HABITUATION OF SWIMMING ACTIVITY IN THE MEDICINAL LEECH

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SUMMARY

Tactile stimulation (light stroking) of a body wall flap attached to the ventral nerve cord of the medicinal leech evokes episodes of swimming activity. This swimming response undergoes habituation, involving changes in swim initiation and swim maintenance.

1. Repeated stimulation of the body wall flap evoked swimming activity between three and 39 times before this response failed. During repetitive stimulation, the length of swim episodes decreased by about 50%. The number of swim episodes which could be elicited was not correlated with swim episode length.

2. Following habituation, swim initiation showed significant spontaneous recovery, but swim episode length returned only to 60% of control values. In preparations where spontaneous recovery was followed by rehabituation, the number of swim episodes elicited declined with each habituation-recovery sequence.

3. Additional stimulation immediately following habituation trials had a dual effect: recovery of the swimming response was delayed, but the lengths of swim episodes following spontaneous recovery were increased.

4. Pinching the body wall flap immediately restored the swimming response in an habituated preparation.

5. Swim initiation habituated more rapidly during stimulation of anterior body wall flaps than during stimulation of mid-body or posterior flaps. However, swim length was independent of this regional variation in swim responsiveness.

6. The number of swim episodes elicited by stimulation of body wall flaps attached to posterior or anterior segments depended upon whether this segment was stimulated before or after other flaps. In contrast, in mid-body segments there was no evidence for such stimulus generalization. The lengths of swim episodes elicited during sequential stimulation of several body wall flaps were independent of the stimulation sequence.

7. We propose that separate processes control swim initiation and swim maintenance. These processes must be repeated in most, if not all, of the segmental ganglia of the leech ventral nerve cord.

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INTRODUCTION

Mechanical stimulation of the leech body wall can evoke a variety of behavioural responses, including curling, bending, shortening and swimming (Gee, 1913). Each of these responses may occur alone or in combination with others as part of a complex behavioural sequence.

Research in leech neurobiology, beginning with the pioneering work of Nicholls & Baylor (1968), indicates that three classes of mechanosensory cells innervating the leech body wall, the T, P and N cells, can account for most of the responses to tactile body wall stimulation. For example, Nicholls & Purves (1970) demonstrated that each of these sensory cells is monosynaptically connected to the L cell, a motor neurone which subserves the shortening reflex in the leech. In addition, Kristan (1982) has shown that co-activation of T and P cells is necessary and sufficient to produce bending. Finally, intracellular stimulation of any one of these three sensory neurones in a dissected preparation can evoke swimming activity (Debski & Friesen, 1982).

Since activation of the same sensory cells can lead to different behaviours, what factors determine which response will be elicited by a stimulus? Gee (1913) suggested that the selection of one behavioural response over another depends upon both the intensity and the site of stimulation. The importance of both of these parameters has been confirmed recently by Kristan, McGirr & Simpson (1982). They found, for instance, that moderate to high intensity electrical stimulation of the anterior leech body wall usually evoked a curling response, while similar stimulation applied to the posterior body wall frequently caused swimming. A low-intensity stimulus at either site usually elicited local bending. Thus, the specific locus and intensity of stimulation correlate positively, albeit imperfectly, with the elicited behaviour.

Since the selection of one behaviour over another in response to tactile stimulation is not entirely a function of stimulus characteristics, we decided to investigate whether some of the variability could be due to plasticity in response selection. In this study we examined only one type of behaviour: swimming evoked by low-intensity tactile stimulation. The experiments were carried out on dissected leech preparations consisting of a body wall flap attached to an otherwise isolated ventral nerve cord. The stimulus was a light stroke applied repeatedly to this body wall flap. As reported earlier (Weeks & Kristan, 1978), such stimulation elicits swimming activity. We found, however, that the response to such repeated stimulation decreases and eventually ceases. The swimming response subsequently recovers spontaneously or can be restored quickly by administration of a strong stimulus. We conclude that the decrement in the behavioural response to the stimulus described here conforms to the operational definition of habituation, since this preparation exhibits at least six of the nine features that characterize habituating systems (Thompson & Spencer, 1966).

MATERIALS AND METHODS

Leeches, *Hirudo medicinalis*, were obtained from a commercial supplier and maintained at room temperature in aquaria filled with approximately 5 cm of an

artificial pond water solution. The leeches were fed every few months on frogs.

The leech is a segmented worm. Its central nervous system consists of a ventral nerve cord composed of 21 segmental ganglia linked by a single median connective and paired lateral connectives. Head and tail brains, consisting of fused ganglia, are located at each end of the nerve cord (Nicholls & Van Essen, 1974). The segmental ganglia are numbered sequentially from 1 to 21 beginning with the ganglion immediately posterior to the head brain (Kristan, Stent & Ort, 1974). Each of the segmental ganglia innervates a well-defined area of the body wall by way of two mixed nerves, the anterior and posterior roots (Nicholls & Baylor, 1968). The posterior root bifurcates near the ganglion to create the posterior-posterior (PP) and the dorsal posterior (DP) nerves (Ort, Kristan & Stent, 1974).

All of the experiments described here were performed on preparations consisting of ganglion 2 to ganglion 19. In most experiments, sensory input to the ventral nerve cord was limited to that derived from stimulation of a body wall flap attached to ganglion 11 *via* the DP nerve. This flap was always at least three segments long, was centred about segment 11, and extended from the dorsal to the ventral midlines. In one experimental series designed to test segmental variation of habituation, body wall flaps remained attached to five segmental ganglia: 3, 7, 11, 15 and 18. In any preparation, all body wall flaps were from the same side of the animal.

Stimulation procedure

Stimulation of the body wall was performed manually with a fine wire loop attached to a wooden handle. The stimulus consisted of one or more light strokes of the body wall flap with this loop, each stroke crossing several annuli. The duration of the stimulus was recorded by creating an electrical marker signal coincident with the stimulus duration. In all experiments, the body wall was stroked repeatedly, with each presentation of the stimulus constituting a 'trial'. The interval between trials was set at 30 s. For each trial, the body wall was stroked until either swimming activity was elicited or 10 s had elapsed. Stimulation was terminated after 10 s because preliminary experiments had shown that preparations which did not swim within this time rarely swam with additional stimulation. When a preparation failed to respond with swimming activity during *two* successive trials, the stimulation sequence was terminated and the response was considered habituated. Following habituation to this criterion, the preparation was either left undisturbed to allow for spontaneous recovery or was dishabituated. Dishabituation was accomplished by pinching the middle annulus, as well as the immediately adjacent annuli, of segment 11 with blunt forceps. Preparations were rehabituated using the same procedures described for the initial habituation. Additional procedural details are presented below in the Results section.

Swim episodes evoked by stroking the body wall were typically 10–15 s in duration; thus the 30-s interval between trials allowed for a 'rest' period of about 5–10 s between the end of a swim episode and the subsequent trial. In a small fraction of the trials (2·4 %), the swim episodes were of such long duration that the preparation had not stopped swimming by the time that it was to be stimulated again. In such instances,

the stimulus was delivered 5 s after swimming had stopped, and consequently, up to 10 s beyond the scheduled stimulation time.

Swimming, like most rhythmic types of behaviour, is a triggered movement that continues in the absence of the stimulus that initiated it. The effect of continued tactile stimulation on swimming activity has not been investigated in detail but preliminary experiments indicated that such stimulation interferes with concurrent activity (E. A. Debski, unpublished observations). This consideration dictated the choice of our stimulus paradigm since our aim in this study was to look at both swim initiation and swim length. Thus, our preparations were stroked only until they swam or had been stroked for 10 s. Strictly speaking, this means that the total amount of stimulation (intensity × duration) delivered on successive trials was not constant, as in most habituation studies. In general, greater stimulus durations were required to evoke swimming on successive trials. Therefore, the use of a paradigm with constant stimulus duration might have resulted in lower swim responsiveness values and more rapid habituation.

Nomenclature

The swimming movements of leeches result from the patterned activity of dorsal and ventral longitudinal muscles. We monitored swimming activity by recording from DP nerves along the ganglion chain. These nerves exhibit repetitive impulse bursts from cell 3, an excitatory motor neurone of the dorsal longitudinal muscles (Kristan *et al.* 1974). For simplicity, the terms 'swimming' and 'swimming activity' are used synonymously in this paper. A swim cycle is the interval between the beginning of successive cell 3 bursts. A swim episode consists of a series of these cycles. In this report, the length of a swim episode, or 'swim length', is defined as the number of cycles in that episode. Similarly, 'swim responsiveness' is defined as the number of swim episodes elicited by body-wall stroke before habituation to criterion.

Data normalization

For most results reported here, the data were combined from several preparations and normalized as follows. In each preparation, the number of cycles in the first swim episode elicited by stroking the body wall was taken as the 100% response level. Subsequent swim lengths were then expressed as a fraction of this initial level. Fig. 3 is a graph of swim length *versus* trial number obtained from 22 experiments. Because there was variability in both the number of swim episodes which could be elicited and the swim length, the abscissa as well as the ordinate are normalized in this graph. The normalization of the abscissa was accomplished by distributing the results (swim lengths) obtained from each experiment into 10 trial bins. The swim length obtained in the initial trial in each experiment (set equal to 100%) was put into trial bin 1. The swim length from the penultimate trial (equal to 0% because habituation to criterion requires *two* trials without a response) was put into trial bin 10. Swim lengths from intermediate trials were distributed into the remaining eight bins. For instance, swim length data from a preparation that swam three times were put into trial bins 1, 4, 7

and 10. The average swim length for each bin was then calculated and plotted as the midpoint of that bin.

RESULTS

Response decrement with repeated stimulation

At first, a light stroke to a body wall flap is an effective stimulus for eliciting swimming activity in a nerve cord-body wall preparation. However, with repeated stimulation, swim length declines and then, quite abruptly, stroking the body wall no longer evokes swimming. In the example shown in Fig. 1, tactile stimulation initially elicited a swim episode of 25 cycles. On subsequent trials, the number of swim cycles per episode decreased monotonically. [In general, greater stimulus durations were required in successive trials to evoke swimming, but surprisingly, this increased stimulation led to shorter swims (not shown)]. On the fifth and sixth trials, swimming could not be elicited.

Preparations differed both in swim responsiveness (range: 3–39 episodes) and in the swim length of initial episodes (range: 5–41 cycles), but nearly all (69 out of 77
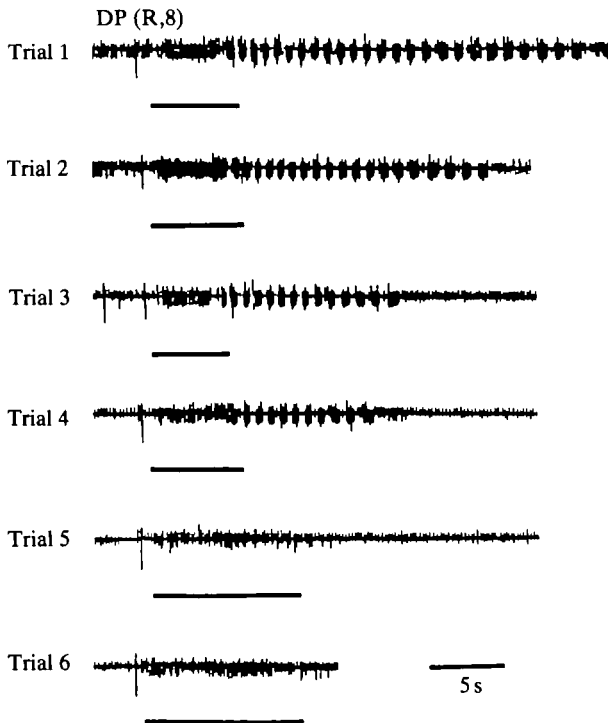

Fig. 1. Habituation of the swimming response. Dorsal posterior (DP) nerve activity from one preparation during sequential trials. The medium-sized spikes are cell 3 impulses. Swimming activity is indicated by bursts of cell 3 spikes in the DP record. Bars indicate the time during which the stimulus was applied. Swimming was elicited during the first four, but not the last two, trials. The stimulation procedure is described in Methods.

preparations) underwent a decline in swim length upon repeated stroking of the body wall. However, this decline was monotonic only occasionally (12 %). In 17 % of our preparations, we observed a 'warming up' phenomenon (Hinde, 1970). That is, the preparations responded on the first trial with a swim episode shorter than those elicited on the next several trials. Following this initial increase, swim length then decreased. In the remaining preparations, the decline in swim length with successive trials showed irregular fluctuations. Results from some experiments (Fig. 2A) suggested that a positive correlation might exist between the maximum swim lengths and swim responsiveness. However, analysis of data from all experiments demonstrates that there is no such correlation (Fig. 2B; $r = -0.007$).

