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IMPACT OF MOVEMENT AND MOVEMENT-RELATED FEEDBACK ON THE LAMPREY CENTRAL PATTERN GENERATOR FOR LOCOMOTION

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Summary

A semi-reduced, minimally restrained lamprey preparation was used to investigate the impact of movement and movement-related feedback during D-glutamate-induced locomotion. The preparation consisted of the trunk alone with the spinal cord exposed to the bathing solution. Two conditions were compared using electromyography or nerve recording: (i) muscle and spinal cord, (ii) spinal cord alone supported by the notochord. Compared with the isolated spinal cord, movement in the presence of muscle consistently and significantly increased the frequency of the motor output and reduced the phase delay among the segments. In moving preparations, coupling among the segments was reduced by two staggered hemisections to permit the strength and direction of intersegmental coupling to be estimated. The estimates revealed that movement increased the total intersegmental coupling strength and increased the proportion of the coupling that was descending over those of the isolated spinal cord.

The effects on the phase and frequency of bursting can be explained in the light of the excitation evoked by bending that we have reported previously. Thus, we demonstrate that movement and movement-related feedback that arise from spinally induced motor patterns can alter the form of the movement and the functional coupling strength among the segments of the lamprey spinal cord.

Key words: movement, spinal cord, oscillator, sensory feedback, intersegmental coordination, lamprey, *Ichthyomyzon unicuspis*, *Petromyzon marinus*.

Introduction

Vertebrate locomotion is a complex motor task that typically requires the integration of neural signals from a spinal pattern generator combined with a variety of sensory feedback signals and descending control signals. The motor pattern must also be matched to the mechanical properties of the body. The multiple sensory and neural factors involved in locomotion have been studied and modeled more extensively in invertebrates than in vertebrates. This is especially the case with legged insects such as the walking stick insect (Cruse et al., 1995) and cockroaches (Delcomyn et al., 1996). Invertebrate systems are far more favorable than vertebrate preparations for this purpose, because the diverse factors can be isolated, identified and manipulated with relative ease. In vertebrates, even though the same level of analysis is considerably more difficult, the interactions between sensory inputs and the locomotor central pattern generator (CPG) in limbed vertebrates have been extensively examined. Especially notable examples come from studies from the laboratories of Jordan (Kriellaars et al., 1994), Prochazka and Pearson (e.g. Hiebert et al., 1996) and Burke (Degtyarenko et al., 1996).

Since the convincing demonstrations of central pattern generation in invertebrates (Wilson, 1961) and in vertebrates (Grillner and Zangger, 1979), it has been known that sensory feedback contributes to the centrally generated motor patterns. Sensory feedback was long ago shown to regulate movement on a cycle-by-cycle basis (Grillner and Wallén, 1977; Grillner and Rossignol, 1978; Andersson and Grillner, 1983; Duysens and Pearson, 1976). This type of regulation can be shown to signal when to terminate a step cycle or can adjust the speed of locomotion to the conditions of the terrain (Andersson et al., 1983) *via*, for example, hip joint angle changes or muscle stretch (Hiebert et al., 1996; Kriellaars et al., 1994). Feedback can also entrain the bursting of the lamprey spinal cord (McClellan and Sigvardt, 1988) on a cycle-by-cycle basis. It has been suggested that this entrainment effect can also improve the performance of swimming in intact lampreys (Di Prisco et al., 1990) and after spinal injury (McClellan, 1990). Sensory feedback acting through the CPG is also known to correct for irregularities and perturbations that arise in the substratum (Forssberg et al., 1977), producing phase-dependent responses to obstacles.

Because the lamprey has achieved some prominence as a model for vertebrate locomotion, it is important that we understand how sensory feedback interacts with the CPG in

this system. It has been known for some time that the lamprey locomotor CPG can be entrained simply by bending the isolated spinal cord by virtue of activation of intrinsic mechanoreceptors called edge cells (Grillner et al., 1984; McClellan and Sigvardt, 1988). We have recently reported that bending the spinal cord during fictive swimming has an additional excitatory effect that is more sustained than originally thought (Kiemel and Cohen, 2001) and can outlast the stimulus by one or more cycles. Such prolonged effects of a defined sensory input have been found in both invertebrates (Pearson, 1981; Katz and Harris-Warrick, 1990; Buschges et al., 1992) and vertebrates (Pearson, 1995; Hiebert and Pearson, 1999). In the turtle *Pseudemys scripta elegans*, a non-specific stimulus can elicit a long-lasting excitability change (Currie and Stein, 1990). A similar change in general excitability is seen in cats after deafferentation (Grillner and Zangger, 1979). However, the role such long-term effects may play during normal movement is not well understood.

In classic work on locusts *Schistocerca gregaria*, Don Wilson (Wilson, 1967) performed a series of experiments to study the interaction between the flight CPG, sensory feedback and movement. He did this by successively removing layers of the animal during flight until he was left only with the isolated nerve cord. However, vertebrate preparations have not afforded such a study. In invertebrates, one can successively remove the components of the animal to demonstrate the relative contribution of the CPG, the various sensory inputs and the movement itself. We present here a first study akin to those of Don Wilson's original experiments, but performed in a vertebrate, the lamprey.

The lamprey is unique among vertebrates for this purpose. The trunk of the animal can be kept functional for some time with muscle still attached to the spinal cord and notochord, while the spinal cord is induced to swim for long periods. The muscle can then be removed and swimming movements can still be elicited while recording now from the spinal cord motor outputs. These two states can thought of much like the isolated spinal cord preparation only with the addition of movement and movement-related sensory feedback. Thus, we have the potential to compare the spinal cord with and without the muscle. For example, using the self-actuated movements, we can examine the role of entrainment when performed by the spinal cord itself. Through the use of acute spinal lesions, we can also compare the spinal cord with and without reduced intersegmental coupling. The strong coupling in the intact spinal cord generally obscures any changes in total coupling strength seen with successive stages of dissection, making the individual components of the ascending and descending coupling undifferentiatable. However, if the coupling strength is reduced, then the estimation methods developed for the lamprey (Kiemel and Cohen, 1998) are reliable and do allow comparison of the total coupling strength present and a relative estimation of the contribution of ascending *versus* descending coupling. The results also demonstrate that the movement itself together with movement-related feedback contribute their own influences to the final motor output during locomotion and that the effects can be attributed to a slowly decaying excitation shown to be evoked by bending movements of the lamprey spinal cord (Kiemel and Cohen, 2001). These changes are described, and the implications of the changes in pattern are discussed and compared with previously published results.

