Multistability in Recurrent Neural Loops Arising From Delay

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Foss, Jennifer and John Milton. Multistability in recurrent neural loops arising from delay. J Neurophysiol 84: 975–985, 2000. The dynamics of a recurrent inhibitory neural loop composed of a periodically spiking Aplysia motoneuron reciprocally connected to a computer are investigated as a function of the time delay, τ, for propagation around the loop. It is shown that for certain choices of τ, multiple qualitatively different neural spike trains co-exist. A mathematical model is constructed for the dynamics of this pulsed-coupled recurrent loop in which all parameters are readily measured experimentally: the phase resetting curve of the neuron for a given simulated postsynaptic current and τ. For choices of the parameters for which multiple spiking patterns co-exist in the experimental paradigm, the model exhibits multistability. Numerical simulations suggest that qualitatively similar results will occur if the motoneuron is replaced by several other types of neurons and that once τ becomes sufficiently long, multistability will be the dominant form of dynamical behavior. These observations suggest that great care must be taken in determining the etiology of qualitative changes in neural spiking patterns, particularly when propagation times around polysynaptic loops are long.

INTRODUCTION

A large body of experimental and theoretical evidence indicates that abrupt and lasting changes in neural spiking patterns can be produced in certain neurons (Canavier et al. 1993, 1994; Fitzhugh 1969; Guttman et al. 1980; Houssgaard et al. 1988; Lechner et al. 1996), simple neural circuits, such as central pattern generators (CPGs) (Canavier et al. 1999; Harmon 1964; Hopfield 1984; Kleinfeld et al. 1990), and large neuronal populations (Kelso 1995; Kelso et al. 1992; Krüse and Stadler 1995; Milton 2000; Wilson and Cowan 1972) by applying a brief perturbation, such as an electrical pulse. This phenomenon is referred to as multistability; each qualitatively different spiking pattern as an attractor. In contrast to the effect of neuromodulators that change neural spiking patterns through their effect on membrane properties such as ion conductances (Benson and Levitan 1983), in a multistable situation changes in spike patterns occur because the effect of the perturbations is to cause a switch between the co-existent attractors. Multistability has been associated with a large variety of cortical functions including memory (Barnes et al. 1997; Cowan 1972; Foss et al. 1996; Mensour and Longtin 1995; Zipser et al. 1993), visual perception (Krüse and Stadler 1995), and motor control (Eurich and Milton 1996). Moreover, it has been suggested that it might be possible to develop therapeutic strategies based on the manipulation of multistability to treat diseases such as epilepsy (Milton 2000).

Multistability also arises in time-delayed feedback mechanisms (an der Heiden and Mackey 1982; Foss et al. 1996, 1997; Ikeda and Matsumoto 1987). In particular, multiple oscillatory attractors have been observed in experiments involving laser optical devices (Aida and Davis 1992; van Tartwijk and Agrawal 1998) and electronic circuits (Foss et al. 1997; Losson et al. 1993). Time delays are an intrinsic property of the nervous system and arise because axonal conduction times and distances between neurons are finite. The propagation time through a polysynaptic loop is even further prolonged by the effects of synaptic mechanisms and neural integration times (Milton 1996). However, relatively little attention has been given to the possibility of time-delay-related multistability by the neurobiological community with the notable exceptions of modeling studies (Foss et al. 1996, 1997; Mackey and an der Heiden 1984; Plant 1981). An essential requirement for multistability to arise in a delayed feedback loop is that the time delay be longer than an intrinsic time scale of the element experiencing feedback, such as the period of an oscillation (Foss et al. 1996, 1997) or a response time (Ikeda and Matsumoto 1987; van Tartwijk and Agrawal 1998). In the CNS, axonal conduction times (Miller 1994; Nunez 1995; Waxman and Bennett 1972) and propagation times through polysynaptic loops (Eurich and Milton 1996; Iijima et al. 1996; Miles et al. 1988; Milton and Longtin 1990; Villa and Abeles 1990; Wright and Sergejew 1991) approach several hundreds of milliseconds. Since neural spike frequencies can exceed 10 Hz (Abeles et al. 1993; Rapp et al. 1985; Richmond and Optican 1990; Zipser et al. 1993), i.e., inter-spike intervals can be less than 100 ms, we anticipate that multistability might occur.

