

known as transparent motion), humans can perceive the two stimuli as distinct provided the directions of motion are separated by at least 10 degrees⁵. Clearly, the center of gravity could not subserve this percept—or else we would always perceive transparent motion as containing only one component of motion at a direction intermediate between the actual directions—but what about the peak(s) of the population response? Does the population response in MT contain separate peaks for each component of a transparent motion stimulus? Do these peaks merge together into one broad peak at the point where the two directions are too close to be resolved?

In an elegant series of experiments, Treue and colleagues¹ tested this hypothesis. Although the responses of MT neurons to both single and multiple stimuli have been well characterized (for review, see ref. 6), it is less clear how the population response varies as a function of the relative directions of the components of multiple stimuli. Treue and colleagues first studied the responses of monkey MT neurons to transparent motion stimuli. Their results show that because these neurons are broadly tuned for direction, the populations of neurons responding to each component of motion overlap quite extensively. For directions separated by less than about 90 degrees, only a single broad peak exists (although when the directions are farther apart, two separate peaks do appear). Importantly, this single peak occurs in monkey MT even when the directions are sufficiently different to be readily distinguishable to human observers (and presumably to the monkeys).

Thus, the relationship between neural activity and perception of the components of transparent motion does not seem to be based on the presence or absence of segregated peaks of activity, as would have been predicted by algorithms that identify peaks of activity (for example, winner-take-all). Rather, the transition from perception of two directions of transparent motion to perception of a single direction of motion must depend on some as-yet unidentified aspect of the shape of the population response in MT.

If the overall shape of the population response is critical to motion perception, then Treue and colleagues reasoned that stimuli that produce population responses having the same shape should produce the same percepts. Based on their recordings using two-component stimuli, Treue and colleagues designed three-component stimuli that should produce the same

population responses as certain two-component stimuli. For example, the population response to a transparent motion stimulus consisting of two components 80 degrees apart should be the same as the response to a motion stimulus with 3 components each 50 degrees apart (see Fig. 3 of ref. 1). If so, and if motion perception relies on this population of neurons, then the direction of motion of these two stimuli should be indistinguishable. They tested this hypothesis in human observers, and found it was indeed the case: these two very different motion stimuli appear perceptually to have the same components (Fig. 1).

A number of issues remain to be resolved. For example, do MT cells actually respond identically to the two- and three-component stimuli? Do the demands of the psychophysical task affect how MT represents motion information? Monkeys can certainly be trained to perform motion tasks like the one used by Treue and colleagues in humans, but there is reason to think that the task itself might influence population responses in MT. In particular, previous work by Treue and others has demonstrated that when an animal is attending to only one of two directions of motion, neurons in MT represent the attended direction much more strongly^{7–9}. Thus, if MT neurons were studied while monkeys performed the psychophysical task used here in human observers, the presence and/or location of peaks in the population response might be different.

Perhaps the most intriguing aspect of this work is the notion that the shape of the population response in MT can be important for motion perception. There are well-defined algorithms for identifying peaks of activity (winner-take-all), or computing the center of gravity (for example, via vector averaging) to arrive at a perceptual judgment or behavioral response, and it is comparatively easy to imagine how neural circuits might perform these calculations (for example, see J.M. Groh, *Soc. Neurosci. Abstr.* 23, 1560, 1997). Yet the findings of Treue and colleagues suggest that perception can be affected by details of the shape of the active population, details that are lost through either of these calculations. Therefore, we need to explore new algorithms for reading population codes.

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ChIPping away at potassium channel regulation

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Kv4 subunits form A-type potassium channels. To replicate native currents, these subunits require additional factors, now shown to be a family of calcium-binding proteins.

In a recent issue of *Nature*, Kenneth Rhodes and colleagues¹ present results that resolve long-standing questions

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concerning the molecular identity of A-type potassium channels. They describe the isolation and characterization of a family of calcium-binding proteins, the KChIPs (K⁺ channel interacting proteins; Fig. 1), that bind to the intracellular amino (N)-terminal domain of cloned Kv4 channels and endow them with many of the properties ascribed to native A-type potassium channels. Co-expression of the KChIPs and cloned

Kv4 subunits increased current densities, shifted the voltage dependence of activation and speeded their recovery from inactivation. Mutagenesis experiments that eliminated the ability of the KChIPs to bind calcium showed that their channel-modulating effects require calcium binding, although the physical association between the interacting proteins was calcium independent.

A-type channels are voltage-dependent potassium channels that activate in the subthreshold range of membrane potentials and completely inactivate during depolarizing pulses, while other voltage-dependent potassium channels are just beginning to activate. As a result, A-type channels influence the time required for membrane depolarization to reach threshold for action potential generation as well as the time between action potential spikes. Thus they are important determinants of the firing frequency in excitable cells such as neurons and cardiac myocytes. Sequence homologies among cloned subunits distinguish several subfamilies of voltage-dependent potassium channels, the Kv subfamilies. The Kv channels are tetramers, and heteromeric channels are assembled only from subunits in the same subfamily. Heterologous expression studies show that members of the Kv4 subfamily form channels similar (but not identical) to native A-type potassium channels². In particular, they demonstrate inactivation mediated by a specialized intracellular domain at the extreme N terminus, the 'ball', which physically occludes the pore when the channel is open. The expression profiles of Kv4 subunits in the CNS and heart are consistent with this role, and Kv4 subunits have been identified as the components of A-type potassium channels in rat neostriatal cholinergic interneurons³ and cardiac ventricular myocytes⁴.