To characterize the average decline in swim length, we normalized the data (see Methods) from a sample of 22 preparations. This decline showed three phases (Fig. 3). First, between trial bins 1 and 5 the decline was rapid. Second, between trial bins 5 and 9 there occurred little if any further decrease in swim length. Finally, the
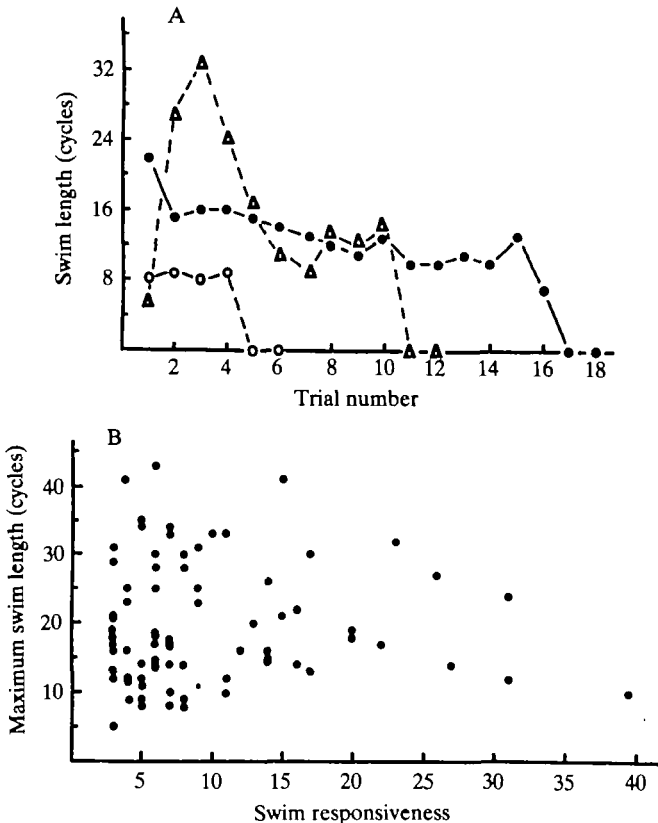

Fig. 2. (A) Swim lengths during habituation to criterion. Sample records from three preparations, each representing a distinct pattern. Most frequently observed was an irregular decrease from an initial maximal swim length (filled circles). Occasionally, preparations reached a maximum later than the first trial and declined from there (open triangles). A few preparations failed to show any decrease in swim length (open circles). (B) Plot of maximum swim length *versus* swim responsiveness for 77 preparations habituated to criterion.

swimming response ceased abruptly, even though the number of swim cycles per episode had declined only to about 50 % of the initial value. The average length of swim episodes immediately before cessation of the response to the stimulus (11·5 cycles; S.E.M. = 0·80) was significantly different from the average length of the initial swim (20·0 cycles; S.E.M. = 1·52; $t = 5·973$; df = 21; $P < 0·0005$).

Habituation is defined as the gradual decline of a behavioural response to a repeated, constant-amplitude stimulus (Kandel, 1976). The decrease and eventual termination of swimming activity elicited by stroking the body wall can be considered an example of habituation. We show below that the nerve cord-body wall preparation displays many of the characteristics associated with habituation in other animals.

Spontaneous recovery

Preparations which have been habituated to stroking of the body wall undergo spontaneous recovery. The time course of this recovery was examined by testing for swim initiation in individual preparations at a single interval following habituation. For different preparations, this interval, or recovery period, was varied from 1 min to 40 min. Recovery from habituation was calculated as the swim responsiveness subsequent to the recovery period expressed as a percentage of the swim responsiveness prior to the initial habituation. For these, as well as for the dishabituation and subzero
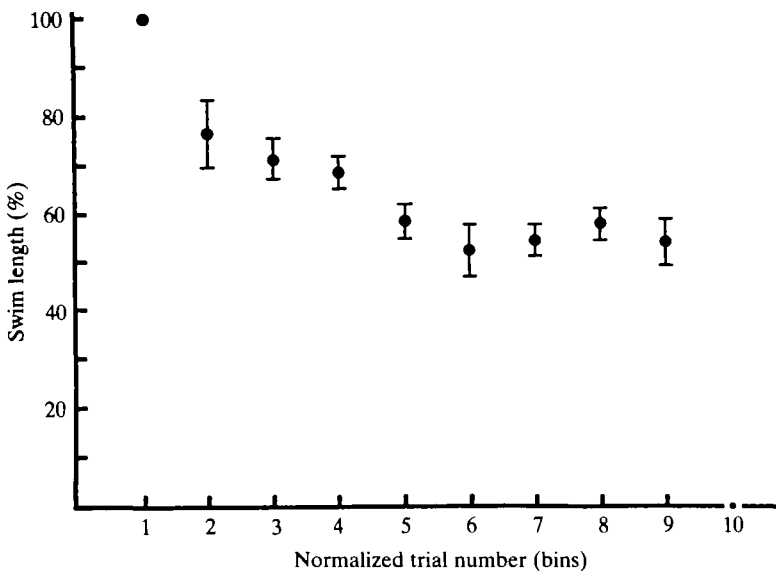

Fig. 3. Average decline in swim length with successive trials. Combined data from 22 preparations. The first swim episode elicited by stroking the body wall is the longest. Thereafter, the swim length declines with each additional trial to plateau at approximately 50 % of the initial value. Normalization of axes is explained in Methods. Bars represent the standard error of the mean. Sample sizes: Bin 1, 22; bin 2, 10; bin 3, 35; bin 4, 19; bin 5, 35; bin 6, 18; bin 7, 31; bin 8, 21; bin 9, 11; bin 10, 22.