Here we investigate the neural spiking patterns that are produced by the delayed neural feedback loop shown in Fig. 1A. This circuit is constructed from a neural oscillator that is subjected to delayed feedback. The neural oscillator may be a single neuron, a CPG, or a neural population; the feedback loop may represent, for example, respectively, a recurrent inhibitory loop in the olfactory cortex or hippocampus, peripheral sensory feedback to a CPG, or feedback from brain stem and subcortical structures to an epileptic cortical region. These feedback loops are pulse coupled, i.e., the coupling is characterized by a pulse-like interaction between the neural oscillator and the loop. This pulse coupling is a direct consequence of the fact that when neurons are physically separated, interactions between them are in the form of discrete synaptic potentials (PSPs) driven by spikes. The critical parameter for determining...
the dynamics of a pulse-coupled neural network is spike timing (see, for example, Judd and Aihara 1993). Consequently all of the physiological processes involved in the transmission of information around the neural loop can be incorporated into a single number, the time delay. To implement this loop experimentally, we developed the recurrently clamped neuron paradigm shown in Fig. 1B: the membrane potential of a spiking Aplysia motoneuron is monitored by a computer. Each time the motoneuron spikes, the computer, after a time delay \( t \), injects an inhibitory current pulse (ICP) to mimic inhibitory postsynaptic currents (IPSCs), which would be caused by natural neural feedback. Although single cells have been found to exhibit multistability, the type of multistability that we study here results entirely from the effect of delay on the feedback loop. As will become apparent, the advantage of studying this feedback loop is that the dynamics can be predicted from experimentally measured parameters. Thus it is possible to directly compare theory and observation.

Our observations are presented in three parts. First, we demonstrate that qualitatively different neural spiking patterns co-exist in the recurrently clamped Aplysia neuron shown in Fig. 1B. This multistability occurs primarily when the time delay, \( \tau \), in the feedback loop is longer than the intrinsic period, \( T \), of the spiking Aplysia neuron. Second we demonstrate that the multistability that occurs in the recurrently clamped neuron can be understood in terms of a mathematical model that incorporates two experimentally measurable parameters: \( \tau \) and the phase resetting properties of the neuron. Finally, based on this model and known phase resetting properties, we predict that the dynamics that occur when the Aplysia motoneuron in the feedback loop is replaced by bursting and beating neurons (Dror et al. 1999; Schindler et al. 1997) will be qualitatively similar. Moreover, numerical simulations show that in all of these cases, as \( \tau \) becomes sufficiently long, multistability becomes the dominant behavior expected for neural oscillators subjected to pulse-coupled delayed feedback.

**METHODS**

**Slowly adapting Aplysia motoneurons**

Slowly adapting buccal motoneurons of Aplysia californica were used. Aplysia care, immobilization, and dissection were carried out as described in Church and Lloyd (1994). Unless otherwise stated, experiments were performed in artificial seawater with high divalent cations (33 mM Ca\(^{2+}\), 165 mM Mg\(^{2+}\)), which raises the firing threshold, thereby reducing spontaneous activity in the ganglion. Typical input resistances were \( \sim 2-5 \) M\( \Omega \).

**Recurrent neural clamping device**

The design of the recurrent neural clamping device is similar in spirit to that previously used to “dynamically” clamp neurons (Sharp et al. 1993a,b) and crayfish stretch receptors (Diez Martinez and Segundo 1983; Kohn et al. 1981; Vibert et al. 1979), except that a time delay, \( \tau \), is explicitly inserted.

Periodic spiking was induced by injecting a depolarizing DC current step (2–6 s) with an AxoClamp 2B amplifier in bridge mode. The smallest DC current (usually 1–20 nA) that caused the neuron to fire repeatedly (inter-spike intervals 20–200 ms) and reliably was used for all subsequent experiments. Under these conditions, the neurons fired several spikes before reaching their highest firing frequency which then gradually declined over 2–5 s. All experiments were started when the change in firing rate was smallest and conducted during the following slow adaptation period.

The membrane potential is monitored through the recording electrode of the AxoClamp. An analog signal proportional to the mem-

![Image](https://i.imgur.com/976JFOSSANDJMILTON.png)
brane potential is passed to a Pentium 66 computer via an A/D board (AD 2210, Real Time Devices). The computer detects neural spikes and when feedback is running, each time the computer detects a spike, at a time \( \tau \) after, it triggers the AxoClamp to inject a single square hyperpolarizing current pulse (ICP; \(-5 \) to \(-25 \) nA) into the neuron, thereby closing the feedback loop. This feedback is allowed to run for lengths of 25–75 times the normal firing period. For the first \( \tau \) amount of time that the feedback is on, there will ordinarily be no pulses because of the delay. During this time, it is possible to simulate various initial conditions of the system by adding a patterned pulse sequence of length \( \tau \). In this way it is possible to identify the co-existing qualitatively different spiking patterns.

**Phase resetting**

Neurons were induced to fire periodically using the same conditions as during the feedback experiments. Phase resetting curves were measured by injecting a single ICP and measuring the effect on the resulting inter-spike interval (see RESULTS). Because of the variation in the neuron’s period from one trial to the next and because the firing rate of the neuron accommodates within a run, each phase resetting and feedback experiment had a control run before it to establish the average spiking frequency of the neuron at that time.