Materials and methods

Trunk and isolated spinal cord/notochord preparation

Young adult lampreys, *Ichthyomyzon unicuspis* and *Petromyzon marinus* (13–15 cm; *N*=18; 17 *I. unicuspis* and one *P. marinus*), were obtained from fishermen along the Mississippi River, USA. The preparation shown in Fig. 1 is a *Petromyzon marinus*. All others data were collected from *Ichthymyzon unicuspis*. The animals were rapidly decapitated and eviscerated. Fifty-five segments beginning just caudal to the gills (segments 12–67 of the total of approximately 100) were further dissected as follows.

For the muscle preparation, the muscle and skin immediately dorsal to the spinal cord were removed together with the tough connective tissue immediately underlying it. This exposed the spinal cord while leaving the dorsal roots largely intact. The dorsal roots remain undamaged because the loose meninges immediately below this connective tissue are left intact in this process, and they protect the majority of the underlying dorsal roots. The skin was peeled from the preparation, leaving the bulk of the muscle afferents intact. This would also leave intact any remaining skin afferents whose receptors were not embedded in the skin proper, although none is known to exist. This peeling procedure removes much of the skin, with only fine connective tissue shreds left behind. It is not known whether they contain afferents. Electromyographic (EMG) electrodes made of Teflon-coated silver wire (diameter 0.12 mm) were threaded through 30 gauge hypodermic needles; they were then inserted into the muscle at or near segments 15 and 30 from the rostral end of the dissected preparation, with both electrodes on the same side.

For the isolated spinal cord, the muscle was completely removed leaving the spinal cord supported by the notochord. Muscle nerve activity was recorded from the same segments as used for EMG recording. Bipolar suction electrodes were placed over the motor nerves of the segments as they course snugly along the outside of the notochord. The nerves are exposed when the muscle is dissected away and are largely undamaged. Moreover, the signals recorded from the nerves at this position are considerably larger than those from the ventral roots inside the spinal canal (A. Cohen, unpublished observation). This recording arrangement is possible because the lamprey does not have a mixed sensory and motor nerve. Under all conditions, the preparation was held in physiological saline (Cohen et al., 1996) and maintained between 12 and 14 °C. In eight *I. unicuspis* preparations, we successfully recorded swimming motor activity under both conditions. Only these eight preparations are discussed further.

For the whole-animal recordings, EMG electrodes were inserted under MS222 anesthesia, as described above. The

animals were allowed to recover from the anaesthetic and tested when fully alert. They were tethered by their electrodes and not allowed to progress. Filming of a reduced trunk preparation (see Fig. 1) was made in the same way, but after the head and tail had been removed and the preparation pinned as for other testing. No electrodes were implanted in the specimen shown in Fig. 1.

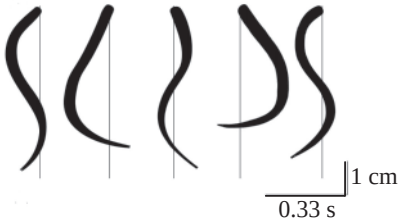

Fig. 1. Movement of a typical muscle preparation. The body is prepared by removing the head, viscera and a small portion of the dorsal musculature, and the skin is peeled away. EMG electrodes are inserted into muscles separated by approximately 15 segments. A small pin inserted through the notochord stabilizes lateral movement, but allows full rotation. Images were recorded on film at 15 frames s^{-1}, with every fifth frame illustrated. To construct the figure, outlines of the preparation in a selected single frame were used to form the shapes. One cycle is illustrated. Lines of equal length are drawn vertically from the pinned end for each image with the lines originating from the pin position.

Hemisections

In nine additional animals, dissected as above, two hemisections were made on opposite sides of the spinal cord at the first stage of dissection. The lesions were made under visual control at segments 22 and 27 from the rostral end of the dissected preparation. It should be noted that the spinal cord is thin (approximately 250 μm in diameter), only approximately 1 mm wide, and that the central canal is visible, so all surgical procedures can easily be made under visual control. To make the lesions, a small longitudinal incision was made along the central canal with a microscalpel tip, and one hemicord was cut with iridectomy scissors inserted from the small central incision to the left or right lateral edge of the spinal cord. An additional hemisection was made five segments caudally on the other side of the spinal cord in the same fashion. Since fibers are known to cross the spinal cord only once relatively close to their soma (Rovainen, 1985; see, for example, Buchanan, 1982; L. Guan, unpublished observations), this pair of lesions has the effect of disrupting the majority of long-range fibers ascending and descending between the two electrodes as well as some short-range axons. Thus, these lesions significantly reduce the total coupling strength (Gormley and Williams, 1999), but mainly interrupt the direct long-range coupling. Long-range coupling can continue to act indirectly, but primarily through the action of the short-range fibers.

The recording electrodes were inserted into the muscle at segments 15 and 30, as in unlesioned preparations. The side of the electrodes relative to the lesion did not affect the measurements and is not noted further. With the long-range coupling reduced, the coupling strength is reduced below the very high values obtained for control spinal cords (Kiemel and Cohen, 1998), thereby allowing reliable estimates of the direction of the remaining coupling (see Fig. 2D). Of the nine animals, the data from two were unstable for some portion of the testing. Thus, we include data from only seven animals.

