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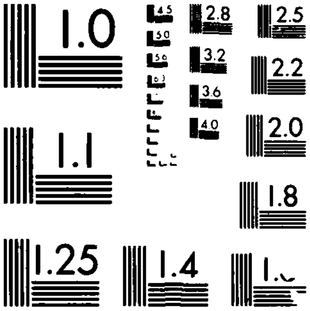

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The Center for the Study of Rhythmic Processes began operation in the academic year 1986-87. It brought together mathematicians and biologists to work on problems of mutual interest. During the first year, the Center focused on two sets of problems. The first involved the structure and function of the intersegmental coordinating system of the vertebrate spinal cord, for which the lamprey provides an excellent model system. A broadly applicable mathematical framework was constructed for analyzing this system, and new mathematical techniques were invented. A new technology was put into use which has the potential of providing information not previously obtainable. Many of the major researchers working on this preparation were consolidated under the auspices of the center, and many new collaborations were formed. The other involved small neural networks, such as the stomatogastric ganglion of the lobster. Detailed mechanisms of function and control were investigated, and mathematical tools were applied to investigate how the circuits change under modulation.

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Center for the Study of Rhythmic Processes

Progress Report - Year 1 (10/1/86 - 10/1/87)

Contents

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3. Eve Marder, Department of Biology, Brandeis University
   Pierre Meyrand, Postdoctoral Fellow
   Robert Zarum, Undergraduate
   James Weimann, Graduate Student

4. Avis Cohen, Neurobiology and Behavior, Cornell University
   Nicholis Mellen, Graduate Student
   Tamara Dobrof, Graduate Student
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II Statement of Work

During the past year, we have established a Center for the Study of Rhythmic Processes. The Center brings together mathematicians and biologists in seminars, workshops and an atmosphere of intense discussion to create an environment in which problems of mutual interest can be most effectively and creatively investigated. The mathematical work entails the use of dynamical systems theory to develop a broadly applicable theoretical framework for explaining the behavior of single oscillators as well as groups of coupled oscillators. The physiology explores the structure of neural networks that can generate oscillatory outputs. Insights from the mathematics are used to help formulate productive experiments, as well as to aid in the interpretation of the results. The results of the experiments are used to inform the development of the mathematical framework.

During the first year, the Center focussed on two sets of problems. One involves the structure and function of the intersegmental coordinating system of the vertebrate spinal cord; the lamprey provides an excellent model system for such an investigation. New mathematical techniques have been invented, and new technology has been put into use which has the potential of providing information not previouly obtainable. Many of the major researchers working on this preparation have been consolidated under the auspices of the Center, and many new collaborations have formed.

The other problem involves small neural networks, such as are found in some invertebrate preparations. Detailed mechanisms of function and control of neural oscillators are being investigated in a system in which there are few cells and the circuitry is near to being understood. Mathematical tools are being applied to investigate how the circuits change under modulation.

III. Mathematics done by Ermentrout and Kopell

Much progress has been made on the projects reported in the letter of May 12. This work is described in some detail in the enclosed expository paper "Coupled Oscillators and the Design of Central Pattern Generators" [1]. There are a large number of related projects that are being worked on simultaneously; the main reason for this is that interactions with the experimentalists have raised questions relevant to the design and interpretation of experiments, and it was important to investigate enough of each to allow the experiments to go on. The projects and their current state of completion is as follows:

Completed : Two expository papers (enclosed), written for different audiences. Structure and Function in an Oscillating Neural Network" is to appear in Computational Neurosciences, E. Schwartz, ed. MIT Press, 1988. Reference [1] is to appear in Math Biosciences and will be reprinted in Nonlinearity in Biology and Medicine, North Holland Publishing Company, 1988

Close to Completion: (available in draft, but not final, form)

1. "Phase transitions and other phenomena in chains of oscillators". This paper explores generalizations of the work in our paper "Symmetry and phaselocking in chains of weakly coupled oscillators" [2], on which most of our work on central pattern generators is based. The generalizations were motivated by the design of feasible experiments, many of which have recently been done, or are scheduled to be performed in the near future. (See Section VI.) The generalizations deal with the effects of local regions of higher frequency, and coupling that is not the same everywhere along the cord. It also explores the implications of coupling for which the downward (caudal) coupling is quite different from the upward (rostral) coupling. New mathematical phenomena have been identified, notably the existence of solutions that exhibit a phase transition as some parameter is varied. (Here a phase transition refers to a large change in the qualitative behavior of the solution that occurs over a tiny change in the parameter if the number of oscillators is large.) These results yield predictions about the split bath experiments (Section VI) as well as giving a framework within which detailed results of those experiments can yield information about differences between rostral and caudal coupling, and differences in natural frequencies along the cord.

2. "Averaged and unaveraged systems of coupled oscillators" This paper has two major themes. The first is that the form of interaction of biological oscillators commonly used in models of forced oscillators, when applied to reciprocally coupled systems, leads to destruction of the rhythmicity. This is very robust, and holds for large classes of models, including standard models [3,4]. Particularly susceptible to such "oscillator death" are oscillators interacting by means of a single large pulse in each

oscillator cycle. The second theme is that this undesirable outcome can be avoided if the interactions take place in a more gradual manner, spread out within the cycle, and we discuss neural networks that may be doing this. Technically, we show that if the interactions are spread out, as above, then the system behaves like one in which the coupling is averaged over the cycle; we show that for such systems, "oscillator death" cannot occur. This work serves to support the assumptions made in [2], in which the method of averaging was used to reduce a seemingly unattackable mathematics problem to one for which analytic conclusions could be drawn.

3. With D. Aronson, "Amplitude response of coupled oscillators" This paper is an investigation of coupled oscillators, each near a Hopf bifurcation, so that the strength of coupling is comparable to the strength of the attraction to the limit cycle. This paper has been substantially increased and improved since the last report. Among the results are the existence of parameter regimes for which the origin is stabilized by the interaction, and is the only stable solution. We are thus able to give an analytical explanation for a phenomena that had been found numerically in several models of chemicals oscillators. We are also able to demonstrate bistability of a phaselocked solution and an unlocked "drift" solution, and bistability of a phaselocked solution and a critical point, phenomena which have not previously been found analytically in coupled oscillators.

In progress:

1. With W. Zhang (graduate student), "Multiple coupling in chains of oscillators". Neurons from a given segment of the lamprey spinal cord have processes that extend for many segments. We have modelled this by considering chains of oscillators in which each oscillator is connected to more than one of its neighbors, as in the leech central pattern generator [5]. We show that the conclusions of [2] still hold for multiple coupling. In addition, we show that the multiple coupling serves to buffer the solutions against variation in frequency of the local oscillators. Finite chains with multiple coupling also appear to exhibit edge effects. These theoretical results help to explain the otherwise puzzling results of A. Cohen on the measured frequencies and phase differences in the isolated spinal lamprey preparation. (See Section V.1 and [1, Section 4G]) The latter theoretical results are preliminary; if they turn out to be robust, they may give unexpectedly explicit predictions about the anatomy of the CPG network. In particular, the mathematics now suggests that the position of the "dips" in phase differences found by Cohen are a measure of how many segments are connected to each local oscillator. More theoretical and experimental work is planned.

2. Work on "Periodically forced chains of oscillators" was motivated by a recent paper of K. Sigvardt and A. McClellan [6], who are now both members of the Center. Sigvardt and McClellan investigated sensory feedback in the isolated lamprey spinal cord preparation, using the mechanoreceptors thought to be so-called "edge cells" on the margins of the spinal cord. They were able to mechanically force a few segments of either end of a portion of a cord, and show that the remaining stationary segments could be entrained by

the intersegmental coordinating system. To understand the details of their results, we modelled the situation as a forced chain of oscillators, and have preliminary analytical and numerical results. We found these results surprising; they assert that it would be very difficult to attain phaselocking, as was seen in the experiments, for a range of forcing frequencies and with forcing at either the rostral or the caudal end, if the rostral and caudal coupling are qualitatively the same. (See Section 5C of [1].) The analysis shows that detailed information about the phase lags from forcing experiments have the potential of yielding a lot of information about the structure of the C.P.G. Because of this, Sigvardt and T. Williams (another new member of the center) have undertaken more detailed entrainment experiments. (See Section VI.)

3. Work on "Nondiscrete chains of oscillators" is motivated by the need to deal with the fact that the lamprey and other vertebrate spinal cords are not composed of discrete oscillating units, as is the leech. Up until now, that has been outside the reach of any modelling efforts. Our work is based on our conjecture, which has both a theoretical and experimental basis, that the oscillating units are composite. (See Section 5D of [2].) Preliminary analytical and numerical results suggest that if the coupling among the neurons within an oscillator are comparable in strength to coupling among different segments, the resulting circuit can behave like a chain of discrete oscillators, while not anatomically having such a modular structure.

4. A collaboration with E. Marder has begun. We are interested in the differential effects of standard conductance changes and voltage dependent conductances on the behavior of burster neurons and networks that contain such neurons. (See Section IV.) Preliminary numerical work on this has been carried out by S. Strogatz, a post-doctoral fellow associated with the center. It has been seen that existing models of bursting cells do not respond appropriately to simulated modulation (e.g., changes in baseline and/or periodicity are incorrect); other models are being explored. The numerical work has recently been taken over by I. Epstein (Department of Chemistry, Brandeis University), who is spending his year as a Guggenheim Fellow visiting the Center.

5. Work has begun on mathematical aspects of the interaction between the neuronal control and the mechanics of swimming in lamprey. This work involves a collaboration with T. Williams, a new member of the Center, who is knowledgeable both about the lamprey [7] and about physics. (See Section VI.) The mathematical work is now being done mainly by S. Laederich, a graduate student at Boston University, under the supervision of N. Kopell.

6. S. Strogatz, a postoctoral fellow with N. Kopell, has been working on questions involving oscillators with stochasticity. With R. Mirollo, he has written a paper, "Phase-locking of oscillators with random intrinsic frequencies", submitted to Physica D.

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IV. Work from the Marder Lab

OVERVIEW

We are interested in understanding how rhythmic motor patterns are produced by the nervous system. We want to define the mechanisms by which rhythmic motor patterns are modulated by neural inputs, sensory information and hormones. We want to know how the activity of single cell neural oscillators is modulated by neurotransmitters and modulators. To address these questions we use the pyloric rhythm of the crustacean stomatogastric ganglion as a model preparation.

1. IN VIVO RECORDINGS OF ONGOING PYLORIC ACTIVITY

One of the aims of the initial proposal was to obtain good quality recordings of the pyloric rhythm in alive and behaving animals. These recordings are intended to provide important baseline data about the frequency and variability of the pyloric rhythm under a variety of physiological conditions. We have successfully started these experiments, and happily find that the crab preparation is eaily amenable to the implantation of chronic recording electrodes, and that data are reasonably easy to obtain in these <u>in vivo</u> recording configurations.

Recordings are made with teflon coated silver wires inserted through the carapace at known sites. (Control experiments were performed to determine where the muscle insertions are located.) Visual control is used for electrode insertion, and recordings are made during the insertion process to enable us to judge when the electrode has made contact with the appropriate muscle. When the electrode is properly positioned, the electrode is fixed in place with surgical cement, and the animal can be allowed to move about. Injections into the animal are made directly into the circulation in the region of the heart.

Figure 1 shows an example of recordings made from two muscles before and after injection of proctolin. These recordings clearly indicate that proctolin injection into the intact and behaving animal produces dramatic increases in the frequency of the pyloric rhythm, as well as also turning on activity in the gastric rhythm. Figure 2 shows a plot of the effects of proctolin injection of pyloric frequency taken from records such as those in Figure 1.

These experiments are in progress at this time. These experiments are being carried out by Mr. Robert Zarum, an undergraduate who is currently working on this project for his Senior Honors Thesis with the help of Dr. Pierre Meyrand, postdoctoral fellow. The methodology for the collection and analysis of these data is now in place.

Figure 1.

EMG recordings from behaving crab. cpv1 muscle recording shows the activity of the pyloric rhythm. gm1 muscle recording shows the activity of the gastric mill rhythm. Proctolin injection into the heart at t=0.

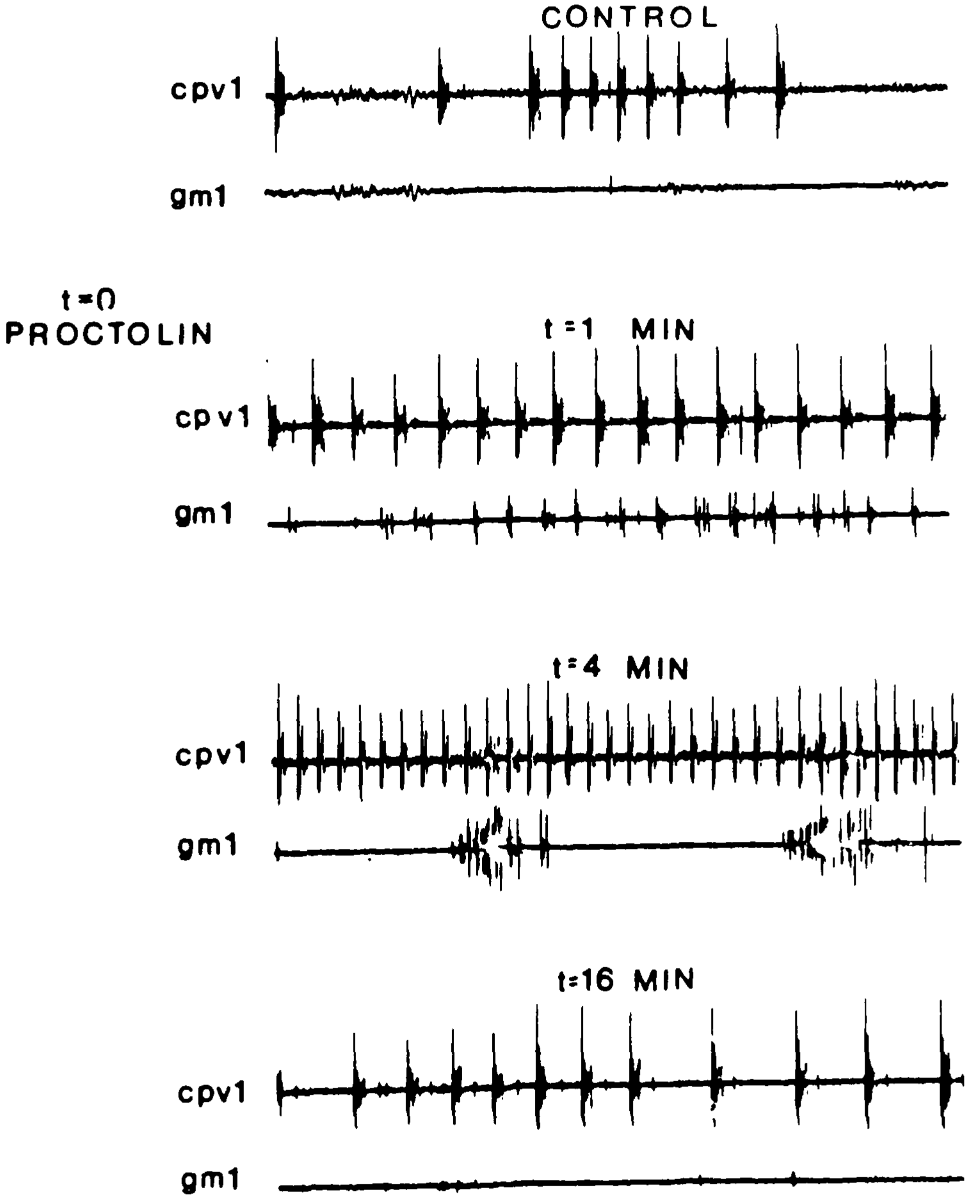
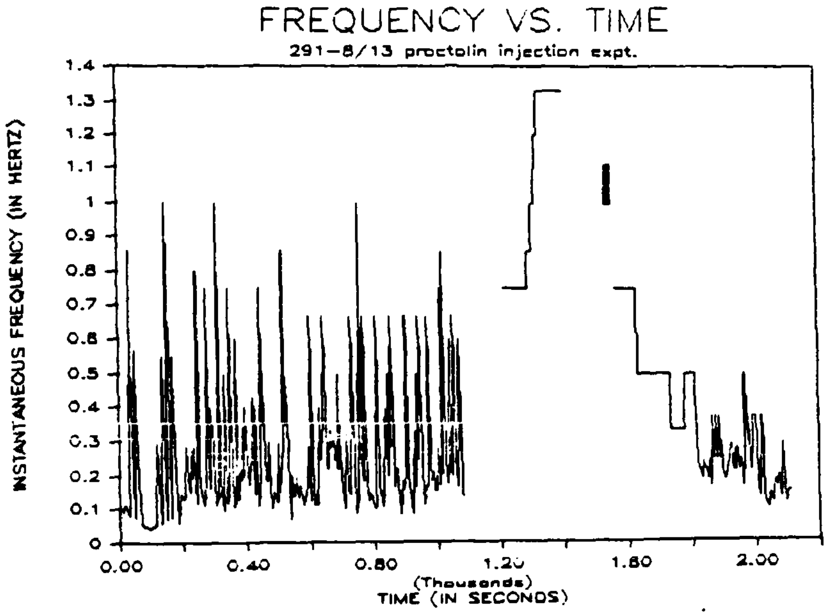


Figure 2.

