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Intra- and inter-subunit cooperativity in activation of BK channels by Ca^{2+}.

Qian X, Niu X, and Magleby KL. *J Gen Physiol* 128: 389–404, 2006.

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Question: What contributes to the highly cooperative activation of large conductance Ca^{2+}-activated K^+ (BK) channels by Ca^{2+}?

Background: BK channels are activated synergistically by membrane depolarization and elevated intracellular Ca^{2+} concentrations. Activated BK channels hyperpolarize cells, which closes calcium channels to prevent further increases of calcium. Thus BK channels couple membrane potential and Ca^{2+} to cellular excitability. Essential in this coupling feature of BK channels is that the activation of BK channels by Ca^{2+} is highly cooperative, such that small changes in $[Ca^{2+}]$ can elicit large changes in channel activity, which in turn can have a large impact on membrane potential and cellular excitability. The mechanism underlying the high cooperativity of the activation remains unclear, but the high Hill coefficients for Ca^{2+} activation of BK channels suggest that a minimum of 2–5 Ca^{2+} ions must bind for full activation. Consistent with such a large number of bound Ca^{2+}, BK channels exist as tetramers, in which each subunit has two different types of high-affinity Ca^{2+} sensors.

Observations: To explore the interactions between the two different types of high-affinity Ca^{2+} sensors, Qian et al. compared Ca^{2+}-dependent activation of BK channels in which the sensors were expressed in eight different combinations. Interestingly, when each of the four subunits had only one type of the Ca^{2+} sensor, regardless of whether the sensors were the same or different, the sensors had roughly equivalent effects. However, when the four Ca^{2+} sensors were present with two sensors on each of two subunits and no sensors on the remaining two subunits, channel activity was much higher than when there was one Ca^{2+} sensor per subunit, indicating positive intra-subunit cooperativity.

Significance: Intra-subunit cooperativity of Ca^{2+} sensors contributes to the highly cooperative activation of BK channels by Ca^{2+}. Although it remains to be determined how the cooperativity between sensors on the same subunit is achieved structurally, these results have widespread implications for cell physiology, since the high cooperativity plays a major role in tuning the response to Ca^{2+}.

Action of TFII-I outside the nucleus as an inhibitor of agonist-induced calcium entry.

Caraveo G, van Rossum DB, Patterson RL, Snyder SH, and Desiderio S. *Science* 314: 122–125, 2006.

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Question: Does the transcription factor TFII-I function outside the nucleus to regulate Ca^{2+} influx?

Background: Agonist-induced activation of phospholipase C (PLC) leads to Ca^{2+} release from intracellular stores and an influx of Ca^{2+} through transient receptor potential (TRP) channels. PLC-γ promotes Ca^{2+} entry by stimulating the expression of TRPC3 channels in the plasma membrane. PLC-γ is uniquely structured with a partial pleckstrin homology (PH) domain, which binds to a PH-like domain in TRPC3 channels. PLC-γ also binds the transcription factor TFII-I, which suggests there may be a functional link between TFII-I and TRPC3 channels.

Observations: Caraveo et al. determined that TFII-I and PLC-γ associated with each other in vivo; TFII-I binds to the Src homology 2 domain on PLC-γ and via an interaction between the PH-like domain of TFII-I and the PH domain in PLC-γ. TFII-I was also found to function as a negative regulator of agonist-induced Ca^{2+} entry by antagonizing PLC-γ through multiple interactions and thus controlling TRPC3 accumulation at the cell surface.

Significance: These results reveal a unique function of a transcription factor outside the nucleus. Perhaps more importantly though, these results may help to elucidate the etiology of Williams-Beuren syndrome, which is a genetic disorder characterized by developmental delay, unusual facial appearance, cardiac abnormalities, and particular cognitive and personality anomalies. The gene for TFII-I is located on chromosome 7, which is in an interval that is deleted in patients with Williams-Beuren syndrome.

Activity- and mTOR-dependent suppression of Kv1.1 channel mRNA translation in dendrites.

Raab-Graham KF, Haddick PC, Jan YN, and Jan LY. *Science* 314: 144–148, 2006.

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Question: How is the expression of Kv1 potassium channels modulated by synaptic activity?

Background: Kv1 channels have primarily been localized to axons but are also expressed in somatodendritic regions of neurons, where they control local excitability of the dendritic branches. The mechanisms responsible for the targeting of Kv1 channel expression to axons are known. In contrast, little is known about the mechanisms that control the targeting of Kv1 channels to dendrites. To this end, the mTOR kinase is known to be important for long-term potentiation and long-term depression. Whether the mTOR pathway regulates Kv1 channel expression was the focus of this report.

Observations: Raab-Graham and colleagues found that the mTOR inhibitor, rapamycin, increased Kv1.1, but not Kv1.4, in the dendritic field of rat CA1 hippocampal slices. This induced increase was also observed in punctate structures in dendrites, but not axons of hippocampal neuronal cultures. They also determined that the dendritic expression of Kv1.1 was due to local synthesis of the protein in the dendrites, and this local dendritic synthesis could be induced by inhibiting mTOR or the NMDA glutamate receptor.

Significance: These findings suggest that dendritic Kv1.1 protein expression in the hippocampus is regulated by local NMDA receptor-mediated synaptic activity. The mechanism by which NMDA mediates this effect is by way of mTOR-induced inhibition of Kv1.1 mRNA translation locally at the dendrite. This represents a novel way in which synaptic activity may regulate the control Kv channels have on the local excitability of dendritic branches.

Imaging intracellular fluorescent proteins at nanometer resolution. Betzig E, Patterson GH, Sougrat R, Lindwasser OW, