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SPONTANEOUS AND ELICITED BAG CELL DISCHARGES IN GONADECTOMIZED *APLYSIA*

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SUMMARY

The neuroendocrine bag cells of the hermaphroditic marine gastropod, *Aplysia*, secrete peptide hormones that induce release of ripe eggs from the ovotestis. The egg string is subsequently deposited on the substrate by means of a complex sequence of rhythmic head and neck movements. Gonadectomy (removal of the ovotestis) was performed in two closely related species of *Aplysia* to prevent completely the synthesis, build-up and release of eggs. Chronically implanted electrodes were used either to monitor spontaneous bag cell discharges (*A. brasiliana*) or to selectively elicit bag cell discharges (*A. californica*) in gonadectomized and mock-operated animals. Gonadectomized animals showed the normal occurrence of spontaneous bag cell discharges in the complete absence of eggs, indicating that feedback from ripe eggs in the ovotestis is not necessary for normal activation of the bag cells. However, gonadectomized animals showed a significant decrease in specific head and neck movements following elicited bag cell discharges. This finding indicates that, once the bag cells fire and the eggs are released, input from the eggs is necessary for normal expression of the behaviour associated with egg deposition.

INTRODUCTION

A description of the functional role of a peptide requires an understanding of the conditions that normally trigger its release as well as the neural circuits and effectors through which the resulting behaviour is expressed (Dismukes & Leibeskind, 1978). In the hermaphroditic marine gastropod *Aplysia* it has long been known that egg laying can be induced by injections of bag cell extracts into reproductively mature individuals (Kupfermann, 1967), but it remains unknown what causes the bag cell to fire. One problem is that spontaneous bag cell discharges are recorded only rarely in isolated ganglia or semi-intact preparations. Electrical stimulation of

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specific central pathways (Kupfermann & Kandel, 1970; Haskins & Blankenship, 1979) and administration of atrial gland extracts (Arch, Smock, Gurvis & McCarthy, 1978; Heller *et al.* 1980; Schlesinger, Babirak & Blankenship, 1981; Painter, Rock & Blankenship, 1983; Nagle & Blankenship, 1983) can trigger bag cell discharges *in vitro* and *in vivo*, but the relevance of these findings to normal bag cell activation remains unclear. When spontaneous bag cell discharges were recorded in intact *A. brasiliana*, by means of cuff electrodes chronically implanted above the bag cell neurites at the base of the pleurovisceral connectives (Pinsker & Dudek, 1977), all episodes of egg laying were preceded by a bag cell discharge, confirming previous suggestions that bag cell activity is necessary for normal ovulation. Furthermore, all spontaneous bag cell discharges were followed by successful egg laying, indicating that the bag cells did not fire unless ripe eggs were available in the ovotestis and suggesting the hypothesis that feedback from the target organ might lead to a bag cell discharge. Feedback from the target organ is believed to play a role in hormone release in a number of mammalian reproductive systems. One example is 'Ferguson's reflex' in which stretching of the uterus leads to increased release of oxytocin from the pituitary which, in turn, causes contraction of the uterus during parturition (Ferguson, 1941; Vasicka, Kumaresan, Han & Kumaresan, 1978). In the case of *Aplysia*, the build-up of eggs could trigger a bag cell discharge or the absence of eggs in the ovotestis could prevent a discharge; either hypothesis predicts that the bag cells will not fire in the absence of eggs in the ovotestis.

Egg-laying behaviour for *A. californica* (Arch & Smock, 1977) was initially described following injections of crude or purified bag cell extracts, and it still remains unclear which patterns of behaviour are produced directly by bag cell hormones and which ones represent effects due to the movement of the released eggs through the reproductive tract. Many studies have shown clearly that elicited bag cell discharges or administration of bag cell hormones can produce a variety of excitatory and inhibitory effects on different identified nerve cells in isolated neural preparations (Branton, Arch, Smock & Mayeri, 1978; Mayeri, Brownell, Branton & Simon, 1979; Mayeri, Brownell & Branton, 1979; Stuart & Strumwasser, 1980), suggesting that some behavioural effects might represent a direct action of the hormone on the nervous system that does not require input from the released eggs. However, these identified neurones have no known relevance to egg laying and no input from egg movement is possible *in vitro*. Furthermore, even when ripe eggs are available in the ovotestis, ovulation does not occur in semi-intact preparations in response to hormone administration or to electrical activation of the bag cells. Thus, the potential contribution of input from the released eggs to the expression of egg-laying behaviour must be analysed in intact animals.

The aims of the present study were to determine (1) whether feedback from eggs in the ovotestis contributes to normal activation of the bag cells, and (2) once the bag cells have fired, whether the lack of input from the eggs that would have been released into the reproductive tract alters the normal expression of egg-laying behaviour. Several new approaches are used to analyse egg-laying behaviour in intact and freely-behaving *Aplysia*: (i) gonadectomy (removal of the ovotestis) is used to

completely prevent synthesis, build-up and release of eggs; (ii) a fine-wire electrode (Parsons, ter Maat & Pinsker, 1983) is used to elicit a normal bag cell discharge selectively; and (iii) chronic EMG recordings are used along with video recordings to monitor head and neck movements during egg deposition. Preliminary reports of some of these findings have appeared (ter Maat, Cobbs & Pinsker, 1983; Ferguson, Parsons, ter Maat & Pinsker, 1984).

MATERIALS AND METHODS

Experimental animals

Two closely-related species of *Aplysia* (200–400 g) were used in these experiments: warm-water *A. brasiliana* were caught in the Gulf of Mexico off the South Texas coast and cold-water *A. californica* were obtained from Alacrity Marine Biological Supplies (Redondo Beach, CA). Animals were maintained in individual perforated cages within a larger aquarium containing circulating artificial sea water (ASW) that was kept at about 20 °C. This is a relatively high temperature for *A. californica* (see Pinsker & Parsons, 1985, for effects of temperature on egg-laying frequency in *A. brasiliana* and *A. californica*). *A. brasiliana* were used in the investigation of the effects of gonadectomy on the occurrence of spontaneous bag cell discharges because this species lays eggs more frequently than *A. californica* under laboratory conditions (Pinsker & Parsons, 1985). Fig. 1 shows a schematic drawing

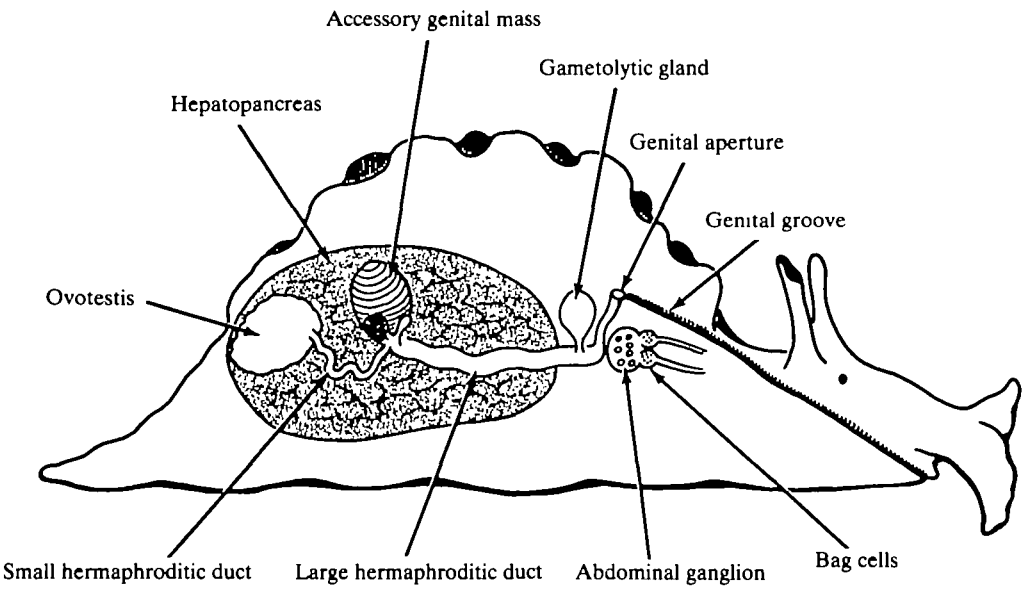

Fig. 1. Schematic drawing of the reproductive tract of *Aplysia*, showing the relative position of the abdominal ganglion (not to scale). The ovotestis is closely apposed to the posterior of the hepatopancreas, and the abdominal ganglion lies ventral to the genital aperture. The distributed nature of the reproductive and nervous systems allows surgical manipulations to gonadectomize animals (completely remove the ovotestis) and implant electrodes on their bag cells.

of the reproductive tract of *Aplysia* (for fuller details see Thompson & Bebbington, 1969; Painter *et al.* 1985) and the relative positions of the ovotestis and the abdominal ganglion.

Surgical procedures

Animals were anaesthetized with magnesium chloride (approx. 25% of body weight) injected into the foot sinuses. Two incisions were made in each animal (one on the right side of the posterior body wall to expose the ovotestis and the other medial to the genital aperture to expose the bag cells) and were later sutured in a double layer. After surgery, animals were injected with sterilized ASW to restore pre-operative body weight. Animals for spontaneous discharges had 6–12 h recovery whereas animals for elicited discharges recovered overnight.

Gonadectomy

In the gonadectomized groups, the membrane overlying the ovotestis was cut, the ovotestis was gently aspirated (taking care not to damage the closely apposed hepatopancreas) and the membrane was closed with sutures. In the control groups, the membrane was cut, the ovotestis was manipulated with a glass probe (but was otherwise left intact) and the membrane was sutured.

Electrode implantation

Either a single-channel cuff electrode (see Pinsker & Eberly, 1982, for details) was implanted at the base of the pleuro-abdominal connectives over the bag cell neurites (primarily to monitor spontaneous bag cell discharges) or a fine-wire electrode (see Parsons *et al.* 1983) was implanted in the connective tissue overlying the bag cell somata (to elicit and monitor discharges). In some animals, EMG cuff electrodes were implanted onto bands of the dorsal longitudinal muscle (DLM) anterior to the left rhinophore. The DLM was exposed by a single incision and a band of muscle fibres placed into the cuff electrode, taking care not to damage surrounding muscle bands. The cuff electrode was then ligatured around the inserted muscle band, and the incision closed with a single layer of sutures. A more detailed description of EMGs during egg deposition will appear (G. P. Ferguson, A. ter Maat & H. M. Pinsker, in preparation).

Spontaneous bag cell discharges

To monitor spontaneous bag cell activity, regular egg layers were selected (animals that had laid eggs four or five times in the previous week). Experimental and mock-operated animals were examined simultaneously. Day 1 began at midnight of the day that surgery was performed and the animals were monitored continuously for 2 days. Because of technical limitations on recording continuously from the bag cells for longer than several days, the electrodes were not implanted in the long-term experiments until 1 week after the surgery on the ovotestis. At the end of the observation period, in those animals in which no spontaneous bag cell discharges had

been recorded, discharges were elicited electrically *via* the cuff or fine-wire electrode (see below) in order to establish proper electrode placement. If no bag cell discharge was elicited with a 30 V stimulus, then the animal's data were discarded (two gonadectomized, two mock-operated). For the mock-operated animals, it was also noted whether they laid eggs during the observation period or after the elicited bag cell discharge.

Elicited bag cell discharges

To elicit a discharge, the amplifier (W.P.I., DAM 5A) was placed momentarily in the 'stimulation' mode and trains of pulses (5 s, 7 Hz, 5–10 ms pulses) were delivered at 1-min intervals, starting at 2 V and increasing in 2-V steps to a maximum of 30 V. At each step, opposite polarities of (monopolar) stimulation were used on alternate trials. With fine-wire stimulation of the bag cells, approx. 15 % of the animals showed evidence of receiving noxious stimulation (inking or body contraction) and their data were rejected.

Behavioural analysis

The present study focused on the rhythmic head and neck movements used to explore (waves) and prepare (undulations) the substrate and to distribute (weaves) and attach (tamps) the egg string. Three of these patterns (undulations, weaves, tamps) have previously been quantified for *A. brasiliana* (Cobbs & Pinsker, 1982*a*). Arch & Smock (1977) have described egg-laying behaviour in *A. californica*; their categories of sweeping, nodding, weaving and tucking are comparable to our categories of waves, undulations, weaves and tamps, respectively. A more complete description of the behaviour in *A. californica* will appear (G. P. Ferguson, D. W. Parsons, A. ter Maat & H. M. Pinsker, in preparation). Time-lapse video recordings from 1 h before to 2 h after an elicited discharge were played back at 9 times speed to score each behaviour independently. For statistical analysis, cumulative counts of each behaviour were totalled for the 1 h before and the 1 h after the discharge.