Plot of the effect of proctolin injection on the frequency of the pyloric rhythm. Note that after injection the frequncy increased and became more regular.



During this next year we will be collecting data from a large number of animals, analyzing these data, and preparing them for publication.

2. COUPLING THE GASTRIC AND PYLORIC OSCILLATORS

The stomatogastric ganglion produces two different rhythmic motor patterns, with very different intrinsic frequencies. The pyloric rhythm, which we have most intensively studied, has a period of about one second, while the gastric rhythm usually displays a period of about 10 seconds. It has been long known that the gastric and pyloric rhythms interact with each other (Mulloney, 1977). This is an example of interactions between two oscillators of very different intrinsic periods, and therefore understanding how these oscillators interact was of interest.

Recently we have been studying the interactions between the gastric and pyloric rhythms in the crab, <u>Cancer borealis</u>. In this species we find that a number of the neurons of the ganglion appear able to function as part of both gastric and pyloric circuits. Actually, it appears that one group of neurons that form a neuronal "network" can be configured into different neuronal circuits with entirely different functions. These data are derived from the work of Dr. P. Meyrand and Mr. J. Weimann, and are currently being prepared for publication.

3. MODULATION OF THE AMPLITUDE AND FREQUENCY OF AN OSCILLATORY NEURON

Many rhythmic processes in the nervous system are thought to be controlled by oscillatory "bursting" neurons (Harris-Warrick and Flamm, 1986). These are neurons that rhythmically depolarize, produce bursts of action potentials and then repolarize. A great deal of work has been expended in trying to understand the ionic mechanisms underlying these endogenously active oscillatory neurons. More recently, research has started in trying to understand how the activity of bursting pacemaker neurons is modulated by neurotransmitters and hormones.

The pyloric rhythm of the stomatogastric ganglion has a timing pacemaker neuron, the Anterior Burster (AB) that can produce bursts of action potentials, even when isolated from other synaptic inputs. We have previously shown that the isolated AB is modulated by amines and peptides (Marder, 1987). We are now interested in comparing the action of different classes of modulatory substances on the AB neuron. Specifically we have become interested in comparing the action of a substance that increases the conductance of the AB neuron as would a classical excitatory transmitter with a substance that modulates the action of voltage-dependent conductances or that acts in a voltage-dependent fashion.

Therefore we have chosen to study the action of two cholinergic agonists on isolated AB neurons. The first substance, nicotine, increases the conductance of the postsynaptic membrane. The second substance, pilocarpine, is a muscarinic agonist, and has unconventional properties, similar to those of muscarinic agonists in many vertebrate preparations (Marder and Paupardin-Tritsch, 1978). We are interested in studying the effects of these two agents on a) burst amplitude b) baseline membrane potential c) burst frequency and d) burst duration. Figure 3 illustrates the basic difference between the action of these two substances on an AB neuron: nicotine depolarizes and excites the AB neuron but does not cause it to produce bursts, while pilocarpine elicits bursting behavior.

Some of these data are included in a paper currently being written (Marder and Meyrand, 1988). As physiologists, we are interested in fully understanding how bursting neurons are modulated, and therefore these data are interesting to us in and of themselves. However, these data are also extremely important to another part of this project, a collaboration with the Kopell group, on the mathematics of the modulation of rhythmically active bursting neurons. This collaboration has already demonstrated that a simple mathematical model that was thought to describe adequately the behavior of an bursting pacemaker neuron (Rinzel) does not account in a satisfactory way for the action of modulatory substances (see Section III).

4. AN EXPERIMENTAL AND THEORETICAL EXPLORATION OF THE DIFFERENCES BETWEEN DIFFERENT KINDS OF MODULATORS ON NEURAL CIRCUITS.

Work on the lobster and crab stomatogastric nervous systems has shown us that the pyloric rhythm is modulated by at least ten different substances, including a number of amines as well as many peptides (Marder, 1987). Interestingly enough, many of these substances appear to act either in a voltage-dependent manner, or by modulating voltage-dependent conductances. This suggested to us that there may be a fundamental reason why modulation of a rhythmically active neural circuit, or for that manner, any neural circuit might be best achieved in this manner. In fact, we have found that when substances that act simply to increase the conductance of one or more of the neurons the pyloric network are applied to the preparation, then it is possible to completely disrupt the normal pyloric rhythm, as illustrated in Figure 4. Therefore, our working hypothesis is that some of the "modulators" that act at the level of the voltge dependent conductances have conditional actions just so that they do not risk disrupting normal rhythmic activity.

Figure 3.

The effects of bath application of 10^{-4} M nicotine and pilocarpine on the isolated AB neuron. Compare the depolarization produced by the nicotine with the induction of oscillations produced by pilocarpine.

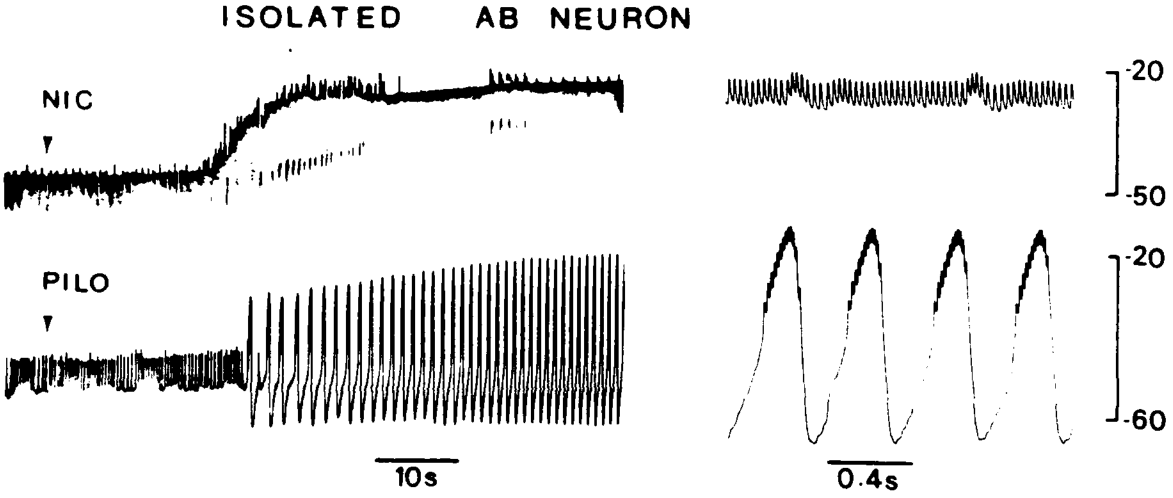
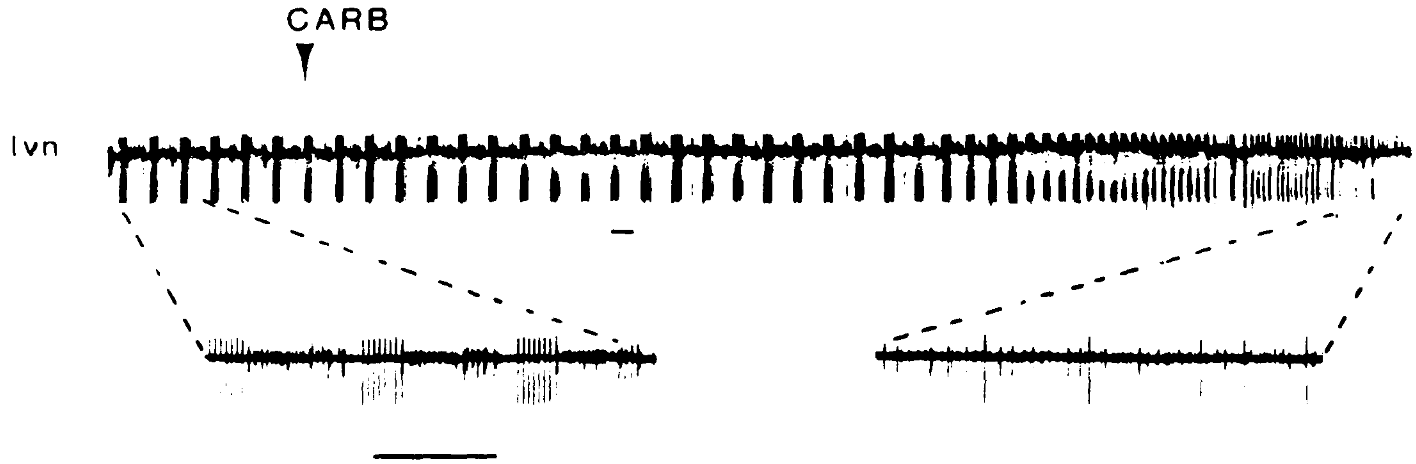


Figure 4.

Application of 10^{-4}M Carbachol disrupts the integrity of the pyloric rhythm.



It is our hope to develop these ideas more fully. We will then test them experimentally as well as is possible. When the model of the bursting neuron responds correctly to the application of modulatory substances we plan to collaborate with the Kopell group to try to develop a formal understanding of the limits of modulation of a rhythmically active network by different classes of modulatory substances.

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V. Work accomplished in the Cohen Laboratory

1. Structure and organization of the central pattern generator for locomotion

A robust conclusion of the mathematical modeling studies has been the fact that it is difficult to obtain uniform phase lags along a chain of oscillators whose natural frequencies differ to any substantial degree. Cohen had shown that the frequencies of the segmental oscillators of the lamprey were typically different and could be very different. The precise nature of the differences was unclear, but the presence of the differences was not. On the other hand, it has been part of the "lore" of the isolated lamprey spinal cord preparation that the phase delays along the cord are constant over a wide range of frequencies of bursting as well as uniform along the cord. However, the evidence for the latter was inadequate either to confirm or deny the contention. Experiments were undertaken by Cohen to examine both the nature of the frequencies of the segmental oscillators along the cord and the precise pattern of phase delays among pairs of oscillators at each level of the cord.

A. Methods

a. Phase lag determinations

Fifty segment cord pieces were dissected out and placed in the bath with 6 recording electrodes to monitor ventral root activity. Recordings were made relative to 2 reference electrodes, one on each side of the cord. The 4 test electrodes were moved along the cord in steps of 3 segments and 25 cycles recorded in each position. The phase lags were measured for each position relative to the reference roots (fig.1). The successive phase lags were subtracted one from the next and the difference divided by the number of segments intervening. This was taken to be the phase lag per segment over the segments between the 2 recording sites. The computed phase lag per segment was plotted against the segment number.

b. Oscillator frequencies

In the same cords, the preferred frequencies of the regional groups of oscillators was determined by two different methods, 1) selective activation by means of cord partition with vaseline seals and 2) cutting the cord in 2 and then 4 pieces.

B. Results

a. Phase lags along the cord

By the method described in Section A it was found that, in fact, the phase delays along the cord were not uniform (fig. 2A). The figure usually given for the phase delays has been an average of 1% of the cycle per segment with no systematic variation in any particular region of the cord.

The delays actually found, although quite variable, had the following pattern. The delays at both edges of the pieces were near 1%, but often rose a few segments farther from the edges. Near the segments 10 from the edges there was a large dip in the delays. Indeed, the phase lags in this region were often negative. Still farther in, the delays rose above 1% briefly and then fell back to the expected value.

To rule out the possibility that this pattern is related to anatomical anomolies in the 50 segment cord region tested, Cohen then did the same type of experiment on cord pieces dissected out 10 segments farther caudally than in the previous experiments. Although dislocated relative to the previous cords, the second set of cord pieces gave essentially the same pattern of dips and rises in roughly the same segment numbers (fig. 2B). Thus, there appears to be some "edge effects" in those segments around 10 from the end of a given piece of cord.

b. Oscillator frequencies

Cohen had suggested on the basis of earlier preliminary results that there was a monotonic gradient of frequencies along the cord. However, she has now found that there can be monotonic gradients, but they are the exception rather than the rule; more common is for the periods to be longer in the end regions and steadily rising or falling in the mid-regions (fig. 3) For reasons that are still unclear the two methods of frequency determination don't always produce the same pattern of results. Thus, although there are differences in the frequencies of the oscillators along the cord, the magnitude and pattern of the differences will only be known after more work, including work to clarify the method best for making the determination.

This work has been reported in an abstract of work to be presented at the American Neurosciences meeting in November, 1987 and a meeting in Florida (NAME), October, 1987. The latter will be accompanied by a paper to be published as part of the preceedings of the meeting, and will include an Appendix by Kopell.

C. Future work on these questions

In new modeling work precipitated by the phase lag data, a pattern similar to the one described has been found to occur with multiple neighbor coupling. In the simulations done to date on the new models, in some parameter ranges the number of segments at which the negative dips occur corresponds to the number of segments connected on each side to a segmental oscillator. Thus, these models predict tentatively that there are coordinating fibers connecting up to 10 contiguous segments. Becuase of the clearness and usefulness of this prediction, it has been decided to continue this line of investigation in greater detail.

Nicholas Mellen, Cohen's graduate student, will do this as a portion of his graduate research. He has designed and is currently building a set-up which will include electrode holders for easy positioning on nearby segments. This will allow measurement of the phase lag over a few segments directly instead of relative to a reference root at a distance; and may thereby reduce the variability in the data as well as speed up the data collection nd analysis. The set-up will also be built with improved partitions for the frequency experiments. It is hoped that this will improve these data and facilitate the experiments.

