Researchers working on neuronal networks are increasingly taking advantage of the ability to overexpress particular molecular components such as channels. What hope do such studies hold for understanding neuronal phenotype and its role in network function?

The overexpression of channel proteins by RNA injection or use of viral vectors is a potentially powerful way of assessing channel function in neuronal networks. In invertebrate networks such as the well-studied crustacean stomatogastric ganglion (STG), the promise is substantial because of the small numbers of neurons participating in its motor-pattern-generating networks and the potent influence of individual neurons on network dynamics [1]. Recent work by the Harris-Warrick laboratory [2,3] using this preparation illustrates both the promise of these methods and the difficulties posed by the complexities of gene expression.

Two channels have been shown to be important determinants of the firing period in pacemaker neurons of the pyloric network of the lobster STG: the transient, low threshold K+ current IA, and the hyperpolarization-activated inward current Ih [4,5]. The genes encoding the channel proteins that underlie these currents have been cloned from a variety of systems, including the STG of spiny lobsters [6–8]. They are the Shaker family and HCN channels, respectively, the lobster versions of which are called lobster Shal and PAIH.

In the bursting neurons of the STG pyloric network, IA delays the onset of the burst phase [4,5], whereas Ih promotes the onset of the transition to the burst — the so-called post-inhibitory rebound [5]. Both currents are modulated by dopamine: IA is down-modulated and Ih is up-modulated [5]. Consistent with the predicted effects of these currents, dopamine advances the phase of neuronal activity in the pyloric network via its effects on target motor neurons. Analysis using the ‘dynamic clamp’ method of recording channel function (see below) and specific channel blockers showed that the effect of dopamine on IA is functionally more important than that on Ih.

In an interesting new study, MacLean et al. [2] overexpressed IA in STG PD neurons by injecting cRNA encoding the lobster Shal protein into single identified PD cells. To the authors’ chagrin, this manipulation did not lead to a change in neuronal or network characteristics, because although IA increased, it was compensated by an increase in Ih, leading to no net change in neuronal properties. PD neurons thus homeostatically regulate the balance between IA and Ih. This regulation, unlike the more familiar activity-dependent homeostatic regulation [9], is independent of activity, because expression of a mutant shal cRNA that produces non-function channels also leads to up-regulation of Ih, and this predictably alters the firing properties of PD neurons. This homeostatic regulation is post-translational, because it is not prevented by transcription blockers. These results set the stage for a look at what happens when Ih channels are over-expressed.

HCN channels have a checkered history, being originally labeled Iq and Ih because of their funny and queer characteristic of opening in response to hyperpolarization [10]. The cloning of genes encoding HCN channel proteins in the late nineties has resulted in their rehabilitation in the minds of most neuroscientists [11,12], and now it is widely recognized that they offer a convenient way of regulating the post-inhibitory rebound and the firing period in a variety of rhythmically active systems, from the mammalian heart to the thalamocortical networks active during delta sleep.

HCN channels open in response to hyperpolarization apparently because the channel gate is articulated with the voltage sensor in such a way that it opens when the sensor is drawn toward the negative inside of the membrane [13]. The voltage sensor is quite conventional, and similar in sequence to those of depolarization-activated channel proteins. Proving that HCN channels have the predicted functional effects in neuronal networks has been more difficult; although pharmacological blockade of HCN channels with Zd7288 has been effective at producing the predicted increase in period in some systems, it has not done so in others [10,14]. Over-expression promises a clean way of determining the functional effects of HCN channels, but the previous work on PD neurons in STG suggested that activity-independent homeostatic regulation might thwart this attempt.

Zhang et al. [3] have now reported that injection of cRNA encoding the HCN channel protein PAIH into PD cells leads to an increased Ih current (Figure 1A), which, in turn, depolarizes the membrane potential and accelerates the onset of post-inhibitory rebound (Figure 1B). These are expected consequences of an increase in Ih, but unexpectedly, no compensatory increase in IA was detected. This result was corroborated by modeling and experiments where PAIH was added to pharmacologically isolated PD neurons by the dynamic clamp — a hybrid technology [15] that allows the current equivalent to any computed conductance to be injected into a neuron (Figure 1C). Thus, it appears that Ih does indeed contribute as predicted to cellular and network properties.

These elegant studies highlight both the strengths and weaknesses of overexpression as a tool for probing neuronal and network activity. The web of molecular interactions that result in neuronal phenotype is complex enough that experimental overexpression can lead to unexpected compensatory changes of the type...
observed when $I_A$ is overexpressed in PD neurons. Such compensatory changes may complicate an experiment, but nevertheless add to our ability to experimentally analyze the genesis of neuronal phenotype. With the overexpression of PAIH in PD neurons, we see the elegance of the technique, as the expression of $I_h$ led to predictable and clear changes in cellular properties. The strength of this demonstration, however, is that it is corroborated with dynamic clamp and modeling studies so that any nagging doubts about how the introduced RNA may alter the expression of unassessed channels are allayed. The most intriguing aspect of the PAIH study [3], however, may be the lack of reciprocal regulation by the $I_h$ channel. The Harris-Warrick lab now has its eleventh Meeting of the European Chemoreception Research Organization.

References