The functional profiles of Kv4 channels expressed in different heterologous cells are variable⁵, suggesting a contribution by factors present in the host cell. This is further supported by differences between Kv4 channels expressed in *Xenopus* oocytes with or without rat brain mRNA. Co-expression of the low-molecular-weight mRNA fraction increases current density, most likely reflecting an increased number of A-type channels. This co-expression also shifts the activation voltage of the channels to more negative potentials and allows faster recovery from inactivation⁶, increasing their similarity to native channels. In addition, a role for calcium in modulating A-type channels has been suggested from recordings of cholinergic neostriatal neurons, where blocking voltage-dependent calcium channels with cadmium shifts the voltage dependence of A-type current activation and inactivation to more depolarized potentials³. Until now, however, the relationship between these observations has remained obscure.

The new report from Rhodes and colleagues¹ reconciles these differences between native A-type potassium channels and cloned Kv4 channels at the molecular level. Using the intracellular N-terminal region of Kv4.3 as bait in a yeast two-hybrid hunt through a rat mid-brain cDNA library, they captured and characterized three members of a family of Kv4 channel interacting proteins (KChIPs). The KChIPs interact selectively with Kv4 subunits, and they are expressed in tissues that also express Kv4 subunits. When co-expressed in heterologous cells, KChIPs were localized with Kv4 subunits. KChIPs were selectively

immunoprecipitated with Kv4 subunits from transfected cells and rat brain membrane preparations. Immunocytochemistry confirmed that the two proteins are colocalized at cellular and subcellular levels. Remarkably, co-expression of KChIPs with Kv4 subunits reconstituted the functional characteristics of native A-type channels, as well as replicating the effects of co-expressing rat brain mRNA on cloned Kv4 subunits. Current densities were increased, the voltage dependence of activation was shifted to more hyperpolarized potentials, and the channels recovered from inactivation much more rapidly.

The KChIPs range in size from 216 to 256 amino acids. The N-terminal domains, ~50 amino acids, vary considerably, but throughout their carboxyl (C)-terminal domains, they share ~70% sequence identity. The conserved regions of the molecules contain four E-F hand calcium-binding motifs. Calcium binding to the KChIPs was confirmed by calcium-dependent mobility shifts. Interestingly, a mutant KChIP-1 that could not bind calcium still inter-

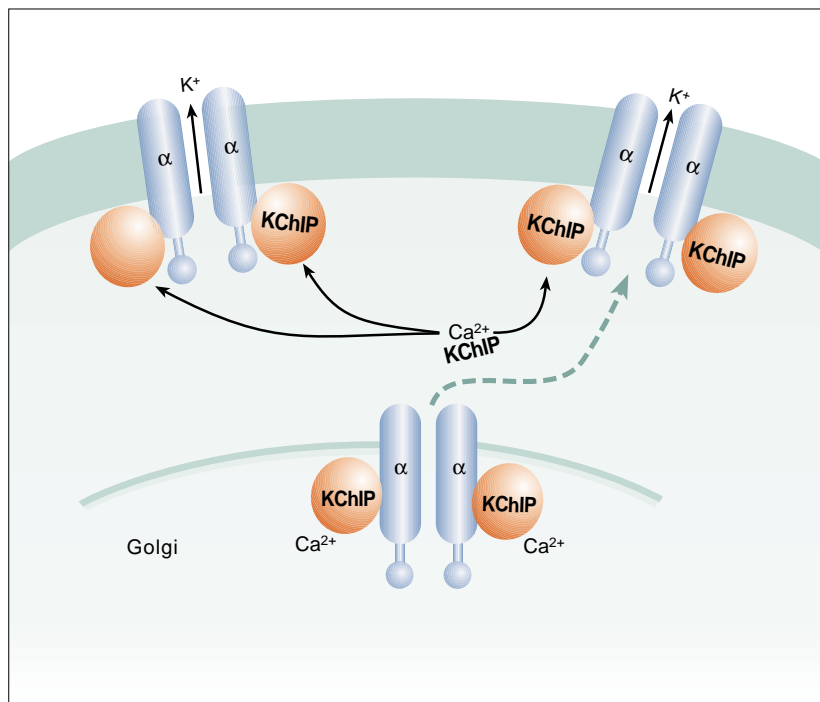


Fig. 1. KChIPs are integral components of A-type potassium channels. The KChIPs bind to the N-terminal domain of Kv4 subunits, close to the membrane, and close to the 'ball' domain that mediates channel inactivation. The physical association between KChIPs and Kv4 subunits does not require calcium binding, but the effects on channel gating are calcium dependent. The KChIPs are also present in the Golgi, where the association with Kv4 channels during their biosynthetic development may regulate the levels of functional A-type potassium channels present in the surface membrane.

acted with Kv4 subunits, suggesting that the association between channels and KChIPs is calcium independent and may be constitutive. However, the mutant KChIP-1 no longer modulated Kv4 channel function.

