transparent and lack structural integrity anterior to retractor insertion. However, the walls of the apex pocket, posterior to retractor insertion, remain comparatively rigid and opaque. This cricetine structure has been described as a 'distensible tissue peninsula' because, in the empty cheek pouch of the Siberian hamster *Phodopus sungorus*, it projects anteriorly, jutting into the pouch lumen, and everts to provide additional pouch capacity only as the pouch is distended posterior to the point of retractor insertion (Ryan, 1986). Our dissections of *Mesocricetus auratus* indicate that in this species, the homologous structure more closely resembles an additional pocket at the apex of the pouch, projecting caudally as an extension between the slips of the retractor, even while the pouch is empty. In a fixed specimen with full pouches, the walls are uniformly thin and transparent, and the apex pocket is indistinguishable from the rest of the pouch (Fig. 2D).

Forelimb use and jaw cycle kinematics during pouching and mastication

All pouching events, whether large or small food items, involved multiple jaw cycles for each food item. Large food items were grasped with the incisors, and co-ordinated alternating jaw cycles and forepaws were used to turn and orient each pellet within the oral cavity. As the mouth was opened, forepaws grasped and turned the pellet, until the jaws closed around it and the forepaws were repositioned to turn the food again at the next jaw opening. Forelimb use ceased, but jaw cycles continued after food was completely within the oral cavity and throughout lateral transport of the food item into the pouch opening. Jaw cycles did not stop until the pellet was within the pouch, lateral to the diastema, and the jaw was able to close completely. The number of jaw cycles required to pouch a large pellet ranged from 3 to more than 25, with mode of 6 (only completely unobstructed views of pellet-pouching are reported in Table 1; many more pouching events took place than were counted in the kinematic analysis of jaw cycles).

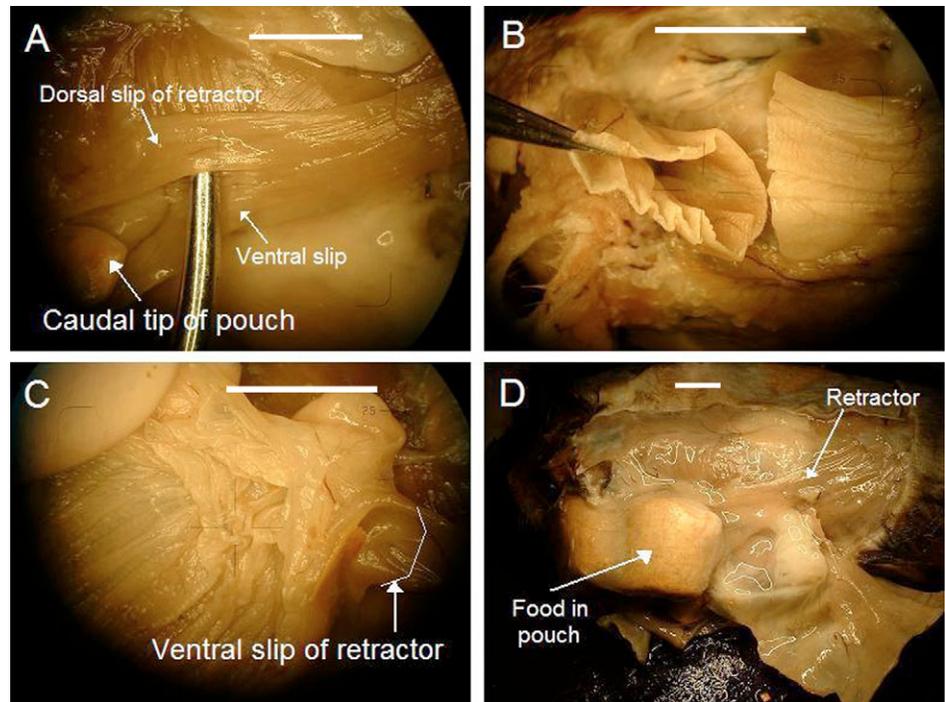


Fig. 2. Dissections of hamster cheek pouch from *M. auratus*. (A) Bifurcation of retractor muscle posterior to caudal end of pouch (apex). (B) Transverse bisection of pouch wall showing longitudinal folds. (C) A longitudinal cut on the lateral wall reveals the morphology of the pouch lumen at the point of retractor insertion, where longitudinal folds end and apex pocket begins. (D) Full cheek pouch. All pictures are from hamsters given nembutol anesthesia ($9 \text{ mg } 100 \text{ g}^{-1}$ body mass) then sacrificed with CO_2 asphyxiation, and fixed in 4% paraformaldehyde solution, except in D, where the animal received no anesthesia before CO_2 in order to maintain the full pouch. Scale bars=1 cm.

Small food items (corn kernels or sunflower seeds with hulls) were picked up with incisors or by surface adhesion to the tongue, and were manipulated within the oral cavity with or without the help of forelimbs. Observable pouching events required 2–12 jaw cycles to complete, with a mode of 2 (again, not all of these events were completely unobstructed).

Complete jaw cycle (CJC) lengths (measured from onset of FO to onset of the next FO, because the precise onsets of SO during pouching were not always clear) were significantly different for the different behaviors ('Total' in Fig. 3), with the mean CJC for pouching seeds [mean=8.41 frames (0.14 s)] taking less time than the CJC for mastication [11.35 frames (0.20 s)] [$F_{(1,12)}=9.54$, $P<0.01$], which took less time than the CJC for pouching pellets (15.49 frames (0.26 s)) [$F_{(1,12)}=18.76$, $P=0.001$]. Although the length of time spent in each phase of the jaw cycle was significantly different for the three different tasks [$F_{(4,24)}=24.25$, $P<0.0001$], *post-hoc* comparisons showed no differences between the length of time spent in FO or FC for chewing vs pouching large or small items [$F_{(2,36)}=0.76$ and 0.62 , and $P=0.47$ and 0.55 , respectively]. Rather, a significant difference was seen in the combined slow phases of the jaw cycle (Fig. 3). The mean slow phase (SC/SO) length for pouching seeds [4.68 frames (0.08 s)] was significantly less than that for mastication [8.14 frames (0.135 s)] [$F_{(1,36)}=76.81$, $P<0.0001$], which was significantly less than the mean SC/SO length for pouching pellets [9.25 frames (0.15 s)] [$F_{(1,36)}=8.01$, $P<0.01$]. Another significant difference between mastication and pouching of large and small items was the existence of a conspicuous pause between the FO and FC phases (Fig. 4). We have not seen a pause during maximum gape reported in mammalian jaw cycle literature, and we refer to it here as the 'gape phase'. The mean length of time spent in gape phase during pouching was significantly longer for large food items [3.04 frames (0.05 s)] than for small [1.09 frames (0.02 s)]

[$t(6)=6.13$, $P<0.001$], and there was no discernible gape phase during mastication (Figs 3 and 4).

Effects of retractor denervation on number of items pouching

The number of items pouching to the denervated side ranged from 4 to 19, with a mean of 13.6 when 30 corn kernels were available, and from 2 to 7, with a mean of 4.4 when 10 were available. Regardless of the number of items available for pouching, the percent of items per visit that were pouching to the denervated side (averaged across visits for each hamster) was 47.63% (s.d.=4.56%) for large items and 48.42% (s.d.=4.72%) for small food items. This value was significantly less than

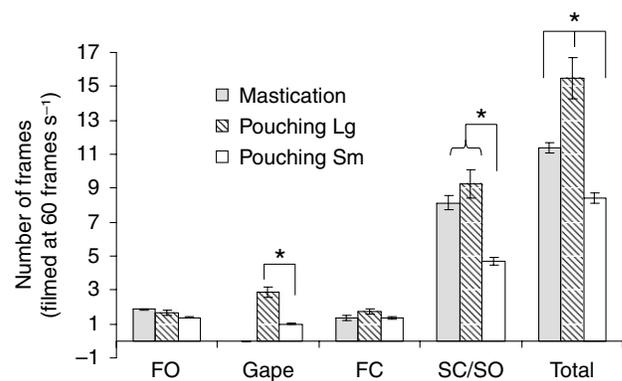


Fig. 3. Mean lengths of jaw cycle phases and total jaw cycles in number of frames (filmed at 60 frames s^{-1}). Gape phase was significantly longer during pouching of large food items than small. SC/SO phases were significantly shorter for pouching of small items than for mastication or pouching of large items. Total jaw cycle length was significantly different for mastication, pouching large food items and pouching small food items. *Bars grouped together are significantly different from each other ($P<0.001$). Lg, large items; Sm, small items.