**RESULTS**

**Multistability**

Figure 2, A and B, shows typical spike trains generated by a recurrently clamped *Aplysia* neuron when \( \tau \) is greater than its normal firing period. Both patterns were observed for the same choice of ICP and delay. They differ in the position of the ICPs and in the spiking frequency. In Fig. 2C, a brief electrical pulse causes a change from the pattern in B to the one in A. After the new pattern is established, a second electrical pulse causes the system to go back to the spiking pattern seen in Fig. 2B. Herein we refer to each stable pattern of neural spiking as an attractor. The observations in Fig. 2, A–C, demonstrate that there is multistability, i.e., at least two attractors are present.

Figure 2D shows the same neuron and \( \tau \), but the timing of the second added pulse is different. In this case no second switch in spiking pattern occurs. This illustrates the finding that the time at which the electrical pulse is added is critically important for causing switches between attractors. Previous studies have shown that randomly timed pulses have a low probability of causing switches between attractors in the bursting R15 neuron of *Aplysia* (Lechner et al. 1996).

**Model**

We developed a mathematical model for the recurrently clamped neuron with the goals of determining the number of attractors which co-exist as a function of \( \tau \) and the timing of electrical pulses to cause switches between the co-existent attractors. Since our focus is pulse-coupled neural oscillators, our model comes from the perspective of the phase resetting properties of neural oscillators. The advantage of this approach is that the phase resetting properties of a neural oscillator can be readily measured experimentally. We first discuss the phase

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**FIG. 2.** Patterns that arise in membrane potential as a function of time for an *Aplysia* motoneuron during delayed feedback stimulation. The neuron is stimulated to fire tonically, period 95.8 ms, with a long step current of size 3 nA. Delayed feedback, delay 3.29 times the normal period, takes the form of rectangular pulses of size \(-15\) nA and width 5 ms, superimposed on the step current. A: a stable high-frequency pattern in which feedback pulses occur early in each inter-spike interval. B: a stable low-frequency pattern in which feedback pulses occur late in each inter-spike interval. The patterns shown in A and B are achieved in the same neuron with all experimental parameters equal by using different starting conditions (see Recurrent loop spiking patterns). C: the same setup as A and B, starting in the same pattern as B. At the 1st *, an extra pulse is applied with the same parameters as the feedback pulses, which causes the system to switch to the pattern in A after some transient behavior. At the 2nd * another extra pulse is given that switches the system back to the original pattern. D: this experiment is the same as the one shown in C except that the relative timing of the second extra pulse is slightly different. After some transient behavior the system remains in the same pattern.
resetting properties of a regularly spiking *Aplysia* buccal motoneuron and then present the model (see also APPENDIX).

**Phase resetting properties**

The effect of a stimulus on spike timing of a periodically firing neuron has extensively been studied both experimentally (Perkel et al. 1964; Pinsker 1977a,b; Schindler et al. 1997) and theoretically (Best 1979; Glass et al. 1984; Winfree 1980). The effect of a perturbation on the dynamics is summarized by the phase resetting curve (PRC) (Glass and Mackey 1988; Glass et al. 1984; Winfree 1980). The PRC is measured by introducing ICPs at different phases, \( \Phi \), and measuring the phase reset, \( \Delta \), which results. The new phase, \( \Phi' \), can be easily calculated from \( \Delta \) by using the relation

\[
\Phi' = \Phi + \Delta
\]

For the data in Fig. 3A, \( \Delta = -0.3 \).

There are several conventions for expressing the phase resetting curve. We have chosen one used frequently in the literature which is a plot of the new phase, \( \Phi' \), versus the old phase, \( \Phi \) (Best 1979; Glass and Mackey 1988; Guevara et al. 1981; Winfree 1980). Figure 4 shows the PRC obtained when the resetting pulses are naturally occurring IPSCs. This PRC was prepared by stimulating an inhibitory interneuron with a step current to cause an IPSP in the periodically firing *Aplysia* motoneuron. The PRC prepared in this manner has the same overall shape as that obtained using the artificially generated ICP (compare Fig. 3B to Fig. 4). These observations suggest that the PRC for the *Aplysia* motoneuron is not very sensitive to the type of stimulus used.

Equation 1 provides a simple means to compute the effect of a stimulus on the phase of the neuron. The stimulus is assumed to be of rectangular shape, and the phase of the stimulus is defined as the phase at which the stimulus pulse ended. The phase of the neuron at the time of occurrence of the stimulus pulse is denoted by \( \Phi_s \), and the phase at the time of occurrence of the next spike is denoted by \( \Phi_{n} \). Using these definitions, the phase difference between the stimulus pulse and the next spike is given by

\[
\Delta = \Phi_{n} - \Phi_{s}
\]

For the data in Fig. 3A, \( \Delta = -0.3 \).

