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BODY HYDRATION AND HAEMOLYMPH OSMOLALITY AFFECT FEEDING AND ITS NEURAL CORRELATE IN THE TERRESTRIAL GASTROPOD, *LIMAX MAXIMUS*

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SUMMARY

1. When terrestrial slugs (*Limax maximus*) are dehydrated to 65–70 % of their initial body weight (IBW) their feeding responsiveness is greatly decreased.

2. There is a 90 % decrease in feeding responsiveness when slugs are injected with hyperosmotic mannitol solution that raises the haemolymph osmolality to that of slugs dehydrated to 65–70 % IBW (i.e. 200 mosmol kg^{-1} H_2O).

3. The duration of the Feeding Motor Programme (FMP) that can be recorded from an isolated CNS-lip preparation is reduced by increasing the osmolality of the saline bathing the preparation. The osmolality of the saline that can modify the FMP corresponds to that of the haemolymph of a slug dehydrated to 65–70 % IBW. The pattern of the motor programme is not affected.

4. A gradual increase in saline osmolality which temporally mimics the progressive increase in haemolymph osmolality of a dehydrating slug also causes a decrease in the duration of the FMP.

5. The neural network underlying the FMP appears to adapt to hyperosmotic saline since the duration of FMP bouts gradually returns to normal levels after long-term exposure (6–8 h).

INTRODUCTION

Homeostasis involves both physiological and behavioural regulation of many body functions. The tendency towards internal equilibrium is achieved by numerous mechanisms ranging from the cellular to organism level. One form of regulation often interacts with another, requiring a compromise between two or more functions. For example, the interaction between body fluid regulation and maintenance of blood nutrient levels is apparent in the relationship between drinking and feeding.

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In most animals food and water intake are directly correlated, particularly when either is restricted; "the 'thirsty' animal doesn't want to eat and the 'hungry' animal doesn't want to drink" (Bolles, 1967). Reduced water intake during food deprivation and the concomitant increase in drinking when food intake is increased have been observed in most animals which have been examined (e.g. Bolles, 1961; Kraly, 1984; for exceptions see Cizek, 1961; Schmidt-Nielsen, Schmidt-Nielsen, Houpt & Jarnum, 1956). Reduced food intake during water deprivation is even more commonly observed (e.g. Collier & Levitsky, 1967; Kutscher, 1969; Levitsky, 1970). In both vertebrates and invertebrates, dehydration-induced reduction of food intake appears to be mediated by the increase in blood osmotic pressure which accompanies dehydration (e.g. Bernays & Chapman, 1974; Hsiao & Langenes, 1971; Hsiao & Trankina, 1969; Yin, Hamilton & Brobeck, 1970).

A change in feeding responsiveness which is associated with changes in blood osmotic pressure might be mediated by osmotic effects on the central nervous system, particularly in molluscs which lack a blood-brain barrier (Lane & Treherne, 1972; Mirolli & Gorman, 1973; Prior, 1981, Sattelle, 1973). Indeed, the sensitivity of isolated gastropod ganglia to changes in saline osmolality are well known (Hughes & Kerkut, 1956; Kerkut & Taylor, 1956). The effects of osmotic stress on neurones reflect an animal's tolerance to osmotic pressure changes (for a review see Prior & Pierce, 1981). These effects include loss of excitability (Pichon & Treherne, 1976), as well as changes in bursting activity and synaptic input to neurones (Prior, 1981).

Terrestrial gastropods are ideal subjects for the study of the interactions between water regulatory functions and feeding behaviour. Their moist skin and the absence of an external shell make terrestrial slugs very susceptible to desiccation. Indeed, slugs experience evaporative loss when the relative humidity falls below 98·9 % (Machin, 1975) and the rate of loss can reach 16 % of their body weight per hour during locomotion (Kunkel, 1916, cited in Howes & Wells, 1934), though much of the loss during locomotion is due to increased pedal mucus. Thus slugs can dehydrate to 60–70 % of their initial body weight within 2 h (Dainton, 1954). However, slugs, by their behaviour, can regulate body hydration by contact-rehydration (Prior, 1982, 1984), huddling into groups (Cook, 1981; Prior, Hume, Varga & Hess, 1983), changing locomotor activity (Dainton, 1954), initiating a pneumostome closure rhythm (Prior *et al.* 1983) and reducing food intake (Phifer & Prior, 1982; Prior, 1983). Many of the neural correlates which underlie feeding behaviour have been described in several gastropods (e.g. Gelperin, Chang & Reingold, 1978; Kater & Rowell, 1973; Kupferman & Cohen, 1971; Rose & Benjamin, 1981; Willows, 1980); therefore the central effects of dehydration can be directly observed by monitoring CNS activity while manipulating the immediate environment of the CNS.

We have examined the interactions between body hydration level, haemolymph osmolality and feeding behaviour in the terrestrial slug, *Limax maximus*, and found that changes in haemolymph osmolality which accompany dehydration and

rehydration can alter feeding motor programme activity. We thus describe a direct effect of changes in osmolality on a neural correlate of a specific behaviour. A preliminary report of a portion of these results has appeared in abstract form (Phifer & Prior, 1982).

MATERIALS AND METHODS

Specimens of *Limax maximus* were collected from sites in and near Lexington, Kentucky, or reared from eggs laid in the laboratory. No experimental differences were observed between laboratory-reared and field-collected slugs. During most of the study, animals were maintained at 20 °C on a 14:10 light:dark cycle. However, in the latter part of the study, slugs were kept in a growth chamber on a 14:10 light:dark cycle and a corresponding temperature cycle of 16 °C lights-on and 12 °C lights-off temperature. Slugs were housed in vented plastic refrigerator boxes (32 × 22 × 6·5 cm) lined with moist paper towelling and were provided with rodent chow (Purina) *ad libitum*.

Slugs can be fasted for over 20 days and still refrain from eating when food is presented (personal observations). Therefore, fasted slugs had to meet a feeding responsiveness criterion before they were used in the behavioural tests of feeding. This criterion was met when a slug made 10 radular rasps (bites) on a piece of dry rodent chow placed directly in front of its head. The criterion was appropriate because slugs which made fewer than 10 bites usually did not continue feeding, and slugs which made more than 10 bites usually continued feeding for several minutes.

One series of behavioural experiments was used to test the effect of air-dehydration on the feeding responsiveness of *Limax maximus*. Slugs were dehydrated by keeping them at room temperature and humidity (18–20 °C, 20–50 % relative humidity) in a plastic Petri dish (15 × 4 cm) lined with dry paper towelling and covered with 2 mm mesh nylon netting. This procedure permitted dehydration to 65–70 % of initial body weight (IBW) within approximately 2 h. Control animals were kept in dishes lined with wet towelling and covered with vented lids for periods equivalent to the dehydration times. These animals remained at 97–98 % IBW. Following the dehydration period, or an equivalent time for control animals, slugs were given one 10-min feeding trial and scored as either feeding or not feeding according to the criterion described above. Approximately one-half of the slugs subsequently were allowed to rehydrate and then were given the reverse treatment in a repeated-measures design. That is, those slugs which were dehydrated in the initial treatment were allowed to remain hydrated prior to the next feeding test and *vice versa*.