experiments (see below), only those preparations whose initial response consisted of three or more swim episodes were used. In addition, preparations which failed to show any swim responsiveness after the recovery period were retested after an extended period. If stimulation then led to swim initiation, this was interpreted as meaning that these preparations initially failed to recover because of prolonged
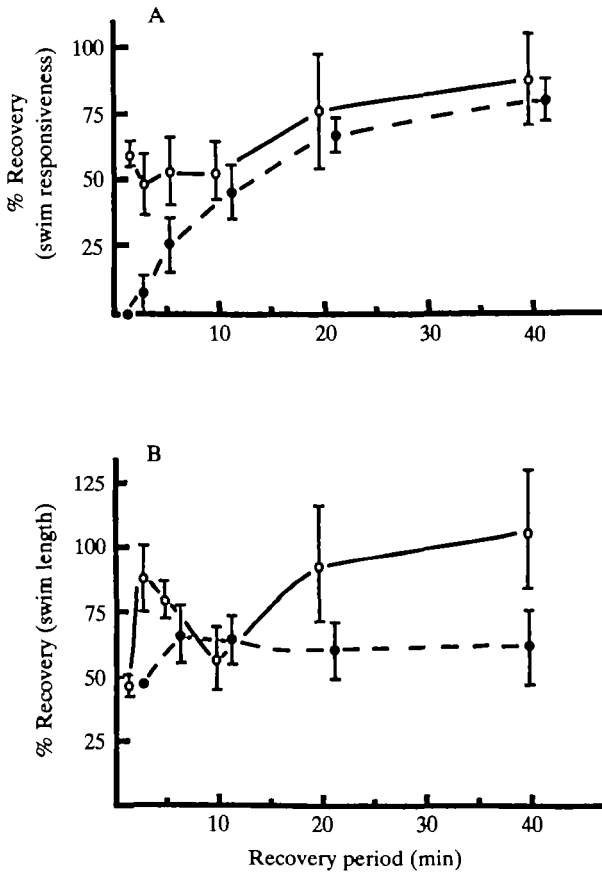

Fig. 4. Spontaneous recovery (filled circles) and dishabituation (open circles). (A) Spontaneous recovery and dishabituation of swim responsiveness. Spontaneous recovery data were obtained from preparations that were habituated and then restimulated after a recovery period. Dishabituation data were taken from preparations that were habituated, pinched, and then restimulated after a recovery period. The ordinate is the ratio of the number of swim episodes obtained after recovery to the number of swim episodes required for the initial habituation, multiplied by 100. Each point represents the mean of six preparations. (B) Spontaneous recovery, dishabituation and swim length. The swim lengths of the preparations included in A are presented as a function of recovery period. The ordinate is the ratio of the number of cycles in the longest swim episode obtained after recovery to the number of cycles in the longest swim episode observed before habituation, multiplied by 100. Only preparations which swam after the recovery period and from which accurate cycle counts could be obtained were included in the graphs. Sample sizes at the 1-, 2·5-, 5-, 10-, 20- and 40-min points for the spontaneous recovery curve were 0, 1, 4, 5, 5 and 5, respectively. For the dishabituation curve, sample sizes were 6, 5, 6, 6, 6 and 6 at these time points. Bars indicate the standard error of the mean. Data are offset slightly for clarity.

habituation, and therefore the 0% recovery achieved during the recovery period was included in the analysis. Only one of the 37 preparations tested for spontaneous recovery failed to initiate swimming in response to stroking during an extended recovery period. Data from this single preparation were discarded.

The average time course of spontaneous recovery from habituation is shown in Fig. 4A (filled circles), in which the swim responsiveness of previously habituated preparations (% recovery) is plotted against the interval allowed for recovery to occur (recovery period). Note that the initially rapid rate of recovery decreases over time. None of the six preparations tested at 1 min after habituation to criterion responded to stroking of the body wall. After recovery periods of 20 or 40 min, all preparations showed some responsiveness.

We also assessed the effect of spontaneous recovery on the length of swim episodes. Fig. 4B (filled circles) illustrates data from the same preparations included in Fig. 4A but compares swim lengths before habituation to those after spontaneous recovery. The ordinate in this graph represents recovery expressed as the ratio of the longest swim episode obtained at the intervals indicated on the abscissa to the longest prehabituation swim episode, multiplied by 100. Only preparations which were responsive during the recovery period were included in this analysis. None of the preparations recovered within 1 min, so there is no point represented on the graph for this time point, and the 2·5 min point was obtained from a single preparation. The other time points are the means of several preparations. The figure reveals that there was no spontaneous recovery in swim length for at least 40 min following habituation. Instead the maximum swim length after rehabituation remained constant at approximately 60% of the prehabituation value.

Dishabituation

Spontaneous recovery of the swimming response to stroking of the body wall is gradual. However, an habituated preparation can be dishabituated at once by pinching the body wall flap that was stroked. Pinching, a more intense stimulus than stroking, usually elicits a swimming episode immediately. Once this episode has ended, the stimulation provided by light stroking of the body wall will once again evoke swimming. The effect of dishabituation on swim responsiveness is depicted in Fig. 4A (open circles). The data were obtained from experiments in which the body wall was pinched 30s after habituation to criterion. The time course of dishabituation was tested by stroking the body wall at several time intervals following the pinch. The recovery period, as for spontaneous recovery, was the time between habituation to criterion and the onset of the test stimulus. The difference between the spontaneous recovery and the dishabituation curves is striking. Although there was no spontaneous recovery at the 1 min time point, dishabituation led to 57% recovery in swim responsiveness. Recovery was constant at approximately 52% for recovery periods up to 10 min. For recovery periods of 10 min or longer, the dishabituation values are nearly indistinguishable from the spontaneous recovery values. Thus the effectiveness of the dishabituating stimulus was observable only at periods of time less than 10 min.

Dishabituation was also examined in terms of swim length (Fig. 4B; open circles). Although pinching appeared to cause eventual recovery of swim length to near control levels, the later time points were not significantly different from those values obtained with spontaneous recovery.

Pinching could also act to enhance swim initiation in unhabituated preparations. Body wall stroking failed initially to elicit swimming in 23 % ($N = 53$) of the preparations tested. Pinching of the body wall in these preparations usually gave rise to a swim episode. Thirty seconds after the pinch, approximately half (52·4 %) initiated swimming in response to stroking of the body wall. Swim responsiveness ranged from 1–10 episodes with a mean of 4·1 episodes (S.E.M. $= 1·1$; $N = 10$). In the absence of this pinch, such preparations never swam in response to tactile stimulation ($N = 6$). Furthermore, after being habituated, five out of seven pinched preparations were successfully dishabituated by repinching the body wall. However, in three of these a stronger dishabituating stimulus was required, i.e. three consecutive pinches had to be delivered before stroking the body wall again initiated swimming. Spontaneous recovery also occurred in two preparations that were tested for this type of recovery. Thus, preliminary indications are that the characteristics of swim initiation in these preparations are identical to those of preparations responsive initially to stroking of the body wall.