First he will test whether the effect still exists on shorter cord pieces (40, 30, 20 and 10 segments long). For long enough pieces the mathematics suggests that the negative dips should remain 10 segments from the end; numeric simulations will be done on short pieces of cord to see if the model matches resultant data. If the modeling holds up in this regard, then this type of experiment will be used to define the regional anatomy of the coordinating system. For example, the experiments described above were all done on rostral pieces of cord. It is unknown whether the more caudal portions of the cord have the negative dips. If they don't then it will suggst that they do not have the same anatomy as the more rostral portions used in the experiments described above.

2. Regeneration

A. Larval lampreys

Studies on larval lampreys were undertaken by Cohen, Mackler and Selzer (1985) to confirm that the functional regeneration was independent of mechanical entrainment. This possibility was raised by Grillner and his associates in an abstract in which they reported that the neuromuscular blocker, D-tubocurarine, disrupted the coordination observed during NMDA induced fictive swimming by formerly transected larval cords. To test whether or not they were correct, Cohen repeated her original experiments with the addition of curare to the bathing solution. She found that curare had no discernible effect on the coordination maintained by regenerated fibers (fig. 4). The only problems that could have accounted for Grillner's negative results were that his group used higher doses of NMDA than she found optimal and that they probably did not wait long enough for the coordination to stabilize (it can take as long as 45 minutes). Cohen's curare work has been submitted for publication to the Journal of Neurobiology.

B. Adult lampreys

There has also been success in keeping the spinal cord lesioned adult lampreys alive. They have now been surviving in adequate numbers (>25%) for 10 months. The critical factor appears to be feeding the animals and employing partial lesions instead of complete transections. The partial lesions allow the animals to feed successfully since they are predatory on free-swimming fish and the partial lesions don't interrupt swimming during the recovery period. Most laboratories that have attempted to keep the animals do not have the facilities to feed their animals and the young adult lampreys used for these experiments simply cannot survive without food on a regular basis. Cohen expects even greater survival in the coming year when arrangements will be made to keep the prey fish available throughout the entire required recovery period. This past year there was a hiatus of two months which precipitated the deaths of several animals.

Tests of the lesioned animals have now begun and have yielded interesting results. The lesions made were of 1) hemicords, 2) lateral tracts, and 3) medial tracts. In isolated control cords, none of these lesions when made acutely disrupted coordination (cohen, 1987). Regenerated fibers of only the medial tracts have consistently produced animals (3 of 3) with fictive swimming coordinated across the lesioned segment after the intact regions were cut acutely in the bath. In all cases, the bursting was never well coordinated. Typically, it would lock for sometime, then drift apart again (fig. 5). Based on modeling studies (Cohen et al., 1982) these observations are consistent with weak coupling, much weaker than that observed in larval cords.

These observations are in contrast to preliminary experiments on hemisected and laterally lesioned cords which lead to much more complex results. To date, with 2 animals tested, the lateral lesions produced one cord with no clear evidence of coupling (fig. 6), but which did produce fictive swimming on each side of the lesion. The hemisected and the other laterally lesioned cord could not be induced to produce any patterned bursting in the segments surrounding the lesion site although the segments at some distance rostral and caudal to the region of the lesion, when cut apart from the area adjacent to the lesioned segments produced stable bursting. The fact that segments at a distance would produce stable bursting while those segments in the vicinity of the lesion would not suggests that the regenerated fibers somehow perturbed the central pattern generator.

C. Future work on regeneration

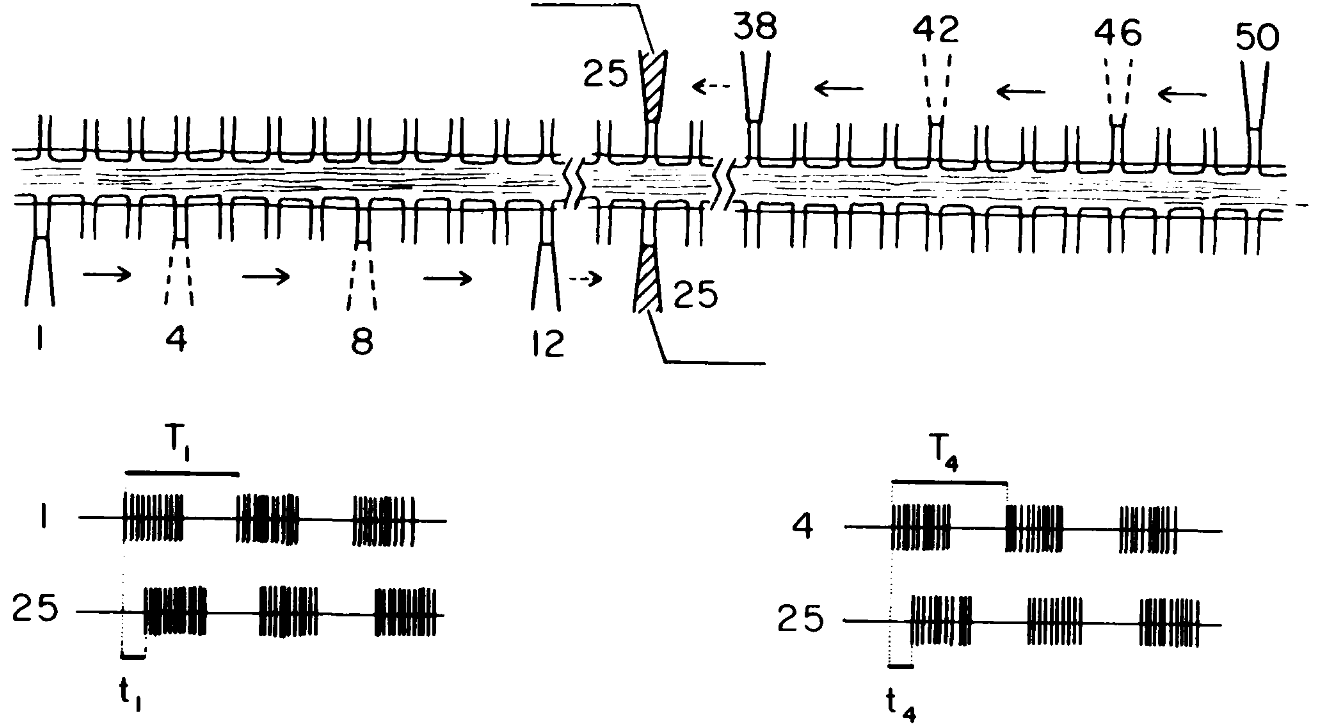
These observations made on Petromyzon marinus are being confirmed with the remaining animals. They are also being extended to include animals of the species Ichthyomyzon unicuspis. Preliminary experiments with this latter species point to its having less trouble producing fictive locomotion regardless of the lesion, but this will be tested directly in 2-3 months when they have had adequate time to recover.

The observations outlined above suggest that the adult lamprey spinal cord can be used as a model system to study the factors underlying both regeneration that restores function and that which leads to disrupted function. Possible disruption by regeneration has been played down by other investigators who have pointed instead to the apparent normalcy of the regenerated axons and their connections (Mackler and Selzer). However, the comparison between the two types of regeneration will be exploited by Cohen's lab. Tamara Dobrof, a graduate in the lab, will be following up on these experiments both anatomically and physiologically. She will learn histological techniques (HRP backfilling) necessary for examining the origin and destination of the regenerated fibers. Dr. Karen Sigvardt, a new member of the Center, has agreed to teach the necessary methods to Dobrof this winter. Since it is intuitively likely that the difference between the lesion results lies in the classes of neurons with fibers in the different tracts and the connections they form upon regeneration, the initial thrust of her work will be to classify the neurons whose fibers pass in the different fiber tracts in control and regenerated cords.

3. Optical recording of neuronal activity through the use of voltage sensitive dyes

The equipment necessary for the optical recording of neuronal activity is now largely assembled. Unfortunately, the analogue to digital converter being built by an electronic technician at Yale University is still incomplete, but has been promised in 3 months. At that time, L.B. Cohen, Yale University, has agreed to take responsibility for writing the programs necessary for taking and analyzing data. He also needs the equipment, and has agreed to share all developments fully with us. All the other equipment has been successfully brought to a state in which recording has been achieved on the lamprey spinal cord. There remain improvements to be made in the recording arrangement, but it is now possible to record neuronal activity optically in Cohen's own laboratory. She will begin to optimize further the conditions to allow measurement of the small signals produced by the lamprey cells post-synaptic to normal and regenerated neurons. Dr. Guan Li of the People's Republic of China, will join her this winter to help in this. Dr. Guan's training in neurophysiological techniques is extensive and she should be able to contribute to the work with a minimum of supervision.

Dr. Peter Brodfuehrer will also work for a short time with Cohen to try to record from leech neurons on the optical set-up. Brodfuehrer did his thesis on leech central pattern generation of locomotion and will try to extend his studies through the use of optical recording techniques. He will begin by screening dyes to find those that have little or no effect on the CPG. The initial testing will be done this winter. If these tests are successful, he and Cohen will submit a small grant application to support his work in her lab on the leech CPG.

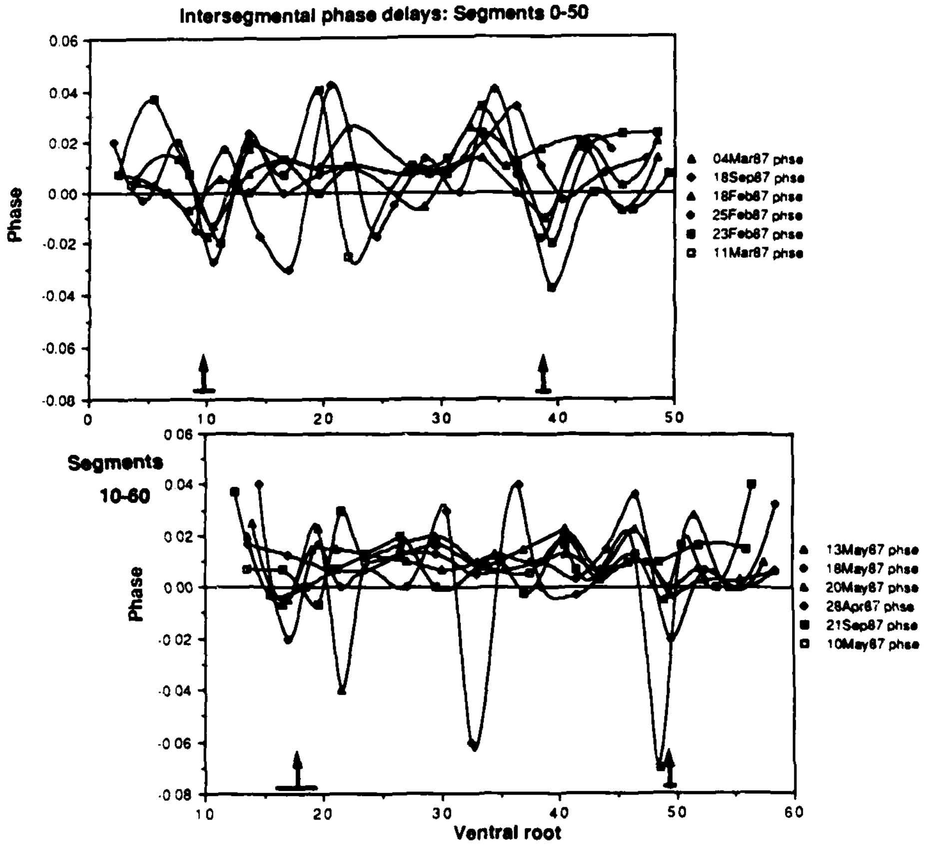


$$\frac{t_1}{T_1} = \phi_{1-25}$$

$$\frac{t_4}{T_4} = \phi_{4-25}$$

$$\frac{\phi_{1-25} - \phi_{4-25}}{3} = \phi_{2.5/\mathrm{seg}}$$

FIG. 1



Intersegmental phase delays: Segments 0-50
Phase
0.06
0.04
0.02
0.00
-0.02
-0.04
-0.06
-0.08
0
10
20
30
40
50
▲ 04Mar87 phse
◆ 18Sep87 phse
▲ 18Feb87 phse
◆ 25Feb87 phse
■ 23Feb87 phse
□ 11Mar87 phse
Segments
10-60
Phase
0.06
0.04
0.02
0.00
-0.02
-0.04
-0.06
-0.08
10
20
30
40
50
60
Ventral root
▲ 13May87 phse
◆ 18May87 phse
▲ 20May87 phse
◆ 28Apr87 phse
■ 21Sep87 phse
□ 10May87 phse

FIG. 2

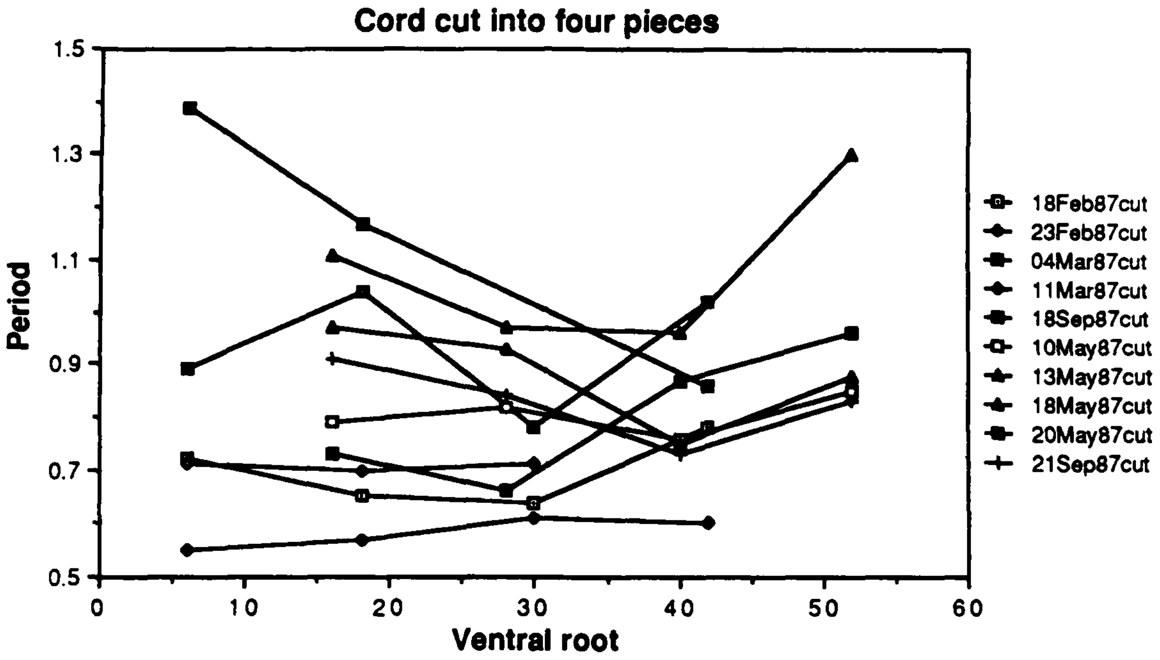

Cord cut into four pieces
Period
1.5
1.3
1.1
0.9
0.7
0.5
0
10
20
30
40
50
60
Ventral root
18Feb87cut
23Feb87cut
04Mar87cut
11Mar87cut
18Sep87cut
10May87cut
13May87cut
18May87cut
20May87cut
21Sep87cut

FIG. 3

Figure 4

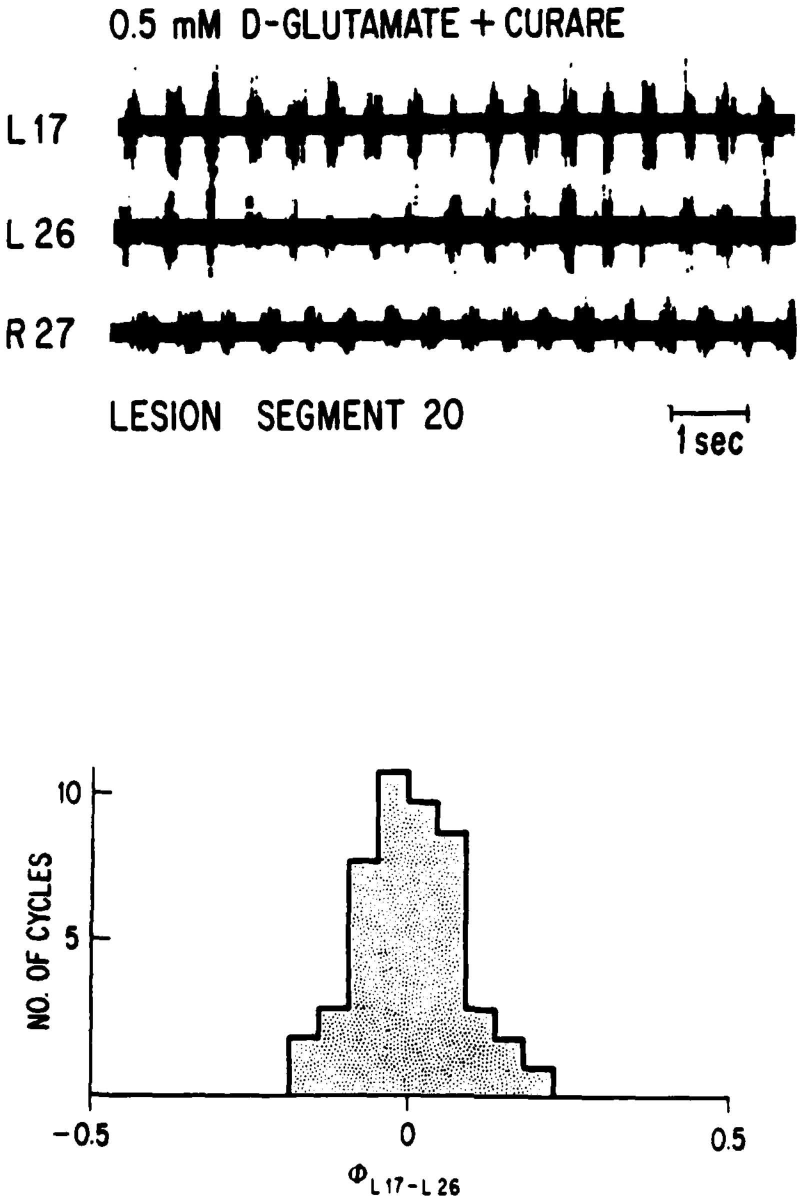


FIG. 4

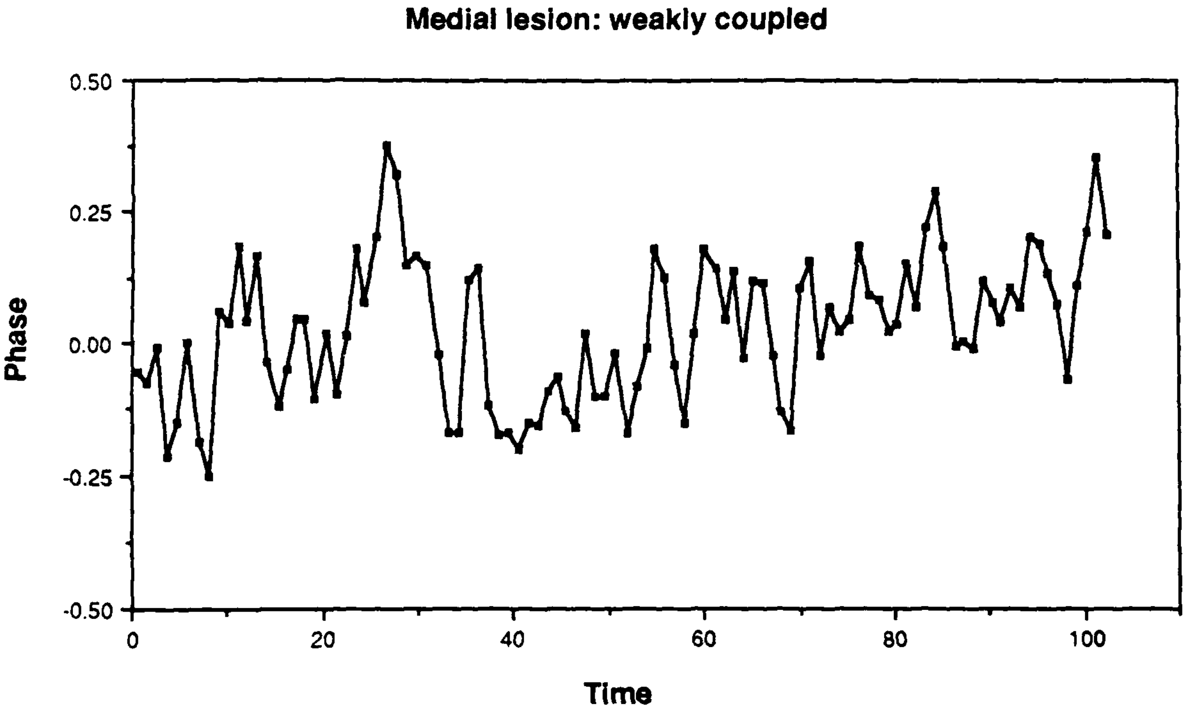

Medial lesion: weakly coupled
Phase
0.50
0.25
0.00
-0.25
-0.50
0
20
40
60
80
100
Time

FIG. 5

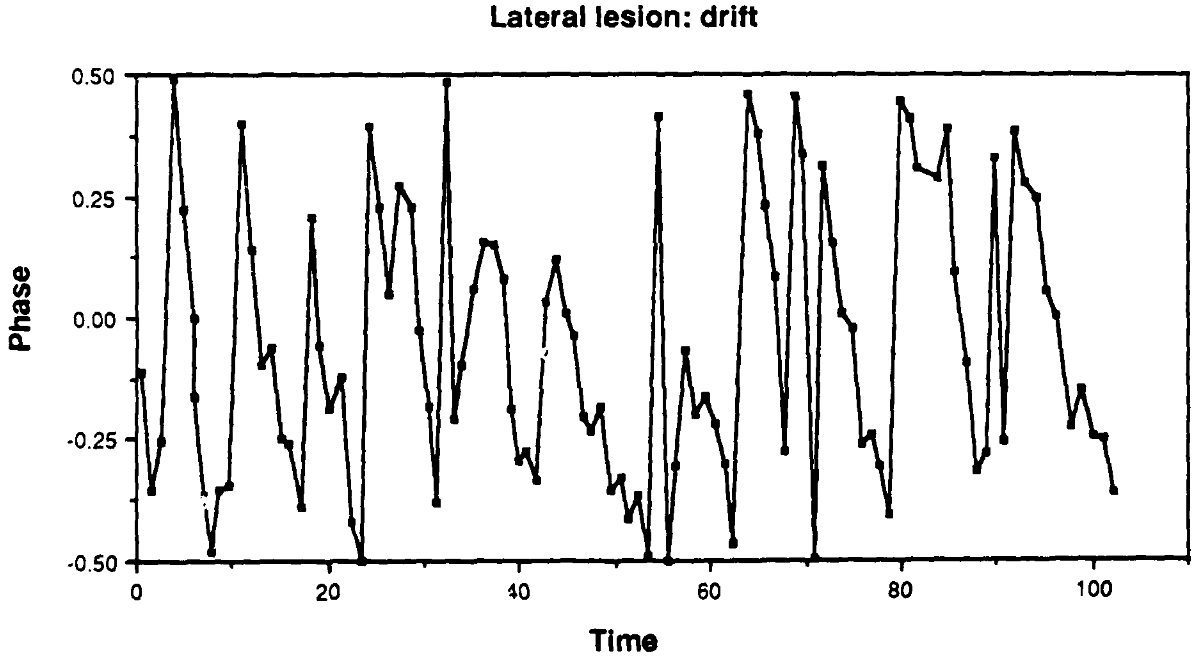

Lateral lesion: drift
Phase
0.50
0.25
0.00
-0.25
-0.50
0
20
40
60
80
100
Time

FIG. 6

FIGURE LEGENDS:

FIGURE 1: Diagram of method for obtaining segment-by-segment phase delays.

FIGURE 2: Segment-by-segment phase delays along 50 segment cord pieces. Upper graph is for segments 0-50 beginning just behind gills. The graphs for each cord vary except for the two rises and dips centered near 10 segments from each end. The arrow indicated the mean of the segment numbers that each cord has a negative phase delay in this region. The horizontal bar is the standard deviation. One cord had no negative dip in the caudal region. Lower graph is the same format but for cord pieces 10 segments caudal to those used to obtain upper graph. Notice the arrows are still about 10 segments from the ends, even though the cord piece is now segments 10-60.

FIGURE 3: Cycle Periods for cord pieces. The mid-segment of the piece is plotted against cycle period. The cords are the same as those used for figure 2 data.

FIGURE 4: Bursting by isolated larval lamprey cords that had been transected. The upper trace is a segment rostral to the lesion; lower trace is caudal to the lesion. Bursting was induced by D-glutamate plus curare. Lower graph is a histogram of the phases which clearly center at a stable value.

FIGURE 5: Phase delays over time of an isolated adult cord. The bursting was by segments rostral and caudal to a lesioned segment in which only regenerated medical tract and gray region were left intact. The phase lags are variable but clearly show regulation which is pulling the activity back and forth to some preferred value.

FIGURE 6: Same as figure 5 except lesioned segment left only lesioned lateral tracts intact. The phase delays demonstrate almost pure drift of rostral and caudal segments.

PAPERS SUBMITTED:

Evidence for functional regeneration in lamprey spinal cord persists in the absence of reflex movements. To: Journal of Neurobiology.

ABSTRACTS SUBMITTED:

Myths and realities of frequency gradients and constant phase lags during lamprey fictive swimming. Society for Neuroscience Abstract.

Evidence for functional regeneration by adult lamprey spinal cords. VA-PVA Symposium.

PAPERS IN PREPARATOIN OR PLANNED:

Studies of the lamprey central pattern generator for locomotion: A close relationship between modeling and experimentation. (Invited for meeting on Dynamic Patterns in Biology).

Evidence for functional regeneration by adult lamprey spinal cords. Science (to be submitted).

Detailed analysis of intersegmental interactions in the isolated spinal cord: Unexpected systematic variations in phase relations. Brain Research or Neuroscience (to be submitted).

V. Work accomplished by Sigvardt and Williams.

1. Control and generation of rhythmicity in the spinal cord. (Alford, Sigvardt and Williams).

In previous work we have demonstrated that rhythmic locomotory output from the spinal cord is elicited by N-methyl-D-aspartate (NMDA) receptor activation (Grillner, et al., 1981). We then showed that there are neurons in the spinal cord that have NMDA-dependent membrane potential oscillations (Sigvardt et al., 1985). It has been hypothesized that inhibitory glycinergic transmission plays an important role in the generation of rhythmicity providing the inhibitory phase of the membrane potential oscillations in ventral horn neurons. However, this year Alford and Williams have demonstrated that the spinal cord networks produce a robust rhythmic output when glycinergic transmission is blocked by strychnine and hypothesize that burst termination is the result of voltage-dependent processes inherent to neurons within the network. Glycinergic transmission would then provide a timing signal to ensure that the networks on the opposite sides of the spinal cord are activated in antiphase. We are now doing experiments to verify the mechanism for burst termination in the presence of strychnine. With glycinergic transmission blocked, the activity on opposite sides of the spinal cord is synchronous. In the coming year, we have planned experiments to determine the locus of this coupling and the neurons that might provide the glycinergic timing signals to the oscillators during naturally- induced activity.

2. Forcing experiments: forced oscillators and intersegmental coupling. (Sigvardt and Williams).

In these experiments a movement-signal was applied to one end of a piece of spinal cord/notochord containing a defined number of segments from a defined anatomical location within the cord and the range of forcing frequencies over which the spinal cord oscillators can be entrained was determined and the intersegmental phase lags measured. It was critical for these experiments that the applied movements were carefully controlled. The movement was produced by a Gould pen motor supplied by the center and controlled by a system designed by Dr. McClellan and me. The usefulness of these experiments for understanding the organization of the spinal locomotory networks depends on the previous observation of McClellan and Sigvardt that mechanoreceptors in the spinal cord transmit movement-related feedback signals onto local oscillators and the changes induced at the local level are transmitted through the intersegmental coordinating system. Therefore, these forcing experiments provide information about both the strength of the feedback system and the intersegmental coordination system. The experiments were designed to address the following questions: 1). What factors influence the range of frequencies over which fictive locomotion can be entrained? a) number of spinal cord segments? b) rostral versus caudal forcing? c) anatomical location (rostral segments versus caudal segments)? 2). Does forcing affect intersegmental coordination (phase lags)? a) are the effects local or global? b) do these changes in intersegmental coordination differ with rostral as compared to caudal forcing? 3). What are the phase relationships between movement and the ventral root bursts? a) Are these relationships related to any of the above factors; e.g. does the relationship between the movement phase and forcing frequency affect the entrainment range? b) How do these relationships compare to those in the intact animal when the feedback loop is unbroken? The experiments were completed this summer and we are now analyzing the data. Plans for further experiments of this type depend on the results of this analysis which will be interpreted within the context of the coupled oscillator theories of Kopell and Ermentrout.

3. Split-bath experiments (Sigvardt, Williams and Kopell).

The coupled oscillator theory developed by Kopell and Ermentrout allows one to make predictions about the effects of various experimental manipulations on the intersegmental coupling within the spinal cord networks. I have done a series of experiments in which intersegmental coupling is examined when the intrinsic frequency of the oscillators in one half of the spinal cord piece is changed relative to that in the other half by bathing the two halfs of the cord in different concentrations of excitatory amino acid. If the inherent frequency of the rostral half of the spinal cord is higher than the caudal half, the phase lags along the cord are longer than normal whereas if the inherent frequency of the caudal half is higher than the rostral half, there is a negative phase lag (backward swimming) at the transition between caudal and rostral and phase lags more rostrally are reduced to zero. We are also planning experiments to raise the inherent frequency in a few segments in the middle part of the cord to see if the waves will propagate outward from this point as the theory predicts. The results of these experiments should provide information about inherent frequency and the strength and timing of ascending and descending coupling in the maintenance of intersegmental coordination.

4. The relationship between muscle activation and movement. (Williams).

In locomotion the movement produced is the result of the pattern of muscle activation generated by the central nervous system. Williams in collaboration with Sten Grillner and his colleagues has examined the match between muscle activation and movement in the lamprey and the trout, two fish that have very different forms of swimming. They have found that the activation of the contractile elements in both animals is approximately in phase with the velocity of that part of the body of the fish that is performing the most work against the water. Dr. Williams has developed a theory relating muscle activation to movement by considering the body of the animal as a damped oscillator with its own natural frequency and then viewing the tension developed by the muscle activation as a driving force extrinsic to the passive oscillator. In this way the phase coupling between the forcing function (muscle activation) and the induced oscillations (movement) at different positions along the body can be compared to the phase coupling in a damped linear oscillator being driven by a simple periodic force at different frequencies. This theory provides an explanation of why the phase between muscle activation and movement changes along the body of the animal. Mathematical questions raised by this conjecture are being investigated by N. Kopell and S. Laederich.