**FIG. 3.** A: membrane voltage as a function of time for a tonically spiking *Aplysia* buccal motoneuron. A 3-nA depolarizing step current was injected into the motoneuron to cause it to fire regularly. The phase of the periodically firing neuron was reset by injecting a single \(-15\)-nA rectangular pulse of duration 5 ms. - - - , the neuron’s membrane potential during a control experiment in which no hyperpolarizing pulse was given (the control trace was translated to line up with the experimental trace at the spike previous to the pulse). Prior to the resetting pulse the spiking period of the neuron was 97.2 ms. The resetting pulse caused the inter-spike interval containing the pulse (\( t_1 - t_0 \) in Eq. 2) to be 1.3 times the normal period \( \Delta(\Phi) = -0.3 \). The resetting pulse was given at phase 0.65 (Eq. 1), the phase after reset was 0.35 (Eq. 3). B: the phase resetting curve for the neuron in A, collected by injecting rectangular pulses as in A at several deferent phases. - - - , the line of no phase resetting (\( \Phi' = \Phi \)). --- , the curve fit with the polynomial-sigmoid function (see APPENDIX) with parameters \( a_0, a_1, a_2, b_1, b_2 \), respectively, \(-0.145, -0.427, 0.0256, 18.5, -0.981 \) (rms error 0.0039).
MULTISTABILITY IN NEURAL LOOPS

FIG. 5. Schematic showing \( \Phi \) as a function of time for a delayed feedback loop. —, the phase; \( \Phi_1, \Phi_2, \ldots \), the positions of spikes. The horizontal — shows a spike, the pulse it generates at a time \( \tau \) later when the phase is \( \Phi_m \). The inter-spike intervals which occur in the delay interval to be considered are \( \Phi_1, \Phi_2, \ldots, \Phi_{n-1} \). The elapsed time between \( S_1 \) and \( \Phi_1 \), and between \( \Phi_{n-1} \) and \( S_2 \), represents the fixed delay interval. The continuation of this construction to \( n \) spikes is shown in Fig. 5. Each time the neuron spikes (indicated by — — —), an ICP is delivered at time \( t \). The numbers \( N_1, N_2, \ldots \) denote the number of ICPs which occur. To illustrate the procedure, a schematic representation is shown in Fig. 5. Each time the neuron spikes (indicated by — — —), an ICP is delivered at time \( \tau \) later (indicated by the horizontal solid line). We denote \( \Phi_n \) as the phase of the \( n \)th ICP, due to a spike \( S_n \). Although it is possible to first calculate the time that the \( n \)th ICP is delivered and then determine \( \Phi_n \), it is much more efficient and straightforward to perform all calculations in the phase domain. The phase at which the ICP is delivered depends on the number and timing of any ICPs that occurred in the interval between \( S_1 \) and \( \Phi_1 \). Except when spikes and ICPs occur, phase increases continuously with time at a rate \( \Delta(\Phi_1) \). The numbers \( C_1, C_2, \ldots \) denote the number of spikes which occur in the delay interval to be considered. The numbers \( C_1, C_2, \ldots \) denote the number of spikes which occur in the delay interval to be considered. Thus the dynamics of the recurrent loop are described by a piecewise defined iterative map in \( \Psi \). The iteration steps are: determine \( k \) from Eq. 8; compute \( \Phi_i \) from \( \Psi_i \); determine \( \Delta(\Phi_i) \) for any given value of \( \tau \). Describing the cells dynamics as a sequence of \( \Psi \) values carries more information than simply giving the sequence of \( \Phi_i \)s. Furthermore, keeping track of past values of \( \Psi \) makes it simple to determine \( k \) without explicitly examining the interval, as was done, for example, to generate Eq. 4.

The basis for multistability in a delayed recurrent loop can be readily appreciated by determining the fixed points, \( \Psi^* \), of Eq. 7 for any given value of \( \tau \). The fixed points, i.e., when all \( \Psi_n = \Psi^* \), are the solutions of the equation

\[
f(\Psi) = g(\Psi)
\]

where

\[
f(\Psi) = \Psi - \frac{\tau}{T}, \quad g(\Psi) = k(\Psi)
\]

and \( k \) is \( \tau \) rounded down to the nearest integer, from Eq. 8. Equation 9 can be solved graphically by plotting \( f(\Psi) \) and \( g(\Psi) \) separately as functions of \( \Psi \) on the same axes (Fig. 6); the horizontal coordinates of the points of intersection correspond to the fixed points, \( \Psi^* \), of Eq. 7. It can be shown that the condition for these solutions to be stable is that \( S^* \), the slope of \( \Delta(\Phi) \) evaluated at the fixed point, satisfies \( -1 < S^* < k^{-1} \) (Foss 1999).