Effect of repeated habituation-recovery sequences

After a preparation is habituated and recovers spontaneously, it can be rehabituated. This procedure can be repeated several times; however, swim responsiveness decreases with each repeated habituation-recovery sequence. Fig. 5A presents data from six preparations which were allowed a 10-min recovery period between habituation sequences. The results of the successive habituation-recovery sequences are expressed as a percentage of the prehabituation response. As shown in this figure, swim responsiveness decreased following each habituation-recovery sequence (stippled bars). However, the number of cycles in the longest swim episode, although less than the initial value, did not show a marked tendency to decrease further with succeeding habituation-recovery sequences (striped bars).

The same trends are evident in data obtained from habituation-dishabituation sequences. The data presented in Fig. 5B were obtained from six preparations pinched within 1 min following habituation. Swim responsiveness decreased rapidly with succeeding habituation-dishabituation sequences (stippled bars). In contrast, following initial habituation, the lengths of the swim episodes remained fairly constant at about 50 % (striped bars).

Subzero effect

In many habituating systems, continued stimulation of a preparation after the response has failed delays the spontaneous recovery of the response (Thompson & Spencer, 1966). This has been termed the 'subzero' effect (Hinde, 1970) and it occurs in our preparations as well. Eighteen preparations were habituated to criterion and

were then subjected to eight additional 10-s stimulus presentations at the normal 30-s interval. (Preparations never responded to additional strokings with swimming, indicating that two consecutive failures was in fact a good criterion for identifying habituation.) Recovery periods were measured from the completion of this stimulation sequence. Spontaneous recovery of swim responsiveness was measured at three different time points and compared to the recovery of preparations stroked to criterion only (Fig. 6A).
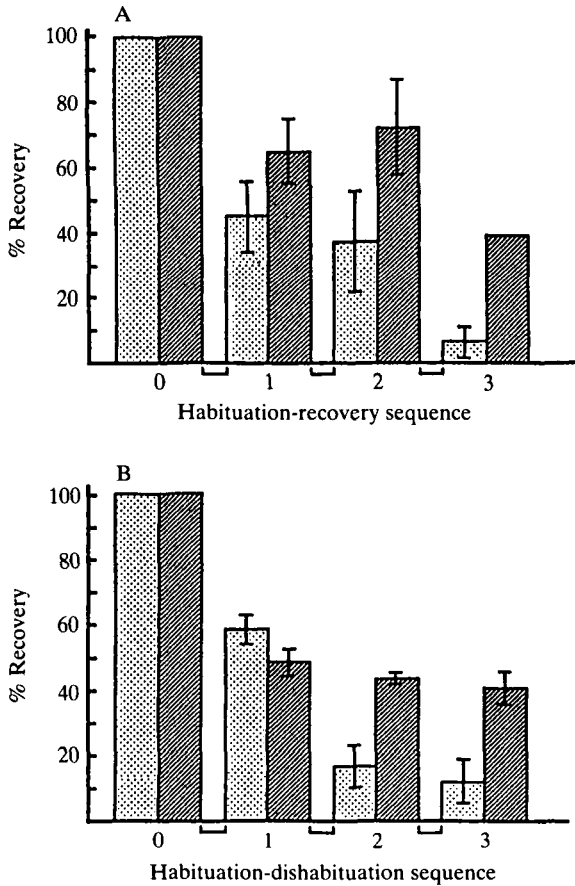

Fig. 5. (A) Repeated habituation-recovery sequences. Swim responsiveness and swim lengths were measured in six preparations following repeated sequences of habituation and spontaneous recovery. The recovery periods following each habituation sequence, shown by the scale bars, were 10 min. Habituation-recovery sequence 0 represents the response of preparations to the initial stimulation sequence. The number of preparations responsive for sequences 1, 2 and 3 was 5, 4 and 1, respectively. (B) Repeated habituation-dishabituation sequences. The recovery of six preparations was measured following repeated sequences of habituation and dishabituation. The recovery periods, shown by the scale bars, were 1 min. Habituation-dishabituation sequence 0 represents the response of preparations to the initial stimulation sequence. The number of preparations responsive for sequences 1, 2 and 3 was 6, 4 and 3, respectively. Stippled bars: number of swim episodes as a percentage of the initial number of swim episodes. Striped bars: number of swim cycles in the longest swim episode as a percentage of the number of swim cycles in the longest initial swim episode. Bars indicate the standard error of the mean.

The additional stimulation had a pronounced effect on swim responsiveness only for short recovery periods. After a 5-min recovery period, only one out of these six subzero preparations responded to stroking of the body wall, compared to four out of the six normal preparations. The responsiveness of the two groups is significantly different at this time point ($t = 1.91$; df = 10; $P < 0.05$). For recovery periods of 10 min and greater, the mean responsiveness for the two groups does not differ significantly.

Analysis of the data in terms of swim length shows that additional stroking, despite delaying recovery, increased the maximum number of cycles in swim episodes (Fig. 6B). After 20 min of recovery, when all preparations tested were responsive, the average longest swim episode of the subzero test group was 114·1 % of the longest episode obtained before habituation (s.e.m. = 13·6). The comparable figure from the group stroked only until habituation to criterion was 60·8 % (s.e.m. = 11·3). The two groups differed significantly at this time point ($t = 2.94$; df = 9; $P < 0.02$). At earlier time points, too few preparations recovered to establish a difference between the two groups ($N = 1$ for 5 min; $N = 3$ for 10 min). However, with a 20-min recovery,
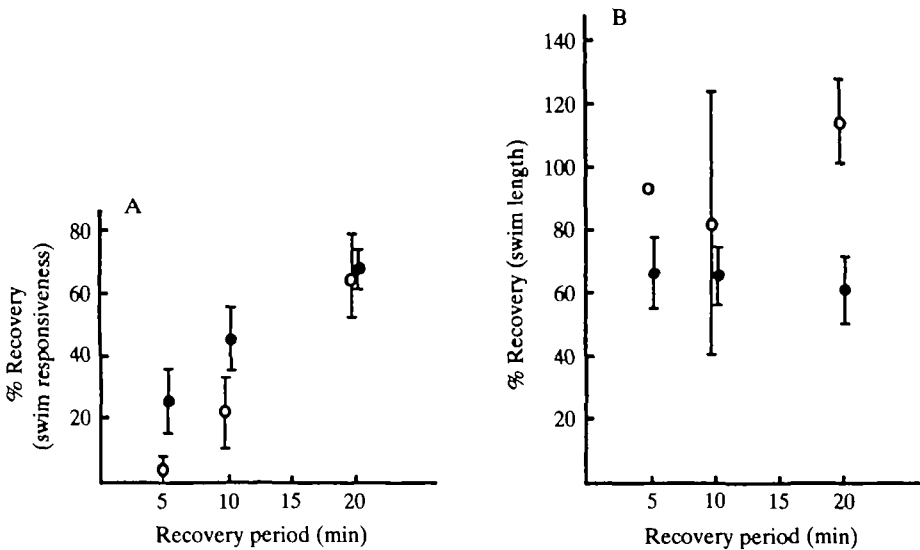