In another series of behavioural experiments, the haemolymph osmolality of the slugs was altered prior to feeding tests. Fasted slugs were randomly assigned to one of four treatment conditions: (1) mannitol injection – a 1 osmol kg^{-1} H_2O solution of mannitol (a non-metabolizable sugar) in 1·0× slug saline (described in Prior & Grega, 1982) was injected into the slug's haemocoel to raise haemolymph

osmolality to specific levels (see Prior, 1983); (2) saline injection – isosmotic 1·0× saline (140 mosmol kg^{-1} H_2O) was injected into slugs to control for volume effects of the injection procedure; (3) needle – as a sham treatment, a needle was inserted through the body wall without injection; (4) time – slugs were allowed to rest unmolested for a time period equivalent to the other procedures. Injections were administered in multiple 0·1- to 0·2-ml doses to avoid rapid increases in body volume. Following the treatment procedures, feeding responsiveness was determined in three, 10-min trials at 20-min intervals. As in the air-dehydration experiments, some of the slugs subsequently were given each of the other treatments at intervals of several days until each animal had received all four treatments (i.e. repeated-measures in a counterbalanced design). Following the initial treatments, subsequent treatments were applied in the order: mannitol, saline, needle and time control.

The Feeding Motor Programme (FMP) was recorded from central nervous system (CNS) preparations to test the effect of saline concentration and osmolality on the neuronal control of feeding. The preparation consisted of the brain and attached lips which were isolated from one another by sealing the lips in small plastic wells with petroleum jelly (see Gelperin *et al.* 1978). Recordings from the CNS were made *via* suction electrodes attached to buccal ganglion nerves. Electrical stimulation of the anterior lip nerve (7-s train of 10-ms pulses at 3 Hz; amplitudes varied between, but not within, experiments) elicited reproducible FMP bouts consisting of coordinated bursts of action potentials in buccal root 1 (BR1), buccal root 2 (BR2), salivary nerve (SN) and the gastric nerve of the buccal ganglion. These bursts of activity drive radula protraction, radula retraction, salivary duct contraction and oesophageal peristalsis, respectively, and therefore are correlates of biting and swallowing in the intact slug. A bite cycle in this CNS-lip preparation consists of action potential bursts in the buccal nerves that correspond to one protraction/retraction cycle. An FMP bout usually consists of 20–60 individual bite cycles and can last several minutes after termination of electrical stimulation to the anterior lip nerve.

FMP bouts were stimulated in slug brains exposed to several experimental conditions. These included acute changes of bathing saline ionic concentration or osmolality, as well as gradual and long-term changes in osmolality. Saline osmolalities were determined by freezing point depression (Advanced Instruments Osmette A).

RESULTS

Dehydration and feeding responsiveness

After a fasting period of 11 days, slugs which satisfied the feeding responsiveness criterion were tested for effects of air-dehydration on feeding. Following dehydration to 66·1 ± 0·9 % IBW ($\bar{x} \pm$ S.E.M.), feeding was not observed in any of the 21 animals in Group 2. However, of the 20 animals in Group 1, which

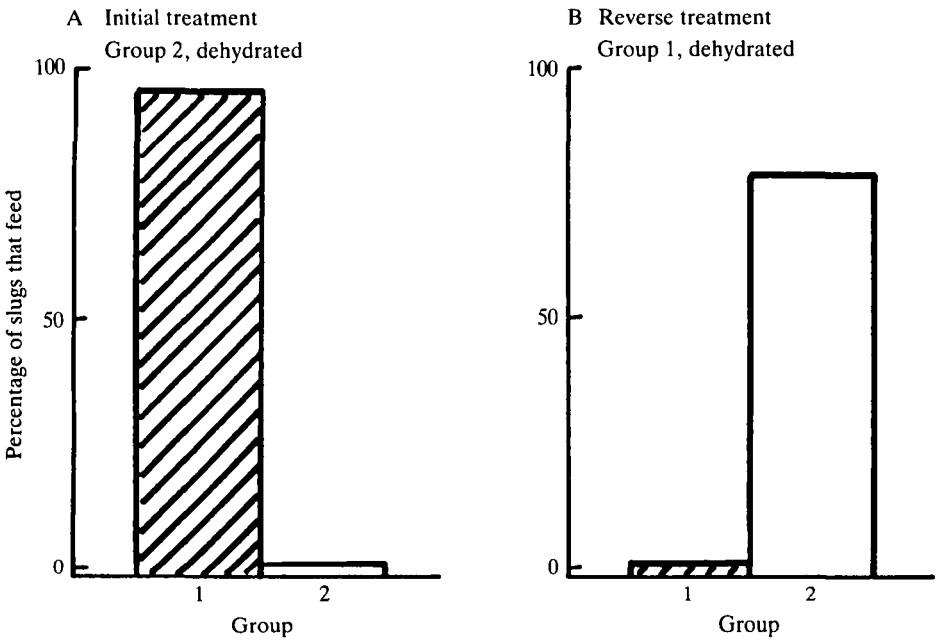

Fig. 1. The effect of dehydration on feeding responsiveness as measured by the percentage of slugs that feed. (A) Slugs in Group 2 ($N=21$) were dehydrated to 65–70 % IBW and slugs in Group 1 ($N=20$) remained fully hydrated. (B) Some of the animals in Fig. 1A were subsequently used in a second experiment but the treatments were reversed. Slugs in Group 1 ($N=11$) were dehydrated and slugs in Group 2 ($N=9$) remained hydrated.

remained hydrated (97·2±1·1 % IBW), 19 slugs fed during a 10-min trial [Fig. 1A, $\chi^2(1)=30{\cdot}0$, $P<0{\cdot}0005$].

Subsequently, the experiment was repeated with approximately one-half of the slugs used previously (after additional fasting of 2–15 days, $\bar{x}=9$ days). However, in this repetition, the treatments were reversed (Fig. 1B). Following dehydration to 63·8±2·0 % IBW, feeding was not observed in any of the 11 animals in Group 1. However, of the 9 animals in Group 2, which remained hydrated (98·1±1·2 % IBW), 7 slugs fed [$\chi^2(1)=11{\cdot}6$, $P<0{\cdot}005$]. Fasted *Limax maximus* thus exhibit a dramatic decrease in feeding responsiveness when air-dehydrated to 65–70 % IBW.

Haemolymph osmolality and feeding responsiveness

The reduction in feeding responsiveness observed in air-dehydrated slugs could have been due to the dehydration-induced decrease in haemolymph volume or the resulting increases in ionic concentration and haemolymph osmolality (Hess & Prior, 1982; Prior *et al.* 1983). A series of injection experiments was used to test the hypothesis that changes in haemolymph osmolality alone can affect feeding responsiveness. After a fasting period of 13 days, slugs which satisfied the feeding responsiveness criterion were injected with mannitol or given one of three control treatments and then tested for feeding responsiveness in three, 10-min

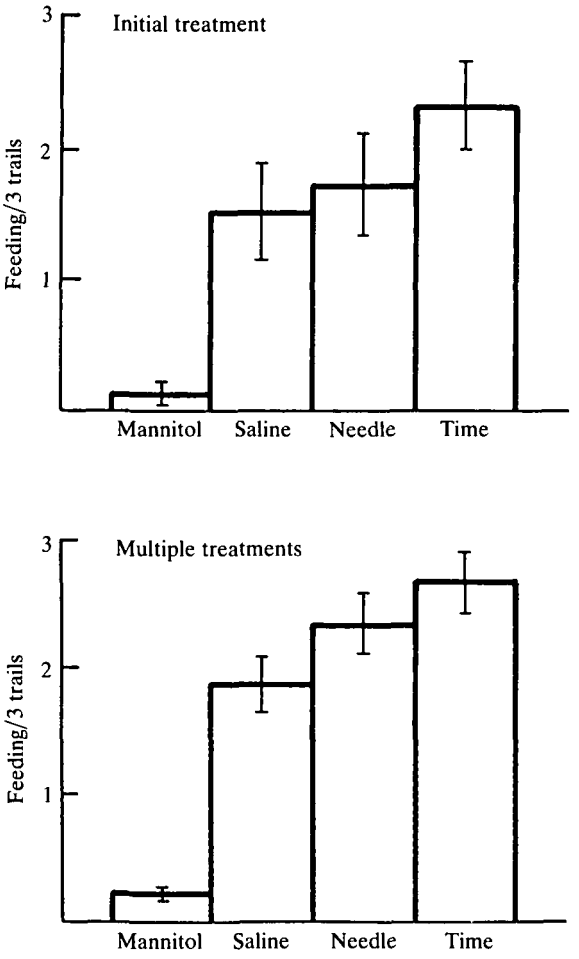

Fig. 2. The effect of changes in haemolymph osmolality on feeding responsiveness. Initial treatment: fasted slugs which were responsive to food received one of four treatments: mannitol injection (calculated to raise haemolymph osmolality to 192–199 mosmol kg^{-1} H_2O), saline injection, needle insertion or an equivalent time delay ($N=10$ slugs per treatment group). Multiple treatments: animals from the initial treatment group ($N=24$) were subsequently given each of the four treatments in counterbalanced order ($\bar{x} \pm$ S.E.M.).

trials at 20-min intervals (Fig. 2, initial treatment). Slugs injected with 1·0 osmol kg^{-1} H_2O mannitol (sufficient to increase haemolymph osmolality from the normal level of 140 mosmol kg^{-1} H_2O to 192–199 mosmol kg^{-1} H_2O, equivalent to the haemolymph osmolality of a slug dehydrated to 65–70 % IBW) fed during fewer of the trials than slugs receiving equivalent volume 1·0× saline injections, sham needle insertions or time controls ($P<0·02$, 0·02, 0·01, respectively; $N=10$ slugs per treatment, Kruskal-Wallis rank sums with Dunn's multiple comparison procedure). No significant differences in feeding responsiveness were observed between any of the three latter groups. Subsequently 24 of these 40 slugs were fasted for an additional 14 days. During this time each slug received each of the other three treatments in counterbalanced order and was

similarly tested for feeding responsiveness (Fig. 2, multiple treatments). As in the initial test with each animal, slugs injected with hyperosmotic mannitol to increase haemolymph osmolality fed during fewer trials than they did under the three control conditions (saline injections, sham needle insertions or time controls; $P<0{\cdot}001$, 0·0001, 0·0001, respectively; distribution-free multiple comparison based on Friedman rank sums). No significant differences in feeding responsiveness were observed between any of the control groups.

If it is the increase in haemolymph osmolality produced by mannitol injections that reduces feeding responsiveness, then dilution of the haemolymph should reverse this reduction. Following an injection of 1·0 osmol kg^{-1} H_2O mannitol sufficient to increase haemolymph osmolality to 190–199 mosmol kg^{-1} H_2O, 14 slugs fed during $1{\cdot}21 \pm 0{\cdot}51$ of 3 feeding trials. After a subsequent injection of 0·1× slug saline (14 mosmol kg^{-1} H_2O), which lowered haemolymph osmolality to 179–183 mosmol kg^{-1} H_2O, the same slugs fed during $2{\cdot}14 \pm 0{\cdot}33$ of 3 feeding trials ($P<0{\cdot}02$, Wilcoxon signed rank test). Thus dilution of the haemolymph restored the feeding responsiveness of the mannitol-injected slugs.

Feeding motor programme

Changes in saline concentration

The effects of dehydration on the Feeding Motor Programme (FMP, Gelperin *et al.* 1978) in *Limax maximus* were assessed by varying the concentration of saline bathing the brain while maintaining a constant saline concentration at the site of electrical stimulation (lip nerves). In five preparations, FMP bouts were stimulated 2–4 times in each of three saline concentrations: 1·0× (140 mosmol kg^{-1} H_2O), 1·5× (195–200 mosmol kg^{-1} H_2O) and a second 1·0×. Three FMP bouts from a typical experiment are shown in Fig. 3. The duration of the FMP bout and the number of individual bite cycles per FMP bout were reduced when the preparation was exposed to 1·5× saline. A slug may eat for a long period at a low bite frequency and consume less than another slug which bites rapidly for a shorter period, therefore, the number of bite cycles per bout was used as the primary measure for comparing FMP bout length in different saline concentrations (Fig. 4). Despite the variation in mean bout length between different slugs, the length was always decreased when saline concentration was increased (1·5×) and returned to near the original level when 1·0× strength saline was replaced. An analysis of variance revealed no significant difference between bout lengths in the first and second 1·0× salines. However, there was a significant difference between bout length in each of the 1·0× salines and in 1·5× saline [first 1·0× *vs* 1·5×, $F(1,8)=13{\cdot}05$, $P<0{\cdot}012$; 1·5× *vs* second 1·0×, $F(1,8)=15{\cdot}25$, $P<0{\cdot}017$]. The actual duration of FMP bouts (in seconds) also decreased when the saline was changed from 1·0× to 1·5× [$F(1,8)=16{\cdot}6$, $P<0{\cdot}014$] and subsequently increased when 1·0× saline was replaced [$F(1,8)=22{\cdot}7$, $P<0{\cdot}006$].

The burst durations and interburst intervals of activity in the salivary burster neurone (SB) were analysed from records taken during FMP bouts and during the

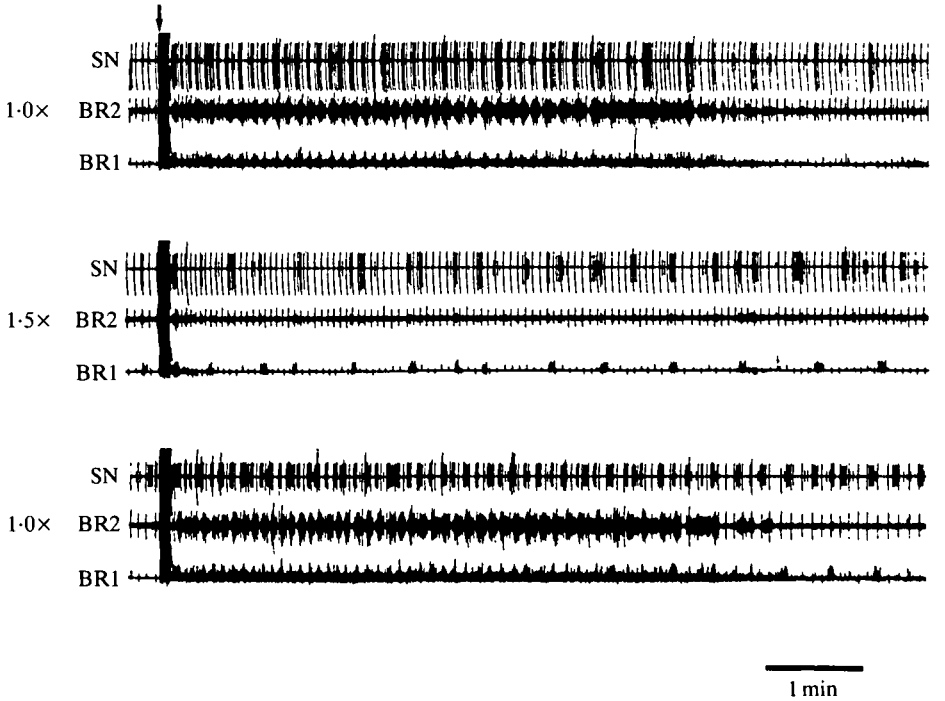

Fig. 3. Example record showing the effect of changes in saline concentration on the Feeding Motor Programme (FMP). FMP bouts were elicited by identical electrical stimulation (arrow) while the brain was bathed in normal saline (1·0×, 140 mosmol kg^{-1} H_2O), concentrated saline (1·5×, 200 mosmol kg^{-1} H_2O), and again in normal saline (1·0×). SN, salivary nerve; BR2, buccal ganglion root 2; BR1, buccal ganglion root 1.

endogenous activity which occurred between bouts. When comparisons of these parameters were made between different saline treatments the only significant difference was between SB burst durations during FMP bouts in 1·5× saline and the second 1·0× saline [$F(1,8)=12{\cdot}97$, $P<0{\cdot}028$]. However, this difference was not upheld in the comparison of the first 1·0× and 1·5× salines. No treatment effects were detected for endogenous SB burst durations or for interburst intervals either during or between FMP bouts.

Changes in saline osmolality

In order to examine whether the effects of saline concentration on FMP bouts were due to ionic and/or osmotic differences between the solutions, hyperosmotic saline (with mannitol, 190–200 mosmol kg^{-1} H_2O) was applied to the CNS-lip preparation instead of 1·5× saline in six preparations (Fig. 5). As in the experiment with saline concentration changes, the number of bite cycles per FMP bout was reversibly decreased when the saline was changed from 1·0× to hyperosmotic saline (1·0× + mannitol). There was a significant difference between the bout length in each of the 1·0× salines and in 1·0× + mannitol [first 1·0× *vs* 1·0× +mannitol, $F(1,10)=29{\cdot}56$, $P<0{\cdot}002$; 1·0× + mannitol *vs* second 1·0×,

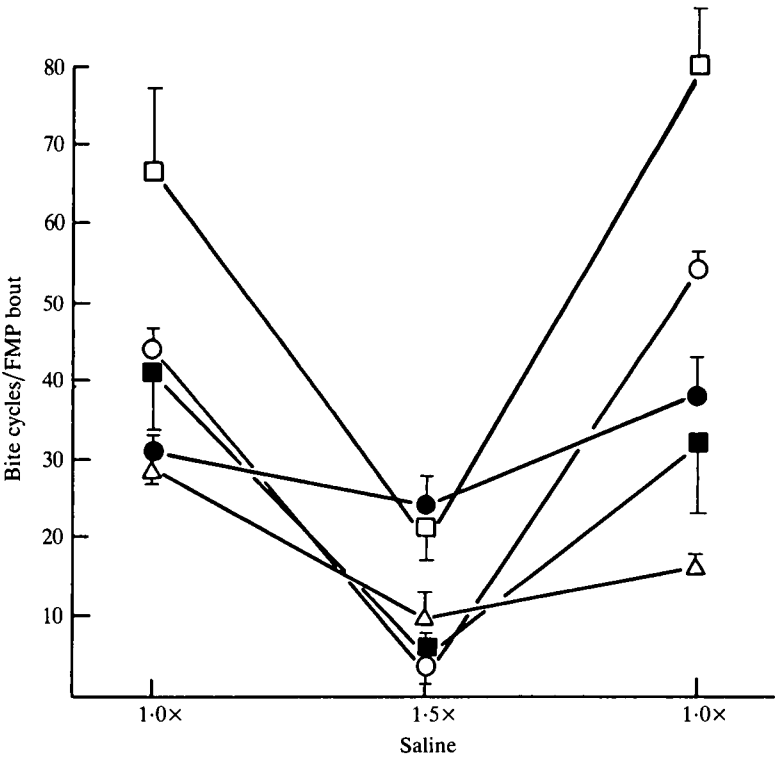

Fig. 4. The effect of saline concentration changes on the number of bite cycles per FMP bout. This figure shows the results of five experiments (five different preparations) in which FMP bouts were elicited by identical electrical stimulation in 1·0×, 1·5× and 1·0× salines. FMP bout lengths for each experiment are plotted as the mean ± S.E.M. number of bite cycles per FMP bout for 2–6 stimulations in each saline.

$F(1,10)=34{\cdot}51$, $P<0{\cdot}001$]. However, no significant difference in the number of bite cycles per bout was observed in the comparison of the first and second 1·0× saline treatments. In addition, the duration of FMP bouts (in seconds) was decreased when the saline was changed from 1·0× to hyperosmotic saline [$F(1,10)=36{\cdot}9$, $P<0{\cdot}0004$] and subsequently increased when 1·0× saline was replaced [$F(1,10)=35{\cdot}5$, $P<0{\cdot}004$].

Several other parameters of neural activity were analysed from records obtained during and between FMP bouts. These parameters included SB burst duration, interburst interval, action potential frequency within SB bursts and action potentials per SB burst. None of these parameters exhibited significant treatment effects when FMP bouts in 1·0× saline were compared to bouts in 1·0× + mannitol. Indeed, the frequencies of bursts during FMP bouts stimulated in 1·0×, 1·5× or hyperosmotic (with mannitol) saline were very similar (Fig. 6). Also no significant variations occurred in the endogenous SB activity recorded in the intervals between FMP bouts. The absence of an effect of saline ionic concentration or osmolality on SB burst activity indicates that the primary effect of these

