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In summary, the preliminary findings reported by Litt et al. (2001) are provocative in that they suggest that quantitative analyses of EEG can be exploited to predict the occurrence of seizures in humans tens of minutes to hours in advance. If correct, this greatly enhances the feasibility of novel therapies for control of seizures. Furthermore, if correct, these findings raise two intriguing questions: what are the cellular and molecular mechanisms underlying EEG rhythms portending seizure onset minutes to hours in advance, and what is the nature of the signal that triggers the seizure onset? It will be exciting (no pun intended) to watch this field unfold in the next several years.

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An End in Sight to a Long TRP

It has been more than three decades since the *Drosophila* transient receptor potential (*trp*) mutant was discovered and named based on its transient rather than maintained response to light (Cosens and Manning, 1969). In the ensuing years, the *trp* gene was cloned (Montell and Rubin, 1989) and shown to encode a novel receptor-operated cation channel (reviewed in Montell, 1999). Two other highly related photoreceptor cell-enriched proteins, TRPL and TRPγ, have been described (reviewed in Montell, 1999; Xu et al., 2000). In addition, many mammalian TRP homologs (TRPC proteins) were identified, thereby spawning a flurry of studies concerned with characterizing the functions and mode of activation of this class of receptor-operated Ca^{2+}-permeable cation channel (reviewed in Harteneck et al., 2000). Despite all the time and progress since the original isolation of *trp*, the basis of the transient light response and delayed recovery from inactivation has remained enigmatic. At least two explanations have been put forward but neither has been completely satisfactory. In the current issue of *Neuron*, Hardie et al. present data supporting an exciting and unexpected mechanism for the *trp* phenotype, depletion of phosphatidylinositol-4,5-bisphosphate (PIP_2), the substrate for phospholipase C (PLC) (Hardie et al., 2001). This study goes a long way toward resolving the *trp* controversy and opens up many lines of experimentation that may finally put this issue to rest.

Despite the impressive array of recent studies on TRP family members, for many years interest in the *trp* locus was rather modest as the basis of the phenotype and the molecular nature of the disrupted gene product were obscure. However, this began to change in the late eighties and early nineties with the finding that the *trp* mutation was associated with a defect in Ca^{2+} influx and the demonstration that the *trp* gene encoded a novel protein with a predicted topology reminiscent of members of the superfamily of voltage- and second messenger-gated ion channels (Montell and Rubin, 1989; Hardie and Minke, 1992). These observations, combined with the evidence that the light-stimulated cation influx is a strictly phospholipase C–dependent phenomenon, raised interest in TRP protein as a candidate for the long-sought store-operated Ca^{2+} entry channel.

Store-operated Ca^{2+} entry appears to be widespread and is implicated in a diversity of processes ranging from T cell activation to fluid secretion and in the modulation of spontaneous neurotransmitter release. This mode of sustained Ca^{2+} entry is so named since it depends on transient release of Ca^{2+} from internal stores via the intracellular cation channel, the inositol-1,4,5-trisphosphate (IP_3) receptor. The release of Ca^{2+} from the stores is coupled to stimulation of PLC, which catalyzes the conversion of PIP_2 to IP_3 and diacylglycerol. Several TRPC proteins appear to be store operated, at least in vitro, although others are not (Harteneck et al., 2000). Moreover, neither TRP nor the heteromultimeric channels, TRPL/TRP and TRPL/TRPγ, is store operated in vivo, as both Ca^{2+} release channels, the IP_3 receptor and the ryanodine receptor, are dispensable for the light response. However, all TRP channels share the common feature of depending on PLC for activity.

Despite the considerable progress in the TRP field over the last few years, no consensus has emerged concerning the basis of the *Drosophila trp* phenotype originally reported in 1969 (Cosens and Manning, 1969). In wild-type flies, the visual response operates through the fastest known G protein–coupled signaling cascade. Only ~10 ms elapse between light stimulation and opening of the TRP channels. Termination of the light stimulus is also rapid and occurs within ~100 ms. The *trp* mutant differs from wild-type in displaying only a transient response to a constant stimulus of bright light. After about ~4 s, *trp* flies are essentially blind and a ~90 s dark adaptation period is required before the flies can exhibit a subsequent response of similar magnitude. According to one proposal, the transient response in *trp* results from depletion of the intracellular Ca^{2+} stores since the stores refill slowly in the absence of the TRP-dependent Ca^{2+} influx (Minke and Selinger, 1991). However, this hypothesis seems untenable in light of the lack of requirement for either of the two Ca^{2+} release channels for the visual response. Another proposal is that the transient light response in *trp* is a consequence of rapid and complete Ca^{2+}-mediated desensitization of the re-

maining channel in *trp* mutant flies. In support of this proposal is the finding that expression of a TRPL derivative, which is defective in calmodulin binding, suppresses the *trp* phenotype (Scott et al., 1997). Furthermore, it was reported that the *trp* phenotype could be suppressed in the absence of extracellular Ca^{2+} (Scott et al., 1997). However, this latter finding has been questioned (Cook and Minke, 1999).

