

## Short Communications

### Shingle-back lizards crush snail shells using temporal summation (tetanus) to increase the force of the adductor muscles

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**Summary.** During the crushing of hard objects, the adductor muscles of *Trachydosaurus* are activated in an unfused tetanus with the tetanic pulses of the several motor units occurring in synchrony.

**Key words.** Muscle; tetanus; mastication; electromyography; lizard.

*Trachydosaurus rugosus*, the shingle-back lizards of Australia have a wide head and a large and complexly pinnate mass of adductor muscles that packs a large number of fibers into an array that achieves a high physiological cross section<sup>1</sup>. Electromyography (EMG) shows that whenever these lizards are feeding on snails (*Helix pomatia*), the jaw adductor musculature is recruited for a longer time during a bite at a hard shell than for

one at the soft contents<sup>2</sup>; however, the magnitudes and frequencies of the EMG spikes differ only slightly among bites. Thus, the mean spike amplitude of an initial crushing bite is only 120% (barely significant at 0.9) that of a normal closing bite, during which only soft material or small pieces of shell are encountered by the closing jaws. The number of EMG spikes coincidentally increases to about 130% (also barely significant at 0.9) above

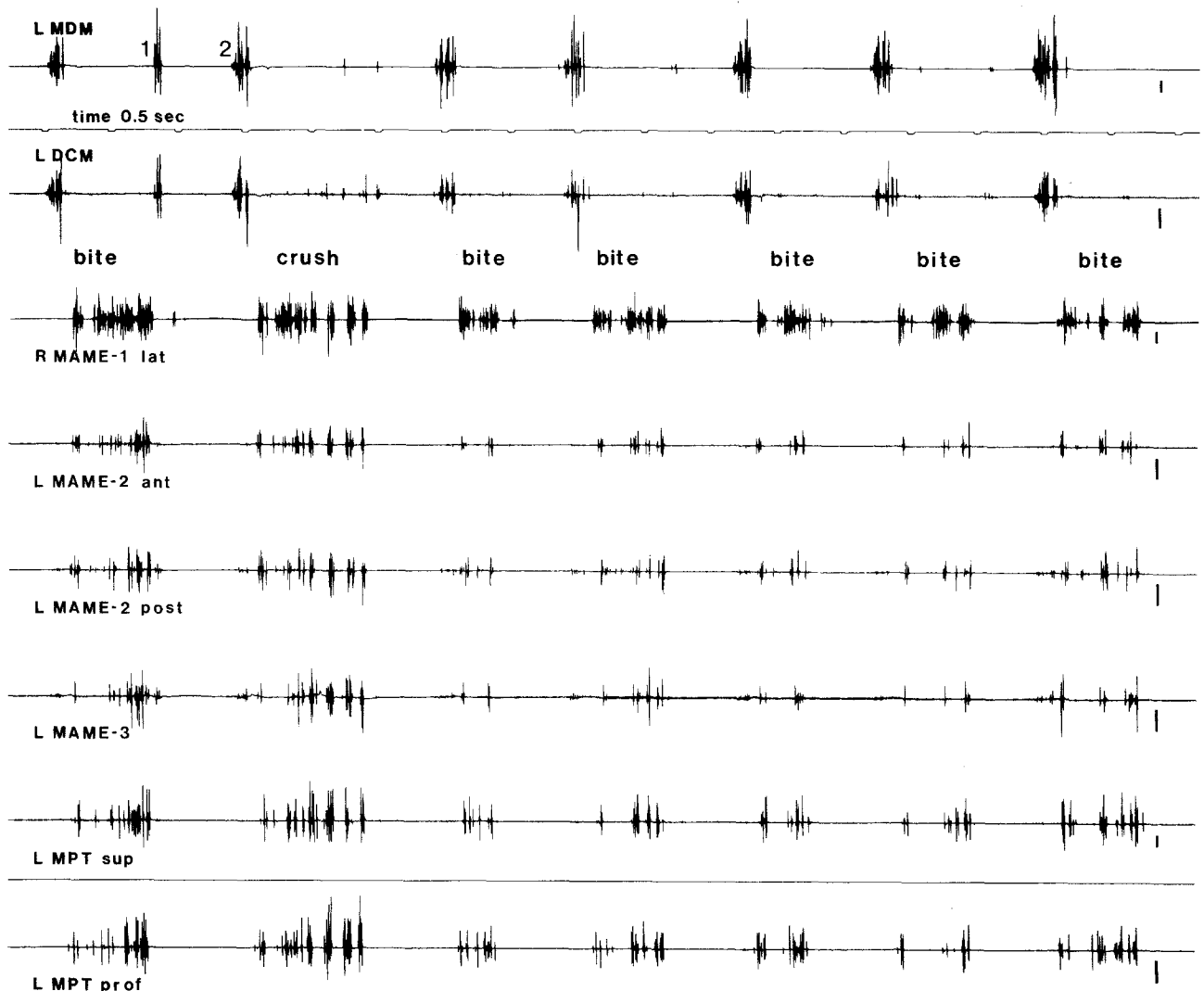


Figure 1. *Trachydosaurus rugosus*. Electromyograms of two jaw openers and six jaw adducting muscles during the start of a snail crushing and masticating sequence. The first bite did not crush the snail. Note that the terminal portion of the crushing phase is accompanied by synchronized pulsatile activation (three pulses) of all adductors, but none of the depressors. The time markers indicate 0.5 s and the vertical bars on the right 0.05 mV. LMDM, left depressor mandibulae; LDCM, left dorsal cervical muscles; RMAME-1, right adductor mandibulae externus 1; RMAME-2, right adductor mandibulae externus 2; LMAME-2, left adductor mandibulae externus 2; LMAME-3, left adductor mandibulae externus 3; LMPTsup, left superficial pterygoideus; LMPTprof, left deep pterygoideus.

the value of the simple closing bite. However, behavioral observations, whenever a lizard crushes the shell of the snail, indicate that the tip of the mandible is then held firmly against the ground, and that the initial bite lasts about twice as long as do succeeding ones.

The EMG signature of the crushing bite also differs from those of most following ones in being discontinuous. The closing sequence includes some 3–10 discrete pulses of spikes at a rate of 6–8 (sometimes up to 12) Hz, separated by gaps roughly as long as the burst length (fig. 1). The pulsatile interval begins half way through the prolonged crushing bite and always terminates before its end. The pulsations occur at equivalent frequency in all of the adductors; their individual pulses always overlap (as if they represent