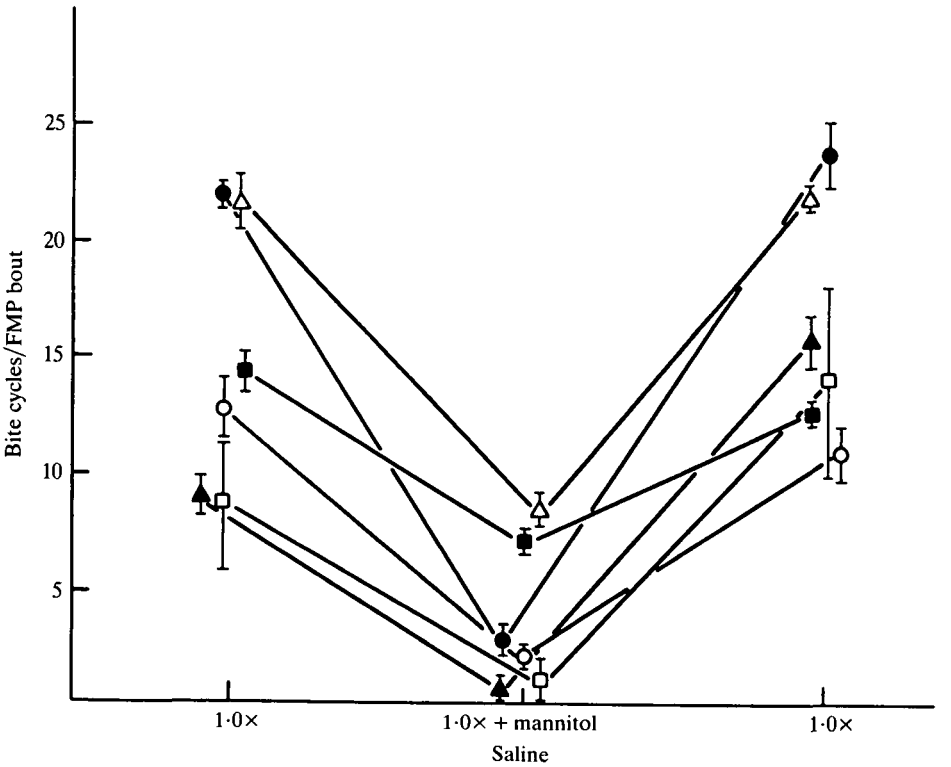

Fig. 5. The effect of saline osmolality changes on the number of bite cycles per FMP bout. This figure shows the results of six experiments (six different preparations) in which FMP bouts were elicited by identical electrical stimulation in 1·0× saline, 1·0× saline plus mannitol (190–200 mosmol kg^{-1} H_2O) and 1·0× saline. FMP bout lengths for each experiment are plotted as the mean ±S.E.M. number of bite cycles per bout for 3–5 stimulations in each saline.

treatments is on the duration of FMP bouts, not on the burst pattern within the bout.

Gradual changes in osmolality

A slug exposed to air-dehydration does not experience abrupt changes in haemolymph osmolality. Approximately 2 h of dehydration is required before haemolymph osmolality reaches 190–200 mosmol kg^{-1} H_2O (i.e. 65 % IBW). In order to mimic the gradual increase in osmolality seen in the intact dehydrating slug, CNS-lip preparations were stimulated while being perfused with saline of progressively greater osmolality. The saline osmolality was slowly increased from 140 to 200 mosmol kg^{-1} H_2O over a period of 1·5–2·5 h. After a brief period of perfusion with the hyperosmotic saline, the osmolality was gradually returned to 140 mosmol kg^{-1} H_2O over 0·6–1·0 h. In four preparations (Fig. 7) the number of bite cycles per FMP bout gradually decreased during the increase in saline osmolality and then returned to initial levels during the subsequent decrease in osmolality. A significant negative correlation ($P<0·02$) between osmolality and

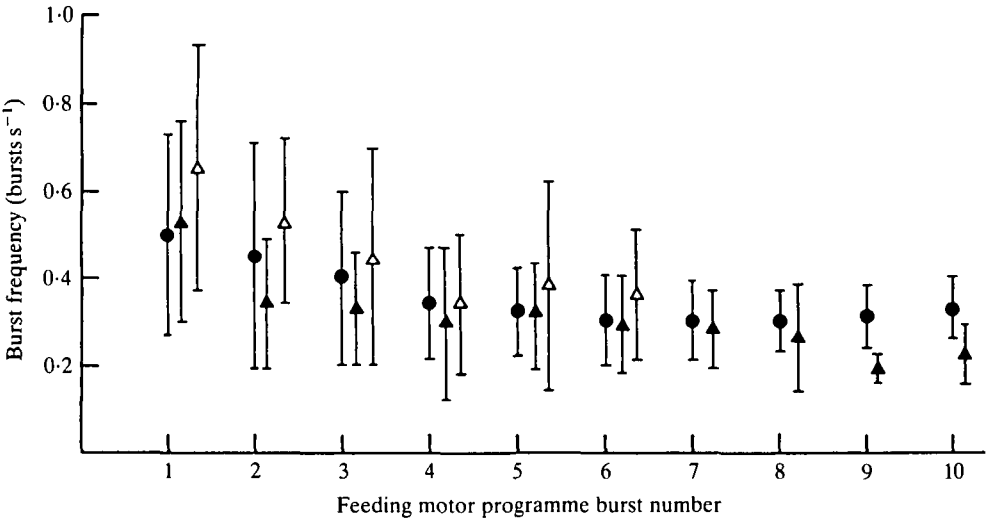

Fig. 6. Burst frequencies for the salivary burster neurone (SB) in salines of different concentration or osmolality. These burst frequencies are plotted against the burst number beginning with the first burst in an FMP bout. Each point represents the $\bar{x} \pm$ S.E.M. for: 16–29 bursts from eight preparations in 1·0× saline (●), 3–9 bursts from four preparations in 1·5× (▲) saline or 3–6 bursts from three preparations in 1·0× +mannitol (△) saline.

bite cycles per FMP bout was obtained in each preparation. The most distinct decreases in FMP bout length appeared to occur when the saline osmolality was raised above approximately 160–175 mosmol kg^{-1} H_2O and this change was reciprocated when the osmolality was subsequently lowered. Fig. 7C illustrates a precipitous increase in FMP bout length that occurred when the decreasing osmolality reached approximately 175 mosmol kg^{-1} H_2O. These results suggest that the nervous system is capable of responding to changes in saline osmolality which are comparable to the haemolymph variations that occur in the intact slug during dehydration.

Long-term hyperosmotic treatment

In some of the experiments utilizing gradual changes in osmolality, the duration of FMP bouts appeared to become progressively longer during extended hyperosmotic treatment (Fig. 7C). This observation suggested that the neural mechanisms which control the FMP might be capable of adapting to hyperosmotic stress. In order to examine this question, FMP bouts were stimulated while the brain was bathed in 1·0× saline plus mannitol (194–218 mosmol kg^{-1} H_2O) for periods of 6–8 h. During this treatment, FMP bouts were elicited every 20 min and the saline was changed every 1–2 h. In each of four experiments (Fig. 8) the number of bite cycles per FMP bout was markedly decreased when hyperosmotic saline was applied and remained so for 2–4 stimulations (40–80 min). The length of the bouts progressively increased throughout the duration of the hyperosmotic treatment as indicated by the regression lines (dashed lines in Fig. 8) and

