From *SIAM News*, Volume 40, Number 2, March 2007

Phase-Resetting Curves and Neuromodulation of Action Potential Dynamics in the Cortex

By Boris Gutkin and Klaus Stiefel

Neurons in the cortex communicate with each other by sending electrical discharges, called action potentials or spikes. Under-standing how such spikes arise from the interplay of voltage-dependent currents was a revolutionary breakthrough, made possible when Hodgkin and Huxley (in the early 1950s) built a mathematical model of such currents. With the Hodgkin–Huxley model, we can explicitly observe the dynamics of the various cross-membrane currents and analyse how they shape the action potential. The model also shows how oscillatory responses are possible when the cell is stimulated by a constant injection of current. In mathematical terms, when the input current is strong enough, the steady state (i.e., the resting potential, etc.) loses stability and a limit cycle appears.

The route to such repetitive spiking can involve different bifurcation mechanisms. Two of the most commonly observed in neurons are the subcritical Hopf bifurcation and the saddle node on an invariant cycle, or SNIC, bifurcation. In the former, a pair of complex eigenvalues cross the real line, giving rise to an unstable limit cycle that in most neural models "bends back" to produce a large-amplitude stable limit cycle. There is a non-zero minimal firing frequency (often, but not necessarily, related to the frequency of the subthreshold oscillations). In the SNIC, a single real eigenvalue changes sign; geometrically, a stable node and a saddle point meet, destroy each other, and leave in their wake a limit cycle. Knowledge of the structure of such bifurcations can help us understand the responses of neurons not only to constant input but to inputs of many other types as well. For example, because the Hopf neurons show subthreshold oscillations, they can act as resonators of synaptic inputs, whereas SNICs act more like integrators.

It is all well and good to determine the bifurcation structure of neural models—in this way, after all, we can directly observe all the dynamical variables, linearize the system of equations, and appeal to powerful theorems, as well as carry out simulations. But can we come close to understanding the bifurcation structure of real neurons? We might expect this to be a very difficult, if not impossible, task. Electrophysiologists can observe only a few state variables. Most often, we record only the voltage of the cells (in the "current clamp") or the total current across the membrane (the "voltage clamp"). We can also tease out some individual current contributions, by blocking them pharmacologically. Bifurcations result from a delicate interplay of all the currents traversing the neural membrane, however; knowing the result of removing just one current or another would not be able to state that we see a bifurcation of one type or another.

Recently, though, we applied to neurons a mathematical technique usually used to characterize nonlinear oscillators: the *phase-resetting method*, which produces a measurement of the *phase-resetting curve* (PRC). By studying how a phase of an oscillator reacts to a brief perturbation, we can characterise its response function and, hence, the internal nonlinear dynamics that produce the oscillation. When such a perturbation is sufficiently weak, the resulting response function can be thought of as a phase analogue of a linear response function and can tell us how the oscillator will react to an arbitrary weak "input." Such analyses have been effective in studies of the mechanisms of synchrony in weakly coupled oscillators, using averaging arguments, and of phase locking in oscillatory circuits, using discrete map methods.

Traditionally, phase-resetting methods have been used in neurobiology to study systems in which oscillations are inherent, such as pattern-generating circuits in the peripheral nervous system. Recently, researchers have begun to apply phase-resetting arguments and analytical methods to cortical neurons and cortical circuits (see [3, 5]). The main rationale for doing so is that neurons in the cortex can show a variety of periodic firing behaviors, from steady firing to complex bursting patterns. In all these situations, we can consider the neurons as a nonlinear oscillator and can thus determine its dynamics by looking at the PRC. Here we apply this analysis to neurons that show steady repetitive firing, the "regular firing" pyramidal neurons.

It was shown previously that the bifurcation that leads to repetitive firing can determine the general shape of the PRC. SNIC, or type I, spike generation is associated with positive PRCs—excitatory inputs, in other words, can only advance the spike times. Oscillators arising via a Hopf bifurcation, by contrast, are associated with sinusoidal PRCs that have both positive and negative regions; in such cells excitatory (positive) inputs can both advance and delay the spike times. In fact, direct measurements in cortical neurons have shown such bimodal PRCs.