Figure 6 illustrates this approach for the case of a recurrent inhibitory loop involving an Aplysia neuron. When \( \tau T < 1 \) (Fig. 6A), having more than one fixed point, and hence multistability, becomes very unlikely unless there is positive resetting (see DISCUSSION). Having more than one fixed point becomes more likely when \( \tau T > 1 \) (Fig. 6B and C). In the case of this Aplysia neuron and many other living and model neurons, the stability condition can be satisfied for more than one critical point for given ranges of \( \tau \) (this communication). Thus it becomes possible to observe multistability experimentally in delayed recurrent loops once the delay becomes sufficiently...
numerically obtain the steady state value of $C$. FOSS AND J. MILTON

the time delay for points of intersection of $f$ and $g$. The delay and all other experimental

initialize the system. Figure 7 shows membrane potential traces for different values of $n$. It should be noted that some neurons and other types of limit cycle oscillators can give rise to behaviors more complicated than multiple stable co-existent fixed points (see, for example, Foss et al. 1997).

Recurrent loop spiking patterns

We used two different strategies to compare the prediction of Eq. 7 to the experimentally measured dynamics of the delayed recurrent loop. First, we determined whether the same initial condition leads to the same neural spiking pattern in the experiment and the model. When two or more spiking patterns co-exist, there must be sets of initial conditions that produce one or the other spiking pattern. In the recurrently clamped neuron, the initial conditions correspond to neural spike patterns of length $t$. In the experiment, for the first $t$ seconds that the feedback is turned on, there are no ICPs (the earliest time that an ICP can occur is $\tau$). During this time it is possible to use the computer to insert a given pulse pattern of length $t$, i.e., to initialize the system. Figure 7 shows membrane potential traces from an Aplysia buccal motoneuron in two consecutive delayed feedback experiments. The delay and all other experimental parameters are the same in both experiments. The first dashed vertical line indicates the time at which feedback was turned on and the time interval between this, and the second dashed vertical line is the “initial condition.” In Fig. 7A, the initial condition corresponds to $\Psi_{n-1} = 2.79$ and $\Psi_{n-2} = 2.79$ (see Fig. 7 legend for how this was determined). Since $k = 2$ (Eq. 8), $\Psi_{n-3}$ does not affect the computation. Thus we can use Eq. 7 to compute $\Psi_{n} = 2.68$. Continuing in this fashion, we numerically obtain the steady-state value of $\Psi = 2.74$. The experimental steady-state $\Psi$ value is 2.80, obtained from averaging the $\Psi$s of several of the ICPs after the system has reached an equilibrium. In Fig. 7B, $\Psi_{n-1} = 2.20$ and $\Psi_{n-2} = 2.20$. $\Psi_{n}$ given by Eq. 7 is 3.33. Iterating repeatedly, we find a steady-state value of $\Psi^* = 3.23$. The experimental $\Psi$ value in this case is 3.27. This exemplifies how the map can be used to predict which attractor will be reached using a given initial condition. It follows from these observations that Eq. 7 can be used to calculate the time(s) at which an ICP should be introduced to cause a switch between attractors.

The patterns shown in Fig. 7 are both fixed points of the map in Eq. 7. The multistability depends on the notion that the delay time can span different numbers of inter-spike intervals (different $N$ values). In Fig. 7A $\tau$ spans two full intervals plus a fraction, while in Fig. 7B it spans three intervals and a fraction (see horizontal bar). This is possible because ICPs fall at different phases in the two patterns, causing different phase resets and thus different sized intervals.

Second we measured the spiking patterns generated by the delayed recurrent loop as a function of $\tau$ and compared these to the predictions of Eq. 7. In particular, we compared the $\Psi$ values at which the ICPs occur as a function of $\tau$. Figure 8A, $\Psi$, gives the prediction using Eq. 7 with the PRC shown in Fig.
were used. For each $t_2$ was 5 ms, size was toneuron was stimulated with a 3-nA current step and square ICPs (duration

series of inter-spike intervals, each of which contains one ICP

Fixed phase resetting curves

The co-existent spike patterns we observed consist of a series of inter-spike intervals, each of which contains one ICP at the same phase (see Figs. 2 and 7). It is possible that an ICP arrives when the spiking neuron is still some distance from its limit cycle trajectory. This is supported by our observation that wave form and inter-spike interval are often slightly different for an interval following one containing an ICP. If this happens, then the PRC will not accurately predict the new phase. To test this hypothesis, we measured the fixed phase resetting curve (FPRC) for the spiking neuron. An FPRC is obtained by repeatedly injecting ICPs at a fixed phase, $\Phi$, and measuring the reset, $\Delta_1$ once a stable situation is attained (Fig. 9A). The FPRC is equivalent to the recurrently clamped neural loop when $\tau < 1$. Figure 9B shows the FPRC for the same spiking neuron shown in Fig. 3. The — is the curve fit through the FPRC. The fact that the PRC and FPRC are not the same (compare — and - - -) has been reported previously for other excitable cells (Kunysz et al. 1997; Lewis et al. 1987).