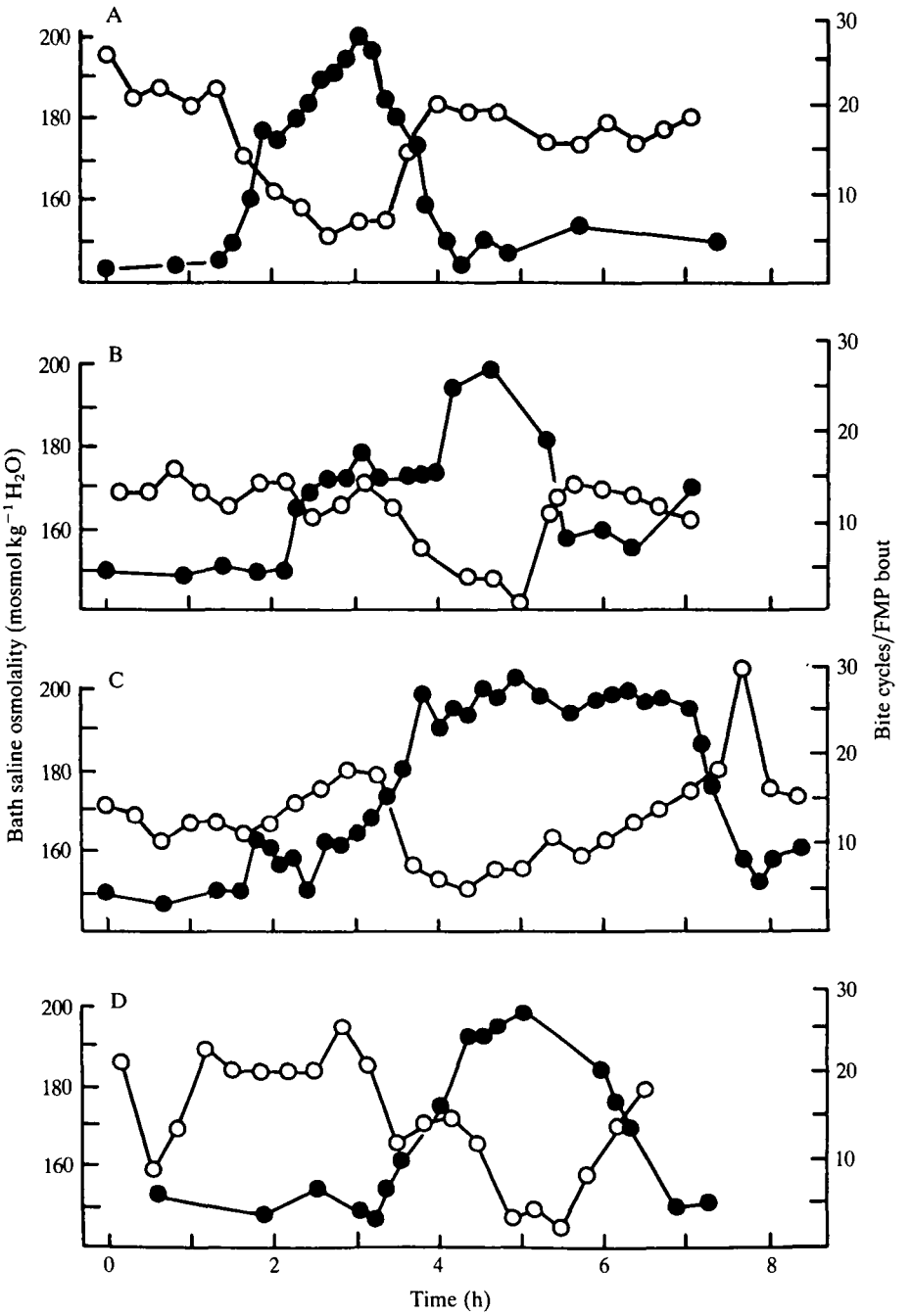

Fig. 7. The relationship between saline osmolality and FMP bout length during gradual osmolality changes. Salines of different osmolality were perfused through a gradient maker gradually increasing and then decreasing the osmolality of the bath saline. The bite cycles per FMP bout (O——O) are plotted against the measured bath osmolality (●——●). Each graph (A–D) represents one experiment on one CNS-lip preparation.

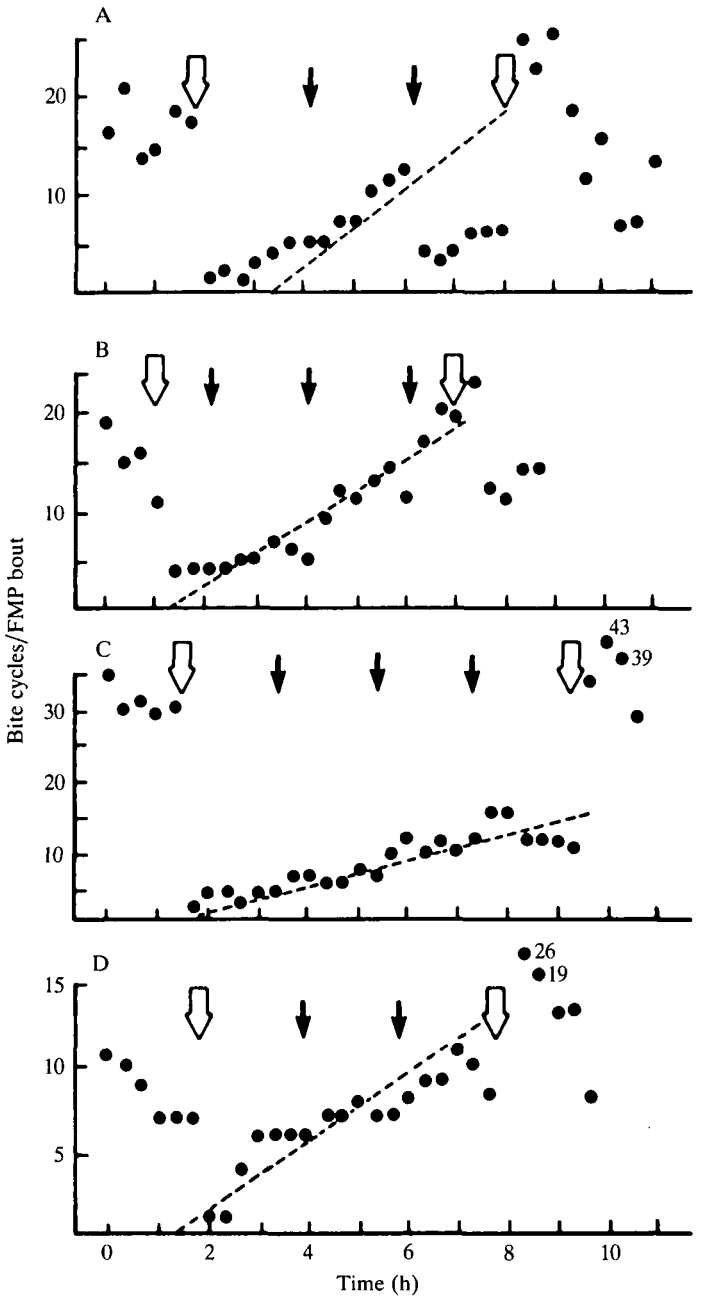

Fig. 8. The effect of long-term hyperosmotic treatment on FMP bout length. At the first open arrow the bath was changed from 1·0× to hyperosmotic saline. At each small arrow, fresh hyperosmotic saline was exchanged for the saline in the bath, and at the second open arrow the saline was changed back to 1·0×. Regression lines are for bout length in bite cycles *versus* time in hyperosmotic saline.

correlation coefficients ($r = 0{\cdot}44$, $0{\cdot}93$, $0{\cdot}88$ and $0{\cdot}74$ for 6A–6D respectively; all correlation coefficients significant to $P < 0{\cdot}05$). This increase is not due to a time-related improvement in the preparations; in six other preparations there were no significant changes in bouts stimulated over 6–8 h in 1·0× saline. These data do not provide any evidence for a sudden increase in bout length; rather the bout length appears to increase gradually with continued exposure to hyperosmotic saline. Thus it seems that the neural network underlying the FMP can adapt to hyperosmotic conditions and that this adaptation occurs gradually over periods ranging from 6 to 8 h or longer.