synchronized firing by multiple motor units), although their onset and cut-off may be poorly synchronized. EMG values for the masticatory movements previously described for reptiles and mammals lacked any pulsatile phase, although crushing of very hard objects does not appear to have been specifically studied. As the frequency values of the pulses fell into the range for which temporal summation (tetanus) has been demonstrated, we tested various stimulation regimes on three anesthetized animals.

The head of each lizard was firmly clamped, a loop of steel wire was hooked over its lower jaw and this was attached to a strain gauge. Current was applied unilaterally between two stainless steel (no. 5 insect pin, approx. 1.5 cm bare surface each) electrodes, inserted one into the anterior and the other into the posterior part of the adductor mandibulae. Square wave pulses (0.5 ms duration) were applied in trains of 3–20 at 4 V. Stimulator and strain gauge signals were displayed simultaneously on a chart and stored on tape. The force values reflect stimulus magnitude and the extent to which the jaws are distended and hence the starting place of the muscles on the length tension curve. As the species was protected and the experiments were acute, we stimulated each animal at relatively low (voltage/current) levels in order to avoid fatiguing the musculature during a minimum of two passages from 2 to 15 Hz and return. Only the maximal stimulation experiments (described below) involved

higher voltages. Whereas the higher voltages produced greater maximum forces they did not modify the time course of the forces produced.

Stimulation at 4 Hz or above produced summation with the unfused tetanic tension values reaching a maximum in the 7–9 Hz range (fig. 2). By the fourth pulse (the mean number observed whenever our *Trachydosaurus* crushed large snails), the force generated at stimulation frequencies of 7–10 would be between 250 and 350% of the twitch value. More prolonged trains led to an unfused tetanus, whereas stimulation at still higher frequencies produced fusion. Stimulation at 5–8 Hz showed a marked Treppe or staircase effect<sup>3</sup> with each of the first few successive stimulus pulses of the train adding an increment of near 50% of the preceding single twitch tension (which tension represents the isometric force for the particular starting length of the muscle fiber). However, trials with large specimens of *Helix* indicated that maximal unilateral stimulation (i.e. increase of the stimulation voltage to induce maximal force generation) only broke the edges of such shells. Apparently, bilateral contraction of the entire adductor musculature (plus action of the subvertebral muscles that add to the closing force by forcing the mandible against the ground) is required for fracturing the center of each snail. (Properly positioned large snails withstood distributed static loads between 66 and 280 Newton depending on the direction of the applied force.)