Statistical comparisons

Nonparametric tests were used to evaluate differences between normal and gonadectomized groups (Mann–Whitney U test) or within groups (Wilcoxon matched-pairs signed-ranks test). On the basis of our previous studies with reproductive tract ligation (Cobbs & Pinsker, 1982*b*), we predicted that the gonadectomized animals would show decreased behavioural changes after the elicited discharge, so one-tailed tests were used for these comparisons. We made no predictions concerning differences before the elicited discharge, so two-tailed tests were used.

RESULTS

Effects of gonadectomy on bag cell activity

To test the hypothesis that feedback correlated with the presence of ripe eggs in the ovotestis induces the bag cells to fire, spontaneous bag cell activity was monitored

Table 1. *Bag cell discharges in gonadectomized and mock-operated* Aplysia brasiliana

	Proportion of animals showing spontaneous bag cell discharges	
	Days 1–2*	Days 8–9*
Gonadectomized	4/4	3/4
Mock-operated	4/4	4/4

*Days after gonadectomy or mock-operation. Animals monitored on days 1 and 2 had the recording electrodes implanted when the surgery on the ovotestis was performed. The animals monitored on days 8 and 9 had recording electrodes implanted on day 7 after gonadectomy or mock-operation.

in animals whose ovotestes were completely removed. To examine short-term effects of gonadectomy, *A. brasiliana* ($N = 8$) were matched on the basis of the frequency of egg laying during the previous week and assigned to the gonadectomized or mock-operated group. In these animals, the surgery to expose and/or remove the ovotestis and to implant the electrodes was done in a single stage. Post-operatively, all of the gonadectomized and mock-operated animals showed spontaneous bag cell discharges (Table 1, days 1–2). Each animal showed a single discharge in the 2 post-operative days of observation, which was comparable to their pre-operative frequency of egg laying. These results indicate clearly that the bag cells can fire in the complete absence of eggs.

Although acute gonadectomy removed any mechanical feedback from the presence of the eggs, it is possible that the ripe eggs and/or the ovotestis secreted a hormone that was still present after 2 days in sufficient concentration to account for the recorded bag cell discharges in the absence of the ovotestis. To determine whether there was a long-term effect of gonadectomy, animals ($N = 8$) were matched on the basis of previous egg laying and were either gonadectomized or mock-operated. After a week, all animals were implanted with a cuff or fine-wire electrode to monitor spontaneous bag cell discharges. Once again there was no obvious difference in the occurrence of spontaneous bag cell discharges between the two groups (Table 1, days 8–9). Fig. 2 shows representative spontaneous bag cell discharges from a long-term gonadectomized and a mock-operated animal. There were no obvious differences between the characteristics of the spontaneous discharges of gonadectomized and mock-operated animals. For example, the durations of the discharges (measured in seven animals in each group) were $15{\cdot}9\,\text{min} \pm 9{\cdot}6$ S.D. for the gonadectomized animals and $14{\cdot}3\,\text{min} \pm 9{\cdot}0$ for the controls. The above findings show that neither mechanical nor hormonal feedback from the target organ is a critical factor in determining whether or not the bag cells fire.

Effects of gonadectomy on egg-laying behaviour

Once the bag cell fire, they secrete peptide hormones that trigger ovulation. The ripe eggs that are released from the ovotestis move through the complex reproductive tract, where they are packaged into a long egg string that eventually reaches the

mouth region and is deposited on the substrate by means of a complex sequence of different rhythmic head and neck movements.

To test the hypothesis that the behaviour resulting from bag cell activity depends on input from the released eggs, bag cell discharges were elicited in gonadectomized animals. Electrical stimulation of bag cell neurites by means of an implanted cuff electrode can elicit a bag cell discharge (Pinsker & Dudek, 1977), but this procedure is non-selective and almost invariably produces noxious side effects such as contractions and inking. The fine-wire method, which is used here for the first time to elicit bag cell discharges in intact animals, is especially useful for investigating behavioural changes caused by bag cell activity because (i) it is a non-invasive procedure (unlike an injection of hormone) that does not disrupt the animal's behaviour; (ii) it is usually quite selective and only the bag cells are stimulated; and (iii) it elicits a physiological input of bag cell hormones, both qualitatively (the different types of peptides) and quantitatively (the amounts released).

A. californica ($N = 20$) that were known egg layers were assigned to the gonadectomized or the mock-operated group. Both groups had a fine-wire electrode implanted above the bag cell somata. In addition, half of the animals in each group had EMG electrodes implanted on comparable bands of the dorsal longitudinal muscle (DLM) that mediates head and neck movements used in egg deposition. In these animals, it was possible to correlate the video recordings of the behaviour with the muscle activity.

In normal animals, four types of rhythmic head and neck movements are associated with egg laying: waves and undulations (which are probably used to explore and prepare the substrate) and weaves and tamps (which are used to distribute and