Due to the inadequacies in the existing theories, Hardie et al. explored a different angle to account for the *trp* phenotype. The transient nature of the *trp* visual response suggests, as pointed out by Minke and Selinger (1991), that some critical component gets exhausted during constant bright illumination. But what might be the identity of such a factor? In view of the absolute requirement for PLC for excitation, Hardie et al. surmised that it might be the PLC substrate PIP_2. However, the challenge to testing this proposal was to devise a strategy to monitor dynamic changes in PIP_2 levels over the same rapid time scale that occurs during phototransduction. To accomplish this goal, the authors came up with the clever idea of using the inwardly rectifying K^+ channel Kir2.1 as a biosensor. Kir2.1 binds directly and preferentially to PIP_2, and this interaction correlates with the open state of the channel. Transgenic flies were generated that expressed human Kir2.1 in photoreceptor cells and the activity of this K^+ channel was assayed in wild-type in the presence or absence of conditions (40 μM La^{3+}) that inhibit TRP but not TRPL channels. Consequently, wild-type photoreceptor cells exposed to 40 μM La^{3+} phenocopy the response typical of the *trp* mutant.

The results were as one would predict if PIP_2 levels were getting depleted in the absence of TRP function. The Kir2.1-dependent current was suppressed in the presence of La^{3+}, and the effect showed an intensity dependence and time course similar to the transient light response in *trp* mutants. Moreover, the recovery of Kir2.1 activity occurred over a similar time course required for recovery of the light response in *trp* photoreceptor cells. The findings that other phospholipids are less effective or completely ineffective in activating Kir2.1 lend support to the notion that PIP_2 levels are declining in a time-dependent manner in *trp* flies.

To provide further support for their model, Hardie et al. examined whether the *trp* phenotype would be enhanced in two mutants that disrupt proteins that are known or proposed to be important in PIP_2 recycling. These include *rdgB*, which disrupts a phosphoinositol transfer protein, and *cds*, which eliminates an enzyme, CDP-diacylglycerol synthase, critical for PIP_2 recycling (reviewed in Montell, 1999). Recovery of light sensitivity was compared after inhibiting TRP with La^{3+}. While inhibition of TRP in wild-type lead to a full recovery after ~90 s, no such recovery was observed in similarly treated *rdgB* or *cds* over the same time interval. The results with the *cds* mutant are particularly notable since *cds* flies, as is the case with *trp*, fail to sustain a response to a continuous light stimulus (Wu et al., 1995). In view of the genetic enhancement of the *trp* phenotype with *rdgB* and *cds*, another worthwhile experiment would be to test for genetics interactions with *rdgA*, a mutant that disrupts another enzyme involved in PIP_2 recycling, DAG kinase.

The studies with the Kir2.1 biosensor and the putative PIP_2 recycling mutants are intriguing as they provide the first indication that depletion of PIP_2 underlies the transient response and >60 s inactivation in *trp* mutants. Nevertheless, the findings are indirect, and follow-up experiments are needed to provide direct evidence that elimination or inhibition of TRP results in a rapid diminution of PIP_2 levels. Such direct evidence would be provided by demonstrating that the *trp* phenotype is suppressed by addition of PIP_2 to the recording pipet. Furthermore, it would be interesting to test whether addition of PIP_2 antibodies, which have been shown to inhibit several types of K^+ channels, phenocopies *trp* in wild-type photoreceptor cells. Although the experiment might be technically challenging, it would also be important to directly compare PIP_2 levels in wild-type and *trp* photoreceptors after exposing the flies to a bright stimulus of long duration.

Given that La^{3+} was used by Hardie et al. to suppress TRP, it cannot be excluded that La^{3+} decreases PIP_2 concentration by inhibiting one or more enzymes that participate in PIP_2 recycling rather than by decreasing Ca^{2+} influx. La^{3+} is known to modulate the activities of a variety of proteins other than TRP. Nevertheless, an argument against this caveat is that the suppression of Kir2.1 in the presence of La^{3+} can be largely reversed by blocking Na^+/Ca^{2+} exchange during light stimulation. Based on these results, an interesting question would be to test whether a mutation in the Na^+/Ca^{2+} exchanger would suppress, at least in part, the *trp* phenotype. A follow-up experiment that would further mitigate concerns about pleotrophic effects of La^{3+} would be to use the Kir2.1 biosensor to assay PIP_2 levels in a *trp* mutant. However, such studies would have to be performed using very young *trp* flies as TRP is required to retain several signaling proteins, including PLC, in the phototransducing organelle (Li and Montell, 2000).

Mutations in *trpl* also result in a time-dependent decline in the response to light (Leung et al., 2000). However, the reduction in response is less pronounced than in *trp* as there is a ~50% decline after 20 s of constant light. Nevertheless, it would be of interest to test whether a diminution in PIP_2 levels also underlies the *trpl* phenotype. The *trp* phenotype may be much more severe than that of *trpl* due to the greater Ca^{2+} selectivity of TRP compared to TRPL/TRPγ heteromultimers.