The demonstration that the tetanus and staircase effect occur in the range of pulsations disclosed by our EMGs provides strong presumptive evidence for the *in vivo* use of tetanic trains for increasing the force generated by the muscle of an intact animal<sup>4</sup>. The argument is strengthened by the demonstration that the forces generated in non-tetanic twitch activation of the adductor muscles are insufficient for crushing snails. Also, characiform and cichlid fishes crush seeds and prey<sup>5</sup> and gekkos grasp and process large insects (grasshoppers) with similar pulsatile EMG signatures although at higher frequencies<sup>6</sup>. The apparent length of each burst seen in *Trachydosaurus* is probably an artifact, reflecting the action of multiple motor units acting slightly out of phase and reaching the electrodes from variable distances. No advantage would accrue closer synchronization of pulses and it is the interpulse rather than the intrapulse frequency that is associated with the tetanus.

The application of the tetanic train after the snail has been firmly grasped, and no further shortening is possible, makes the tetanic force a multiple of the isometric force. Retention of the pulsatile frequency in the range of the staircase effect would permit the animal to reduce the potential shock associated with excessive accelerational forces. It would have obvious benefits in crushing shells or nuts that encounter brittle failure, particularly if coupled with a mechanism for stopping the pulse train as soon as the shell fails. Shock effects on the teeth and contents of the cranial cavity then will be reduced to those required by the level of force required to produce failure of the shell. The relatively gradual loading of the shells (over approx. 400–600 ms, i.e. the duration of four pulses at 8–10 Hz) distributes the incremental forces, so that the shell fails in peripheral bending (freeing the contents) rather than by localized (puncturing) shear. As the snail is firmly held in the jaws, any shock forces imposed upon the lizard's head will be distributed, rather than being concentrated. The pulsatile activation of adductor muscles reported for other reptiles and fishes<sup>5,6</sup> makes it likely that the use of synchronized tetanus represents a general mechanism used by vertebrates crushing hard prey.

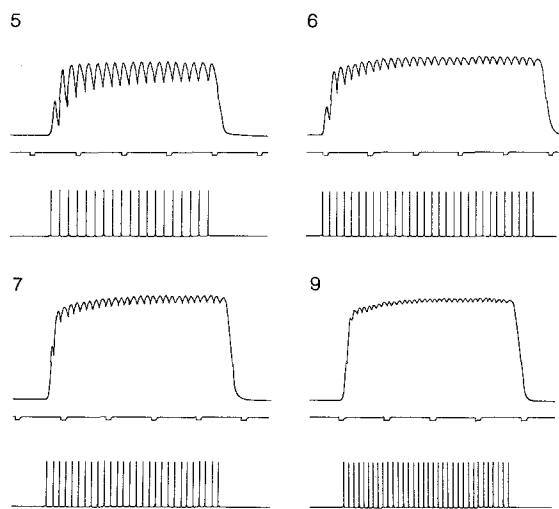


Figure 2. *Trachydosaurus rugosus*. Effect of frequency of submaximal stimulation on the torque generated by the mandibular adductors of a typical animal. The adductor muscles of the right side of the head of an anesthetized lizard were simultaneously stimulated by passage of trains of pulses (numbers indicate pulsatile frequency in Hz) between a pair of low impedance electrodes placed respectively at the front and rear of the muscle mass. Lower trace, stimulation voltage with pulse amplitude 4 V; middle trace, 1 s markings; upper trace, torque measured at the mandibular symphysis. The torque scale is arbitrary as it (but not the nature of the tetanus) is modified by minor changes in electrode position and stimulation voltage.

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### Cadmium-induced changes in renal hemodynamics in the domestic fowl

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**Summary.** Low i. v. doses of cadmium chloride (15 µg Cd) given to pullets resulted in a significant reduction in urine flow (UF), glomerular filtration rate (GFR) and effective renal plasma flow (ERPF). However, in hens treated with the heavy metal chelate FeNa EDTA prior to cadmium treatment no oliguria or reduction in GFR or ERPF was observed.