5. Studies of muscle physiology: the relationship between muscle activation and force generation. (Curtin, Sigvardt and Williams).

In order to fully understand the effects of the changing phase between muscle activation and movement down the length of the body one need to know the relationship between muscle activation, tension, shortening and time. Williams and Sigvardt will work collaboratively this year with Curtin to describe these relationships in lamprey muscle so that future models of the neural basis of movement can include these parameters.

6. Methods of data inspection, display and analysis.

a. Strip chart recorder: All of the experiments done in my laboratory involve recording extracellularly from between 2 and six ventral roots along the length of the spinal cord in the *in vitro* lamprey spinal cord preparation during fictive locomotion to test the effects of various pharmacological manipulations, anatomical lesions and applied movements on the intersegmental coordination along the length of the spinal cord. I had in my laboratory all the equipment necessary to do the experiments except a chart recorder for data display and analysis. Since the value of the these experiments depends on precise quantitative analysis of intersegmental phase lags over many cycles at at least two and as many as six points along the length of the spinal cord, a hard copy of all of the data was needed from which to make measurements of phase lags. The center purchased a refurbished Gould 260 chart recorder and accompanying pen motors and amplifiers from Pacer Scientific Instruments. The hard copy of the data can be made on-line or off-line from my FM tape recorder onto this recorder.

b. ASYST training course. (Sigvardt).

On September 15-17, 1987 I attended a training seminar to learn ASYST. ASYST is a scientific software system that allows acquisition, analysis and presentation of data. Furthermore, ASYST is a programming language that is extremely easy to use to develop simple commands or complex programs for data acquisition and analysis. The acquisition system allows one to collect data from most of the widely used A/D boards as well as to create data files from other systems that have been stored on floppy discs. The analysis package allows one to edit data files and easily do any type of mathematical analysis from simple arithmetic to Fourier analysis, statistics and curve fitting. The graphics package provides for simple plots as well as contour plots and several types of charts.

My attendance at this course was sponsored by the center. I am using ASYST for data analysis in my laboratory and plan to teach the system to the other members of the group. In all of our experiments we collect data on magnetic tape. Then relevant segments of the data can be transferred to floppy disc for data analysis using the ASYST system. Since so much of the data collected is of interest to all the members of the group, we think that it is important to standardize methods of data analysis and display. With ASYST we will be able to send data files back and forth between laboratories on floppy disc and develop programs for data analysis and display that can be used by all of us. In my opinion ASYST is an excellent system because it has so many capabilities, is easy to use and the staff in the technical support office are very helpful.

VI

MANUSCRIPTS IN PRESS

McClellan, A.D., and K.A. Sigvardt. Features of entrainment of spinal pattern generators for locomotor activity in the lamprey. J. Neurosci. (In press).

MANUSCRIPTS SUBMITTED

Sigvardt, K.A. Spinal mechanisms in the control of lamprey swimming. In: *Symposium on Axial Movement Systems: Biomechanical and Neural Control*. E.J. Peterson, Ed. *American Zoologist* (submitted).

Williams, T.L., S. Grillner, V. Smoljaninov and P. Wallen. Different strategies for matching activation and movement in lamprey and trout. *Nature* (*submitted*).

MANUSCRIPTS IN PREPARATION

Sigvardt, K.A. and T. Williams. The effects of segment "load", location and directionality on entrainment of the locomotory output of the *in vitro* lamprey spinal cord. Planned for submission to *J. Neurophysiology*.

Alford, S. and T. Williams. Rhythm generation in the absence of glycine-mediated inhibition is mediated by NMDA-receptor activation. Planned for submission to *Nature*.

Sigvardt, K.A., A.D. McClellan, and M.P. Remler. Effects of putative neurotransmitters on generation and entrainment of the spinal locomotory pattern generators in the lamprey. Planned for submission to *Brain Research*.

Alford, S., K.A. Sigvardt and T.L. Williams. Mechanisms for burst termination in motor neurons in the absence of glycine-mediated inhibition. Planned for submission to *J. Neuroscience*.

Sigvardt, K.A. and N. Kopell. Split baths and coupled oscillators: implications for intersegmental coordination. Planned for submission to *J. Neurophysiology*.

Kopell, N., G. Ermentrout, K.A. Sigvardt and T.L. Williams. Coupled oscillator theory and forcing experiments. Planned for submission to *J. Neurophysiology*.

Sigvardt, K.A., A.D. McClellan and T.L. Williams. Excitatory amino acid block reveals strength of entrainment signals. Planned for submission to *Brain Research*.

Alford, S., K.A. Sigvardt and T. Williams. Brainstem activation of spinal locomotory rhythms by NMDA-receptor activation. Planned for submission to *Brain Research*.

Williams, T. Experimental and theoretical studies of segmental coupling in lamprey locomotion. Planned for submission to J. Theoretical Biology.

Curtin, N., K. Sigvardt and T. Williams. Studies on the physiology of muscle in the lamprey *Ichthyomyzon unicuspis*. Planned for submission to J. Comparative Physiology.

VII. Coordinating activities of the Center.

1. Consolidation and Collaboration. Through the efforts of the Center, most of the major researchers (west of Stockholm) investigating the central pattern generator of the lamprey are working together in a joint effort to understand this important model system. Three such investigators have joined the center in the past year: K. Sigvardt, T. Williams and A. McClellan. Many collaborations between members of the center have formed over the year, with joint work performed and research papers planned. These include

a. Entrainment experiments. Sigvardt and Williams have worked together to generate new data. (See Section VI.) A joint paper with Kopell and Ermentrout is planned about the implications of the data.

b. Split bath experiments. Sigvardt has performed some of these experiments, and is planning others. (See Section VI.) A joint paper with Kopell and Ermentrout is planned about the theoretical implications.

c. Kopell plans to write an Appendix to a paper by Cohen discussing the theoretical implications of the edge effects found in her data on phase lags. (See Sections III and V.)

d. McClellan has agreed to provide Williams with some data on mechanical properties of intact, spinalized and dead lampreys, information that is important in the theories of Williams and her mathematical collaborators.

e. Marder is working with Kopell and Epstein on questions relating to the effects of neuromodulators on the output of the lobster stomatogastric central pattern generator. (See Sections III and IV.)

2. Training of graduate students, postdoctoral associates and Center personnel was shared by the members of the Center. For example

a. S. Alfords, a graduate student of Williams, is spending time in the laboratory of Sigvardt, working on a new preparation which will avoid some of the technical problems of working with the isolated spinal cord. So is M. Dobrof, a student of Cohen, who will learn some techniques in histology.

b. The Center sponsored the attendance of Sigvard at a software training course, in which she learned techniques valuable to other members of the Center, and which she expects to teach to other members.

c. S. Laederich, a graduate student of Kopell, is working on mathematical issues raised by the theories of Williams.

3. The funding of equipment, training and travel costs of new members of the Center was accomplished by reallocation of some of the money in the core budget.

4. All but one member of the center convened from Aug. 25 to Aug. 28 to take part in an informal workshop. This workshop functioned for the Center as a large laboratory meeting, in which recent data and unanswered questions were discussed in detail. Some of the themes of that meeting are in the Informal Agenda, in the Appendix.

5. The Center sponsored a widely publicized and well-attended seminar series on "Dynamical Systems in Biology." Speakers were S. Strogatz (Boston University), N.Kopell (Boston University), J. Rinzel (N.I.H.), D. Tank (A.T.&T Bell Labs), S. Geman (Brown University), S. Grossberg (Boston University), G.B. Ermentrout (University of Pittsburgh), C. Peskin (Courant Institute), A. Perelson (Los Alamos) and T. Sejnowski (Johns Hopkins). This series emphasized oscillatory phenomena and network phenomena (neural networks and immunological networks).

6. The Center hosted many visitors, in addition to the ones listed above. Visitors this academic year included D. Ruelle (Institute dec Hautes Etudes Scientifiques), D. Aronson (University of Wisconson), P. Getting (University of Iowa), T. Williams (St. Georges Medical School),K. Sigvard, (University of California, Davis), A. Stretton (University of Wisconsin), R. Eisenberg (Rush Medical Center) and H. Maturana (University of Santiago). C. Rovainen (Washington University Medical School) spent his Fall sabbatical in Boston, and was an adjunct member of the Center. Another such adjunct member, who partipates in most center activities, is S. Giszter, (Bizzi Lab, M.I.T.). Some of these visits were the occasion of the gathering of outlying members of the Center for mini-workshops.

Appendix

Center Workshop - Tues Aug. 25 - Fri Aug. 28

Informal Agenda

1. Overview of lamprey swimming work. What can the reduced preparation tell us? Problems and pitfalls of reduced preparations. Issues of central vs. peripheral control, role of higher structures. Where are various kinds of regulation performed? Etc. This discussion is meant to introduce these topics, but not exhaust them (or us!); we will come back to them as necessary.

2. Some relevant mathematics. An informal tutorial on some mathematics of oscillators, concentrating on results that appear to be directly relevant to experiments that have been done, or in principle can be done.

3. Detailed discussion of experimental results on the isolated spinal cord. Interpretation of results. Issues involving manipulation of data. Discussion of further experiments that would be useful.

    A. Structure of intersegmental coordinating system, frequency and lesion experiments
    B. Entrainment experiments
    C. Effects of changes in pharmacology on phase-lags
    D. Intracellular work, discussion of effects of NMDA and strychnine

4. Initiation of motor activity at different levels.

5. Neuromodulation of small neural networks. This is work motivated mostly by work on invertebrates, such as the lobster stomatogastric ganglion. However, principles appear to be emerging that are likely to be more generally relevant.

6. Regeneration experiments. Central vs. peripheral factors in behavioral recovery.

7. Biomechanics and muscle. Neural waves and mechanical waves. Interpretation, modelling, discussion of further experiments

Participants: N. Kopell, A.H. Cohen, T. Williams, K. Sigvardt, A. McClennan, S. Laederich, S. Alfords, M. Baker, N. Mellon, M. Dobrof, S. Strogatz, E. Marder, S. Giszter.

AFOSR-TR-89-1657

appendix

COUPLED OSCILLATORS AND THE DESIGN OF CENTRAL PATTERN GENERATORS

N. Kopell, Boston University
G.B. Ermentrout, University of Pittsburgh

Abstract:

Much can be deduced about the behavior of chains of oscillators under minimal assumptions about the nature of the oscillators or the coupling. This paper reviews work on such chains, and provides a framework within which implications may be drawn about the neural networks that govern undulatory locomotion in lower vertebrates.

To appear in Math. Biosciences, 1988
and in Nonlinearity in Biology and Medicine,
North Holland Publishing Company, 1988

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Running title: Oscillators and the Design of CPG's

1. INTRODUCTION

One strategy in the study of neural networks is to attempt to find every cell which partipates in the network, and learn all of the major properties of such cells and their interactions. Such a strategy is widely espoused [1]. For small networks, as occur in some invertebrates [2-4], this is a feasible program, which is meeting some success. For large networks, such as those governing even relatively simple processes in vertebrates, it is unlikely that that it can be carried out any time in the foreseeable future; another strategy is required. This is particularly true when the network in question consists of elements that have complex dynamics such as oscillations, so that the behavior of the ensemble is not easily predictable from knowledge of the components and their connections.

Fortunately, much of the behavior of nonlinear oscillators, when forced or coupled to one another, is independent of at least some of the detail of the structure of the oscillators and their coupling. Indeed, mathematics provides some tools, and a framework, for sorting out which details are in fact important; within this framework, it is possible to draw implications from the kind of phenomenological observations that are, or potentially may be, available.

One area in which this point of view can be useful is the study of central pattern generators (CPG's). These are neural networks thought to govern a range of stereotypic motions, including such rhythmic activities as walking, running, swimming, chewing and breathing. It is widely believed that they contain neurons or neural subnetworks that, in the appropriate physiological conditions, are capable of endogenous oscillations [5,6]. The mathematical question to be understood is how these oscillators self-organize to form the appropriate spatio-temporal pattern of signals to the muscles.

We shall concentrate on the CPG's of the lamprey and other fish-like animals. The lamprey CPG is one of the most studied, since it is a vertebrate having a relatively simple spinal cord which can be dissected out and kept alive for further study [7]. It provides a preparation in which rhythmic output can be elicited even when all influence of sensory feedback and descending control from the brain are removed. Furthermore, the relatively simple geometry of the spinal cord also helps in the analysis.

2. THE FISH SWIMMING CPG.

Fish of many species propel themselves through water by rhythmic undulations [8]. The undulations are caused by contractions that pass down the axial muscles (with muscles on the opposite sides of the fish 180° out of phase). These contractions are in turn directed by activity in motoneurons, whose outputs emerge from special positions along the spinal cord called "ventral roots" that are arranged periodically along the cord. Measurements of the activity from these ventral roots, and from innervated muscles, have been made in several species. They have been found to be qualitatively similar to each other and to the measurements from the isolated spinal cord of the lamprey [8,9].

The measurements taken at a ventral root show rhythmic voltage changes (bursts of activity) at a uniform frequency, with a phase lag averaging 1% per segment between any two roots; the lags are approximately proportional to the distance between the roots. Thus, there are travelling waves of electrical activity having an approximately constant wave speed. (Recent data involving finer measurements suggest that, at least in the isolated cord, the phase lags per segment are not so constant along the cord [10,11]). The swimming speed of an intact fish, which is proportional to the frequency of oscillation, may vary over an order of magnitude [8]. A very striking observation is that the wavelength of the electrical and resulting mechanical waves remains constant, at about one body length, independent of the speed of swimming, and this is true as well of the isolated spinal cord [9]. Though this is obviously useful for the mechanical and hydrodynamical properties of the animal, it is not a-priori clear how such regulation is accomplished, and it is one of the achievements of the mathematics to suggest how this can be done.

The work described in this paper is addressed to the question of how the network self-organizes in order to help produce the appropriate phase-lags. (The lack of complete constancy of the phase lags in the <u>isolated</u> cord suggests that the mechanics and sensory feedback may also be important in regulating the lags; this requires further work.) The above observation about constancy of the wave-length constrains the possible models. For example, two mechanisms that are ruled out have waves of activity due to transmission delay or synaptic delay. Since those delays are generally time independent, if the frequency of the oscillations is increased, the delay will occupy a larger fraction of the oscillator cycle, and the phase lags between any two points will increase; this has the effect of decreasing the wave-length. We also note that the constancy of the wave length is a unusual property of nonlinear waves; it is absent in the waves of reaction-diffusion equations generated by local oscillations [12] or waves in nerve-conduction equations [13]. Another constraining observation is that the wave-producing mechanism is highly distributed; any sufficiently long section of the spinal cord can "fictively swim". Finally, an intact animal is capable of temporarily swimming backwards (in which case the electrical wave passes from tail to head), and this is occasionally seen even in an isolated spinal cord [9,11].