Induction of swimming

Under both conditions, the preparation was induced to burst by bath application of 0.25–0.5 mmol l^{-1} D-glutamate; the concentration that produced the most stable swimming for a given preparation was continued throughout the entire experiment. Swimming was induced in a round 14 cm diameter dish filled to cover the body and exposed spinal cord. Given the size of the animals, the dish easily allowed full movement of the trunk, which measured approximately 5–7 cm (see Fig. 1 for an illustration of a muscle preparation moving). When testing the effect of the muscle, the body was permitted to move unobstructed, except for a single small pin inserted through the ventral edge of the notochord at either the rostral or caudal end of the body. The pin maintained the body upright while still allowing it to swivel easily with only minimal drag on the bottom of the dish. Fig. 1 illustrates the movement of the preparation when pinned. In some cases, the body was left free to move with no pin. In these cases, the movement was often asymmetric because the body leaned either to the left or right side and pushed against the bottom of the dish. However, motor output could be measured for coupling strength estimates.

Data analysis

At least 500 cycles of motor activity from the rostral and caudal recording locations during each bout were digitized at 2.5–5.0 kHz using Spike Studio. Spikes and bursts were detected as in Mellen et al. (Mellen et al., 1995). For each burst, its time of occurrence (burst time) was calculated as the average of the times of all spikes contained in the burst.

The rostral and caudal sequences of burst times were analyzed by fitting parameters in a stochastic model of two coupled phase oscillators using the method previously described in detail by Kiemel and Cohen (Kiemel and Cohen, 1998). The method exploits the small cycle-to-cycle fluctuations in periods and intersegmental delays to determine the impact of one oscillator on the other. To summarize briefly, the model is:

$$\frac{d\Theta_1}{dt} = \omega_1(t) + H_1(\Theta_2 - \Theta_1) + \sigma_1\xi_1(t) + \frac{dZ_1(t)}{dt}, \quad (1)$$

$$\frac{d\Theta_2}{dt} = \omega_2(t) + H_2(\Theta_1 - \Theta_2) + \sigma_2\xi_2(t) + \frac{dZ_2(t)}{dt}, \quad (2)$$

where

$$H_j(x) = \frac{\alpha_j}{2\pi}\sin[2\pi(x - \psi_j)]. \quad (3)$$

The stochastic variables Θ_1 and Θ_2 are the absolute phases (mod 1) of the rostral and caudal oscillators, respectively, t is

time, $\omega_1(t)$ and $\omega_2(t)$ are quadratic polynomials describing slowly varying uncoupled frequencies, α_1 and α_2 are coupling strengths, ψ_1 and ψ_2 are preferred phases, σ_1 and σ_2 are noise levels, $\xi_1(t)$ and $\xi_2(t)$ are independent white-noise processes (Arnold, 1974) and $Z_1(t)$ and $Z_2(t)$ are independent compound Poisson processes (Sørensen, 1991) representing (typically rare) discontinuous jumps in absolute phase. H_1 and H_2 are coupling functions (we use the variable x as the argument when defining these functions). The model is nonlinear because of the nonlinearity of the coupling functions H_j.

Model parameters were estimated using the method of maximum likelihood (Harvey, 1990). The likelihood function was computed under the assumption that, at every experimentally measured burst time, the absolute value of the corresponding oscillator is at zero (mod 1) plus some random measurement error. Thus, all model parameters were estimated indirectly on the basis of the burst times.

After fitting model parameters to the data, the slowly varying uncoupled frequencies $\omega_1(t)$ and $\omega_2(t)$ were fixed at the frequency at the mid-point of the bout, $\omega_1(T/2)$ and $\omega_2(T/2)$, where T is the duration of the data sequence. Additional measures describing the data were derived from the model parameters: the average coupled frequencies $\overline{\omega_1}=E[\omega_1(T/2)+H_1(\Theta_2-\Theta_1)]$ and $\overline{\omega_2}=E[\omega_2(T/2)+H_2(\Theta_1-\Theta_2)]$ (where E stands for 'expected value'); the average relative phase $\overline{\phi}=E(\Theta_1-\Theta_2)$, computed using circular statistics; the average absolute ascending and descending coupling strengths $\overline{\alpha_1}=E[|H_1'(\Theta_2-\Theta_1)|]$ and $\overline{\alpha_2}=E[|H_2'(\Theta_1-\Theta_2)|]$ (primes denote differentiation); and the average absolute coupling strength $\overline{\alpha}=E[|H_1'(\Theta_2-\Theta_1)+H_2'(\Theta_1-\Theta_2)|]$. Coupling strength $\overline{\alpha}$ was transformed to lie between 0 and 1 by defining the coupling strength parameter $\beta=1-\exp(-\overline{\alpha}/\overline{\omega})$, where $\overline{\omega}=(\overline{\omega_1}+\overline{\omega_2})/2$. For phase-locked bursting, β is roughly the fraction of a relative phase perturbation that is, on average, corrected after one cycle period. Zero coupling strength corresponds to $\beta=0$, and infinite coupling strength corresponds to $\beta=1$. The relative strength of ascending and descending coupling was described using the ascending strength fraction $\gamma=\overline{\alpha_1}/(\overline{\alpha_1}+\overline{\alpha_2})$.

Approximate 95 % confidence intervals were computed using the Hessian matrix (Harvey, 1990). In cases in which the estimated coupling strength was infinite ($\beta=1$), it was not possible to compute confidence intervals. The confidence intervals for some parameters were large. For example, parameters describing the uncoupled frequencies were large, because uncoupled frequencies cannot be accurately estimated when coupling is present. However, the measures we present here, such as coupled frequencies, typically have small to medium confidence intervals.

Statistical analyses were performed on five measures: the average of the rostral and caudal frequencies $\overline{\omega}$; the rostral/caudal frequency ratio $\overline{\omega_1}/\overline{\omega_2}$; the phase lag per segment $\overline{\phi}/n$, where n is the distance in segments between the rostral and caudal outputs; the coupling strength parameter β; and the ascending strength fraction γ. Comparisons of means were made using t-tests, with values of $P<0.05$ deemed significant and values of $P<0.10$ deemed marginally significant. These statistical tests do not rely on the confidence intervals of the individual measurements.