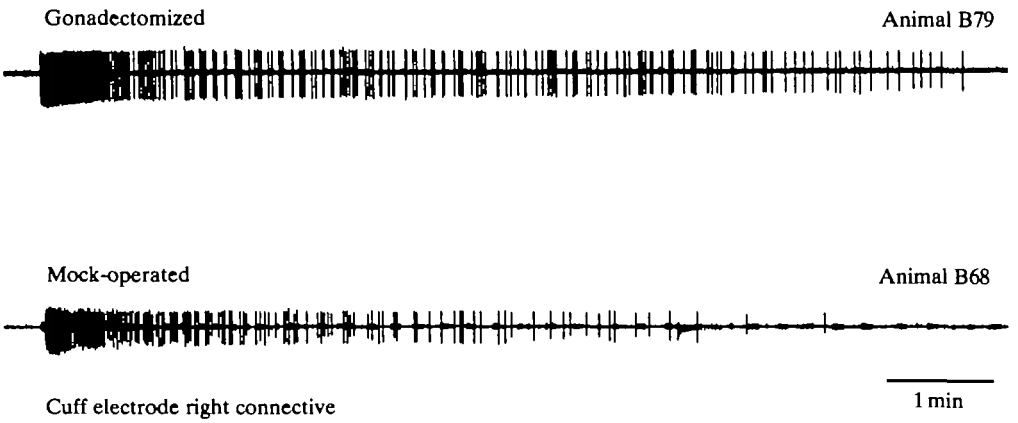

Fig. 2. Cuff electrode recordings of spontaneous bag cell discharges from a gonadectomized (top trace) and a mock-operated (bottom trace) *Aplysia brasiliana* on post-operative day 9. Both animals show typical bag cell activity. At the onset of the discharge, there is a brief period of high-frequency firing that is probably associated with the release of sufficient hormone to trigger ovulation. The frequency of firing gradually decreases until the end of the discharge (8.8 and 7·4 min in the gonadectomized and mock-operated animals, respectively). The mock-operated animal began to deposit eggs on the substrate 28 min after the onset of the discharge.

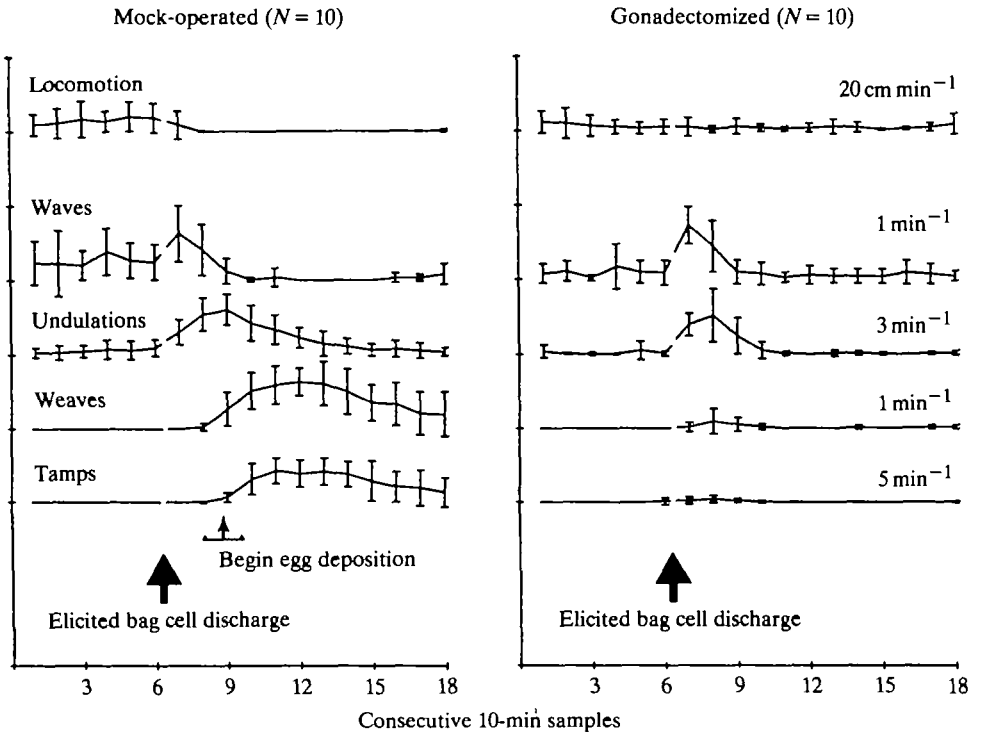

Fig. 3. Behaviour of normal (mock-operated) and gonadectomized *Aplysia californica* 1 h before and 2 h after elicited bag cell discharges (large arrow). The mean frequency per min (±S.D.) is shown for consecutive 10-min samples. The scale (rate min^{-1}) for each behaviour is shown at the extreme right: this number indicates the value of a single division on the left-hand ordinate of each graph. Note that the exploratory and preparatory movements (waves and undulations) have a relatively high level before the elicited discharge. In the mock-operated animals, the eggs first appear on the substrate with an average latency of 29 min after the elicited discharge (small arrow, with S.D.). The short latency for the onset of egg deposition in *A. californica* is probably due to the relatively high temperature (approx 20°C) at which these experiments were conducted. Weaves and tamps increased as the eggs appeared in normal animals and continued until the egg mass was completely deposited, whereas gonadectomized animals showed very few weaves and tamps.

attach the egg string). Fig. 3 (left) plots the average frequency of each behaviour per minute for consecutive 10-min samples before and after the elicited bag cell discharge in normal *A. californica*. The within and between group statistical comparisons for this experiment (based on cumulative counts for each behaviour in the hour before and the hour after the elicited discharge) are shown in Table 2. The normal (mock-operated) animals stopped locomoting shortly after the discharge and showed exploratory and preparatory head and neck movements (a slight, but insignificant increase in waves, followed by a large increase in undulations). The undulations reached a maximum and began to decrease by the time that the eggs appeared on the substrate (latency = 28·8 min ± 7·7, $N = 10$). Weaves and tamps began as the eggs appeared and continued until the egg mass was completely deposited.

In the gonadectomized animals, there appeared to be somewhat less activity for each type of behaviour prior to the elicited discharge (Fig. 3, right) compared to the activity in normal animals (Fig. 3, left), but only one difference, for waves, was significant (Table 2). There was no difference in the stimulus intensity required to activate the bag cells in the normal (9·4 V ± 3·0) and gonadectomized (9·8 V ± 2·6) groups. Also, there was no difference in the duration of elicited bag cell discharges in gonadectomized (9·2 min ± 3·1, $N = 7$) and mock-operated (11·9 min ± 3·2, $N = 7$) animals. Unlike the mock-operated animals, the gonadectomized animals failed to show a significant decrease in locomotion following the elicited discharge. The gonadectomized animals showed a large increase in waves from before to after the discharge and the total wave activity during the hour after the discharge was not significantly different from that of the controls. Like the normal animals, the gonadectomized animals showed a large increase in the frequency of undulations shortly after the elicited discharge. However, the gonadectomized animals did not appear to maintain the undulations and showed significantly fewer undulations in the first hour after the discharge than the normal animals. Thus, gonadectomy had relatively little effect on exploratory and preparatory patterns of behaviour that are not specific to egg deposition.

The most striking differences between the two groups were in the weaves and tamps. Many of the gonadectomized animals showed no increase at all in either of these movements (6/10 showed no weaves after the discharge and 4/10 showed no tamps) and the few that did occur were closer in time to the elicited bag cell discharge than in normal animals. Furthermore, there was no overlap between the post-discharge weaves and tamps of the control and the gonadectomized animals. These

Table 2. *Statistical comparisons before and after elicited bag cell discharges*

Comparisons	Locomotion	Behaviour patterns Waves	Undulations	Weaves	Tamps
1 h post- *vs* 1 h pre-					
mock-operated (1-tailed)	Decrease $P = 0{\cdot}01$	Increase NS	Increase $P < 0{\cdot}005$	Increase $P < 0{\cdot}005$	Increase $P < 0{\cdot}005$
gonadectomized (1-tailed)	NS	$P < 0{\cdot}025$	$P < 0{\cdot}005$	*	$P < 0{\cdot}025$
Gonadectomized *vs* Mock-operated	Gonadectomized = More	Less	Less	Less	Less
1 h pre- (2-tailed)	NS	$P = 0{\cdot}05$	NS	NS	NS
1 h post- (1-tailed)	NS	NS	$P < 0{\cdot}001$	$P < 0{\cdot}001$	$P < 0{\cdot}001$

Within group comparisons (1 h post- *vs* 1 h pre-) used Wilcoxon Tests and between group comparisons (gonadectomized *vs* mock-operated) used Mann–Whitney U-tests. Scores were cumulative counts for the 60 min before (pre) and the 60 min after (post) an elicited bag cell discharge (see Fig. 2 for average counts for each 10-min sample).

* The majority of gonadectomized animals (6/10) showed no weaves either before or after the discharge, so there were too few scores to evaluate the significance of the post-discharge increase.

NS, not significant.

findings indicate that input from the eggs is necessary for the full expression of the behaviour associated with egg deposition, particularly the rhythmic head and neck movements (weaves and tamps) used to distribute and attach the egg string.

The relative absence of weaves and tamps in the gonadectomized animals suggested that the rhythmic neuronal activity that normally mediated these movements would also be absent. Fig. 4 shows chronically recorded EMGs in a representative control (left) and gonadectomized (right) animal at selected times before and after the elicited bag cell discharge. Both animals showed relatively small amplitude EMG activity prior to the discharge, usually associated with concurrent movements of the head and neck. Shortly after the discharge was elicited, the activity increased and there were bursts of excitatory junctional potentials (EJPs) in both animals during waves and undulations. This activity continued until the eggs reached the lips in the mock-operated animal (latency about 42 min) or until about 36 min after the elicited discharge in the gonadectomized animal. In the control animal, the muscle became especially active just before egg deposition started and there were larger amplitude, phasic bursts of EJPs that continued until egg laying was completed. This increase in EMG activity was associated with the marked increase in weaves and tamps that were used to deposit the eggs. However, as predicted from the behavioural evidence, the gonadectomized animal did not show this increase in muscle activity and the EMG became quiescent when the animal would normally have been laying eggs (average time to cessation of EMG was 29·8 min ± 5·3 in four gonadectomized animals). The absence of the EJPs at this phase indicates that the central motoneurones that normally became rhythmically active when both bag cell hormone and egg movement were present remained silent when only the hormone was present.

DISCUSSION

The question of what causes the bag cells to fire remains unanswered. The present results indicate clearly that neither the physical presence of the eggs nor some hormonal factor associated with the eggs (or released from the ovotestis) are necessary for the normal frequency of occurrence of bag cell activity in *A. brasiliana*. The hypothesis that copulation might trigger bag cell activity, thereby enhancing the likelihood of fertilization, has also been disproved for *A. californica* (Blankenship *et al.* 1983). Egg laying by one *A. californica* tends to induce egg laying in other animals sharing the same water (Audesirk, 1977; see also review of Audesirk & Audesirk, 1985) and applying a freshly laid egg mass to the oral veil of *A. californica* can induce egg laying (E. Mayeri, personal communication). These findings suggest that contact with a freshly laid egg mass may be sufficient to activate the bag cells, although it is not necessary because isolated animals lay eggs.

Although gonadectomized animals show normal spontaneous bag cell activity, the present results indicate that they do not show normal egg-laying behaviour following elicited bag cell discharges. Thus, once the bag cells fire, some type of input from the released eggs plays a critical role in determining the subsequent expression of the behavioural sequences. Under normal circumstances, spontaneous bag cell discharges occur only when ripe eggs are present in the ovotestis (Pinsker & Dudek,