would be expected by chance for large items [$t(15)=2.07$, one-tailed $P<0.05$], but not for small items [$t(15)=1.34$, one-tailed $P=0.10$]. The effect size (d) for large items was 0.52.

Hamsters engaged in what appeared to be a compensatory neck-stretching behavior while pouching to the denervated side, protracting the lower jaw and head repeatedly in the direction opposite the pouch being used. This behavior also occurred while pouching to the intact side, but appeared to involve more repetitions when pouching to the denervated side. The difference could not be quantified because it varied extensively with the body position of the hamster (e.g. the extent to which the body was supported by hind limbs or both hind and forelimbs) and the number of items previously pouching (neck-

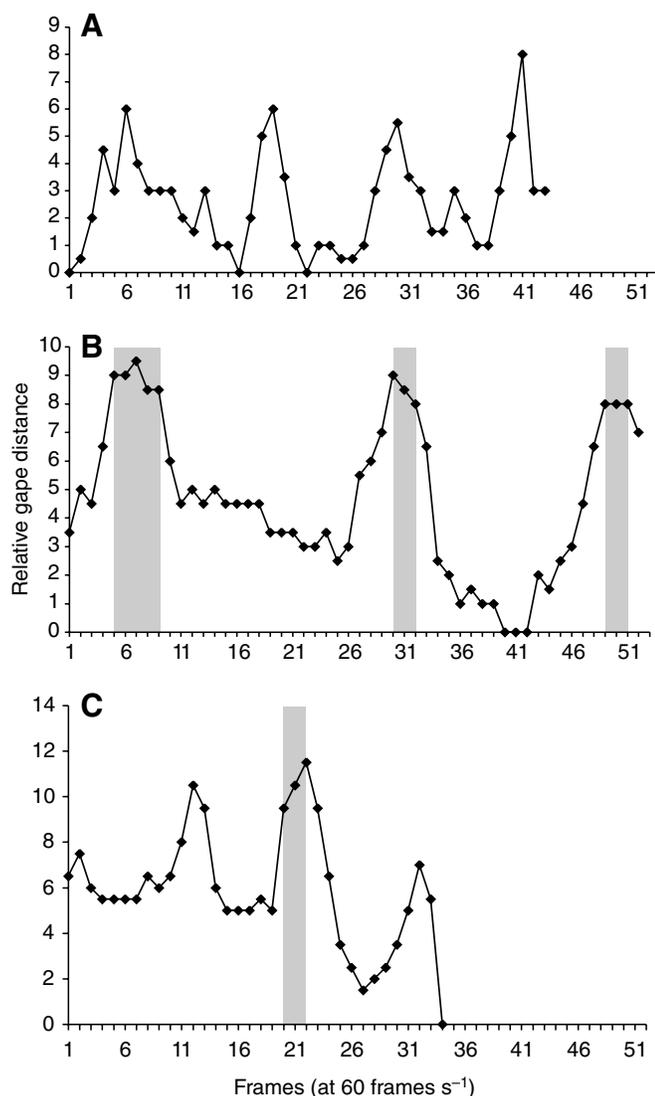


Fig. 4. Kinematic profiles of relative gape distance over time for one male hamster during (A) mastication, (B) pouching large food items and (C) pouching small food items, measured in arbitrary scale units (a function of image size on the video display, to the nearest 0.5 unit) using points on the lower jaw and rostrum. Measurements end where views were obstructed (A,B) or where the pouching event ended (C). Shaded areas at maximum gape distance (B,C) illustrate 'gape phase'. Note that '0' refers to the minimum gape distance for each set of measurements, and in B, does not indicate full occlusion.

stretching behavior appeared to increase as the pouch was filled).