The cells participating in the CPG have been only partially identified [14,15]. Nevertheless, there is much indirect evidence for the existence of coupling in both the rostral (toward the head) and caudal (toward the tail) directions. The most compelling evidence involves the recent experiments of Sigvardt and McClellan [16], who exploited the existence of mechanoreceptors in the spinal cord to force the electrical activity at either the rostral or caudal end. By cutting the direct connections from the mechanoreceptor to all but the very end that was being mechanically forced, they insured that any longer range effects were through the intersegmental coordinating system; they found that forcing at either end of a long portion of the cord could entrain the oscillations of the entire piece, thus proving that the coordinating system provides signals in both directions. In other evidence, work of N. Dale shows that if only a portion of the cord is chemically excited, the excitation can pass either caudal or rostral [14]. Finally, when a portion of a cord is cut into two pieces, the frequency of the pieces are in general different from that of the larger piece, suggesting that that the removed connections had effects in both the rostral and caudal directions [10].

For the above reasons, any mathematical description of the CPG must take into account the two-way coupling. The lamprey cord is known to have several coupling systems, operating at different distances [17]. We shall concentrate on the short range coupling, partly because we believe that it will turn out to be the most important for the self-organization, but also because a thorough investigation of the properties and limitations of short-range coupling can give clues to the reasons for the existence of the longer range systems. Further hypotheses on the coupling will be discussed in more detail in Section 4.

We shall treat the network as a chain of discrete oscillators. (A more complete description for some animals, including lamprey, would be a pair of such chains. However, since the analogous oscillators on opposite sides of the cord generally operate in antiphase, it usually suffices to consider just one chain.) We note here, however, that the existence of discrete oscillators, each presumably (though not necessarily) associated with a segment in the spinal cord, has not been established in the lamprey. In the leech, the spinal cord is segmented, and a single segment has been shown to be capable of oscillation [18]; the lamprey spinal cord is not itself segmented, and no piece smaller than 3 segments (of the associated musculature) has yet been shown to be competent to oscillate. Extensions of the work on discrete oscillators, discussed in Section 5D, go in the direction of showing how an unsegmented array of oscillators can behave like a discrete chain.

3. A PAIR OF SIMPLE MATHEMATICAL DESCRIPTIONS.

A. Sine-coupled phase oscillators.

The earliest attempt to model some of the features of the fish swimming C.P.G. was based on the idea that a gradient in the natural frequencies of the oscillators of a chain can cause the formation of travelling waves in the chain [19]. To show this, Cohen et al. used a simple and completely solvable set of equations. The oscillators are represented by phases θ_k, $k = 1,..,N+1$, which, in the absence of interactions, satisfy the equations $\theta_k' = \omega_k$, where ω_k is the frequency of the k^{th} oscillator. The full coupled equations are

$$(1) \qquad \theta_k' = \omega_k + B \sin(\theta_{k+1} - \theta_k) + B \sin(\theta_{k-1} - \theta_k)$$

where B is a measure of the strength of coupling. Travelling waves are phaselocked solutions to (1), i.e., ones for which the phase differences $\phi_k \equiv \theta_{k+1} - \theta_k$ are independent of time; for systems of the form (1), this is equivalent to solutions for which the frequency θ_k' is the same for each k and is independent of time. The equations for the phase differences $\{\phi_k\}$ in a phaselocked solution then satisfy

$$(2) \qquad 0 = \underset{\sim}{\Delta} + B\,\underset{\sim}{M}\,\underset{\sim}{\sin}\,\underset{\sim}{\phi}$$

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where $\underset{\sim}{\phi} = (\phi_1,...\phi_N)$, $\underset{\sim}{\Delta} = (\Delta_1,...,\Delta_N)$, Δ_k is the frequency difference $\omega_{k+1} - \omega_k$, $\underset{\sim}{\sin}\,\underset{\sim}{\phi} = (\sin \phi_1,...,\sin \phi_N)$ and $\underset{\sim}{M}$ is the $N \times N$ tri-digonal matrix with -2 along the main diagonal and 1 along the other two diagonals.

Since $\underset{\sim}{M}$ is invertible, (2) can be solved for any choice of $\{\Delta_i\}$, provided that every component of $B^{-1}\underset{\sim}{M}^{-1}(\underset{\sim}{\Delta})$ is less than one in absolute value. It can be shown that this requirement implies, for a gradient (in which $\{\Delta_k\}$ all have the same sign), that the $\{\Delta_k\}$ must scale like $O(1/N^2)$, i.e., the total frequency difference that can be sustained and still have phaselocking scales like $O(1/N)$.

It is easy to show that, in the presence of a gradient, the solutions to (2), if they exist, do not correspond to constant speed waves, i.e., solutions with ϕ_k independent of k and non-zero [19]. If the oscillators are identical, the solution is synchrony, i.e., $\phi_k \equiv 0$; thus frequency differences are required to get any travelling wave at all.

B. Chains with one-way coupling

Another way to couple oscillators in a chain so as to get a system that is easy to analyze is to assume that the coupling is only in one direction. For such a chain, the analysis of the ensemble reduces to the analysis of any two successive oscillators, with the first driving the second, which drives the third, etc. If the forcing is done by a localized stimulus, such as a rapid pulse, and if the resulting trajectories return very quickly to the limit cycle, the behavior of each pair of forced oscillators can be analyzed using "phase response curve" theory [20,21].

A phase response curve for a forced oscillator gives the change in phase (after the transients have died away) due to the stimulus as a function of the phase at which the stimulus is given. From such a curve, it is possible to calculate at what frequencies the stimulus may be given in order to entrain the oscillator. In general the phase of the forced oscillator at which the stimulus appears when there is entrainment varies with the frequency of the forcing, and can also be calculated from the PRC [22]. Even if the forcing oscillator is identical to the forced one, the phase difference between them need not be zero.

A chain of oscillators, each forcing its successor by a single pulse and whose transients are very rapid as above, can be analyzed using PRC theory. One sees immediately that such a chain can support a constant speed (i.e., constant phase-difference) travelling wave, provided that the oscillators are identical. Furthermore, the wave can go in either direction, i.e., it needn't be in the same direction as the coupling; the direction depends on the shape of the PRC. This direction is unchanged by the presence of a small frequency gradient which, for sine-coupling discussed above, would make the waves travel in the opposite direction. However, for a sufficiently large adverse gradient, the wave can reverse direction. (For a still larger gradient, there is no locking at all.) Such a model has been applied to understand the so-called "metachronal waves" in the swimmerets of crayfish [23].

Both of the above descriptions are useful for understanding some of the properties of the fish swimming CPG, but neither is wholly adequate. As was discussed in Section 2, the coupling is in both directions, not a unilateral forcing. Although the PRC framework can be used to describe two-way coupling, the ease of analysis vanishes, and solutions must be found numerically. More crucially, the PRC framework deals with forcing or coupling by pulses; it cannot deal with coupling that is more spread out within each cycle, with multiple pulses per cycle [4] or graded release of transmitters [24,25]. Indeed, there is mathematical evidence that, unless the coupling is spread out as above, then if the coupling is two-way, there may be no rhythmic activity at all. This is discussed in more detail in Section 4F and 5D.

The major difficulty with the first description is the specificity of the coupling; it is difficult to tell a-priori which of the conclusions that are drawn depend on this particular coupling. As will be seen below, the features that make this example easy to analyze also make it sufficiently "non-generic" that it is a bad guide to intuition. (For example, the conclusion that locking fails for large N unless the total frequency differences scale like 1/N turns out to be generically wrong. Other, more qualitative differences are discussed in Section 4B.) We shall present below a framework that includes the above examples, while avoiding the undesirable restrictions.

4. A VERY GENERAL MATHEMATICAL FRAMEWORK

A. The equations:

Since the composition of any fish swimming network is largely unknown, and the networks can be expected to vary among species, it is desirable to build a theory that is as robust as possible. For the oscillators themselves, our framework is almost completely general. Each oscillator can be any system, a single neuron or a subnetwork, that can be described by an ordinary differential equation having a stable periodic limit cycle. The state variables of these equations can be vectors of arbitrary size, including any variables, such as membrane potentials, firing rates, channel openings, etc., that may be relevant. The oscillators at different positions along the chain need not be the same (for example, they need not have the same frequency), but the changes must be gradual along the chain. The large amount of generality about the oscillators reflects the fact that the behavior of the system is insensitive to the details of the oscillators.

By contrast, as will be seen in this paper, some of the details of the coupling are quite important. We shall start with a few hypotheses; these are needed to carry out the analysis, but we also believe that they represent important constraints on the design of the network, as we argue below in Section 4F and 5D. We shall not assume that the coupling among the oscillators is only between nearest neighbors; indeed, the coupling to somewhat more distant neighbors turns out to have important design implications. (See Sections 4G and 5A.)

We write below the form of the equations to be discussed. For simplicity, they are written only for nearest neighbor coupling; if there is additional coupling, more terms must be added.

$$(3) \qquad X_k' = F_k(X_k) + s_k G^+(X_{k+1}, X_k) + s_{k-1} G^-(X_{k-1}, X_k).$$

In (3), $X_k' = F_k(X_k)$ represents the equation of the oscillator at the k^{th} position. The $\{s_k\}$ are positive numbers representing the strength of the coupling, which is allowed to vary (gradually) along the chain. $s_k G^+$ and $s_{k-1} G^-$ represent, respectively, ascending and descending coupling, which need not be the same. To add coupling with other neighbors, we include terms of the form $G_i^{\pm}(X_{k\pm i}, X_k)$, multiplied by appropriate strengths.

So far the coupling is almost completely general. We now add two hypotheses. The first one is that all the state variables of the oscillators are completely determined by the phases of the oscillators. This is always true, provided that the coupling is not so strong as to overcome the tendency of limit cycle oscillators to maintain their wave-form and amplitudes, and is a fairly benign hypothesis. (See [26] for more details.)

The second is by no means so automatic, and is therefore much more interesting. It is assumed that if the coupling terms in (3) are replaced by their averages over a cycle of the k^{th} oscillation, then the resulting equations have the same phaselocking behavior as (3). If the coupling is "weak", it follows from so-called "averaging theory" that this hypothesis can be made without loss of generality [27]; for large coupling, it need not hold. However, if the coupling signals are sufficiently dispersed around the cycle, instead of being concentrated in a small "window" of phases, the hypothesis can hold even for strong coupling [28]. Furthermore, as mentioned in Section 3B, current numerical and analytical evidence indicates that, when coupled in both directions by pulses, the chain (or even a pair) of oscillators may stop all rhythmic activity. [28]. Thus, we believe that this hypothesis is more than a mathematical convenience. Other hypotheses to be discussed below also emerge as conclusions of the mathematical investigation, i.e., they are further restrictions that are necessary, within this framework, to get the observed behavior, and therefore also constitute possible restrictions on design.

Under the above hypotheses, the full equations (3) can be reduced to a much smaller set of equations describing the interactions of the phases of the oscillators [26,29]. If θ_k is the phase of the k^{th} oscillator, then after averaging, these equations have the form :

$$(4) \qquad \theta_k' = \omega_k + s_k H^+(\theta_{k+1} - \theta_K) + s_{k-1} H^-(\theta_{k-1} - \theta_k)$$

[26,29]. Here, H^+ and H^- are scalar, 2π-periodic functions, and ω_k is the frequency of the k^{th} oscillator. The ability to reduce to phase equations is the first hypothesis; the fact that these equations have the form (4), with the coupling terms dependent only on the differences of the phases, is a consequence of the averaging procedure. Note that equations (2) are of this form, with $s_k \equiv 1$, $H^+(\cdot) = H^-(\cdot) = \sin(\cdot)$. We shall discuss below the behavior of the nearest neighbor equations (4), and return in Section G to the more general case.

B. Electrotonic versus "synaptic" coupling

No further hypotheses are needed in order to be able to analyze the above equations. However, because of the generality of the framework, it becomes possible to see the importance of certain further distinctions. We focus now on the mathematical differences between electrotonic coupling and coupling due to chemical synapses.

For electrotonic coupling (or, equivalently, bulk diffusion coupling), the coupling terms $G^{\pm}(X,\bar{X})$ vanish if $X = \bar{X}$; for the averaged equations, this implies that $H^{\pm}(0) = 0$. By contrast, if two neuronal oscillators are coupled via the release of transmitter substances, they can continue to influence one another even when they are in the same state, and they can, on the average, slow each other down or speed each other up. For the averaged equations, this corresponds, respectively, to $H^{\pm}(0) < 0$ and $H^{\pm}(0) > 0$. (Even if $H^{+}(0)$ and $H^{-}(0)$ have opposite signs, the frequency in a chain of more than two identical oscillators will not in general be the same as the natural frequency. See Section 4C.) We shall refer to coupling which has the property $H^{\pm}(0) \neq 0$ as "synaptic coupling", with the understanding that this is a property that can be held by oscillating subnetworks as well as individual neurons.

Synaptic coupling is important when oscillators are arranged in a linear geometry because it forces the existence of non-zero phase differences among the oscillators. To see this, we let $\phi_k \equiv \theta_{k+1} - \theta_k$, and use (4) to get

$$\text{(5a)} \qquad \phi_k' = \Delta_k + s_{k+1}H^{+}(\phi_{k+1}) - s_k H^{+}(\phi_k) + s_k H^{-}(-\phi_k) - s_{k-1}H^{-}(-\phi_{k-1}).$$

Here $\Delta_k = \omega_{k+1} - \omega_k$, the frequency difference between successive oscillators. Because the oscillators on the ends are connected to only one neighbor each, the first and last equations of (5a) differ from the above in missing the first or last term; since the $\{s_k\}$ are positive, this means that

$$\text{(5b)} \qquad H^{-}(-\phi_0) = 0 = H^{+}(\phi_{N+1}).$$

Phaselocked solutions to (3) or (4) correspond to critical points of (5), i.e., solutions in which the phase differences are independent of time. If there is diffusive coupling of identical oscillators ($\Delta_k = 0$ for all k), and the strengths of coupling do not depend upon k, the network oscillates synchronously, i.e., $\phi_k = 0$ for all k. (Equation (2) can be derived from equations of the form (3) having diffusive coupling.) When the coupling is synaptic, synchrony is impossible even for the above simple case $\Delta_k \equiv 0$, $s_k \equiv 1$: it is easily seen that, because of (5b), $\phi_k = 0$ does not solve the first or last equation of (5a). Thus, synaptic coupling forces non-zero boundary conditions. As will be seen below, this effects the behavior far away from the edges. We believe that synaptic coupling is the primary mechanism for the production of the waves of electrical activity, as we explain further in Section 5.

We remark that the synaptic coupling condition is the analogue for (4) or (5) of the mechanism in Section 3B that produced produced travelling waves ($\phi_k \neq 0$) even with identical oscillators. That is, consider a pair of oscillators having period 1, coupled in one direction by pulses, and let $\theta = 0$ denote the phase at which the coupling pulse is emitted. Then a non-zero phase difference ϕ is produced if the PRC function $\Delta(\theta)$ has a zero $\theta_0 \neq 0$ with $0 < \Delta'(\theta_0) < 2$ for stability; if $\Delta(0) = 0$ and $0 < \Delta'(0) < 2$, synchrony will occur. If the coupling by pulses is not too strong, then averaging theory may be applied, and a function H^- computed as in (4). It can then be shown that the condition $H^-(0) \neq 0$ is equivalent to $\Delta(0) \neq 0$. (See [22] and [30] for more details.)