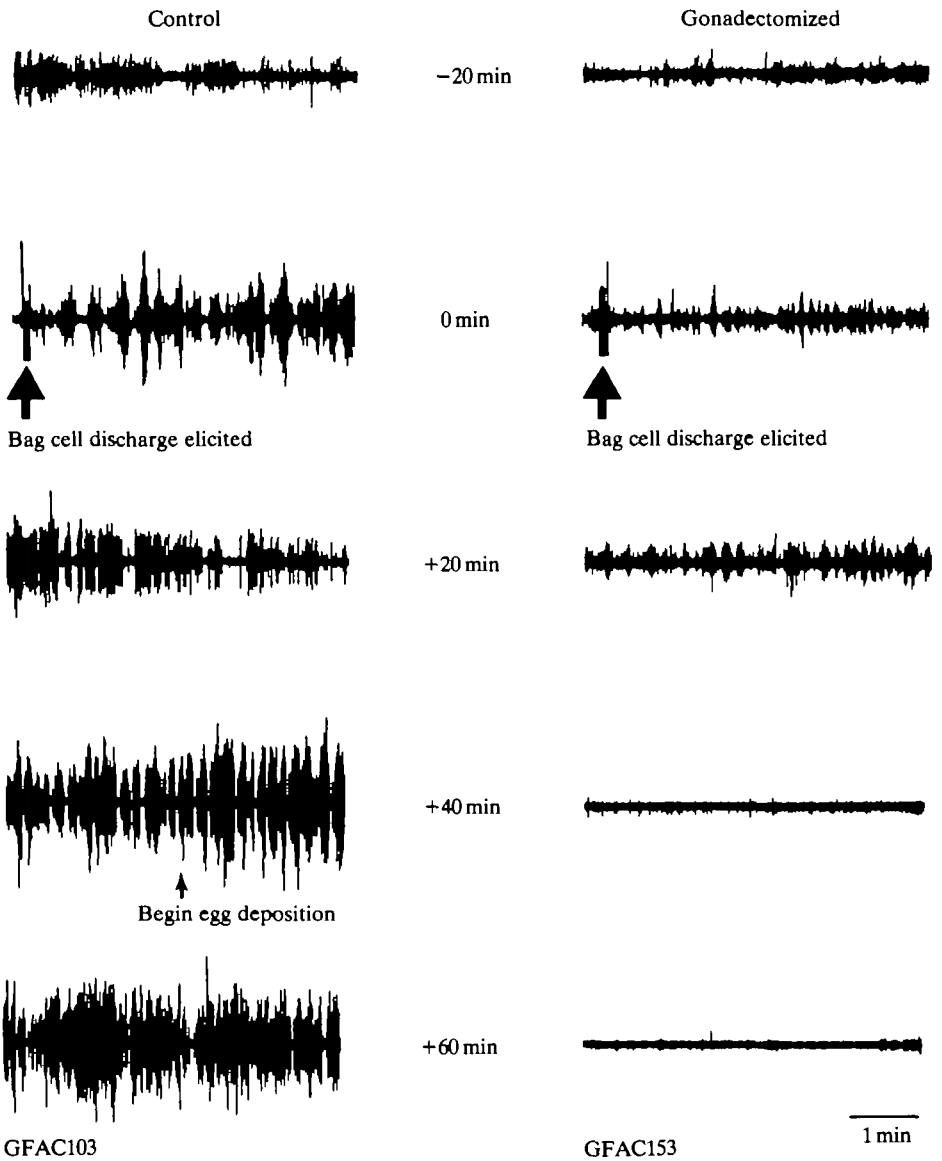

Fig. 4. Selected 5-min samples of EMG activity recorded from bands of the left anterior dorsal longitudinal muscle (DLM) of a control and a gonadectomized animal. Before the elicited bag cell discharge (−20 min), there was some relatively small amplitude DLM activity in both animals. Immediately after the bag cell discharge (0 min) there were phasic bursts of EJPs in the DLM of both animals which were correlated with rhythmic waves and undulations of the head and neck. This muscle activity continued (+20 min) in both animals while they were showing these exploratory and preparatory movements. Just prior to eggs appearing on the lips of the control animal (+40 min), phasic bursts of larger amplitude EJPs, associated with tamps and weaves, were recorded. This activity continued (+60 min) until egg deposition was complete, and the animal stopped tamping and weaving (not illustrated). By contrast, the gonadectomized animal did not show the DLM activity associated with deposition of eggs (+40 min, +60 min), but instead became quiescent when it would normally have been laying eggs.

1977) so that input from the released eggs is always present and normal egg-deposition behaviour occurs. However, artificially removing the eggs and eliciting a normal bag cell discharge showed that the movements used to distribute and attach the eggs were almost completely abolished. We conclude that a bag cell discharge initiates two phases of egg-laying behaviour in an intact animal: a 'preparation' or appetitive phase (consisting of waves and undulations) that does not depend upon egg movement, followed by a 'deposition' or consummatory phase (consisting of weaves and tamps) which occurs only when the eggs have reached the end of the genital groove. Presumably, the preparation phase is initiated by a direct effect of bag cell hormone(s) on the nervous system whereas the deposition phase is not triggered directly by the hormone(s) but requires input from the eggs.

In previous experiments involving injections of bag cell extracts, blocking egg movement by ligating the reproductive tract near the ovotestis markedly decreased egg-laying behaviour, which was partially restored by introducing artificial eggs through a cannula in the reproductive tract (Cobbs & Pinsker, 1982*b*). However, methodological problems limited the conclusions that could be drawn from the ligation experiments. For example, the build-up of released eggs within the reproductive tract (behind the ligature) could have produced noxious stimulation that impaired behaviour and injected bag cell extracts could have been both qualitatively and quantitatively different from the substances normally released by the bag cells. In the present gonadectomy experiments, egg movements were abolished without ligating the reproductive tract and normal bag cell discharges were elicited selectively in intact animals. Surprisingly, gonadectomy proved to be a relatively benign procedure and these animals survived as well as mock-operated animals. There were also no obvious effects on behaviour other than the effects upon the egg-laying movements. For example, gonadectomized *A. californica* housed in pairs continued to copulate as either males or females. Also, the present studies included EMG recordings as a first step in the neuronal analysis of egg-laying behaviour. Thus, the present study overcame limitations of the earlier study and indicated clearly that normal behaviour and neuronal output were elicited by the hormone only when the eggs were present.

The contribution of eggs to the expression of reproductive behaviour has also been documented in other species. In the freshwater snail, *Lymnaea stagnalis*, egg laying is normally induced by an ovulatory peptide hormone released by a discharge of the Caudo-Dorsal Cells (de Vlieger, Kits, ter Maat & Lodder, 1980; ter Maat, Lodder & Wilbrink, 1983), but egg-laying behaviour patterns following hormone injections occur only in snails that subsequently lay eggs (Goldschmeding, Wilbrink & ter Maat, 1983). Similarly, spawning behaviour in female goldfish (normally stimulated by the male) does not occur unless there is input from eggs in the ovarian cavities: spawning behaviour ceases in females stripped of their eggs but can be reinstated if eggs are injected into the ovarian cavities in the presence of oestrogen (reviewed by Slater, 1978).