Electrophysiology

At no point did stimulation of the retractor muscle result in significant repositioning of the corn kernel. However, once it was completely within the pouch, repeated retractor tetanus did contribute to very slight caudal movement (approximately 1 mm) and reorientation of the kernel longitudinally. Further stimulation of the retractor had no effect on the position of the corn kernel. When the entire kernel was within the pouch, mechanical pressure applied with the blunt probe through the pouch aperture (simulating an additional food item being added to a partially filled pouch) was not, by itself, effective in pushing the kernel caudally. Instead, both the kernel and the pouch walls responded to mechanical pressure. The posterior portion of the cheek pouch merely folded, and the kernel and pouch walls rotated laterally. When pressure was removed, the kernel remained in its original position, just inside the pouch aperture. Thus, neither mechanical pressure nor repeated retractor tetanus alone had any effect on kernel position. However, when applied simultaneously, the longitudinal extension produced by retractor stimulation provided rigidity to the pouch walls and exerted lateral pressure on the kernel, reorienting the longitudinal axis of the kernel with that of the pouch, so that only slight mechanical pressure was quite effective in moving the kernel caudally within the pouch (see movie 1 in supplementary material).

Effect of retractor denervation on pouching efficiency

For the first pellet pouching on each side, the mean number of frames between lateral shift and mouth closing was 47.1 (0.79 s) to pouches with intact innervation of the retractor muscle, and 49.4 (0.82 s) to pouches with denervated retractors. For the second pellet pouching to each side, means were 36.9 frames (0.62 s) and 73.8 (1.23 s), respectively (Fig. 5A). The main effect of denervation was significant [$F=6.02$, $P<0.05$], and *post-hoc* comparisons revealed that the second pellet pouching took significantly longer to move from its lateral shift to completely within the pouch on the denervated side than on the sham-operated side [$F=9.73$, $P<0.05$]. The interaction was not significant [$F=3.93$, $P=0.08$].

The mean number of frames to complete interval B (mouth closing to end, Fig. 5B) was 48.3 (0.81 s) for the first pellet pouching to the intact side, and 52.9 (0.88 s) for the first pellet pouching to the denervated side. For the second pellet, these means were 59.7 frames (1.0 s) and 68.9 frames (1.15 s), respectively. Thus, there was no significant effect of denervation on the length of interval B [$F=1.32$, $P=0.28$], and no significant interaction [$F=0.06$, $P=0.82$].

The main effect of denervation on the mean total length of time to pouch pellets (lateral shift to end, Fig. 5C) was significant [$F=9.02$, $P<0.05$]. For the second pellet, the mean number of frames to complete an entire pouching event was significantly greater for the denervated side when compared to the intact side [136.1 frames (2.27 s) and 96.8 frames (1.61 s)] [$F=11.69$, $P<0.05$]. This was not true for the first pellet pouching [means=102.3 and 95.4 frames (1.71 and 1.59 s)] [$F=0.36$, $P=0.56$]. The interaction was not significant [$F=3.58$, $P=0.09$].

Discussion

Several important behavioral and morphological results emerge from this research. Unlike externally pouched kangaroo rats (*Dipodomys deserti*), Syrian hamsters coordinate the use of forelimbs with multiple jaw cycles for each food item transported through the pouch opening, with less or no forelimb involvement for small food items. The kinematic pattern of jaw cycles during pouching suggests substantial overlap with masticatory motor patterns, with two important distinctions, described below. The retractor muscle plays an active role in pouch filling. Taking a cross-disciplinary, integrative approach to the study of appetitive behavior, we stress that the rodent cheek pouch is a functionally isolated appetitive specialization, and that future research may shed light on several important questions, including the evolution of novel appetitive systems and the enigmatic lack of post-fast hyperphagia in this and closely related species.

General kinematics

Nikolai and Bramble filmed pouching in a heteromyid, *Dipodomys deserti*, and reported that coordination of jaw abduction with ipsilateral forelimb adduction allows *D. deserti* to simultaneously relax the pouch aperture and push food items into the pouch, at a remarkable speed of 9 cycles s^{-1} (Nikolai and Bramble, 1983). The co-opting of the feeding apparatus (jaws) to assist the forelimbs in filling external cheek pouches in a high-speed, repetitive pattern suggested that internally pouched rodents might also co-opt elements of feeding behavior to assist in pouch filling, perhaps to an even greater extent, given the location of the pouch aperture within the oral cavity. Indeed, *Mesocricetus* utilized at least two, and in some cases more than 25, jaw cycles to pouch one food item. Fewer jaw cycles were used for smaller items, suggesting a functional link between jaw activity and pouching efficiency.

We also expected decreased involvement of the forelimbs in internally pouched rodents, as the tongue is in a better position to apply pressure to the food item, and that any forelimb use would be more likely to be contralateral to the pouch being used, due to the angle of entry. One previous researcher (Gorniak, 1977) captured pouching behavior of Syrian hamsters on film while examining masticatory jaw cycles, and reported little or no forelimb usage for small food items (sunflower seeds), and much more forelimb use for large lab pellets. Our observations confirm and extend those of Gorniak. Small food items were often pouched without any forelimb use. Instead, lingual surface adhesion and/or the incisors were used to pick up sunflower seeds and corn kernels, combined with tongue and jaw motion to work each seed into the pouch opening. Forelimbs were used simultaneously with jaw cycles to orient larger food items (chow pellets cut to approximately 2.5 g) within the oral cavity. Once oriented, pressure from the contralateral forelimb appeared to be favored over the ipsilateral, although this was not objectively assessed due to difficulty quantifying the degree of contact between food and manus. Thus, the mechanics of external and internal cheek pouch use in these two representative species are fundamentally different, but with at least one important similarity: both have incorporated cyclic jaw activity to assist in pouch filling, to varying degrees. The coordination of jaw activity and rhythmic forelimb motor

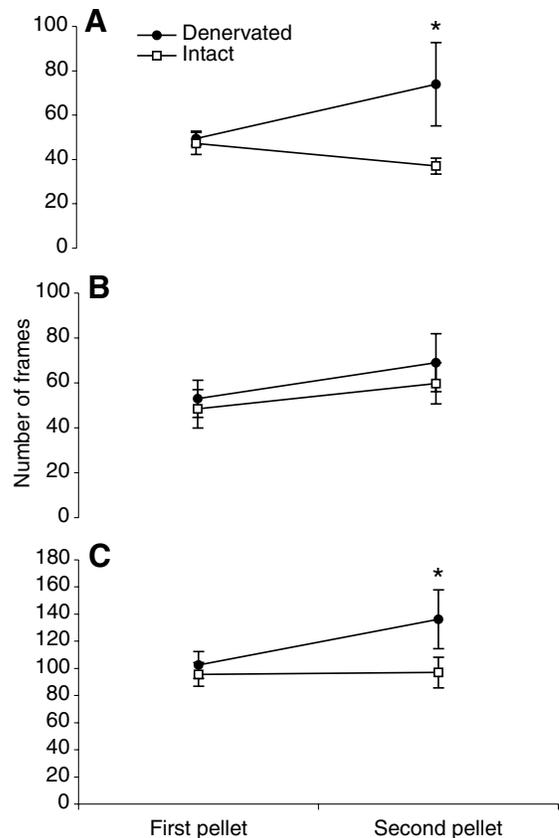


Fig. 5. Mean number of frames (1 frame=1/60 s) required to completely pouch each of two pellets to a cheek pouch with a denervated (closed circles) or an intact (open squares) retractor muscle. (A) Interval A, from lateral shift of the pellet to mouth closed. (B) Interval B, from mouth closed to end of pouching event. (C) Complete event (Intervals A+B). *Values significantly different from each other ($P < 0.05$) for second pellet, intact vs denervated retractor.

patterns appears necessary for externally pouched rodents, but not for Syrian hamsters, especially when pouching small food items, which are the main staple of Syrian hamster diet (Siegel, 1985).