C. Continuum equations and how they help

It is difficult to find analytically even the time-independent solutions to (5). However, it can be shown [29,31] that the latter solutions are arbitrarily well approximated, for N large, by solutions to an associated continuum equation

(6a) $$0 = \beta(x) + [s(x)f(\varphi)]_x + \frac{1}{N}[s(x)g(\varphi)]_{xx}.$$

Here, $0 \leq x \leq 1$, and $\varphi(x)$ is a continous function whose discretization is the unknown phase differences $\{\varphi_k\}$, i.e., $\varphi_k \approx \varphi(k/N)$. Similarly, if $\beta_k = \Delta_k/N$, then $\{\beta_k\}$ and $\{s_k\}$ are discretizations of some continuous functions $\beta(x)$ and $s(x)$. The 2π-periodic scalar valued functions f and g are computed from H^+ and H^-; for example, if $H^+ = H^- \equiv H$, then f is the even part of H and g is the odd part of H. For a relatively simple class of oscillators and such isotropic coupling, f and g have the form $f = A\cos(\varphi)$ and $g = B\sin(\varphi)$, for some A,B [26]. In the anisotropic case, f and g are related to H^+ and H^- by $(f+g)(\varphi) = H^+(\varphi)$ and $(f-g)(\varphi) = H^-(-\varphi)$. (See [29] for details.) The end conditions (5b) translate into boundary conditions

(6b) $$\begin{aligned} \varphi &= \varphi_L \quad \text{at } x = 0, \text{ where } H^-(-\varphi_L) = 0 \\ \varphi &= \varphi_R \quad \text{at } x = 1, \text{ where } H^+(\varphi_R) = 0. \end{aligned}$$

Equations (6) form a two-point, singularly perturbed boundary value problem. Such problems need not have any solutions, and solutions need not be unique. Nevertheless, it can be shown for these equations that if the range of natural frequencies $\{\omega_k\}$ is not too large, and some not very restrictive technical hypotheses are satisfied, then there is indeed exactly one solution, and there is a stable solution to (5) which converges to this as N grows without bound. One of these hypotheses is a "genuine nonlinearity" condition, i.e., $f'' \neq 0$ over the part of its domain where the solution turns out to lie. The sign of f" plays a role discussed below. We note that the continuum equation (6) is not obtained from (5) by a standard continuum limit, but is more closely related to the idea in physics of a "thermodynamic limit". (See [29] for more details.)

The importance of the equations (6) is that they are much easier to solve analytically than (5), and it is even easier (though often still not trivial) to get approximate solutions to (6). Thus it is possible to use these equations as a kind of "calculus" to figure out the properties of the solutions to (5). To do this, we note that many singular perturbation problems, including (6), have solutions that behave over much of the domain as if the small term (in this case $(1/N)[s(x)g(r)]_{xx}$) were absent. Since the omission of that term leaves a first order equation that cannot solve both boundary conditions, there is a region, called a transition layer, in which that term is significant. Such a layer is usually, but not always, near a boundary, and is then called a "boundary layer".

If the frequencies are monotone (so $\beta(x) \geq 0$ or ≤ 0 for all x) and the $\{s_k\}$ are constant, there is generically a boundary layer at only one end [29]. Then the solution is very close over most of the domain to the solution to the "outer equation"

$$0 = \beta(x) + [s(x)f(r)]_x \tag{7)}$$

satisfying the boundary condition at the other end. Under some robust conditions, discussed in Section 4D, there may be boundary layers at both ends; in this case the solution to (6) is very close to a solution to (7) satisfying a condition at an interior point instead of at a boundary [31].

The choice of boundary condition to be satisfied by the outer equation determines much of the solution. For example, if the oscillators all have the same natural frequency, and the strengths s_k are independent of k, then the solutions $\varphi(x)$ to (7) are constant functions. Thus the boundary condition determines the value of the constant function. If that boundary value is negative, so $\varphi_k = \theta_{k+1} - \theta_k < 0$, there is a wave that goes in the direction of increasing k (forward swimming); if the boundary value is positive, the wave goes in the direction of decreasing k (backward swimming). Furthermore, if the boundary conditions are of opposite signs, as is true if H^+ and H^- are multiples of one another, then the position of the boundary layer determines the direction of swimming. (The boundary conditions need not have opposite signs, in which case the boundary layer may be much smaller, or even nonexistent.) We also note that if $\max|H^+| \to 0$, so that the coupling becomes only descending, then the outer solution approaches the full solution to the uni-directional coupling problem [31].

The boundary condition to be satisfied also determines the frequency. For example, if (6b) is satisfied at $x = 0$, it can be shown that the phaselocked frequency converges, as $N \to \infty$, to the right hand side of (4), with $k = 1$ and $\theta_2 - \theta_1$ replaced by φ_L; a similar computation holds for the other cases. Note that, in this case, the sign of $H^+(\varphi_L)$ determines whether the frequency of the ensemble will be higher or lower than the frequency of the first oscillator; when the coupling is synaptic, it will not be the same as the latter. (If the boundary condition at $x = 1$ is satisfied, it is the sign of $H^-(-\varphi_R)$ that is relevant.)

When there is only one boundary layer, its position is determined in a complicated way from the signs of $\varphi_L - \varphi_T$, $\varphi_R - \varphi_T$, f'' and $Q = 2[f(\varphi_L) - f(\varphi_R)] - \int_0^1 \beta(y)dy$, where the latter measures a balance between the overall frequency gradient and the amount of anisotropy. If H^+ and H^- are multiples of one another, and there is no frequency gradient, it can be shown that for $f'' > 0$, it requires stronger coupling in the forward direction to have forward moving waves; for $f'' < 0$, the opposite is true. (See [29].)

D. The effect of "pacemakers" and "lesions"

If coupling strengths are constant and frequencies are monotonic, then the outer solution (almost) always satisfies one of the two boundary conditions. (The exceptions are special cases, such as identical oscillators and $H^+ = H^-$, which contain symmetries that can be perturbed away.) However, if there is a region in which the frequencies are sufficiently different from the ambient local frequencies a new phenomenon may occur. For simplicity, we describe the almost-isotropic case; the generalizations are in [31]. If the local frequency is sufficiently higher (if $f'' > 0$, or lower if $f'' < 0$), then the outer solution satisfies a condition at the point of maximum frequency instead of at one of the boundaries, and there are in general boundary layers at both ends. (See Figure 1a-c). In this case, waves propagate outwards from the point of highest frequency (if $f'' > 0$, or inwards if $f'' < 0$.). In (1), corresponding to diffusive coupling, this "pacemaker effect" occurs whenever a state of uniform frequency is perturbed by local increase. When there is synaptic coupling, in general there is a threshold for this effect to happen; for smaller local increases in local frequency, the direction of the wave is unchanged, so the synaptic coupling acts to buffer the wave direction against perturbations in frequency. The frequency of the ensemble is also unchanged (to lowest order in 1/N) until the threshold is reached, and then it changes ~~discontinuously~~ (in the limit as $N \to \infty$). Thus, the crossing of this threshold corresponds to a kind of phase transition for the solution [31]. For sufficiently large local increases or decreases in frequency, locking is lost.

Similar effects are found if instead of, or in addition to, nonmonotonic frequencies, we allow the coupling strengths to be locally reduced. (This corresponds to lesions in the spinal cord.) However, the effect of such a "lesion" depends greatly on the context; for example, in a chain of oscillators having a gradient in frequency, the same lesion could act to either locally increase or locally decrease the phase differences, depending on the position of the chain at which the lesion is made. For large enough lesions (but not too large to prevent locking), the waves propagate outwards from the lesion as for pacemakers. See [31] for more details.

F. Averaging and oscillator death

We return now to the hypothesis introduced in Section 3 that the phaselocking behavior of the chain of oscillators remains qualitatively the same if the coupling terms of the k^{th} equation are replaced by ones that are averaged over the cycle of the k^{th} oscillator. As mentioned above, this hypothesis does not hold automatically, except when the coupling is "sufficiently weak". The natural question is then: what is the behavior when the hypothesis is violated?

Numerical simulations and analytic examples indicate that when the hypothesis is badly violated, e.g., when the coupling is by a single strong pulse per oscillator per cycle, the system may cease to oscillate [28]. In its simplest form, this phenomena occurs for a pair of oscillators coupled symmetrically; it is not exhibited by a forced oscillator. A simple example [32] is as follows: Let θ_1 and θ_2 be phases of a pair of oscillators, and suppose that each emits a pulse $P(\theta_i) \geq 0$ with maximal amplitude around $\theta_i = 0$. (See Figure 2.) The effect of the this pulse is presumed to depend on the phases at which it is received, that is, the full equations are

$$\theta_1' = \omega_1 + \alpha P(\theta_2) R(\theta_1) \qquad (8)$$

$$\theta_2' = \omega_2 + \alpha P(\theta_1) R(\theta_2)$$

where α is a measure of the strength of the coupling. The function R plays the same role as the phase response curve described in Section 3. For e.g., $R(\theta) = A \cos(\psi) - B \sin(\psi)$, $B > 0$, and α small, (8) has a stable limit cycle. It is easy to show by phase plane methods that for ω_i close to one another, as α increases, the frequency of the limit cycle goes to zero and a stable critical point emerges. (For $\omega_1 = \omega_2$, it is even easier to check this for solutions satisfying $\theta_1 \equiv \theta_2$.)

This "death" of rhythmicity appears to be very robust. That is, it can be shown that, for large classes of pairs of oscillators whose interactions depend on the phases as well as the phase differences of the oscillators, a sufficient strength of interaction will make the oscillation disappear [28]. The disappearance involves the creation of a stable time-independent solution for the pair, instead of the stable limit cycle. In models that depend on amplitudes as well as phases, such critical points are also found. The same phenomena occur in chains of oscillators.

Since this outcome is clearly to be avoided by networks that must compute an oscillatory pattern, it is natural to ask how an oscillatory network can be designed that will not fail in the above manner. The key observation is that such a failure is due to the non-uniformity in local frequency along each of the cycles; the interactions cause the oscillators to slow down at some portion of the cycle, and, for large enough interactions, to stop. Thus, a design that keeps the local frequency of each oscillator more uniform allows for larger interactions without risking failure. Such uniformization can be accomplished by having interactions between oscillators occur via coupling signals that are dispersed around the cycle, with all (or at least most) of the signals operating in a consistent way (i.e., to phase-advance or phase-delay the receiving oscillator). This is discussed further in Section 5D.

G. Multiple coupling

Most of the results described above for nearest neighbor coupling still hold in the more general situation of multiple neighbor coupling. However, we believe that some added effects of multiple neighbor coupling are of considerable importance.

Multiple coupling is modelled by the addition to (3) of terms of the form $G_i^{\pm}(X_{k\pm i}, X_k)$, multiplied by appropriate strengths. After averaging, this leads to terms in (4) of the form $H_i^{\pm}(\theta_{k\pm i} - \theta_k)$. As in the nearest neighbor case, a continuum equation can be associated with the discrete equation for the phase differences. Away from the boundaries, it has been shown that this continuum equation and the discrete system behave in a similar manner [33]. If K neighbors are connected, the continuum equation has the same form as

(6), with $f(\varphi)$ and $g(\varphi)$ replaced by $\bar{f}(\varphi)$ and $\bar{g}(\varphi)$, where

$$\begin{aligned} \bar{f}(\varphi) &= f_1(\varphi) + \ldots + f_K(K\varphi) \\ \bar{g}(\varphi) &= g_1(\varphi) + \ldots + g_K(K\varphi) \end{aligned} \tag{9}$$

and f_i, g_i are computed from the connections to the i^{th} neighbor. Under very general conditions on the $\{f_i\}$ and $\{g_i\}$, the resulting functions $\bar{f}$ and $\bar{g}$ have the property that for the analogue of the outer equation (7), the effect of frequency differences on phase lags is considerably smaller than when there is nearest neighbor coupling; that is, the multiple coupling acts in such a way as to buffer the phase lags against possible variation in frequency [33]. It also allows for larger frequency differences without loss of coordination in the chain.

The boundary condition to be satisfied by the solution to the new "outer equation" is less understood. If (5b) is replaced by the analogous conditions, namely, the omission of all terms corresponding to oscillators with indices outside 1,...,N, then the resulting solution appears numerically to satisfy a boundary condition close to that of (6b), with $H^+(\varphi)$ replaced by $(\bar{f}+\bar{g})(\varphi)$ and $H^-(-\varphi)$ replaced by $(\bar{f}-\bar{g})(\varphi)$. (This results in values $\bar{\varphi}_L$ and $\bar{\varphi}_R$ that are closer to zero than for nearest neighbor coupling.) However, numerical simulations and formal computations on the discrete equations indicate that, when there is multiple coupling, boundary layers are to be expected on both edges, and these layers can be oscillatory [33].

In some simulations done with a chain of identical oscillators, multiply coupled, there was a pronounced dip in the value of the phase lag at a position corresponding to the number of neighbors coupled. The H^+ and H^- for those simulations were chosen to be compatible with the forcing experiments of Sigvardt and McClellan [16] (see Section 5C). The simulation results resemble the experimentally measured lags, which show such a dip at a distance of about 10 segments from each end of the portion of the cord measured [10]; the effect appears to be almost independent of the position of this portion within the cord. Both theory and experiment need more work to make the results definitive.

5. A FEW CONCLUSIONS AND SOME SPECULATIONS

A. Phase lags and frequency differences

In Section 4, we saw that synaptic coupling implies that there are non-zero phase lags, even if the oscillators and the coupling strengths are all the same. Indeed, we saw that the solution in this case has ϕ_k almost constant (except near a boundary layer). This corresponds to constant speed swimming, with the direction of the wave dependent on the sign of the ϕ_k. Thus, the mathematics shows that, if there is synaptic coupling, constant phase lags can be achieved by regulating the frequencies and coupling strengths to be uniform.

The mathematics also shows that *unless* the frequencies and coupling strengths are uniform, the phase lags cannot be expected to be uniform. In a very early attempt to model the travelling waves in the cord, it was suggested that the waves were due to a gradient in natural frequencies, for which there was some preliminary evidence [19]. According to the above mathematics, if there *is* a gradient in natural frequencies, and if there is not too much anisotropy in the coupling, the earlier model is correct in its assertion that there is a travelling wave starting from the high frequency end. (This is true whether or not the coupling is synaptic. If there is sufficient anisotropy, the direction of the wave may be reversed.) However, these waves never have phase lags that are independent of k, so the existence of a frequency gradient cannot be construed as the primary cause of the constant phase lag waves. Indeed, recent measurements of the phase lag made on portions of the lamprey spinal cord show that there are frequency differences among different parts of each isolated spinal cord, with no constant pattern in the variation from one animal to another [10]. There is also preliminary evidence that the phase lags vary less than might be expected from such frequency variation if there were only nearest-neighbor coupling [10]. These results are consistent with the conjecture that the lamprey cord is highly multiply coupled, and that the multiple coupling performs the buffering function discussed in Section 4G. Simulations involving multiple coupling also predicts edge effects, which were seen in experimentally measured lags [10] (see section 4G). We also recall that synaptic coupling, as opposed to diffusive coupling, buffers the system from allowing small changes in frequency to locally or globally change the direction of wave propagation. For another physiological system for which these ideas are relevent, see [34.].