Results

Impact of movement and movement-related feedback: spinal cord intact

Overall comparison across conditions

With the rostral end pinned (Fig. 1), the movement of this preparation is very similar to that seen in intact swimming animals, as shown by Williams and her colleagues (Williams et al., 1995; Bowtell and Williams, 1994). Note, for example, that the amplitude of the movement at the caudal end is larger than that at the rostral end and that a smooth traveling wave traverses the body. It is unclear why almost a full wavelength is seen despite the fact that only half the body is left and despite the fact that the phase delays are shortened in the muscle condition to less than 1 % of the cycle period. Presumably, this full wavelength is the result of mechanical factors such as the absence of skin pulling against the contractions, but this remains to be determined.

The movements of the preparations with intact spinal cords were highly stereotyped under the two sets of conditions. Five different measures describing the motor pattern are plotted in each of the two conditions: (i) trunk with muscle; (ii) isolated spinal cord and notochord (the spinal cord condition). All five measures were obtained by fitting a model of two coupled oscillators to the data (see Materials and methods). Similar values of the first three measures, those involving frequency and relative phase, can be obtained using conventional correlational methods. The last two measures (β and γ) involve coupling strength and their estimation requires some cycle-to-cycle variability in the bursting pattern. However, the estimation method is able to distinguish between changes in coupling strength and changes in variability (Kiemel and Cohen, 1998).

Changes in frequency and phase

There was clear variation in the absolute frequencies of the different preparations. However, despite this variation, the most striking result was that in all eight animals the frequency in the muscle condition was greater than the frequency of the isolated spinal cord (Fig. 2A). The mean frequency of the isolated spinal cords (0.84±0.12 Hz, mean ± S.E.M.; Fig. 3A) was significantly less than the mean frequency in the muscle condition (1.54±0.24 Hz) (t-test, $P<0.01$).

The ratio of rostral frequency to caudal frequency for each preparation was usually close to 1, and neither of the mean ratios (muscle 1.00±0.02; spinal cord 1.01±0.04; Fig. 3B) was significantly different from 1. When this ratio was not equal to 1, the frequencies of the two parts of the spinal cord were not phase-locked; each had its own frequency and the phase lags were highly variable. If the ratio was 1, as seen here, and there was phase-locked activity, then the phase lags were highly stable.

In all eight animals, the phase lag per segment was less in

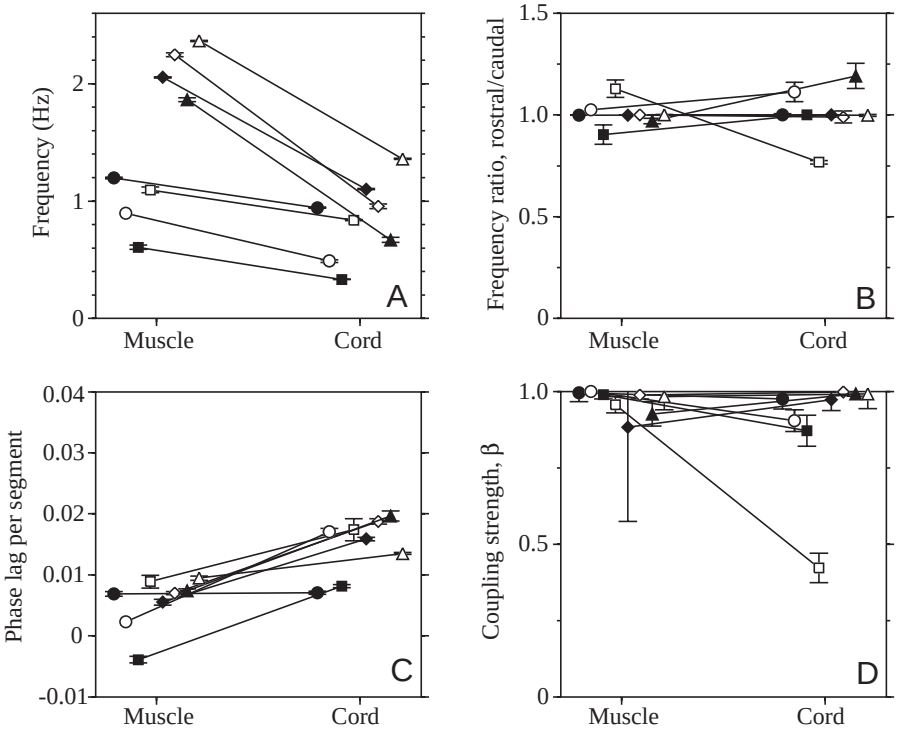

Fig. 2. Results from the eight individual animals with intact spinal cords and rostrally pinned that were successfully tested in both conditions: trunk with muscle (Muscle) and isolated spinal cord and notochord (Cord). Each symbol represents the same animal in all graphs. (A) $\overline{\omega}$, the average of the rostral and caudal bursting frequencies. In cases in which the rostral and caudal outputs had different frequencies, the average of the two frequencies is plotted. (B) $\overline{\omega_1}/\overline{\omega_2}$, the rostral/caudal frequency ratio. (C) $\overline{\phi}/n$, the phase lag per segment between rostral and caudal outputs. (D) β, the coupling strength parameter describing the functional coupling strength between rostral and caudal recording locations. The value β=0 corresponds to no coupling, and the value β=1 corresponds to infinite strength coupling. Error bars represent approximate 95% confidence intervals. In one case (open circles in the muscle condition), we were unable to compute confidence intervals because β=1.

the muscle condition than in the isolated spinal cord (Fig. 2C). The mean phase lag (Fig. 3C) in the muscle condition (0.0055±0.0015) was significantly less than the mean lag in the spinal cord condition (0.0147±0.0017; $P<0.01$). Thus, the presence of muscle and associated movement seems always to critically reduce the phase. It is unclear why the phase delay with the muscle is shorter than the phase delay in intact animals (for the intact values, see Wallén and Williams, 1984). Presumably, the difference comes from interactions with the skin and its afferents or the descending control systems, neither of which is available to the muscle preparation.