Jaw cycle kinematics

Kinematic profiles of mastication (grinding of lab chow pellets) and pouching of large and small food items suggest similar patterns of jaw displacement, even though complete jaw cycle lengths vary for these three behaviors (Fig. 3). *Dipodomys* reportedly completes about nine pouching events (hence, 9 jaw cycles) per second when pouching millet seed (Nikolai and Bramble, 1983). The kinematics of pouching jaw cycles in *Dipodomys* were not described; thus whether or not they resemble masticatory jaw cycles is unknown. Modulation of the jaw cycle (decrease in the length of the slow phase portion) has been observed during gnawing behavior in rodents, including Syrian hamsters (Gorniak, 1977), although kinematic plots of jaw displacement during gnawing were not reported. Our comparison of pouching to mastication shows a relative decrease in the slow phase portion of the jaw cycle during pouching of small food items, but not large items (Figs 3 and

4). The duration of slow phases remains substantial even for small items, and pouching slow phases appear to mimic the pattern of vertical jaw displacement for masticatory slow phases, regardless of the size of the item being pouching (Fig. 4). Comparisons with the slow phases of the gnawing jaw cycle have not been made, as data were not available. For large items, the increase in the slow phase portions of each pouching jaw cycle might be due to forelimb use; after food is grasped between mandible and maxilla (beginning of SC), forepaws must release the food, reposition, and regain control of the food before the next FO may begin. Forelimbs are minimally or not at all involved in pouching of small food items. Instead, the tongue appears to play a significant role in orienting food and pushing it laterally toward the pouch aperture, although this was difficult to quantify on video due to decreased resolution within the darkness of the oral cavity.

In *Mesocricetus*, masticatory jaw cycles occur at a rate of about 4–6 s⁻¹, depending on the consistency of the food (Gorniak, 1977). Our measurements using 60 frames s⁻¹ video produced estimates of 0.18 s cycle⁻¹ for lab chow pellets, or about 5 cycles s⁻¹, verifying the validity of our frame-counting technique to measure jaw cycle kinematics. For pouching large items, the mean length of a complete jaw cycle (CJC) was longer than that during mastication (Figs 3 and 4). More cycles were required than for small items, and mean CJC for smaller items was significantly shorter than the masticatory (grinding) CJC mean. In their natural habitat, on the steppe-plains of Aleppo, Syria, these hamsters hoard small seeds, grains and insects (Jacobs, 1945; Siegel, 1985), and our research confirms that pouching small items is a much more efficient way of collecting resources than chewing. First, it requires an average of 3.5 jaw cycles to pouch a small food item the size of a corn kernel, whereas just one bite (from a lab chow pellet) requires an average of nine masticatory cycles to chew before swallowing (Table 1). Second, the average small-item pouching jaw cycle takes about 67% of the time required to complete a masticatory cycle, resulting in a conservative estimate of 3:1, time to consume one bite vs time to pouch an entire item the size of a corn kernel (~0.8 g). Pouching large food items is similarly advantageous. Even though it requires more time per cycle (and more cycles) than chewing, the net gain in food availability overwhelmingly favors pouching a 2.5-g food item over chewing a single bite, weighing about 0.1 g.

We also consistently observed an extended pause (gape phase) between FO and FC phases during pouching, but not during chewing. This differs from the 'gape plateau' that has been reported during lingual feeding in iguanian lizards (Schwenk and Throckmorton, 1989) and anurans (e.g. Gray and Nishikawa, 1995), in that prey capture cycles are isolated, single jaw cycles that are likely to be influenced by sensory feedback. Isolated extensions of the period between FO and FC have also been reported in humans, where chewing is interrupted to clear food particles from the teeth using the tongue (Hiemae et al., 1996). The extended gape phase during pouching is unique because it is a repeated (cyclic) pause between FO and FC during what appear to be otherwise normal chewing cycles (Figs 3 and 4); however, it might also be accounted for by activity of the tongue. During mammalian mastication, CPG-mediated movements of the tongue and hyoid are linked to jaw

