Investigation of Serotonin Effects in the Lamprey Spinal Locomotor System

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1 Introduction

A major goal in modelling the lamprey spinal locomotor CPG is to explain how it produces intersegmental phase lags that are both uniform along the cord and frequency-invariant. The frequency-invariance of the phase delay has led researchers to model it at the highest level as a chain of phase-coupled oscillators. In these models, the phase lags between oscillators are determined both by the uncoupled preferred frequencies of the segmental oscillators and the nature of the coupling. However, the relative contribution of these two variables is often difficult to determine experimentally.

Experiments have demonstrated that there is a uniform phase lag of approximately 1% per segment in all cords. When a cord is cut into shorter sections, the burst frequencies of the isolated sections may be similar or may vary by as much as 65% from the frequency of the entire cord [1]. When there is a frequency difference, the rostral end is more often faster, but sometimes the reverse is seen. Although there is a high degree of variability between animals, observed relative differences in preferred frequency along the cord are consistent over time within a single animal. The frequency experiments have some problems in interpretation because the cuts are likely to damage adjacent tissue. However, they are suggestive, and we are currently repeating them using partitions and pharmacological blockade of activity instead of cutting.

The modelling community appears to be converging on the view that coupling properties, and not frequency differences, control the intersegmental phase lags. However, this leaves open the question of what role, if any, is played by the observed differences in preferred frequency along the cord and the non-uniform frequency response to modulatory inputs. We propose to study this problem using an interdisciplinary approach that combines biological experimentation with simulation and analysis of models. Existing models will be analyzed to define their behavior in the presence of systematic variations in the uncoupled frequency of the individual oscillators. Experiments will more accurately measure the preferred frequency differences and manipulate them so their effects on phase lags can be determined. The experimental technique is in some ways similar to split-bath experiments, but uses bath application of serotonin to the entire preparation to produce a more continuous and therefore biologically more natural frequency gradient.

2 Proposed experimental work

The purpose of the proposed experimental work is to more precisely measure the phase behavior of the lamprey spinal CPG in the presence of frequency differences of varying magnitude in the preferred frequencies of the individual oscillators. Combined with further analysis of behavioral properties of chains of phase-coupled oscillators, these measurements should help to determine which models more closely correspond to the behavior of the biological system. The experiments will make use of pharmacological agents to manipulate serotonergic systems within the cord; serotonin is an endogenous neurotransmitter that, when bath applied to the entire spinal cord, produces an increased rostral-to-caudal gradient in the preferred frequency along the cord [2]. This technique will be combined with an inhibitory pharmacological manipulation that allows us to reversibly isolate small sections of the cord.

2.1 Serotonin effects

Cohen and Harris-Warrick showed that a reduction in frequency to approximately 5% of its control value occurs gradually over a range of serotonin concentrations between 10^{-8} and 10^{-6} molar [2]. Over the same range of concentrations, the phase lags between segments increase to roughly 3.5 times their original value. At an intermediate serotonin concentration, at which phase lags have more than doubled over their control values, cutting the cord into three segments reveals an increased slowing of the caudal over the rostral end of the cord, indicating a greater sensitivity to serotonin in the caudal cord.

Recently, others have exploited 5HT's ability in a similar type of experiment [3], but their results have left some significant unanswered questions. It has been hypothesized that the increased phase lags seen when serotonin is added to the bath are, at least in part, due to the resulting increase in the preferred frequency gradient along the cord. However, the concentration dependence of these effects has not been systematically studied, and the relationship between changes in preferred frequency and increases in the phase lags is not known.

2.2 Possible explanations for serotonin effects

If the increased phase lags seen with serotonin are, in fact, produced by the increased frequency gradient, then the degree of change of both frequency gradients and phase lags should be related to each other over the operative range of serotonin concentrations. Further experimental data is needed to determine whether this is the case.

The introduction of a linear frequency gradient in nearest-neighbor models with uniform coupling strength results in increased phase lags in the middle of the cord relative to the ends (for example, see [4].) Other models may produce other characteristic variations in phase lag in response to a gradient in the preferred frequency; characterizing these is a topic for further modelling work (see below.) Previous experimental results with serotonin indicate that it produces a similar phase lag in the rostral and caudal halves of the cord, but the measurement was inadequate to know whether the phase lags were, indeed, uniform along the entire cord.

If variations in preferred frequency along the cord are merely a problem to be overcome by the coupling, then a mechanism should exist in the lamprey to make phase lags relatively insensitive to them. In this case, you would expect phase lags to remain unchanged in the presence of serotonin-induced frequency differences as well, up to some limit that is larger than the frequency differences seen under control conditions. If, on the other hand, frequency differences are actively used in some way to modulate phase lags, you would instead expect to find local differences in phase lags corresponding to the intrinsic differences in the uncoupled frequency response, or a local mechanism that compensates for them without a resulting insensitivity to externally induced changes.

2.3 Experiments

2.3.1 Relationship between frequency and phase lag in the presence of serotonin

To experimentally distinguish between different mathematical models, more detailed data is needed on the relationship between changes in serotonin concentration, in the phase lags, and in the differences in preferred frequencies along the cord. Previous experiments using serotonin have revealed the presence of these effects, but a systematic study has not been done.