A critical question raised by the current work pertains to the basis for the decline in PIP_2 levels as a consequence of inhibiting the TRP-dependent Ca^{2+} influx. In the current work, the authors propose that disruption of the TRP-dependent Ca^{2+} influx results in much lower PIP_2 levels due to the combination of two effects. These include a decrease in the activity of Ca^{2+}-dependent facilitation of one or more enzymes that participate in rapid PIP_2 recycling and a reduction in Ca^{2+}-mediated feedback inhibition of PLC. Ca^{2+}-mediated inhibition of PLC could occur due to feedback inhibition of rhodopsin or the G protein or as a consequence of a direct effect of Ca^{2+} on PLC. There is a precedent for Ca^{2+}-induced inhibition of PLC, although the concept of Ca^{2+}-mediated facilitation of PIP_2 recycling is quite speculative. The regulation of the hydrolysis and recycling rates of PIP_2 levels appear to have a profound impact on the visual response. The short latency in *Drosophila* photo-

transduction appears to be achieved in part by high hydrolysis rates of PIP_2. Without Ca^{2+}-mediated regulation of PIP_2 metabolism, Hardie et al. estimate that a single photon of light might deplete all of the PIP_2 in four microvilli rather than the PIP_2 in the immediate vicinity of the photoactivated rhodopsin.

Finally, one of the important contributions of the current study is the demonstration that Kir2.1 can be an effective biosensor for PIP_2 in vivo. It will be important to establish a dose response curve comparing the activities of Kir2.1 over a range of PIP_2 concentrations. In addition, the work by Hardie et al. raises the possibility that mutations in mammalian TRPC proteins may lead to phenotypic consequences due in part to a reduction in PIP_2 levels. Recently, a mouse knockout of TRPC4 has been reported resulting in an impairment in vasorelaxation. Thus, the means are now available to begin testing this intriguing possibility.

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Rapid-Fire Synaptic Vesicle Exocytosis

The amount of electrical signaling in the brain depends, in large part, on the rate at which neurotransmitters are released from presynaptic terminals. At a given synapse, the net rate of neurotransmitter release is the product of the number of presynaptic release sites (termed active zones) and the rate at which synaptic vesicles can fuse with the plasma membrane at each release site. Therefore, it is important to determine how fast single active zones can release transmitters. Measurements of the rates of vesicle fusion have been made at a number of synapses. For example, at hippocampal synapses there appears to be a requisite refractory period of at least 20 ms between fusion of one vesicle and the next at a single release site (Stevens and Wang, 1995), with a maximum rate of 15–30 fusion events per second per release site (Stevens and Tsujimoto, 1995). In contrast, sensory neurons—such as retinal bipolar or saccular hair cells—are capable of fusing 500 vesicles per second per release site (Parsons et al., 1994; von Gersdorff et al., 1996). This high release rate may be supported by the synaptic "ribbons" that appear to provide a constant supply of releasable vesicles. The lack of these ribbons could then account for the slower maximal rate of vesicle release at more conventional synapses. Alternatively, because postsynaptic responses are typically used to determine the number of vesicles released at central synapses, the disparity could arise from limitations in measuring vesicular release rates.

In this issue of *Neuron*, Sun and Wu (2001) directly measure the number of synaptic vesicles undergoing fusion with the presynaptic plasma membrane during neurotransmitter release. They employed the patch-clamp method to measure membrane capacitance changes that arise from incorporation of vesicular membrane following fusion. Although such measurements have been made at the squid giant synapse (Gillespie, 1979) and goldfish retinal terminals (von Gersdorff et al., 1996), it had not previously been possible to perform such measurements at mammalian central synapses. Sun and Wu were able to accomplish this feat by examining the large presynaptic terminal at the calyx of Held. These synapses arise from the terminations of globular bushy cell axons upon principal neurons in the medial nucleus of the trapezoid body. Because the calyx is so large, it is accessible for patch-clamp recordings in brain stem slices and has been widely used in recent electrophysiological analyses of transmitter release (Forsythe, 1994; Borst et al., 1995; Wang and Kaczmarek 1998).

Kinetics of Vesicle Fusion

With this approach, Sun and Wu were able to resolve several properties of neurotransmitter release at the calyx of Held. First, they measure the number of synaptic vesicles that are ready for release and the rate at which these vesicles are released. The size of this readily releasable pool was determined to be ~3000–5000 synaptic vesicles. This number is much larger than the previous estimates of the releasable pool (600–1800 vesicles) that were based solely on measurements of excitatory postsynaptic currents (EPSCs) arising from the release of glutamate onto the postsynaptic principal neurons (Wu and Borst, 1999; Schneggenburger and Neher, 2000). Such measurements will underestimate the size of the releasable vesicle pool if the postsynaptic receptors are saturated or desensitized during the period of transmitter release. In fact, Sun and Wu showed that EPSC amplitudes are completely saturated when capacitance measurements reach only 35% of their maximum value. Thus, it appears that the contents of ~1/3 of the releasable vesicle pool are sufficient to saturate all the postsynaptic glutamate receptors at the calyceal synapse. This conclusion was supported by experiments employing kynurenate, a drug that reduces glutamate receptor saturation. Given that the calyx has ~500 active