B. Some physiological mechanisms

It is now easy to envision some general physiological mechanisms which can accomplish the regulation of the phase lags as swimming speed (which is proportional to the frequency of the phaselocked ensemble [8]) is changed. As discussed above, the uniform phase lag that is produced when the oscillators are all the same is determined by the relevant boundary condition (6). Thus, any change that preserves that boundary condition also preserves the phase lags. In particular, if the natural frequencies are uniformly increased, without changing the coupling, there is no change in the equation or the boundary conditions for the phase lags. Such uniform changes could be produced by a uniform increase in descending or ascending tonic drive, and is consistent with known physiology. We also note that the regulation of phase lags under an increase in frequency for all oscillators can take place even if the frequencies are not uniform along the cord.

Similarly, the mathematics suggests mechanisms for changing the direction of swimming. This direction depends on the sign of the r_k. If r_L and r_R have opposite signs, this depends on the position of the boundary layer, which in turn depends on the relative strength of the forward and backward coupling. (See Section 4.) Thus, the direction of swimming may be changed by changing the bias in the coupling that determines the position of the boundary layer; there is no need for a separate network to govern backward swimming. (If both boundary conditions r_L and r_R are negative, which requires significant anisotropy in the coupling, it would take a larger change to produce backward swimming; H^+ and/or H^- must be changed so that r_L and/or r_R is positive.) Backward swimming can also be produced by appropriate changes in local frequency. The above mechanisms can, in principal be differentiated from one another by experiment, but the existing data is not sufficiently fine. (See the next section for some evidence in that direction.)

C. Experimental Manipulations

The mathematical framework allows the prediction of the outcome of various experimental manipulations. One set of manipulations that can be made changes the intrinsic frequency in part of the cord; this is done by partitioning the bath in which the spinal cord is held, and using the dependence of the frequency on the concentration of the excitatory amino acid that is used to evoke the fictive swimming behavior. The mathematics predicts, for example, that if there is a local maximum in frequency that is sufficiently different from the other neighboring intrinsic frequencies, then waves can propagate outwards from that point; thus, a stretch of cord can "swim backwards". One way in which such a local maximum is created is if the natural frequencies at a given concentration have a rostral to caudal gradient (which is frequently seen), and if the frequency of the caudal half is artificially changed. An experiment involving raising the frequency of the caudal half has been done by K. Sigvardt [11], and the backward swimming has been observed. Experiments involving directly raising the frequency in a very small region so as to create a local maximum in frequency have not yet been done.

Another experimental manipulation for which there is at least some preliminary data involves mechanical forcing of the isolated spinal cord. (See Section 2.) Numerical simulations of this forcing produced unexpected results: We found that if H^+ and H^- are multiples of one another (which implies that ν_L and ν_R are of different signs), then phaselocking was difficult to achieve with forcing at one of the ends. For example, if the oscillators have uniform frequency, and the unforced waves travel in a caudal direction with a boundary layer at the caudal end, then forcing at the caudal end (by any frequency different from that of the non-forced phaselocked ensemble) appears to lead to loss of locking, especially if the ascending coupling is weaker than the descending coupling. If H^+ and H^- are such that ν_L and ν_R are of the same sign (e.g., negative, which corresponds to waves in the caudal direction as above), then the forcing at the caudal end does not appear to prevent entrainment, and significant differences in frequency between the unforced phaselocked ensemble frequency and the forcing frequency can be sustained without the loss of locking. It can be shown analytically [35] that if ν_L and ν_R are both negative, then forcing at the caudal end at higher than the unforced frequency of the locked chain causes the phase lags to tend toward or beyond zero, and forcing at the rostral end at higher frequencies causes the lags to become larger in absolute value. (The opposite is true for ν_L and $\nu_R > 0$.) This experiment has been done on the isolated lamprey cord, and locking has been found for a significant range of forcing frequencies, with forcing at either end [16]. Furthermore, the lags behaved as described above for $\nu_L, \nu_R < 0$. There was a much larger effect for caudal forcing, which is consistent with having a stronger descending coupling.

One way to achieve the kind of anisotropy in which ν_L and ν_R have the same sign is to have differences in timing between the coupling signals of H^+

and those of H^- [31]; such differences have been found in the lamprey cord, along with differences in strength, for the part of the coupling due to excitatory synapses [14]. (The ascending excitatory coupling appears to be much weaker.) It is also possible that crossed inhibitory coupling plays a larger role in either the ascending or descending coupling. Since forced chains of oscillators with much weaker ascending coupling do not easily phaselock to a range of forcing frequencies at the caudal end, crossed inhibitory coupling may be important in the ascending coupling. More work on forced chains of oscillators is in progress.

The anisotropy suggested by the forcing experiments have several implications. One is that, if H^+ and H^- are thought of as separate parts of the coordinating system, each alone would produce waves in the caudal direction. Another implication is that backward swimming in the lamprey requires more than a small perturbation in the bias of the coupling. (See the previous section.)

D. Averaging and circuit design

In Section 4F, we saw that oscillators coupled in both directions by means of signals localized within the oscillator cycle may lose rhythmicity and go to a stable steady state, and that this can be avoided by having coupling signals that are more dispersed within the cycle. Numerical work shows that even if the number of coupling signals per cycle is small (e.g., 3 or 4), oscillator death can be prevented [28]. A neuronal oscillator with many coupling signals can easily be constructed if the oscillator is composite, i.e., composed of a set of neurons (each of which may or may not be competent to oscillate) and which can burst at different times in the cycle. The leech segmental oscillator has this property; identified cells in the oscillator have bursts with midpoints, relative to some arbitrary zero, of 0, 90, 180, and 270 degrees respectively [4]; more recently identified cells burst with midpoints near 45 and 240 degrees [36].

As mentioned in Section 2, the lamprey spinal cord is not segmented; there is no anatomical evidence of "unit segmental oscillators" as there is in the leech. However, the above notion of composite oscillators (having cells of many types) can be generalized to be compatible with an unsegmented cord. That is, a description of the cord as a chain of discrete composite oscillators assumes that the coupling among the cells of a particular unit oscillator is very strong compared to the coupling between units. If this restriction is relaxed, we have a set-up in which small portions are competent to oscillate, and there is no clear distinction between intra-oscillator and inter-oscillator coupling.

It is therefore of interest to understand mathematically what indeed does happen when this restriction is relaxed. It is possible to construct composite oscillators, e.g. arranged in a ring, with phase differences within the components of the composite oscillator [37]. Preliminary work has shown that when such composites are coupled, with each element coupled to its neighboring analogues with strengths comparable to the intra-oscillator coupling, the entire ensemble behaves like the chains of discrete oscillators that we have been discussing. It remains to show that the this is also true for more generally constructed composite oscillators.

E. Developmental implications

The above mathematics has implications for the embryological development of motor behavior in fish. Early coordinated motion called "C-coils" in fish (and in all vertebrates) involves simultaneous contractions of muscles on one side of the spinal cord. Somewhat later in development, fish embryos display travelling waves (and S-waves, which involve antiphase coupling of units on opposite sides of the cord) [38]. According to the mathematics, C-coils are to be expected if the neural or muscular units are coordinated electrotonically. Evidence for such early electrotonic coupling has been found [39,40]. For neural units coupled "synaptically", the expected behavior is travelling waves. Thus the mathematics suggests that the change from the C-coils to travelling waves corresponds to the development of appropriate functional synapses. Furthermore, since co-functioning of presynaptic and post-synaptic cells is thought to be useful in the strengthening of synapses, the initial electrotonic coordination may be an important prerequisite for the later organization.

F. Future directions

The work described above concerns the effects of local coupling within a spinal cord isolated from effects of brain stem control and sensory feedback. It was shown that such organization is capable of generating the most basic elements of the stereotypic motion, and of performing certain kinds of regulation (maintaining phase lags while increasing the swimming speed, buffering somewhat against the effects on phase lags of differences in local frequency). However, the phase lags in the isolated cord are not constant along the cord (they average about 1% per cycle but can vary from below 0.05% to above 2% per cycle in different portions of the same cord.) Indeed, the mathematics says that unless the local frequencies and coupling strengths are very carefully regulated, such differences in phase lags are to be expected. In addition, it has not yet been fully investigated how efficiently the very reduced system can respond to the kinds of perturbations that would occur in vivo.

It is unlikely that the regulation of phase lags in the freely behaving animal is performed at the level of the brain. There are two more local levels that have not been taken into consideration in the above analysis. The first involves again just the isolated cord; in addition to the multiple, but local, coupling, there is also believed to be more long-range coupling [17]. We conjecture that the latter coupling may play a role in the correction of inappropriate phase lags. That is, if frequency differences are too large to be buffered by the multiple coupling, or there is a transient perturbation that disturbs the phase lags, long distance coupling can measure and correct a "mismatch" between the actual and desired lags, especially if the desired lag between the two points is approximately 180°; this type of correction is difficult to perform by local interactions, since the phase difference between successive oscillators is so small. The role of this long range coupling during fictive swimming may perhaps be assessed experimentally, since the fibers for long-range coordination lie in a different tract than a portion of the short range connections, and hence can be lesioned without totally eliminating short-range coupling.

The other major source of local regulation is through proprioceptive feedback. Though there is an extensive literature on the hydrodynamics of fish swimming [41-44], until recently, there has been relatively little work on the mechanics of swimming and its relationship to the electrical activity [44-45]. The mechanics is linked to the electrical activity of the spinal cord through mechanoreceptors in the spinal cord [46]. We conjecture that the resultant feedback smooths out the irregularities in the phase lags seen in the isolated spinal cord preparation.

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FIGURE CAPTIONS

1. Schematic drawings of the solutions to (6) with $H^{+} = H^{-} = A \cos \varphi + B \sin \varphi$, $A < 0$.

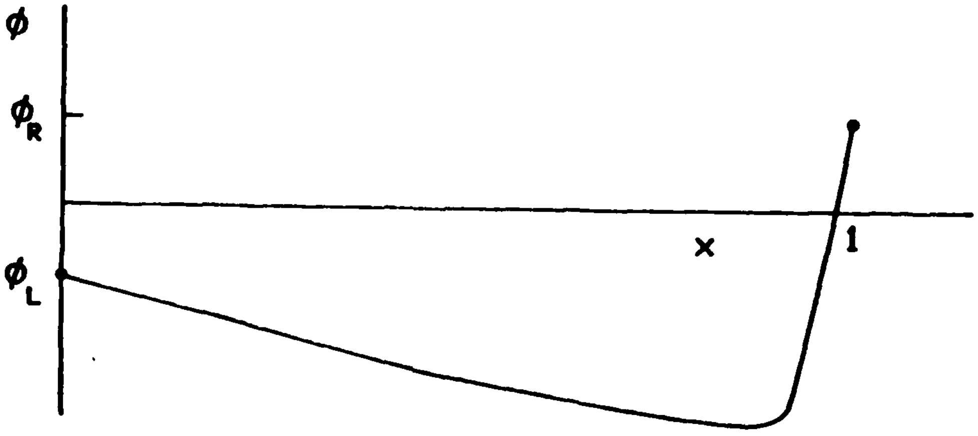
   a) $\beta(x) \equiv -\beta < 0$

   b) $\beta(x) = -\beta + p_1(x)$ where $p_1(x)$ is a small positive bump function with a maximum at x_0. The added frequency has an effect on phase differences only on the support of p_1.

   c) $\beta(x) = -\beta + p_2(x)$, where $p_2(x)$ is a sufficiently large bump function. For $x < x_0$ and away from the boundary, $\varphi(x) > 0$, so the wave goes backward.

   The details of the solutions depend on the signs of φ_L, φ_R, f'' and Q.

2. A pulse function, centered at $\theta = 0$.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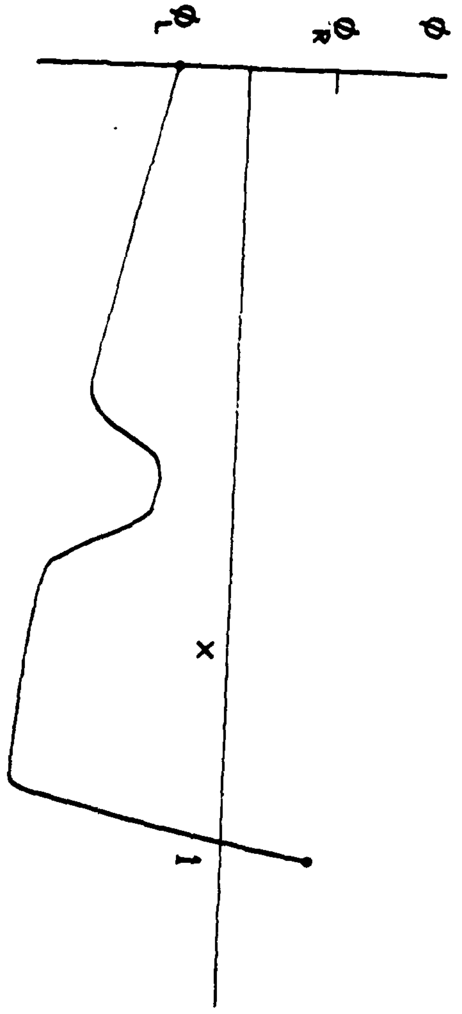
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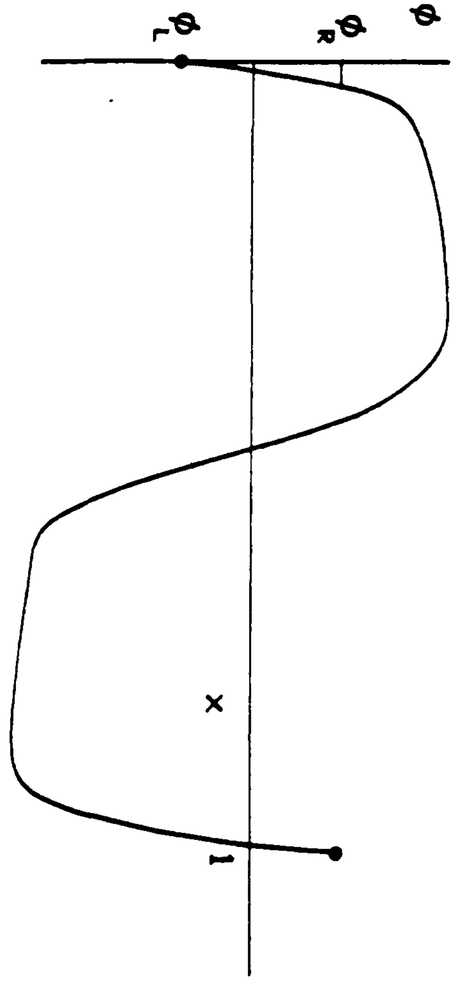
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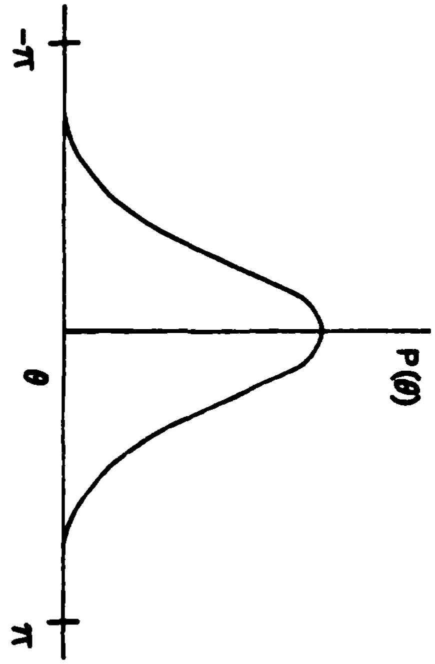
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