Changes in intersegmental coupling strength

Fig. 2D shows the parameter β describing the functional coupling strength between the rostral and caudal recording locations. All coupling strengths were fairly strong (β>0.87) except for one value in the spinal cord condition. The difference in means between the muscle and spinal cord conditions (Fig. 3D) was not significant.

With very strong total coupling, as is typically found in the intact spinal cords with and without muscle, the values for the ascending strength fraction γ are often unreliable and are therefore not shown (see Discussion).

Impact of movement and movement-related feedback: spinal cord with two staggered hemisections

Changes in frequency and phase

Fig. 4 shows traces from one animal under three conditions: the fully intact animal (Fig. 4A) prior to any dissection, the reduced muscle condition with brain and tail removed (Fig. 4B, see Materials and methods), stimulated to swim with D-

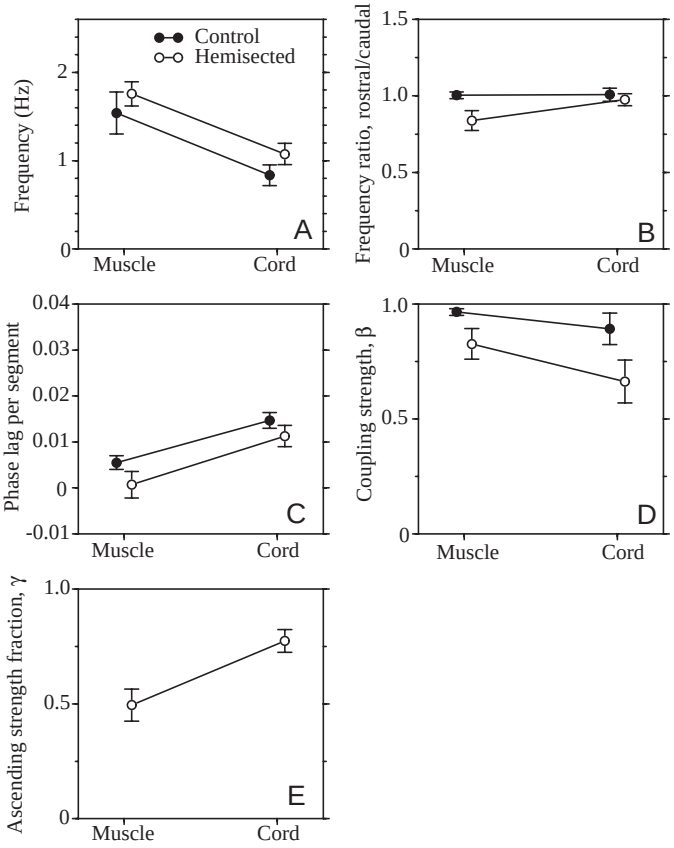

Fig. 3. Means across all animals; values are means ± S.E.M. Intact spinal cords are designated with filled symbols (N=8; summary of data from Fig. 2) and hemisected spinal cords with open symbols (N=7; summary of data from Fig. 5). Other details as in Fig. 2.

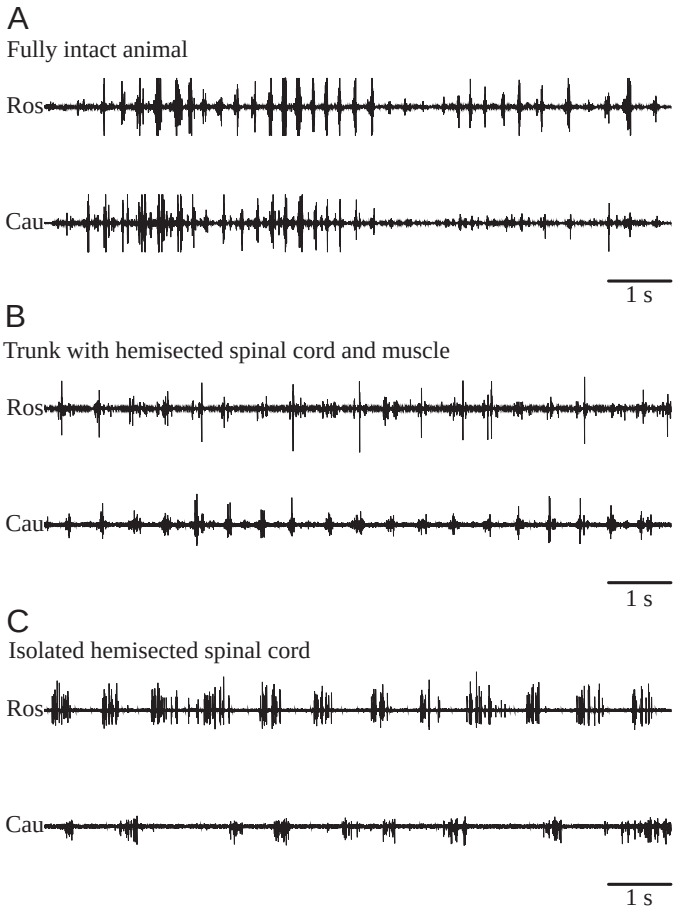

Fig. 4. EMG recordings from a single animal under three conditions. (A) Intact animal swimming. (B) Trunk with muscle and spinal cord with two staggered hemisections. (C) Isolated spinal cord with two staggered hemisections. Values for this animal are represented by the filled squares in Fig. 5. See text for further details. Ros, rostral; Cau, caudal.

glutamate, and the isolated spinal cord stimulated to fictive swimming with D-glutamate (Fig. 4C). After decapitation, two staggered hemisections were made in the spinal cord as described in the Materials and methods section. Note that there is considerable cycle-to-cycle variability in the intact swimming. This type of variability in amplitude and burst duration is lost in the muscle preparation. In the isolated spinal cord, the segments are poorly coupled as a consequence of the two hemisections, as demonstrated by a coupling strength of β=0.25, leading to bursting with irregular periods but uniform amplitude.