Despite the apparently complete adaptation of the FMP network to hyperosmotic conditions, when 1·0× saline was replaced each preparation exhibited a long spontaneous bout (44–62 bite cycles, not shown in Fig. 8) that was not observed at any other time. Further, the lengths of the first stimulated bouts in the replaced 1·0× saline often greatly exceeded the bouts in the initial 1·0× saline, revealing a possible rebound effect following long-term hyperosmotic treatment.

DISCUSSION

Haemolymph osmolality and feeding behaviour

Limax maximus exhibits a dramatic dehydration-induced reduction in feeding responsiveness (Fig. 1) that is similar to that observed by Prior (1983) in *Limax pseudoflavus* and *Lehmannia valentiana*. In all three species, dehydration to 60–70 % IBW results in almost complete abolition of feeding. Feeding responsiveness is, however, re-established when the slugs are rehydrated by either contact-rehydration or injection of dilute saline. The effect of air-dehydration can be mimicked by injections of hyperosmotic mannitol that raise haemolymph osmolality to about 200 mosmol kg^{-1} H_2O, which corresponds to that of a slug dehydrated to 65 % IBW (Fig. 2).

Similar dehydration effects have been reported in a variety of other species. For example, a 35 % increase in haemolymph osmolality in locust nymphs resulted in a 90 % reduction in meal size (Bernays & Chapman, 1974). Similarly, among vertebrate species, when rats were water-deprived for 1 day there was a 2 % increase in serum osmolality (e.g. Rolls, Wood & Rolls, 1980) which was sufficient to elicit a 45 % decrease in mean daily food intake (Collier & Levitsky, 1967). In addition, when dehydrated rats were allowed to dilute their blood by drinking, there was a 0·6 % decrease in serum osmolality and a 90 % decrease in the mean latency to feed (Deaux & Kakolewski, 1971).

Thus, regardless of the relative sensitivity of various species to dehydration, it appears that the accompanying increase in blood osmolality mediates a general reduction in feeding responsiveness.

Feeding motor programme

Because gastropods lack an impermeable blood-brain barrier (Lane & Treherne, 1972; Mirolli & Gorman, 1973; Prior, 1981; Sattelle, 1973), changes in

haemolymph osmolality could have a direct effect on the neuronal network underlying feeding. Indeed, increases in either ionic concentration or osmolality of the saline bathing a CNS preparation result in a reduction in the duration of FMP bouts (Figs 4,5). Furthermore, the reduction in FMP bout duration is completely reversed when saline ionic concentration or osmolality is lowered to the level found in fully hydrated slugs. The reversibility of this effect on the CNS corresponds with the observations in intact slugs that decreases in feeding responsiveness, which are induced by air-dehydration (Fig. 1, and Prior, 1983) or increased blood osmolality (Fig. 2, and Prior, 1983), can be reversed by rehydration of the slug, either by drinking or injection of dilute saline.

Although exposure of the CNS preparation to 200 mosmol kg^{-1} H_2O saline would appear to be a generally disruptive stress, none of the various parameters of SB burst activity was significantly affected in a consistent manner. For example, Fig. 6 illustrates the similarity of instantaneous SB burst frequency during FMP bouts in hyperosmotic and normal salines. Thus it appears that the primary effect of increases in saline ionic concentration or osmolality is on the duration of an FMP bout rather than on the ongoing pattern of the neural programme. In addition, since bouts were elicited in all salines with similar efficacy, increased osmolality seems to alter the events involved in termination of the bouts.

When saline osmolality was gradually increased the reduction in the duration of FMP bouts was similar to that observed during acute changes (Fig. 7). Also, the reduction in FMP duration occurred gradually as the saline osmolality was increased. These results suggest that the neuronal network which mediates the FMP is similarly affected by both abrupt experimental changes in osmolality and the gradual changes that occur during air-dehydration in the intact animal. Therefore the changes in the FMP associated with variations in osmolality not only represent the possible responses of the network, but also the probable responses. In this experiment, as in the acute saline changes, the presence of the FMP in all salines points to termination of bouts as the locus of osmotic effects.

Even dehydrated slugs will feed after sufficient food-deprivation (personal observations); therefore one might predict that the neural mechanisms underlying feeding could adapt to hyperosmotic conditions. When FMP bouts were stimulated repeatedly in hyperosmotic saline the duration of the bouts gradually increased until, after 6–8 h, the bouts sometimes equalled those observed in 1·0× saline (Fig. 8). This response is similar to that observed in cardiac ganglion follower neurones of *Limulus* (Prior & Pierce, 1981) and the spontaneous activity of the SB neurone of *Limax pseudoflavus* (Prior, 1981) and may reflect the time courses of various cell volume regulatory mechanisms. We have initiated an investigation of the osmotic responses of several feeding motoneurones (e.g. B7, B9, SB; Prior & Gelperin, 1977) and a modulatory interneurone (MGC; Gelperin, 1981) in order to examine the neuronal mechanism underlying the osmotic control of feeding responsiveness.

In conclusion, as in many other animals, dehydration of the slug causes a decrease in feeding responsiveness and this effect is mediated by an increase in

blood osmolality. Furthermore, this increase in osmolality appears to act directly on the central nervous system to effect early termination of feeding bouts.

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REFERENCES

BERNAYS, E. A. & CHAPMAN, R. F. (1974). Changes in haemolymph osmotic pressure in *Locusta migratoria* larvae in relation to feeding. *J. Entomol.* **48**, 149–155.

BOLLES, R. C. (1961). The interaction of hunger and thirst in the rat. *J. comp. Physiol. Psychol.* **54**, 580–584.

BOLLES, R. C. (1967). *Theory of Motivation.* New York: Harper & Row.

CIZEK, L. J. (1961). Relationship between food and water ingestion in the rabbit. *Am. J. Physiol.* **201**, 557–566.

COLLIER, G. & LEVITSKY, D. (1967). Defense of water balance in rats: behavioral and physiological responses to depletion. *J. comp. Physiol. Psychol.* **64**, 59–67.

COOK, A. (1981). Huddling and the control of water loss by the slug *Limax pseudoflavus* Evans. *Anim. Behav.* **29**, 289–298.