It is suggested that the renal changes following the i. v. administration of cadmium to diuretic hens and alleviated in hens primed with the heavy metal chelate may result from changes in glomerular hemodynamics.

**Key words.** Glomerular filtration rate; effective renal plasma flow; FeNa EDTA chelate.

Long term exposure of humans and mammals to cadmium leads to profound changes in renal function<sup>1</sup>. In the present study we have investigated the short term effects of cadmium on renal homeostasis by monitoring glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) in pullets.

Twelve Rhode Island Red Cross Light Sussex hens of approximately the same age (14 weeks) and average weight (1.3 ± 0.2 kg) were used in this study. One group of birds was treated with cadmium and a second group was infused over the experimental period (1.5 h) with a heavy metal chelate, the ferric sodium salt of ethylene diamine tetra-acetic acid (FeNa EDTA), and then injected with cadmium.

The left brachial vein of each hen was cannulated for the continuous infusion (0.5 ml/min<sup>-1</sup>) of isotonic saline solution (0.93% NaCl) containing an admixture of inulin (0.25%), para-amino hippuric acid (0.025%), and mannitol (10%). After establishing an adequate rate of urine flow local anesthetic (xylocaine) was applied around the cloaca prior to cannulation of the ureters of each hen.

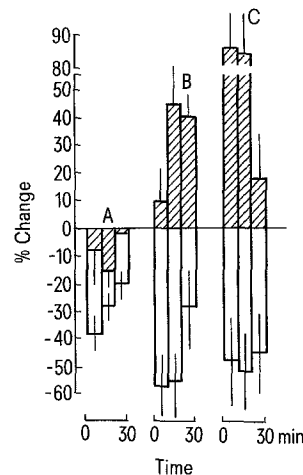
In the first group of birds (n = 7) six consecutive 10-min urine samples were obtained 1 h after the start of the saline infusion (control samples) and continued for 30 min after a single injection of 25 µg cadmium chloride (15.34 µg Cd). This procedure was repeated for the second group of pullets (n = 5) except that each bird was infused at a slow constant rate (0.1 ml/min<sup>-1</sup>) with the heavy metal chelate (1% FeNa EDTA) 1 h before and 30 min after a single cadmium injection (15 µg Cd).

In both groups of hens blood samples (2 ml) were taken at the midpoint of each urine collection period.

Following cadmium treatment in group one pullets a significant reduction (p < 0.05) in urine flow (1.1 ± 0.12 to 0.77 ± 0.22 ml/min) was observed, reflecting an approximate 30% decline (fig.) from control values over the experimental period. In addition GFR was found to decrease significantly (p < 0.02) by about 50% compared with that observed before cadmium treatment (3.12 ± 0.4 to 1.67 ± 0.28 ml/min). Statistical analysis of these data revealed a positive significant correlation (r = 0.59; n = 70; p < 0.002) between urine flow and GFR. However, in group two pullets, pretreated with the heavy metal chelate and then cadmium, no pronounced oliguria was observed and this may be related to the 30% increase in GFR over the 30-min period of cadmium intoxication (fig.).

The renal clearance of PAH (ERPF) was shown to decline significantly (13.7 ± 2.06 to 6.9 ± 2.29 ml/min: p < 0.02) in poisoned hens indicating an overall decline of about 50%, 30 min after cadmium treatment. Based on the equation  $RBF = RPF \cdot (1 - \text{hematocrit})^{-1.2}$  and assuming a cardiac output in similar sized hens of 430 ml/min<sup>3</sup> the fraction of cardiac output to the kidneys was calculated to be reduced by half in cadmium treated hens. Moreover, in pullets treated with FeNa EDTA and cadmium, ERPF increased significantly (p < 0.02) by about 60% above control values (fig.).

The changes observed following cadmium intoxication in both groups of birds were not attributable to the infusion of either isotonic saline solution or FeNa EDTA, since in separate experiments, when these constituents were infused continuously over a 3-h period, no marked differences were noted in urine flow, GFR or ERPF.



% change in urine flow (A), glomerular filtration rate (B), and renal plasma flow (C) (mean ± SEM) in hens treated with a 25 µg CdCl<sub>2</sub> (□) and b CdCl<sub>2</sub> + FeNa EDTA (▨). 0% refers to controls for both groups before cadmium treatment. Each 0-30-min block represents results of consecutive 10-min sampling.