That excitatory inputs can have an inhibitory effect is itself a surprising idea, one that goes against the accepted view of how synaptic inputs affect spikes. Electro-physiologists (and most neural network modelers) would say that an excitatory input always advances the spike. Our analysis has revealed that a SNIC model can have a bimodal PRC when it includes sufficiently strong potassium currents. This makes sense, in that the negative portions of the PRC always occur in the interspike intervals immediately following spikes. Potassium currents are, in fact, activated by the spikes and may thus be responsible for the negative part of the PRC.

Further analysing the models, we showed that slow potassium currents that activate via depolarising voltages (and produce so-called spike-frequency adaptation) do produce sinusoidal PRCs. This is so because these slow potassium currents actually convert the type I spike generation into type II—changing the bifurcation structure from SNIC to Hopf (see [2]). One such current, the muscarine-sensitive potassium current (IM), is expressed by the pyramidal neurons in the cortex and controls, in addition to the spike-frequency adaptation, the resting potential of the cell. Such current can be regulated by neuromodulators: Acetylcholine, for example, blocks this current through a complex second-messenger cascade and can cause the pyramidal neurons to fire. This was known previously, but our theory predicted a new role for cholinergic neuromodulation—a change in the bifurcation and hence in the dynamics underlying the generation of spikes.

We set out to prove or disprove this theoretical hypothesis by conducting experiments in slices of rat neocortex. A slice is a reduced electrophysiological preparation, typically between 0.2 and 0.5 mm thick, that can be kept alive in a glass dish (in vitro) for several hours. In such a

slice, the transmembrane voltage of individual neurons can be measured and currents can be injected with glass electrodes (the "patch-clamp" technique). We established such patch-clamp recordings in pyramidal (excitatory, principal) neurons and depolarised them to above the firing threshold. We then perturbed the regular firing at random intervals to determine the PRC. We found type I and type II PRCs.

At the end of this first stage of the experiment, we applied carbachol, an analogue of acetylcholine. As one of the most important neuromodulators in the cortex, acetylcholine alters the basic mode of this neural structure. High levels of acetylcholine are found when an animal is awake and vigilant. As predicted by the aforementioned theoretical work, acetylcholine, most likely via the inactivation of IM, changed the PRC of the majority of neurons that initially showed type II to type I.

This is an intriguing result, as the type of PRC affects the synchronization properties of a neuron, and acetylcholine is known to alter the types of synchronous oscillations occurring in the cortex. Using the mathematical phase-resetting technique, we have been able to understand how neuromodulators related to cognitive states (such as attention, motivation, emotion, awareness) of the organism affect the machinery by which neurons in the cortex produce spikes. The next frontier is to understand how such mechanistic changes result in functional outcomes—how does neuromodulation change the processing of information in cortical cells and networks?

References and Further Reading

[1] B. Ermentrout, *Type I membranes, phase resetting curves and synchrony*, Neural Comput., 8 (1996), 979–1001.

[2] B. Ermentrout, M. Pascal, and B. Gutkin, *The effects of spike frequency adaptation and negative feedback on the synchronization of neural oscillators,* Neural Comput., 13:6 (2001), 285-310.

[3] B.S. Gutkin, G.B. Ermentrout, and A.D. Reyes, *Phase-response curves give the responses of neurons to transient inputs*, J. Neurophysiol., 94:2 (2005), 1623–35.

[4] E.M. Izhikevich, *Dynamical Systems in Neuroscience: The Geometry of Excitability and Bursting*, MIT Press, Cambridge, Massachusetts, 2007.

[5] A.D. Reyes and E.E. Fetz, *Effects of transient depolarizing potentials on the firing rate of cat neocortical neurons*, J. Neurophysiol., 69:5 (1993), 1673–83.

[6] A.T. Winfree, *The Geometry of Biological Time*, Springer–Verlag, Berlin, 1980.

Boris Gutkin is a member of the Group for Neural Theory in the Département des Etudes Cognitives, Ecole Normale Supérieure, and the Collège de France. Klaus Stiefel is a researcher in the Theoretical and Experimental Neurobiology Unit, Okinawa Institute of Science and Technology, Japan.