activity, such that the tongue begins to retract just prior to the transition from FO to FC (Hiemae and Palmer, 2001). It is possible that the extended gape phase in hamsters is necessary to allow the tongue sufficient time to exert a lateral force on the food item while the jaw is open. If this is true, it might also contribute to the considerably faster pouching rate in *D. deserti* (Nikolai and Bramble, 1983), because the tongue in externally pouching rodents is presumably not involved in pouch filling. A detailed report of the kinematics of masticatory jaw cycles in Syrian hamsters (Gorniak, 1977) gives no indication of any anomalies between FO and FC, and our measurements of masticatory kinematics are in general agreement. Gape phase was only observed during pouching, and was significantly longer for large food items than for small (Fig. 3). Because FO and FC phases were quite similar during pouching and mastication, and SC/SO phases accounted for similar proportions of the total jaw cycle and showed similar patterns of vertical jaw displacement during mastication and pouching, it is reasonable to suggest that pouching jaw cycles have co-opted the kinematics of feeding jaw cycles, and are likely to be controlled by a modified or modulated masticatory CPG, the former implying separate central programs for pouching vs chewing jaw cycles.

Hamsters chew food asymmetrically (Gorniak, 1977), unlike most other rodents, including rats. Rats chew bilaterally, with symmetric pressure and both sides of the mouth actively chewing food at the same time (Weijts, 1975), while hamsters have an active and an inactive chewing side, with much more lateral translation of the mandible during the power stroke. Having an active chewing side is beneficial for internally pouching rodents, as it allows them to force ground food particles medially, by lingual motion of the mandible on the active side (Gorniak, 1977). Were hamsters to chew bilaterally, food on one side of the mouth would move medially, but food on the other side would simultaneously be pushed laterally, toward the cheek pouch opening. Gorniak observed no instances of food entering the pouch during chewing. Although we did not quantify this aspect, our videos indicate a large lateral swing of the mandible at the end of many pouching events, especially for large items, and in the direction of the pouch being used. Further comparative studies are needed to test whether lateral translations during chewing and an asymmetric chewing pattern are more common among internally pouching rodents than in other rodents.

The role of the retractor muscle

We have shown that the retractor muscle, regionally distinct from the feeding apparatus in Syrian hamsters, and previously thought to provide only structural support or to function in pouch emptying (Priddy and Brodie, 1948), actually cooperates with jaw musculature to accomplish the goal of filling the cheek pouch. The cheek pouch is an oblong, thin-walled sac with very few longitudinal muscle fibers in the lateral wall. Structural support for the insertion of food items is almost completely lacking, as evidenced by the folding of pouch walls when food was inserted during electrophysiological observations (see movie 1 in supplementary material). By stretching the pouch along its longitudinal axis, contraction of the retractor muscle provides structural integrity to the pouch walls, allowing

previously pouched food to shift caudally in response to the mechanical pressure of additional food as it is added. Without this stretching, food that is already within the pouch would block the entry of additional items. Longitudinal stretching of the pouch walls also orients food that is already within the pouch longitudinally, so that any points such as those quite often found on seeds are safely directed toward the back or front, rather than at the sides of the pouch walls. These conclusions must be considered in light of the fact that our subject was anaesthetized. Thus, any contributions of other muscles associated with the pouch opening and pouch wall, i.e. the mechanical forces that would act on subsequent food items as they are added to the pouch, were not observable. Nevertheless, a functional role for longitudinal stretching of the pouch wall is also implicated by the 'neck-stretching' behavior that was observed toward the end of many pouching events.

The topology of retractor innervation is highly variable, and ranges from fairly simple bifurcation of the spinal accessory nerve at two points to complex anastomoses between the branches (Segal et al., 2000). The failure of retractor denervation to affect the number of food items pouched might therefore be attributed to incomplete denervation procedures. However, the behavioral effects of retractor denervation were evident when pouching efficiency was observed with respect to temporal efficiency and the sequence of items pouched. Denervation had no effect on the time required to pouch the first of two food items, but significantly slowed the pouching of the second food item, particularly the time required to shift the item through the diastema and the pouch opening to the point where the jaw could be closed (Fig. 5). This result is in keeping with our functional hypothesis based on electrophysiological observations, and confirms that retractor denervations were sufficient to elicit behavioral effects. Additionally, this hypothesis gives functional significance to the increased rigidity of the walls of the apex pocket, because the pocket is located posterior to the insertion of retractor muscle slips (Fig. 2A,C) and therefore cannot rely on retractor contraction to maintain rigidity as food is pushed into it.