Egg laying in *Aplysia* has been described as a neuroendocrine fixed action pattern, i.e. an innate stereotyped behavioural array that is mediated by one or more

neuroactive peptides (see reviews of Scheller *et al.* 1983; Scheller & Axel, 1984). A bag cell discharge causes the coordinated release of at least four different peptides. One of these, egg-laying hormone (ELH), is a slowly degrading peptide that enters the circulation and causes release of eggs by a direct effect on the ovotestis (Dudek & Tobe, 1978; Rothman, Weir & Dudek, 1983). Three of the peptides (ELH, alpha bag cell factor, beta bag cell factor) are also believed to be neuroactive, each producing specific changes in the firing patterns of different identified neurones in isolated abdominal ganglia (Branton *et al.* 1978; Rothman *et al.* 1983). Each of the peptides released locally by the bag cells appears to account for a limited subset of the abdominal ganglion neuronal effects produced by a bag cell discharge. The abdominal ganglion neurones that have been used to assay the effects of different bag cell peptides have no established role in egg deposition and apparently normal egg laying can be elicited after complete removal of the abdominal ganglion (Strumwasser, Schlechte & Bower, 1972; Arch *et al.* 1978; Blankenship, Rock & Schlesinger, 1982). The circuits that mediate the different rhythmic head and neck movements that normally follow a bag cell discharge have not yet been identified. One model to explain such rhythmic behaviour is that each different type of behaviour is mediated by a different central pattern generator (CPG) located in the head ganglia. ELH can alter the firing patterns of units in the head ganglia (Stuart & Strumwasser, 1980; Ram, 1983), but alpha bag cell peptide degrades rapidly and probably does not enter the circulation. Synthetic ELH-lysine-amide injections produce normal egg-laying behaviour (Strumwasser, 1984) and synthetic alpha bag cell peptide injections do not produce any egg-laying behaviour unless the injection also elicits a bag cell discharge (J. E. Blankenship, personal communication). Thus, it is theoretically possible that each behaviour could be activated by a different peptide, but the present evidence suggests that ELH alone may be responsible. Whether one or more peptides are involved, our results suggest that activity in certain CPGs (those producing undulations and waves) may depend solely upon the release of the appropriate peptide, whereas activity in other CPGs (those producing weaves and tamps) may also require input from the eggs in addition to the appropriate peptide. However, an alternative model that does not involve central pattern generation is also possible. According to this model, weaves and tamps could occur in a purely reflexive manner as a consequence of the egg movement, and the role of the peptide (presumably ELH) in generating these movements might be simply to induce ovulation (see Arch & Smock, 1977; Stuart & Strumwasser, 1980, for discussions of this point). To address these questions directly, it will be necessary to identify the specific circuits that mediate the different egg-laying movements.

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REFERENCES

ARCH, S. & SMOCK, T. (1977). Egg-laying behavior in *Aplysia californica*. *Behav. Biol.* **19**, 45–54.

ARCH, S., SMOCK, T., GURVIS, R. & MCCARTHY, C. J. (1978). Atrial gland induction of the egg-laying response in *Aplysia californica*. *Comp. Physiol.* **128**, 67–70.

AUDESIRK, T. E. (1977). Chemoreception in *Aplysia californica*. III. Evidence for pheromones influencing reproductive behavior. *Behav. Biol.* **20**, 235–243.

AUSDESIRK, T. & AUDESIRK, G. (1985). Behavior of gastropod molluscs. In *Physiology of the Mollusca*, vol. 8, *Neurobiology and Behavior* (ed. A. O. D. Willows). New York: Academic Press (in press).

BLANKENSHIP, J. E., ROCK, M. K., ROBBINS, L. C., LIVINGSTON, C. A. & LEHMAN, H. K. (1983). Aspects of copulatory behavior and peptide control of egg laying in *Aplysia*. *Fedn Proc. Fedn Am. Socs exp. Biol.* **42**, 96–100.

BLANKENSHIP, J. E., ROCK, M. K. & SCHLESINGER, D. H. (1982). Structure and function of peptides from a neuroendocrine system controlling egg-laying behavior in *Aplysia*. In *Proteins in the Nervous System: Structure and Function* (ed. B. Haber, R. J. Perez-Polo & J. D. Coulter), pp. 159–177. New York: Alan R. Liss, Inc.

BRANTON, W. D., ARCH, S., SMOCK, T. & MAYERI, E. (1978). Evidence for mediation of a neuronal interaction by a behaviorally active peptide. *Proc. natn. Acad. Sci. U.S.A.* **75**, 5732–5736.

COBBS, J. S. & PINSKER, H. M. (1982*a*). Role of bag cells in egg deposition of *Aplysia brasiliana*: I. Comparison of normal and elicited behaviors. *J. comp. Physiol.* **147**A, 523–536.

COBBS, J. S. & PINSKER, H. M. (1982*b*). Role of bag cells in egg deposition of *Aplysia brasiliana*: II. Contribution of egg movement to elicited behaviors. *J. comp. Physiol.* **147**A, 537–546.

DE VLIEGER, T. A., KITS, K. S., TER MAAT, A. & LODDER, J. D. (1980). Morphology and electrophysiology of the ovulation hormone producing neuro-endocrine cells of the freshwater snail *Lymnaea stagnalis* (L). *J. exp. Biol.* **84**, 259–271.

DISMUKES, R. K. & LEIBESKIND, J. C. (1978). How much can psychopharmacology tell us about the role of neuropeptides in behavior? *Neurosci. Res. Prog. Bull.* **16**, 493–497.

DUDEK, F. E. & TOBE, S. S. (1978). Bag cell peptide acts directly on ovotestis of *Aplysia californica*: basis for an *in vitro* bioassay. *Gen. comp. Endocr.* **36**, 618–627.

FERGUSON, G. P., PARSONS, D. W., TER MAAT, A. & PINSKER, H. M. (1984). Bag cell activity and egg laying in *Aplysia*. *Soc. Neurosci. Abstr.* **10**, 150.

FERGUSON, J. K. W. (1941). A study of the motility of the intact uterus at term. *Surgery Gynecol. Obstet.* **73**, 359–366.

GOLDSCHMEDING, J. T., WILBRINK, M. & TER MAAT, A. (1983). The role of the ovulation hormone in the control of egg laying in *Lymnaea stagnalis*. In *Molluscan Neuro-Endocrinology* (ed. J. Lever & H. H. Boer), pp. 251–255. Amsterdam: North-Holland Publishing Co.

HASKINS, J. T. & BLANKENSHIP, J. E. (1979). Interactions between bilateral clusters of neuro-endocrine cells in *Aplysia*. *J. Neurophysiol.* **42**, 356–367.

HELLER, E., KACZMAREK, L., HUNKAPILLAR, M., HOOD, L. & STRUMWASSER, F. (1980). Purification and primary structure of two neuroactive peptides that cause bag cell afterdischarge and egg-laying in *Aplysia*. *Proc. natn. Acad. Sci. U.S.A.* **77**, 2328–2332.