Fig. 5 illustrates the results from seven animals with staggered hemisections. As with the intact spinal cords, the frequency of the isolated spinal cord was lower than the frequency in the muscle condition in all animals (Fig. 5A). The mean frequency of the isolated spinal cords (1.07±0.12 Hz) was significantly lower than the mean frequency in the muscle condition (1.76±0.14 Hz; P<0.01) (Fig. 3A).

Unlike the preparations with intact spinal cords, the mean rostral/caudal frequency ratio for the muscle condition (0.84±0.06) was significantly less than 1 (P<0.05), indicating a clear tendency for the caudal frequency to be faster than the rostral frequency (Fig. 5B). The mean ratio for the isolated spinal cords (0.98±0.04) was marginally significantly greater than the mean in the muscle condition (P<0.10) and not significantly different from 1.

As in the unhemisected animals, the mean phase lag per segment in isolated spinal cords (0.0113±0.0023) was significantly greater than the mean lag in the muscle condition (0.0007±0.0029; P<0.05) (Fig. 3C, Fig. 5C).

Changes in intersegmental coupling strength

Comparison of the estimated coupling strengths across those seven animals for which data were stable enough for the maximum likelihood method to be accurate for bursting data (see Kiemel and Cohen, 1998) revealed the following changes across both conditions. Coupling strengths were stronger in the muscle condition than in the spinal cord condition in six of the seven animals (Fig. 5D). However, the mean of value β in the muscle condition (0.83±0.07) was not significantly greater than the mean value in the spinal cord condition (0.66±0.09) (Fig. 3D). This was apparently due to the seventh animal (open diamonds in Fig. 5D), which had the smallest β value in the muscle condition but the largest β value in the spinal cord condition. (Without this animal, the difference was significant, P<0.05.) This animal was also exceptional in that the 1:2 rostral:caudal frequency ratio in the muscle condition changed to a 1:1 ratio in the spinal cord condition (open diamonds in Fig. 5B). The mean value of β in the hemisectioned animals in both conditions was smaller than the corresponding mean in the unhemisectioned animals, with the difference being significant in the muscle condition (P<0.05) and marginally significant in the spinal cord condition (P<0.10).

For all seven animals, γ for the isolated spinal cord was greater than 0.5, indicating that, with two staggered hemisections, the estimated ascending coupling was stronger than estimated descending coupling. Also, in all animals, γ in the isolated spinal cord was greater than γ in the muscle condition. The mean value of γ in the spinal cord condition (0.77±0.05) was significantly greater than 0.5 (P<0.01) and significantly greater than the mean value of γ in the muscle condition (0.49±0.07; P<0.01) (Fig. 3E).

The mean values for the hemisected and non-hemisected across all animals and both conditions are compared in Fig. 3. The hemisected animals are the open symbols.

Discussion

For an analysis of the impact of movement and its related feedback on the locomotor CPG, ideally, one would like to compare a single spinal cord under conditions that permit or prevent movement while removing some layers of the sensorium without deterioration of the preparation. The lamprey is a model vertebrate system in which just this experiment can be performed. The whole body minus the brain, viscera and skin, with only minor additional dissection, can be induced to swim for long periods. There are also only minimal changes in the

status of the preparation over a period of days. Thus, one can perform a comparison within a single animal of the impact of movement as well as the impact of a particular class of sensory inputs generated by the preparation itself. This type of analysis was performed in the locust by Wilson (Wilson, 1961), but vertebrate preparations are less amenable to such types of manipulation. Wilson (Wilson, 1961) found that locusts behaved similarly to the lampreys reported here. Indeed, his study is remarkable for the similarities it shows with the current discussion of the relative roles of sensory feedback and movement. His conclusions are similar except that they are in a context in which CPGs had not yet been accepted, 'Eliminating sensation from the wing and motor innervation of the dorsal longitudinal muscles has the definite effect of lowering the frequency of the wing-beat cycle.' … 'This reduction of input did not, however, upset the basic pattern of wing movements including wing twisting and segmental phase differences. This surprising result led to the hypothesis of a built-in central pattern which is not dependent upon peripheral feedback loops for its basic operation, but which is modified by such input. This input apparently increases frequency as well as affecting small changes in pattern which control flight' (Wilson, 1961, p. 480).

We show here that, when allowed to move, the rhythm induced pharmacologically in a lamprey preparation without head or viscera is typically of higher frequency than that of the respective isolated spinal cord. With muscle attached, the phase delay has a tendency to be shorter than that of the isolated spinal cord, and the presence of movement can increase the intersegmental coupling in a preparation that has had its coupling reduced surgically. It is likely that the coupling is also stronger in preparations without lesions, but we are unable to measure differences in the strengths of coupling among those conditions that have coupling so strong that it is near the asymptotic limits of our calculations.

Proposed mechanism for changes in frequency and phase with movement and movement-related feedback

One possible source of the changes in the bursting characteristics reported here, i.e. an increase in oscillator frequency in the presence of movement, is the slowly decaying excitation observed by Kiemel and Cohen (Kiemel and Cohen, 2001). Following periodic bending of one end of an isolated spinal cord, the bursting frequency was initially above its baseline value, slowly returning to the baseline on the time scale of one or more cycles. Such an effect can produce an amplitude-dependent bias towards increasing local uncoupled oscillator frequencies during movement (Kiemel and Cohen, 2001).