DAINTON, B. H. (1954). The activity of slugs. I. The induction of activity by changing temperatures. *J. exp. Biol.* **31**, 165–187.

DEAUX, E. & KAKOLEWSKI, J. W. (1971). Character of osmotic changes resulting in the initiation of eating. *J. comp. Physiol. Psychol.* **74**, 248–253.

GELPERIN, A. (1981). Synaptic modulation by identified serotonin neurons. In *Serotonin Neurotransmission and Behaviour*, (eds B. L. Jacobs & A. Gelperin), pp. 288–304. Cambridge: MIT Press.

GELPERIN, A., CHANG, J. J. & REINGOLD, S. C. (1978). Feeding motor program in *Limax*. I. Neuromuscular correlates and control by chemosensory input. *J. Neurobiol.* **9**, 285–300.

HESS, S. D. & PRIOR, D. J. (1982). Effects of dehydration-induced increases in hemolymph osmolality on identified pedal ganglion neurons in the slug, *Limax maximus*. *Am. Zool.* **22**, 891.

HOWES, N. H. & WELLS, G. P. (1934). The water relations of snails and slugs. I. Weight rhythms in *Arion ater* L. and *Limax flavus* L. *J. exp. Biol.* **11**, 344–351.

HUGHES, G. M. & KERKUT, G. A. (1956). Electrical activity in a slug ganglion in relation to the concentration of Locke solution. *J. exp. Biol.* **33**, 282–294.

HSIAO, S. & LANGENES, D. J. (1971). Liquid intake, initiation and amount of eating as determined by osmolality of drinking liquids. *Physiol. Behav.* **7**, 233–237.

HSIAO, S. & TRANKINA, F. (1969). Thirst-hunger interaction. I. Effects of body-fluid restoration on food and water intake in water-deprived rats. *J. comp. Physiol. Psychol.* **69**, 448–453.

KATER, S. B. & ROWELL, C. H. F. (1973). Integration of sensory and centrally programmed components in generation of cyclical feeding activity of *Helisoma trivolis*. *J. Neurophysiol.* **36**, 142–155.

KERKUT, G. A. & TAYLOR, B. J. R. (1956). The sensitivity of the pedal ganglion of the slug to osmotic pressure changes. *J. exp. Biol.* **33**, 493–501.

KRALY, F. S. (1984). The physiology of drinking elicited by eating. *Psychol. Rev.* **91**, 478–490.

KUNKEL, K. (1916). *Zur Biologie der Lungenschnecken.* Heidelberg: Carl Winter. (Cited in Howes & Wells, 1934).

KUPFERMAN, I. & COHEN, J. (1971). The control of feeding by identified neurons in the buccal ganglion of *Aplysia*. *Am. Zool.* **11**, 667.

KUTSCHER, C. L. (1969). Species differences in the interaction of feeding and drinking. *Ann. N.Y. Acad. Sci.* **157**, 539–552.

LANE, N. J. & TREHERNE, J. E. (1972). Accessibility of the central nervous connectives of *Anodonta cygnea* to a compound of large molecular weight. *J. exp. Biol.* **56**, 493–499.

LEVITSKY, D. A. (1970). Feeding patterns of rats in response to fasts and changes in environmental conditions. *Physiol. Behav.* **5**, 291–300.

MACHIN, J. (1975). Water relationships. In *Pulmonates*, Vol. 1, (eds V. Fretter & J. Peake), pp. 105–163. New York: Academic Press.

MIROLLI, M. & GORMAN, A. L. F. (1973). The extracellular space of a simple molluscan nervous system and its permeability to potassium. *J. exp. Biol.* **58**, 423–425.

PHIFER, C. B. & PRIOR, D. J. (1982). Dehydration induced modification of feeding and its neural correlate in the slug, *Limax maximus*. *Neurosci. Abstr.* **8**, 901.

PICHON, Y. & TREHERNE, J. E. (1976). The effects of osmotic stress on the electrical properties of the axons of a marinè osmoconformer. (*Maia squinado*, Brachyura:Crustacea). *J. exp. Biol.* **65**, 553–563.

PRIOR, D. J. (1981). Hydration related behaviour and the effects of osmotic stress on motor function in the slugs, *Limax maximus* and *Limax pseudoflavus*. In *Advances in Physiological Science*, Vol. 23, *Neurobiology of Invertebrates*, (ed. J. Salanki), pp. 131–145. Oxford: Pergamon Press.

PRIOR, D. J. (1982). Osmotic control of drinking behavior in terrestrial slugs. *Am. Zool.* **22**, 978.

PRIOR, D. J. (1983). Hydration-induced modulation of feeding responsiveness in terrestrial slugs. *J. exp. Zool.* **227**, 15–22.

PRIOR, D. J. (1984). Analysis of contact-rehydration in terrestrial gastropods: osmotic control of drinking behaviour. *J. exp. Biol.* **111**, 63–73.

PRIOR, D. J. & GELPERIN, A. (1977). Autoactive molluscan neuron: reflex function and synaptic modulation during feeding in the terrestrial slug, *Limax maximus*. *J. comp. Physiol.* **114**, 217–232.

PRIOR, D. J. & GREGA, D. S. (1982). Effects of temperature on the endogenous activity and synaptic interactions of the salivary burster neurones in the terrestrial slug, *Limax maximus*. *J. exp. Biol.* **98**, 415–428.

PRIOR, D. J., HUME, M., VARGA, D. & HESS, S. D. (1983). Physiological and behavioural aspects of water balance and respiratory function in the terrestrial slug, *Limax maximus*. *J. exp. Biol.* **104**, 111–127.

PRIOR, D. J. & PIERCE, S. K. (1981). Adaptation and tolerance of invertebrate nervous systems to osmotic stress. *J. exp. Zool.* **215**, 237–245.

ROLLS, B. J., WOOD, R. J. & ROLLS, E. T. (1980). Thirst: the initiation, maintenance, and termination of drinking. In *Progress in Psychobiology and Physiological Psychology*, Vol. 9, (eds J. M. Sprague & A. N. Epstein), pp. 263–321. New York: Academic Press.

ROSE, R. M. & BENJAMIN, P. R. (1981). Interneuronal control of feeding in the pond snail *Lymnaea stagnalis*. II. The interneuronal mechanism generating feeding cycles. *J. exp. Biol.* **92**, 203–228.

SATTELLE, D. B. (1973). Potassium movements in a central nervous ganglion of *Limnaea stagnalis* (L.)(Gastropoda:Pulmonata). *J. exp. Biol.* **58**, 15–28.

SCHMIDT-NIELSEN, B., SCHMIDT-NIELSEN, K., HOUPT, T. R. & JARNUM, S. A. (1956). Water balance in the camel. *Am. J. Physiol.* **185**, 185–194.

WILLOWS, A. O. D. (1980). Physiological basis of feeding behavior in *Tritonia diomedea*. II. Neuronal mechanisms. *J. Neurophysiol.* **44**, 849–861.

YIN, T. H., HAMILTON, C. L. & BROBECK, J. R. (1970). Effect of body fluid disturbance on feeding in the rat. *Am. J. Physiol.* **218**, 1054–1059.