KUPFERMANN, I. (1967). Stimulation of egg-laying: possible neuroendocrine functions of bag cells of abdominal ganglion of *Aplysia californica*. *Nature, Lond.* **216**, 814–815.

KUPFERMANN, I. & KANDEL, E. R. (1970). Electrophysiological properties and functional interconnections of two symmetrical neurosecretory clusters (bag cells) in abdominal ganglion of *Aplysia*. *J. Neurophysiol.* **33**, 865–876.

MAYERI, E., BROWNELL, P. & BRANTON, W. D. (1979). Multiple, prolonged action of neuro-endocrine "bag cells" on neurons in *Aplysia*. II. Effects on beating pacemaker and silent neurons. *J. Neurophysiol.* **42**, 1185–1197.

MAYERI, E., BROWNELL, P., BRANTON, W. D. & SIMON, S. B. (1979). Multiple, prolonged action of neuroendocrine "bag cells" on neurons in *Aplysia*. I. Effects on bursting pacemaker neurons. *J. Neurophysiol.* **42**, 1165–1184.

NAGLE, G. T. & BLANKENSHIP, J. E. (1983). Differential sensitivity of ovotestis and bag cells to atrial gland peptides: egg-laying without bag cell after discharge in intact *Aplysia*. In *Molluscan Neuro-Endocrinology* (ed. J. Lever & H. H. Boer), p. 180. Amsterdam: North-Holland Publishing Company.

PAINTER, S. D., KALMAN, V. K., NAGLE, G. T., ZUCKERMAN, R. A. & BLANKENSHIP, J. E. (1985). The anatomy and functional morphology of the large hermaphroditic duct of three species of *Aplysia*, with special reference to the atrial gland. *J. Morph.* **186**, 167–194.

PAINTER, S. D., ROCK, M. K. & BLANKENSHIP, J. E. (1983). Lesion studies indicate possible sites of action of atrial gland extracts for inducing bag cell afterdischarge in *Aplysia*. In *Molluscan Neuro-Endocrinology* (ed. J. Lever & H. H. Boer), pp. 91–92. Amsterdam: North-Holland Publishing Company.

PARSONS, D. W., TER MAAT, A. & PINSKER, H. M. (1983). Selective recording and stimulation of individual identified neurons in freely-behaving *Aplysia*. *Science* **221**, 1203–1206.

PINSKER, H. M. & DUDEK, F. E. (1977). Bag cell control of egg laying in freely behaving *Aplysia*. *Science* **197**, 490–493.

PINSKER, H. M. & EBERLY, L. B. (1982). Whole nerve cuff electrodes in neuroethological studies. *J. electrophysiol. Tech.* **8**, 88–101.

PINSKER, H. M. & PARSONS, D. W. (1985). Temperature dependence of egg laying in *Aplysia brasiliana* and *A. californica*. *J. comp. Physiol.* B **156**, 21–27.

RAM, J. L. (1983). Neuropeptide activation of an identifiable buccal ganglion motoneuron in *Aplysia*. *Brain Res.* **288**, 177–186.

ROTHMAN, B. S., MAYERI, E., BROWN, R. O., YUAN, P.-M. & SHIVELY, J. E. (1983). Primary structure and neuronal effects of α-bag cell peptide, a second candidate neurotransmitter encoded by a single gene in bag cell neurons of *Aplysia*. *Proc. natn. Acad. Sci. U.S.A.* **80**, 5733–5737.

ROTHMAN, B. S., WEIR, G. & DUDEK, F. E. (1983). Egg-laying hormone: direct action on the ovotestis of *Aplysia*. *Gen. comp. Endocr.* **52**, 134–141.

SCHELLER, R. H. & AXEL, R. (1984). How genes control an innate behavior. *Scient. Am.* **250**, 54–62.

SCHELLER, R. H., JACKSON, J. F., MCALLISTER, L. B., ROTHMAN, B. S., MAYERI, E. & AXEL, R. (1983). A single gene encodes multiple neuropeptides mediating a stereotyped behavior. *Cell* **32**, 7–22.

SCHLESINGER, D. H., BABIRAK, S. P. & BLANKENSHIP, J. E. (1981). Primary structure of an egg laying peptide from the atrial gland of *Aplysia californica*. In *Symposium on Neurohypophyseal Peptide Hormones and Other Biological Active Peptides* (ed. D. H. Schlesinger), pp. 137–150. New York: Elsevier North-Holland, Inc.

SLATER, P. J. B. (1978). *Sex Hormones and Behaviour*. Baltimore: University Park Press, 55 pp.

STRUMWASSER, F. (1984). The structure of the commands for a neuropeptide-mediated behavior, egg-laying, in an opisthobranch mollusc. In *Biosynthesis, Metabolism and Mode of Action of Invertebrate Hormones* (ed. J. Hoffman & M. Porchet), pp. 36–43. Berlin: Springer-Verlag.

STRUMWASSER, F., SCHLECHTE, F. R. & BOWER, S. (1972). Distributed circadian oscillators in the nervous system of *Aplysia*. *Fedn Proc. Fedn Am. Socs exp. Biol.* **31**, 405.

STUART, D. K. & STRUMWASSER, F. (1980). Neuronal sites of action of a neurosecretory peptide, egg-laying hormone, in *Aplysia californica*. *J. Neurophysiol.* **43**, 499–519.

TER MAAT, A., COBBS, J. S. & PINSKER, H. M. (1983). Control of egg deposition in intact *Aplysia brasiliana*. In *Molluscan Neuro-Endocrinology* (ed. J. Lever & H. H. Boer), pp. 237–242. Amsterdam: North-Holland Publishing Company.

TER MAAT, A., LODDER, J. C. & WILBRINK, W. (1983). Induction of egg laying in the pond snail *Lymnaea stagnalis* by environmental stimulation of the release of ovulation hormone from the Caudo-Dorsal Cells. *Int. J. Invert. Reprod.* **6**, 239–247.

THOMPSON, T. E. & BEBBINGTON, A. (1969). Structure and function of the reproductive organs of three species of *Aplysia* (Gastropoda: Opisthobranchia). *Malacologia* **7**, 347–380.

VASICKA, A., KUMARESAN, P., HAN, G. S. & KUMARESAN, M. (1978). Plasma oxytocin in initiation of labor. *Am. J. Obstet. Gynec.* **130**, 263–273.