On the basis of the findings of Kiemel and Cohen (Kiemel and Cohen, 2001), we suggest the following working hypothesis to explain the data on frequency and relative phase we have presented: movement increases the uncoupled frequencies of the local oscillators, with the amount of the increase being directly related to the amplitude of movement. The uncoupled frequency of an oscillator in this context refers to the frequency that would be observed under the same set of conditions if the intersegmental coupling were eliminated. When the trunk preparation is pinned, the amplitude of movement appears smaller near the pin than at the tail; the movement grows as it moves caudally (Fig. 1). Thus, given the hypothesis stated above, movement would non-uniformly increase the uncoupled frequencies of the oscillators, with the increase being greatest at the caudal end. In typical models of chains of coupled oscillators, such as phase models (Kopell and Ermentrout, 1986), such a non-uniform increase in uncoupled frequencies applied to a phase-locked chain would (i) increase the coupled frequency of the chain and (ii) decrease the rostral-to-caudal phase lags between the oscillators. In addition, if the non-uniformity of the effect were sufficiently large compared with coupling strength, then phase-locking in

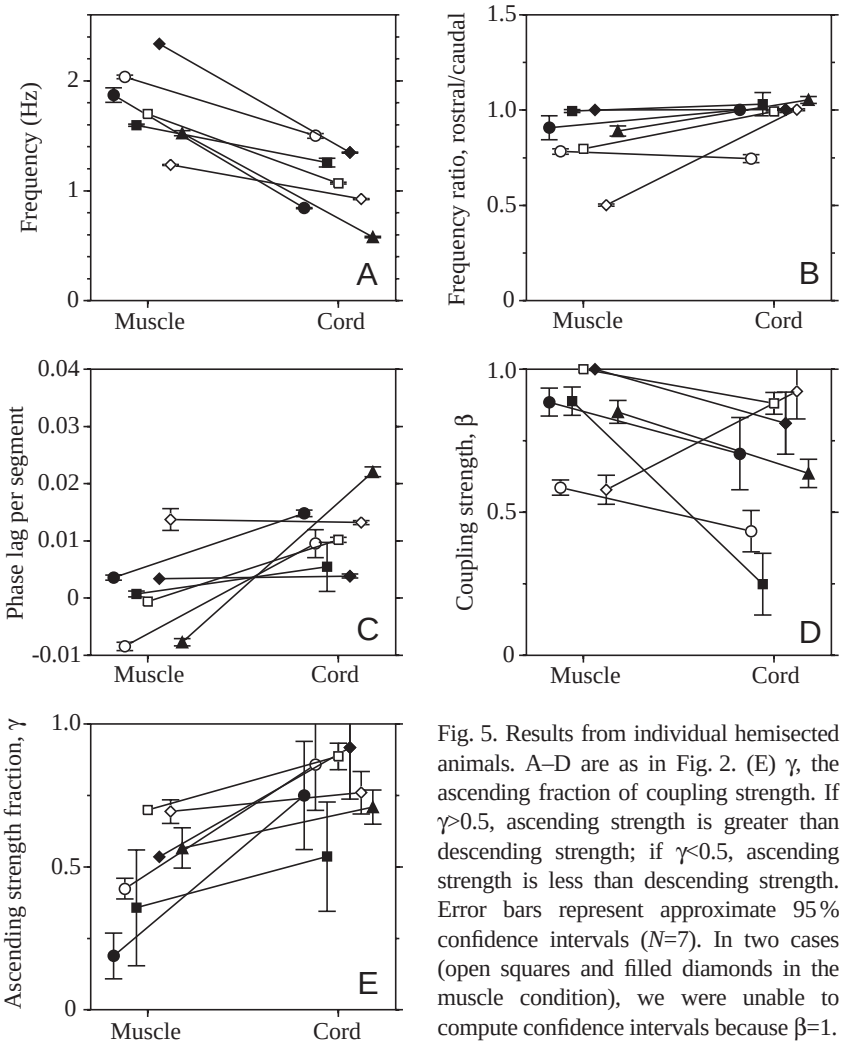

Fig. 5. Results from individual hemisected animals. A–D are as in Fig. 2. (E) γ, the ascending fraction of coupling strength. If $\gamma>0.5$, ascending strength is greater than descending strength; if $\gamma<0.5$, ascending strength is less than descending strength. Error bars represent approximate 95% confidence intervals ($N=7$). In two cases (open squares and filled diamonds in the muscle condition), we were unable to compute confidence intervals because $\beta=1$.

the chain would be lost and the coupled frequencies of the caudal oscillators would be greater than the coupled frequencies of the rostral oscillators.

We have observed all three of these effects in our data. (i) The frequencies in the condition with movement were on average significantly higher than the frequencies of the isolated spinal cords in both the unhemisected and hemisected animals (Fig. 2A, Fig. 3A, Fig. 5A). This increase in frequency with movement was seen in every animal when comparing the muscle and spinal cord conditions. (ii) The phase lags per segment of the two conditions with movement were on average less than the phase lags of the isolated spinal cords for both the unhemisected and hemisected animals, with the difference being statistically significant (Fig. 2C, Fig. 3C, Fig. 5C). Finally, in the hemisected animals, the rostral/caudal frequency ratios in the two conditions with movement were on average significantly less than 1 and at least marginally significantly less than the ratios in the isolated spinal cords (Fig. 5B). Thus, the caudal frequencies are on average higher than the rostral frequencies in the presence of movement, but not in the absence of movement. It is reasonable, therefore, to suppose that movement is the critical ingredient in causing these changes across both muscle and spinal cord conditions.

However, phasic effects will also be present during ongoing movement, and the two-way interaction between neural activity and muscle movement is sufficiently complex that modeling would be required to appreciate fully the effects that slowly decaying excitation might have during ongoing swimming.

It is unclear why the phase delays in moving preparations with intact spinal cords are smaller than in intact animals. The typical value for intact animals is 1 % per segment (as reported by Wallén and Williams, 1984). This difference, among other possibilities, could be due to a mechanical effect of the skin or to the activity of some skin afferents. However, this remains to be determined.