In Fig. 8B, — gives the prediction of Eq. 7 using the FPRC shown in Fig. 9B. In Fig. 8B, ● are the same experimental points shown in Fig. 8A. Clearly the agreement between prediction and observation is better when repeated fixed phase stimuli rather than single stimuli are used to compute $\Delta_1(\Phi)$ for the map. These observations indicate that in the delayed recurrent loop the spiking Aplysia neuron has not quite returned to its limit cycle attractor before the next ICP arrives.

Other PRCs

Figure 10 shows the PRCs (left) and the predicted dynamics of a recurrent pulse-coupled neural loop using the PRCs for a

3B for the time delay up to four times the period. In the figure, for each value of $\tau$, several different initial conditions, varying systematically in frequency, were used to start numerical iteration of the map. For this PRC, all solutions represent periodic firing patterns, i.e., the firing patterns differ only by their rate. As can be seen, for $\tau$ between 2.0 and 2.3 or 2.95 and 3.7, two firing patterns are predicted to co-exist. The ● in Fig. 8A represent the experimental observations for the same neuron and ICP (examples of 2 of the coexisting neural spiking frequencies are shown in Figs. 2 and 7). As predicted by the model, two different neural spiking frequencies exist for ranges of $\tau$.

Although the agreement between observation and prediction shown in Fig. 7A is quite good, it can be seen that Eq. 7 systematically gives lower predictions for the $\Psi$s than is observed experimentally. In the next section, we examine one possible explanation for this discrepancy.

Fixed phase resetting curves

The co-existent spike patterns we observed consist of a series of inter-spike intervals, each of which contains one ICP.
tonically firing *Aplysia* buccal motoneuron reset by square hyperpolarizing current pulses (A), model of a bursting R15 *Aplysia* neuron reset by simulated inhibitory synaptic currents (Dror et al. 1999) (B), tonically firing rat cortical neuron reset by square hyperpolarizing current pulses (Schindler et al. 1997) (C), and Morris-Lecar model, a two variable model of a periodically firing cell, reset by a square hyperpolarizing current pulse (Rinzel and Ermentrout 1998) (D).

The structure of the steady-state phases as a function of \( \tau \) are remarkably similar for all the cases. This is surprising since the PRCs are quite dissimilar. Once the time delay is greater than the period for neural spiking (i.e., \( \pi T \gg 1 \)), it becomes possible for multiple spiking patterns to co-exist over certain ranges of \( \tau \). Regions in which multiple spiking patterns co-exist alternate with regions in which only one spiking pattern is possible. As \( \tau \) increases, the regions for which multiple spiking patterns co-exist become larger while those for which only one spiking pattern exists become smaller. Figure 11 demonstrates using the recurrently clamped Morris-Lecar model that once \( \pi T > 5 \), multiple attractors exist for all \( \tau \)’s. Similar results were obtained for the other three types of neurons.

**DISCUSSION**

In our model of the recurrently clamped neuron, we have made use of the fact that a neural circuit composed of spatially separated elements must be pulse-coupled. This assumption enables the dynamics of the recurrent loop to be modeled using the PRC measured from the isolated spiking neuron. Thus instead of developing a model based on, for example, detailed descriptions of the various currents across the neuronal membrane (see, for example, Foss et al. 1996), the model can be based on the experimentally measured PRC. The major advantage of the recurrently clamped neural loop is that we are able to quantitatively compare prediction to observation. This is precisely the type of experimental paradigm we needed to be able to conclusively demonstrate that multistability could arise in the nervous system simply because time delays in neural circuits are sufficiently long. However, our results should not be interpreted as implying that a recurrent pulse-coupled inhibitory neural loop that includes a limit cycle oscillator is the only type of delayed neural feedback mechanism capable of producing multistability. Mathematical and computer studies suggest that there are a large number of neurobiologically relevant types of feedback mechanisms which can generate
multistability (see for example, an der Heiden and Mackey 1982; Campbell et al. 1995; Eurich and Milton 1996; Foss et al. 1996; Ikeda and Matsumoto 1987; Milton and Foss 1997). The demonstration that such mechanisms are also relevant to the occurrence of multistability in the living nervous system awaits the development of appropriate experimental paradigms and methods to evaluate all of the required parameters.