Fig. 6. Subzero effect. One group of preparations was stimulated to criterion (filled circles) and a second group was stimulated eight additional times (open circles). The rate of spontaneous recovery of the two groups was then measured. (A) Swim responsiveness. Recovery is defined as the number of swim episodes following a recovery period expressed as a percentage of the number of swim episodes before habituation to criterion. The two groups are significantly different at the 5-min time point ($t = 1.91$; df = 10; $P < 0.05$). Each point is the average from six experiments. (B) Swim length. Recovery is defined as the maximal swim length following a recovery period expressed as a percentage of the maximal swim length before habituation to criterion. The two groups are significantly different at the 20-min time point ($t = 2.94$; df = 9; $P < 0.02$). For the 'subzero' group, $N = 1$, 3 and 6 for the 5-, 10- and 20-min time points respectively. For the group habituated to criterion, $N = 4$, 5 and 5 for the 5-, 10- and 20-min time points respectively. Bars indicate the standard error of the mean.

additional stroking clearly increased the maximum length of subsequent swim episodes.

Response of different body segments

Kristan *et al.* (1982) showed that intact leeches swim readily in response to electrical stimulation of posterior or mid-body segments. Stimulation of the anterior region of the leech, however, usually did not lead to swimming. This result, and other behavioural observations, prompted them to postulate that tactile mechanosensory cells have regionally specified interneuronal targets. To examine regional specialization of leech swim initiation, we tested the responsiveness of anterior, mid-body and posterior body segments to body-wall stimulation.

Twenty-seven preparations, each having body wall flaps attached *via* a DP nerve to ganglia 3, 7, 11, 15 and 18, were used. The flaps were habituated in a random order. Stimulation of a flap began 30 s after habituation of the previous flap.

As shown in Fig. 7, swimming initiated by stimulation of segment 11 showed less rapid habituation than did stimulation of the other four segments tested. While habituation of segment 11 occurred after an average of 6·4 swim episodes, habituation of segment 3 occurred after only 1·7 episodes, making this segment the least responsive of those tested. The swim responsiveness of segments 7, 11 and 15 was significantly greater than that of segment 3; that of segments 11 and 15 was significantly greater than that of segment 18 (Table 1).
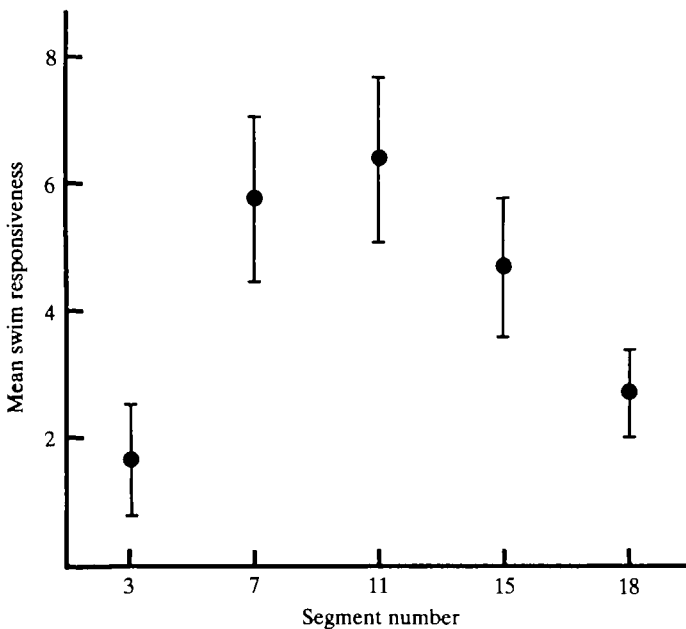
Fig. 7. Regional variation in the swim responsiveness of five body regions (segments 3, 7, 11, 15 and 18). Each body region was habituated to criterion by stroking the body wall. The data were obtained from 27 preparations in which the body wall flaps were habituated in random order.

For segment 3, we found that stroking of the body wall in 17 out of 27, or 63 %, of the preparations failed to elicit swimming. These failures were expected because similar results were obtained in experiments on intact animals (Kristan *et al.* 1982). However, the fact that 10 dissected leech preparations *did swim* in response to stimulation of segment 3 indicates that mechanosensory cells at least as far anterior as ganglion 3 have access to the swimming oscillator, although this access is less effective here than in more posterior ganglia.

Despite marked regional differences in responsiveness, we found, somewhat surprisingly, no demonstrable differences in the length of swim episodes evoked by stimulation of different segments. Table 2, which illustrates this point, was constructed as follows. The data from the experiment illustrated in Fig. 7 were used to obtain the subset of preparations that swam, for example, in response to stimulation of both segment 3 and segment 11. Within this subset, we compared the number of cycles in the longest swim episode elicited by stimulation of each of these two segments. We repeated this procedure for all subsets of preparations which swam in

Table 1. *Response of different body segments*

Segment number	Mean swim responsiveness	t Value† when compared to segment				
		3	7	11	15	18
3	1·7 ± 0·9*	—	2·56‡	3·33‡	2·32‡	1·67
7	5·8 ± 1·3	—	—	0·59	0·61	1·92
11	6·4 ± 1·3	—	—	—	0·40	2·38‡
15	4·7 ± 1·1	—	—	—	—	2·68‡
18	2·7 ± 0·7	—	—	—	—	—

* Standard error.
† Calculated for dependent samples.
‡ Significantly different, $P < 0.05$ (two-tailed distribution; df = 26).