Changes in intersegmental coordination with movement and movement-related feedback

Change in intersegmental coordination caused by movement-related feedback has recently been demonstrated in the leech *Hirado medicinalis* (Yu et al., 1999). Indeed, these authors found that mechanical coupling was sufficient to bridge a gap in the nerve cord. In that system, there was a dispute as to whether or not such mechanical coordination could occur (for discussion, see Yu et al., 1999), but Yu et al. (Yu et al., 1999) clearly showed that it could. McClellan has shown that mechanosensory input can contribute to intersegmental coordination in larval *P. marinus* lampreys with spinal lesions (McClellan, 1994). There was no attempt in the study of McClellan to be more than qualitative. In the present study, we are able to go beyond the qualitative measures using the method developed by Kiemel and Cohen (Kiemel and Cohen, 1998).

The notion of functional coupling strength we use here is based on a model of two coupled phase oscillators (see Materials and methods). Systems of coupled phase oscillators have often been used as simple models for the lamprey CPG (Cohen et al., 1982; Kopell and Ermentrout, 1986; Williams et al., 1990; Hagevik and McClellan, 1994). Our estimates of coupling strength have the advantage that they are consistent with this existing theoretical framework. Simpler measures, such as cross-correlation (Perkel et al., 1967), coherence (Brillinger, 1992; Miller and Sigvardt, 1998) and the variability of phase values (Kriellaars et al., 1994) are useful for ascertaining whether coupling exists between neural oscillators, or from an external oscillator to a neural oscillator, and for analyzing the dynamic relationship between the oscillators. However, these measures are greatly affected by factors other than coupling strength, such as the level of cycle-to-cycle variation in oscillator frequencies. Therefore, they are better thought of as measures of coordination rather than as measures of coupling strength. Our estimates of coupling strength are only mildly affected by the level of frequency variation (Kiemel and Cohen, 1998). We have found that our method gives useful estimates of coupling strength when applied to simulated data from models other than the phase model (T. Kiemel and A. Cohen, unpublished work).

We have used two parameters to describe coupling strength. The parameter β describes the total coupling strength, and the ascending strength fraction γ describes the relative strength of ascending and descending coupling. When the two oscillators are phase-locked, β is roughly the fraction of a relative-phase perturbation that is, on average, corrected after one cycle. The parameter γ depends on the extent to which each oscillator participates in the correction.

The parameter β can be estimated with some finite precision, which depends on factors such as the number of cycles of activity analyzed. If coupling is so strong that β is indistinguishable from 1, then further increases in coupling strength cannot be detected. Since unhemisected isolated spinal cords typically are strongly coupled, our ability to detect any additional coupling present in the muscle condition is limited. Thus, not much can be concluded from the result that coupling strength β was not, on average, significantly stronger in the muscle condition than in the spinal cord condition in animals with unhemisected spinal cords (Fig. 2D, Fig. 3D). Looking at individual animals, it is suggestive that, in the three animals with the weakest coupling in the spinal cord condition, coupling strength was greater in the muscle condition.

Further evidence for the idea that the presence of muscle and its attendant movement adds to functional coupling strength can be seen in animals whose spinal cords were weakened by a pair of staggered hemisections (Fig. 5D). Here, six of the seven animals have stronger estimated coupling in the muscle condition than in the spinal cord condition. The exception is an animal whose rostral:caudal frequency ratio was 1:2 in the muscle condition and 1:1 in the spinal cord condition (Fig. 5B). This dramatic change in frequency ratio complicates the interpretation of the change in estimated coupling strength, making its estimated values unreliable.

Strong coupling, in addition to limiting our ability to detect

changes in coupling strength, also limits our ability to estimate the relative strength of ascending and descending coupling. When coupling in the spinal cord is weakened with hemisections, estimates of relative strength are more reliable. In all hemisected animals, the ascending strength fraction γ was lower in the muscle condition than in the isolated spinal cord, with the mean value of γ in the muscle condition significantly lower than the mean value in the spinal cord condition (Fig. 3E, Fig. 5E). This suggests that the coupling added by movement is predominantly in the descending direction. Such a descending bias may be due simply to the fact that the mechanical traveling wave progresses in the descending direction, sequentially activating stretch receptors. However, given the complexities involved with biomechanics, fluid dynamics and their interaction with the neural activity, modeling would be required to give a convincing argument.

Beyond comparison between conditions, it is important to note that the value of γ for all isolated hemisected spinal cords was greater than 0.5. Thus, the functional coupling provided by the short fibers that remain following the two staggered hemisections is stronger in the ascending direction than in the descending direction. A similar conclusion was reached by Williams et al. (Williams et al., 1990), who interpreted experimental data from bending experiments in terms of a phase model with only short-distance coupling and argued that coupling is stronger in the ascending direction. A key difference is that we have eliminated the majority of long connections and thus are not making any suggestion about the directionality of coupling when long fibers are present.

Concluding remarks

We show here that movement and sensory feedback may alter several aspects of an animal's motor behavior. Historically, there has been debate about the potential for sensory feedback to influence movements, such as locomotion, in which CPGs play a major role (see Pearson, 1981). From the beginning, CPGs have been shown to be responsive to sensory feedback on a cycle-by-cycle basis, being entrained by some critical sensory receptors (Rossignol et al., 1988). There have been suggestions of a greater magnitude of influence in the work of Pearson and his colleagues (e.g. Pearson, 1995; Wolf and Pearson, 1988; Cruse et al., 1995). The data presented here demonstrate that movement and its related sensory feedback can alter the frequency, change the phase delays, increase and alter the intersegmental coupling and have an impact on the preferred relative frequencies among the segments. The changes in the motor patterns combined with the changes in coupling strength are likely significantly to alter the overall motor behavior of the intact animal compared with that provided simply by the central circuits for locomotion. It is impossible in this preparation to separate the relative roles of sensory feedback *versus* movement. For example, it is possible that movement and its resultant mechanical forces are responsible for the changes in intersegmental coordination and that sensory feedback is playing an insignificant part in this change. While evidence for an impact of movement and sensory feedback has been provided in the studies of invertebrates, it has been more difficult to confirm across a range of conditions in a vertebrate preparation. We show here that movement and perhaps sensory feedback contribute to the final output pattern in locomotion. A final estimation of the relative roles of the individual components remained to be determined.

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