General discussion and additional perspectives

Comparative studies of cheek pouch functional morphology are warranted, and might address several important questions. First, Siberian (*Phodopus sungorus*), Turkish (*Mesocricetus brandti*) and Syrian hamsters do not exhibit post-fast hyperphagia (Bartness et al., 1995; Rowland, 1982; Silverman and Zucker, 1976). While most animals quickly compensate for body fat loss after a fast by overeating as soon as food becomes available, these hamsters do not. Instead, they drastically increase hoarding behavior, and continue to consume the same amounts, at the same intervals, as before the fast. Post-fast increases in hoarding behavior are attenuated by treatment with the hormone leptin (Buckley and Schneider, 2003), which decreases food intake in a wide variety of species including, but not limited to, hamsters (Wade et al., 1997), rats (Seeley et al., 1996) and arctic ground squirrels (Ormseth et al., 1996). Leptin's ability to decrease food intake in rats appears to be due to effects on appetitive rather than consummatory behavior (Ammar et al., 2000), an idea supported by exogenous leptin's attenuation of food-deprivation-induced hoarding in hamsters.

Consummatory behavior (as measured by food intake) in Syrian hamsters is regularized and does not increase in response to restricted feeding schedules (Phillips et al., 1989). Indeed, food-deprived hamsters significantly decrease food intake when given the opportunity to hoard food, compared to *ad libitum* fed controls (Buckley and Schneider, 2003). It is therefore quite possible that the overwhelming survival advantage of pouching food rather than consuming it has produced hunger signals that inhibit consummatory behavior in favor of food hoarding, resulting in a lack of post-fast hyperphagia in Turkish, Syrian and Siberian hamsters. This hypothesis would be marginally supported if other cricetine rodents and distant relatives with similar pouching kinematics and pouch capacity (e.g. some sciurids) also fail to exhibit post-fast hyperphagia, and more so if closely related sister taxa without pouches do show compensatory hyperphagia after food deprivation.

Second, the morphology of the apex pocket in *M. auratus* suggests a more derived homolog of the distensible tissue peninsula of *P. sungorus* (Ryan, 1986). Recent molecular evidence supports the idea that *Phodopus* is basal to *Mesocricetus* (Neumann et al., 2006). Partial DNA sequences of two mitochondrial and one nuclear gene suggest that *M. newtoni* and *M. brandti* are sister taxa that split from the *M. auratus/raddei* group in the Pliocene. Both molecular data and current ranges suggest that *M. auratus* and *M. raddei* separated in the lower/mid Pleistocene (Neumann et al., 2006). Detailed descriptions of the apex pocket in these and other species of hamsters (especially *Phodopus* and *Cricetulus*) will provide morphological evidence to better understand the evolution of cricetines.

Third, extensive morphological (Ryan, 1986), developmental (Luckett, 1985) and genetic evidence (Sarich, 1985) supports the independent emergence of cheek pouches in rodents no less than three times. In sciurids, the retractor is derived from the facial musculature; in crecetids, from the trapezius, and in geomyoids, from both facial and trapezius (Ryan, 1986). Within this phylogenetic context, the coordinated activity of the retractor and jaw musculature during pouching in *Mesocricetus* suggests that cheek pouch use might provide an excellent model system for studying the evolution of an appetitive behavior with regionally distinct, functionally dependent anatomical units that evolved with minimal selection pressure due to participation in behaviors unrelated to appetite. In other words, if the retractor can be shown to facilitate pouch use in *P. sungorus*, *D. deserti* and other species, then rodent cheek pouches might serve as an ideal model system for the study of functional integration of anatomically separate elements in an evolutionary context.

List of abbreviations

FO	fast open
FC	fast close
SC	slow close
SO	slow open
CJC	complete jaw cycle
CPG	central pattern generator

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