Table 2. *Intersegmental comparison of swim length*

Segment pair $X_1 - X_2$	Mean maximal swim length X_1	Mean maximal swim length X_2	N	t-Value†
3–7	18·1 ± 2·1*	17·6 ± 3·1*	8	0·36
3–11	17·3 ± 1·8	16·8 ± 2·5	10	0·23
3–15	17·3 ± 1·8	19·4 ± 5·4	10	0·53
3–18	17·4 ± 2·0	17·0 ± 1·8	9	0·34
7–11	15·2 ± 1·4	14·8 ± 1·3	22	0·33
7–15	14·8 ± 1·6	17·4 ± 3·0	18	1·43
7–18	15·8 ± 1·6	14·6 ± 1·2	17	1·00
11–15	14·9 ± 1·3	16·8 ± 2·6	22	0·78
11–18	15·0 ± 1·3	14·2 ± 1·1	22	0·65
15–18	17·9 ± 3·1	14·6 ± 1·2	18	1·34

* Standard error.
† Calculated for dependent samples.

response to stimulation of segment 11 and to stimulation of the segment of interest. This approach ensured that the desired comparisons were not influenced by sample variation. From these data it is clear that stimulation of any segment resulted in a swim length as long as that given by segment 11. This analysis was then extended to cover all possible pair combinations. In no case was the swim length given by one segment significantly different from that given by another. Therefore swim length is independent of regional variation in swim responsiveness.

Stimulus generalization

Habituation of a series of body flaps affected swim initiation *via* stimulation only of the most anterior and posterior flaps. Table 3 provides a comparison between the swim responsiveness of a given flap stroked first in a stimulation sequence and its swim responsiveness when stroked last. The data for this table come from the experiments used in Fig. 7. Here, however, the data are divided into subsets based on the order of stimulation. Thus, when flap 11 was stroked before other flaps, 6·0 swimming episodes could be elicited ($N = 5$). If flap 11 was stroked after the other four flaps had been habituated, the average number of episodes elicited was 4·7 ($N = 6$). The two figures are not significantly different. Likewise, for flaps attached to ganglia 7 and 15, there is no difference in the results obtained in the two experimental conditions, first *versus* last. Thus, these segments are unaffected by the stimulation of the other ones. Flaps attached to ganglia 3 and 18, however, yielded different results. There was a significant decrease in the number of episodes elicited when each of these segments was stroked last as opposed to when it was stroked first (segment 3: $t = 2·213$; df $= 10$; $P < 0·05$; segment 18: $t = 3·038$; df $= 7$; $P < 0·01$). The difference between segments 3 and 18 and the other three segments tested may reflect a segmental specialization of the animal.

Table 3. *Stimulus generalization*

	Swim responsiveness (mean number of swim episodes)	
	Stimulated first	Stimulated last
Segment 3†	2·2 ± 1·0*‡ (N = 6)	0·0 ± 0·0 (N = 6)
Segment 7	4·3 ± 2·3 (N = 4)	7·3 ± 2·9 (N = 8)
Segment 11	6·0 ± 2·1‡ (N = 5)	4·7 ± 1·9 (N = 6)
Segment 15	5·5 ± 1·7‡ (N = 4)	5·0 ± 1·2 (N = 3)
Segment 18†	3·6 ± 0·9 (N = 5)	0·5 ± 0·3 (N = 4)

* Standard error.
† Significantly different, $P < 0·05$.
‡ One preparation in each of these groups was not included in this analysis because its swim responsiveness was five standard deviations or more above the average of the group.

Habituation of a series of body wall flaps does not affect the length of swimming evoked by stimulation of another flap. Since swim length is independent of the stimulus locus (Table 2), the length of swim episodes elicited by stimulation of any body wall flap first are comparable to those of any flap stimulated last. The average length of the longest swim episode evoked by all segments stimulated first was 15·5 cycles (S.E.M. = 1·9; N = 15). The average length of the longest episode given by all segments stimulated last in the same preparations was 14·9 (S.E.M. = 1·4). The two numbers are not significantly different (t = 0·545; df = 14). Therefore, the stimulation and habituation of four of these body wall flaps does not affect significantly the swim lengths obtained by stimulation of the remaining flap.

DISCUSSION

Habituation in the leech

Gee (1913) provided the first demonstration that habituation occurs in leeches. He found that when a shadow passed over *Dina microstoma*, or when the table upon which they were placed was jarred, the animals momentarily interrupted their ventilatory movements. Upon repetitive presentations of either type of stimulation, the leeches eventually failed to respond, that is, their ventilatory rhythm continued despite presentation of the stimulus. When shadows were used as the stimulus, habituation usually occurred within a few trials; with vibratory stimulation, 9–11 trials were required. Kaiser (1954) later showed that the rate of habituation of the shadow reflex is inversely proportional to the intensity of the light used. This report, however, is the first systematic investigation of an habituating response in the leech.

Nine characteristics have been ascribed to habituating behaviour (Thompson & Spencer, 1966). We have found that six of these characteristics are found in the swimming response elicited by repetitive tactile body wall stimulation: reduction in response amplitude, spontaneous recovery, the subzero stimulation effect, increased rate of habituation with repeated habituation-recovery sequences, dishabituation and habituation of dishabituation. No tests were performed to ascertain if two other features are characteristic of this response, namely, more rapid habituation with a weaker stimulus and more rapid habituation with more frequent stimulation.

The last feature noted by Thompson & Spencer is that habituation can generalize to affect other parts of the body. We specifically tested for this in our preparation, but found it only in segments 3 and 18. The number of swim episodes elicited from segments 7, 11 and 15 was not dependent upon whether these flaps were stimulated before or after other ones. In addition, the average swim length of the longest episode obtained by stimulation of any flap, including segments 3 and 18, did not decrease after habituation of another flap. Consequently, except in the anterior and posterior of the leech, for the segments examined in these experiments, habituation of the swim response to body wall stimulation occurs independently in each segment. The absence of generalization has been documented in many other habituating systems, including *Aplysia* (Kandel, 1976).

Preliminary experiments with de-brained but otherwise intact leeches indicate that they too habituate to a stroking stimulus that initially elicits swimming (A. Chandler & W. O. Friesen, unpublished observations). This nearly intact preparation exhibits many of the properties described in this paper, including an abrupt cessation of the swim response when the swim length has decreased to 50 % of its initial value and dishabituation by body wall pinch. Thus, habituation appears to be a process present in the intact animal.