Sequence alignments show that KChIPs belong to the recoverin family of calcium-binding proteins that includes the *Drosophila* protein frequenin, which regulates transmitter release, and mammalian homologs NCS-1 (neuronal calcium sensor-1) and hippocalcin⁷. Although KChIPs 1 and 2 have not been previously described, KChIP-3 is identical to DREAM (downstream regulatory element antagonist modulator), which is reported to regulate transcription in a calcium-dependent manner⁸. KChIP-3 is also identical to calsenilin, a protein that resides in the ER and Golgi and interacts with the C-terminal domains of the presenilins, transmembrane proteins found in the same subcellular compartments. Mutations in the presenilin genes account for 40 percent of early-onset familial Alzheimer disease and sensitize neuronal cells to apoptosis, possibly by disrupting intracellular calcium levels⁹. In this regard, it is tempting to speculate that the increased current density (thought to reflect an increased number of channels in the membrane) induced by co-expression of KChIPs with Kv4 subunits may reflect effects on membrane trafficking, which could be related to the interactions between KChIP-3 and presenilins in the *trans* Golgi.

Taken together, the results suggest that KChIPs are integral parts of Kv4 channels, both in the plasma membrane and during their biosynthetic journey through the *trans* Golgi. On elevation of intracellular calcium levels, such as during an action potential, calcium binding to KChIPs induces a conformational alteration that is rapidly transduced to the channel, resulting in altered gating. The previous localization of KChIP-3 to the *trans* Golgi suggests that the increased current densities may result from regulated cell-surface expression of the channel complexes.

Several questions await further experiments. For example, just how does calcium binding to the KChIP result in altered channel activity? Which amino acids in the N-terminal domain of Kv4 subunits interact with KChIPs? Does the interacting domain of Kv4 overlap with binding sites for other proteins? How selective are the different

KChIPs for the various Kv4 subunits? What other proteins might KChIPs bring into the A-type channel complex?

This is the latest in a series of findings suggesting that intracellular calcium signaling can modulate membrane potential. Fluctuations in intracellular calcium levels have long been appreciated as an important modulator of ion channel activity. A generally accepted model posits that second messenger systems, such as calcium-sensitive protein kinases or phosphatases, alter the phosphorylation status of the channel, affecting channel activity and cellular excitability¹⁰. These are relatively slow processes that require calcium interaction with the signaling molecule and subsequent interaction with the ion channel.

Recently, several reports have suggested a faster calcium signaling process by demonstrating that calmodulin is constitutively bound to the ion-conducting α subunits of voltage-dependent calcium channels (VDCCs) and small conductance calcium-activated potassium channels (SK channels). In both cases, compelling evidence supports a model in which calmodulin is an integral part of the channel complex, and calcium binding to calmodulin induces structural alterations in calmodulin, which are transduced into conformational changes in the channel proteins that alter their function^{11,12}. Local signaling induced by calcium entry may be much more rapid than second-messenger-mediated processes, and can respond rapidly to discrete, localized alterations in intracellular calcium. For example, VDCCs are the likely source of the calcium ions that would bind to channel-associated calmodulin, and the precise distance between SK channels and VDCCs or intracellular calcium release sites strongly affects the dynamics of burst frequency. The work by Rhodes and colleagues¹ suggests that KChIPs are an integral component of A-type potassium channels, acting as direct calcium sensors that affect channel gating properties, analogous to the role of calmodulin for SK channels and VDCCs.

The KChIPs join an expanding list of proteins that bind to potassium channels and influence their activity, but do not contribute to ion conduction. Among the voltage-dependent potassium channels, the intracellular N-terminal domain has been shown to mediate heteromeric subunit assembly, restrict-

ing associations to members of the same subfamily. The N-terminal region also interacts with the Kv β proteins, some of which endow inactivation that is modulated by protein kinases¹³. More recently, two alternatively spliced proteins, ZIP1 and ZIP2, have been identified that act as molecular bridges, linking the β subunits to protein kinase C ζ (ref. 14). At their C termini, many voltage-dependent potassium channels bind to PDZ-containing proteins, such as PSD-95/SAP90 family members, to modulate the distribution and surface expression of the potassium channels, which is affected by the presence of a β subunit at the N-terminal domain in some cases¹⁵. These PDZ-containing proteins, in turn, interact with other putative regulatory molecules. The emerging picture suggests that the α subunits of potassium channels are embedded in large, multimeric protein complexes with components that sense a wide range of metabolic signals. Indeed, the ZIP proteins, which do not themselves interact with the α subunits, begin to define a larger microdomain, a molecular neighborhood, in which the channels reside. The two-hybrid screen can be used with each newly identified resident to determine the next nearest neighbor.

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