The tonically firing Aplysia neuron used in our experiment does not by itself exhibit multistability under our experimental conditions. The multistability observed is due to the time delay in the loop. Ikeda and Matsumoto (1987) were among the first to draw attention to the observation that multistability could arise in a delayed feedback mechanism once the time delay became longer than a critical time scale. This time scale depends on the nature of the delayed feedback mechanism under consideration. For example, for a feedback control mechanism of the form

$$\dot{x}(t) + \alpha x(t) = f(x(t - \tau))$$

where $\alpha$ is a constant and $f$ describes the time-delayed feedback, the condition for multistability is that the delay must exceed the response time, i.e., $\tau > \alpha^{-1}$ (Aida and Davis 1992; Ikeda and Matsumoto 1987; van Tartwijk and Agrawal 1998). For the pulse-coupled neural recurrent loop, the relevant time scale is the intrinsic period of the spiking neuron, $T$ (Foss et al. 1996, 1997). Figure 6 illustrates that multistability is unlikely to occur for significant parameter ranges when $\tau < T$. The requirement for simultaneous solutions of Eq. 7, regardless of stability, is that the function $g(\Phi)$ intersect the function $f(\Phi)$ in at least two places. For many types of phase resetting curves, only one intersection occurs if $\tau < T$. However, a notable exception occurs when $\Delta(\Phi)$ contains a region of positive resetting. Many neurons, including the Aplysia motoneuron, have phase resetting curves which contain regions of positive resetting, most frequently at low phase (Dror et al. 1999; Glass and Mackey 1988; Pinsker 1977a,b; Schindler et al. 1997). In this case, there can be more than one fixed point for $\tau < T$, when $\tau$ is slightly $<1$. If $T' < T$ is the shortest interspike interval a neuron exhibits due to perturbations, the necessary (but not sufficient) condition for multistability is that $\pi T' > 1$.

It is not difficult to imagine how the condition that $\tau > T$ might be satisfied in large polysynaptic recurrent neural loops. In such loops, many factors can contribute to the delay and consequently the propagation time through the loop may be considerably longer than would be estimated from the conduction velocities. For example, the latency of the pupil light reflex is $\sim 300$ ms compared with $\sim 20$ ms, which would be estimated from the conduction velocities and the length of the loop (Milton 1996). However, it is important to note that it is not the absolute magnitude of $\tau$ which is relevant, but rather its value relative to another time scale. Thus if $T$ is short, the condition for multistability, i.e., $\pi T' > 1$, may be satisfied even in recurrent inhibitory loops composed of two neurons (Foss et al. 1996).

The PRCs we measured for the Aplysia buccal motoneurons can contain regions of phase advance as well as phase delay. The phase advances which sometimes occur when ICPs are given early in the cycle (i.e., $\Phi < 0.1$) have been suggested to arise because of the deactivation of a voltage-gated $K^+$ conductance (Lytton and Sejnowski 1991); consequently the outward $K^+$ current decreases sufficiently to allow the cell to reach threshold earlier. The phase delays observed when ICPs are given later in the cycle reflect the effects of the brief change in stimulating current on the time required for the membrane to recharge. For many PRCs, an abrupt change in $\Phi'$ occurs for $\Phi$ between 0.8 and 1.0 (see Fig. 10A). Since the PRC for a limit cycle oscillator is theoretically predicted to be a continuous function of $\Phi$ (Glass and Mackey 1988; Glass et al. 1984; Winfree 1980), we choose to fit the PRC with a continuous function (see Appendix). For many excitable cells, the change in $\Phi'$ following a change in $\Phi$ occurs so abruptly that the PRC is often fit with a piecewise defined function (Graves et al. 1986; Guevara et al. 1981). In our experiments, the question of the continuity of the PRC is not that important because attractors do not take on values of $\Phi$ in the steep, potentially discontinuous region of the PRC. Other investigators have found complex dynamics, not including multistability, when stimuli are repeatedly applied with delay $\tau < T$ falling in this transition region of the PRC (Kunszy et al. 1997). We were unable to convince ourselves that such behavior exists in our paradigm since the noise level is quite high.