Swimming in the leech

The patterned contraction and relaxation of dorsal and ventral longitudinal muscles produce the swimming movements of the medicinal leech. This pattern of activity is imposed upon the muscles by motor neurones, which are themselves driven by a group of interneurones located within the central nervous system (Friesen, Poon & Stent, 1978; Poon, Friesen & Stent, 1978). These interneurones are part of a central pattern generator (CPG) which generates the neural equivalent of swimming in the isolated cord.

Besides the actual generation of the rhythm, the act of swimming includes three controlling processes: (1) initiation, (2) maintenance and (3) termination. Since swim responsiveness is dependent upon the swim initiation system and swim length is determined by the state of the swim maintenance system, our experiments provide some insight into these two processes. We have shown that each of the segments tested has access to the swim oscillator *via* the swim initiation and swim maintenance pathways. However, the observation that stimulation of some segments elicits fewer swim episodes than others means that there are segmental differences in swim initiation circuitry. Such segmental specialization appears to be absent from the swim maintenance process, since the length of swim episodes is independent of the stimulus locus. Thus swim initiation and swim maintenance are at least partly independent. A second argument for some separation of the swim initiation and swim maintenance pathways is that swim responsiveness is not correlated with swim length (Fig. 2B). Since the maintenance of swimming activity is not predicted by the resistance of the initiation circuit to failure it is likely that two separate points of control exist.
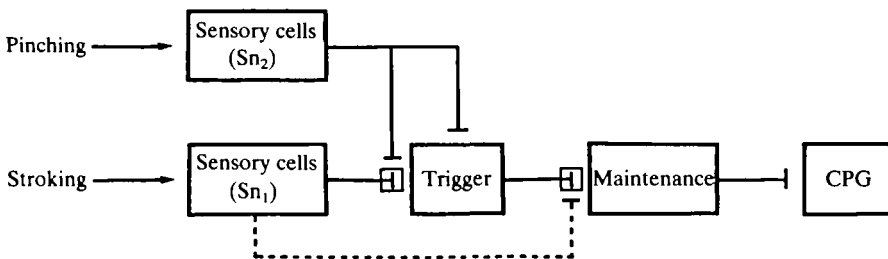

Fig. 8. Summary diagram of the interactions underlying swim initiation and swim maintenance in the leech. Excitation to the trigger initiates swimming; excitation level to the maintenance circuit determines the length of that swim. 'T' connection, excitatory connection. Squares indicate labile connections. The dashed line indicates that the effect is evident only when Sn_1 stimulation does not lead to swimming. CPG, central pattern generator.

As a working model, we view the processes controlling leech swimming as composed of two distinct components: a switch which acts to trigger swim initiation and an excitatory network which maintains swimming activity. This idea is represented diagrammatically in Fig. 8. The elements in the diagram are sensory cells activated by stroking (Sn_1) and by pinching (Sn_2) the body wall, a trigger unit, a swim maintenance circuit and the CPG for swimming. When Sn_1 is activated *via* body wall stimulation, it excites the trigger to above threshold. For simplicity, we assume that the trigger produces a constant output in response to suprathreshold stimulation. The output generated from the trigger activates the swim maintenance circuit which in turn drives the swim oscillator. No specific swim terminating circuit is included in this diagram; instead, swim episodes end because of an assumed decay in the excitation provided by the maintenance system. Variations in the duration of a swim episode are determined solely by the strength of the connection between the trigger and the maintenance system.

Our results have been included in the diagram as follows. Since swimming eventually cannot be elicited by stroking the body wall (Fig. 3), there must be a labile connection in the circuit controlling swim initiation. In the diagram, this point occurs between Sn_1 and the trigger. During repetitive stroking of the body wall, the effectiveness of this connection decreases until activity in Sn_1 can no longer activate the trigger, and therefore swimming can no longer be elicited by this route. Likewise, the number of cycles in a swim episode declines with repeated stimulation (also Fig. 3), indicating that a connection in the circuit controlling swim maintenance must be decreasing in strength. Since we assume that the duration of excitation from the maintenance circuit depends on the strength of input from the trigger, the connection between the trigger and the maintenance circuit must be labile. This ensures that the state of this labile connection is determined by the number of times the circuit is activated rather than by the number of cycles the maintenance circuit generates.

The rates of depression in the two labile connections are not identical. Specifically, the connection in the initiation part of the circuit must become ineffective more rapidly than that in the maintenance one. This difference in depression rates accounts for our finding that swim initiation declines to zero before swim length decreases by more than 50 %. Spontaneous recovery in these connections also is not identical, for the Sn_1-to-trigger connection recovers to approximately 50 % of its prehabituated value within 10 min, while the trigger-to-maintenance system connection exhibits no spontaneous recovery even after 40 min.

The Sn_2 cells, which are activated by pinching, can also initiate swimming by virtue of their connection to the trigger. Additionally, they must have the capacity to facilitate transmission between Sn_1 and the trigger to account for dishabituation. The mechanism by which the efficacy of this labile connection is restored as the result of Sn_2 cell activation is unknown. Since the neuronal system generating leech swimming is sensitive to serotonin levels (Willard, 1981), one possibility is that serotonin serves as a synaptic modulator at this point. Additional depression of the Sn_1-to-trigger connection caused by activation of Sn_1 after habituation can account for the delayed recovery of swim responsiveness in the subzero effect. To explain the greater swim

lengths observed following recovery from subzero stimulation, it is necessary to postulate that the Sn_1 cells act as well to enhance the efficacy of the trigger-to-swim maintenance connection. This enhancement is evident only when the Sn_1 cells can no longer initiate swimming.

Finally, control of swimming *via* light tactile stimulation appears to be organized within segments, since habituation in one segment has no effect on swim initiation (with the exception of segments 3 and 18) or maintenance in other segments. Thus, the Sn_1 and Sn_2 cells, the trigger and the labile connections that underlie habituation represent segmental entities that occur all along the nerve cord.

As yet, the circuits we identify by function have not been linked conclusively with identified neurones of the segmental nervous system. However, among the leech cells whose properties have been well characterized, there do exist candidates for some of these circuit elements. It is likely, for example, that Sn_1 is a T cell and that Sn_2 is an N cell. These sensory cells can produce swimming activity when stimulated intracellularly. Additionally their swim-evoking ability fatigues rapidly (E. A. Debski & W. O. Friesen, in preparation). Weeks & Kristan (1978) have proposed that cell 204, a segmental interneurone whose depolarization initiates swimming activity in isolated nerve cords, plays a major role in swim initiation and swim maintenance. Its role in the system described in this paper is currently under investigation.

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