We propose to use pharmacological means to isolate groups of segments from one another, so that differences in preferred frequency along the cord can be systematically measured in the presence of differing concentrations of D-glutamate and serotonin. The technique we use to block activity (inhibitory solution: GABA + glycine + low Ca^{+2}) is completely reversible, and is therefore an improvement over the previously used technique of cutting the cord.

Phase lags will be measured in a long section of cord under control conditions ("fictive swimming" induced by bath-applied D-glutamate alone) and in the presence of a range of serotonin concentrations in addition to D-glutamate. Measurements will be taken at six segments spaced out along the cord, so that differences in the phase lags between the middle and the ends of the cord can be detected if they are present.

The frequency response of different parts of the cord will be measured using the same set of solutions. The bath will be subdivided into separate compartments using bridges to pharmacologically isolate short sections of cord, and the preferred frequency of each section will be measured while inhibiting the other sections with the inhibitory solution.

Since serotonin's effects are mediated by a second-messenger system, it is relatively slow-acting. Its coupling effects should therefore decay more slowly than coupling effects that are mediated only by fast neurotransmitters. A second set of experiments is proposed to test for the presence of slow coupling effects, which, if present, may be due to serotonin or to other neuromodulators[1]

We know there are differences from one animal to another in the variations in preferred frequency along the cord. If these differences are compensated for by changes in the coupling properties, then differences in experimental properties such as the uniformity of phase lags in the presence of serotonin should correlate with differences in the relative preferred frequencies seen in the cord under control conditions.

Cords that are found to have significant rostro-caudal differences in preferred frequency will be used for the coupling experiments. The bath will be separated into three compartments using bridges. The entire cord will initially be bathed in D-glutamate to initiate fictive swimming. Either a cell inhibitor or an axonal conduction block will then be used to isolate sections of cord from one another. If a cell inhibitor is used, each section will be sequentially isolated by inhibiting the other two, and the dynamic effects on the active section will be monitored as it moves to its new preferred frequency. If a conduction block is used, the center section will be inhibited to isolate the rostral and caudal ends from each other, and their frequencies will be monitored as they drift apart.

In the presence of slow coupling, newly isolated sections of cord should settle gradually to their new preferred frequency over many cycles, whereas if only fast coupling is present the transition should occur within one or two cycles. Serotonin reuptake blockers and antagonists [2] can be used to determine the extent to which slow coupling, if present, is mediated by serotonin.

3 Proposed modelling work

Mathematical models of the lamprey CPG have traditionally used one of two approaches to producing the phase lags along the cord. One approach is to use differences in the preferred frequency of the oscillators in the chain to produce phase lags with a synchronous coupling function [4]. The other is to introduce non-synchronous coupling (e.g. a non-zero preferred phase shift) to produce a phase lag with uniform preferred frequencies [5].

However, models which rely on non-synchronous coupling to produce phase lags typically do not exhibit uniform phase lags in the presence of variations in preferred

[1] For the purposes of this discussion, neuromodulators are defined as interneuronal messengers whose time course of action is slow relative to the burst frequency.

[2] Grillner's colleagues have identified a serotonin antagonist for the system and have agreed to share the information with us.

frequency, and models which rely on frequency differences to produce phase lags fail to exhibit any phase lag at all when the frequencies are uniform along the cord. Also, nearest-neighbor models generally do not maintain uniform phase lags in the presence of continuous variations in preferred frequency, relying instead on differences between the two end oscillators and the rest of the chain. The phase response to a preferred frequency gradient in models with long-range coupling is only known for a few special cases.

Experimentally induced gradients in preferred frequency produce corresponding changes in the phase lags. However, frequency differences seen in cutting experiments are more difficult to explain. Natural frequency differences will be re-examined as described above; if these experiments yield similar results, then a more sophisticated model may prove necessary to reproduce the experimental results.

There may also be inter-animal variability in the mechanisms used to produce intersegmental phase lags, where the relative predominance of coupling-induced and frequency-induced phase lags is determined by genetic differences or a combination of genetic and developmental effects. Studying these inter-animal differences may also shed light on the causes of observed inconsistencies in responses to forcing [6]. There have also been major inconsistencies reported in responses to imposed frequency differences [3, 7, 8], which could perhaps be explained either by individual differences or variations in frequency or coupling along the cord.

These issues will be addressed through computer simulation and mathematical analysis of different phase-coupled oscillator models. The proposed modelling work is outlined below.

1. Determine which of the current models best fit, or cannot be ruled out by, old and new experimental data. Possible models include nearest-neighbor models and long-range coupling with a coupling strength that decays either inversely with distance or exponentially, and with tuned or untuned phase lags.

2. Further test these models to determine their behavior in the presence of variations in preferred frequency along the cord. Compare the simulation results with the new data on serotonin effects.

3. If experimental and simulation results indicate the need, develop a more sophisticated model that combines non-synchronous coupling with a mechanism to utilize or compensate for differences in the preferred frequency of individual oscillators.

4. Study how individual differences might develop (e.g. through a learning process.) Examine the question of which coupling and frequency differences would be expected to co-occur in a given animal.

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