The PRC is a powerful tool to study the effects of perturbations on biological oscillators (Winfree 1980). Since the PRC can be measured, predictions based on the PRC are directly applicable to the interpretation of real world phenomena. For example, the PRC has been used to understand the etiology of certain cardiac arrhythmias (Guevara et al. 1981) and the interaction between the respiratory oscillator and a mechanical ventilator in an intubated subject (Graves et al. 1986). Mathematical models for the nervous system that emphasize the generic properties of PRCs have been used to study fictive swimming in the lamprey (Ermentrout and Kopell 1994; Kopell 1995), the effects of periodic inhibitory stimulation of cortical slices (Schindler et al. 1997), and the occurrence of multistability in ring circuit models for CPGs (Canavier et al. 1999; Dror et al. 1999) and to develop models to describe the behavior of large population of neurons (Hoppensteadt and Izhikevich 1997). The observations in Fig. 10 indicate that for many neural oscillators the PRCs can be very different, yet the dynamics of the recurrent loop remain markedly similar. Thus we conclude that the occurrence of multistability in a delayed recurrent neural loop is a robust phenomenon. Indeed we observed multistability in 23 of 26 Aplysia motoneurons that were studied in the recurrently clamped neural loop.

In our experimental paradigm, the timing of the ICPs is precisely controlled. It is reasonable to question whether spike timing could be sufficiently precise so that the dynamics we observe could be seen in the normally functioning nervous system. Each of the attractors that arise in a multistable delayed recurrent loop has associated with it a basin of attraction (Foss et al. 1996; Milton and Foss 1997). This means that the spiking patterns have a certain stability to perturbations in the timing of ICPs. Thus the issue of the precision of spike timing is not crucial. Of course, certain fluctuations in spike timing can cause switches between basins of attraction (Foss et al. 1997). Noise-induced switching between basins of attraction has been suggested to underlie the fluctuations observed in human postural sway (Eurich and Milton 1996) and the changing perceptions of visually ambiguous figures (Krüse and Stadler 1995).

Multistability can arise at many different levels within the nervous system. For example, in motor control multistability can arise at the level of the alpha-motoneuron (Hounsgaard et al. 1988) and at the level of local neuromuscular feedback control.
loops (Campbell et al. 1995) or be a property of integrative control systems such as the control of human posture (Eurich and Milton 1996). Here we have emphasized the occurrence of multistability in recurrent neural loops. It has been suggested that every neuron within the CNS can be connected to every other neuron by a pathway involving just a few synapses (Guyton 1976; Watts and Strogatz 1998). Thus from a macroscopic point of view the nervous system has a recurrently looped structure. Currently emphasized recurrent neural loops include the multiple pathways involved in the control of movement (Beuter et al. 1989), reciprocal thalamo-cortico loops involved in epileptic seizures (Gutnick and Prince 1972) and regulation of states of arousal (Conteras et al. 1996), the limbic nervous system loops related to memory (Barnes et al. 1997) and epileptic seizures (Schwartzkroin and McIntyre 1997), and the cortical-basal ganglia-thalamus-brain stem-cortical loops, which participate in the control of movement and act as gate keepers for the propagation of epileptic seizures (Proctor and Gale 1997). As neural loops become longer, at some point the propagation time around the loop must exceed the typical inter-spike interval. Our observations suggest that once this occurs, multistability becomes probable. The recognition that multistability can be an emergent property of the integrative organization of the nervous system has implications ranging from understanding higher cortical processing to the design of therapeutic strategies for brain diseases.

APPENDIX: CURVE FITTING

To incorporate $\Delta(\Phi)$ into our model, it must be expressed as a function or a lookup table. The effect of noise and the measurement error made using the raw data for a lookup table problematic. The small number of data points and their unequal spacing made conventional smoothing algorithms unusable, and simple linear interpolation created artifacts in the map results due to the nondifferentiable nature of the $\Delta$ function resulting from such interpolation. Therefore we chose to fit functions to the experimental data. Phase reset data points measured from *Aplysia* motoneurons were empirically fit with a function formed by the product of a parabola and sigmoid, i.e.

$$
\Delta(\Phi) = \frac{a_0 + a_1 \Phi + a_2 \Phi^2}{1 + \exp(b_1(\Phi + b_2))}
$$

(A1)

using a least-squares analysis (rms errors are given in the figure legends). In figures in which fit PRCs are shown, we have plotted $\Delta + \Phi$ versus $\Phi$. We experimented with other functional fits, such as high order polynomials (i.e., $n > 3$) and piecewise fits, such as splines. These alternate choices did not qualitatively affect our findings. PRCs (and hence also $\Delta$) frequently have abrupt transitions (see remarks in Discussion) that make them difficult to fit with simple polynomial or exponential fits. Using the parabola-sigmoid function allowed all of the $\Delta$s measured to be fit by a continuous function of the same form, yet still capture their characteristic shape.

Given the time constraints of experiments (both the phase resetting curve and the delayed feedback experiments must be done on the same neuron) and the noise level in our preparation, it was impractical to adequately explore the phase resetting values for phases close to 1. Thus the discontinuity of the curve fits at $\Phi = 1$ does not affect the accuracy of our map’s predictions.

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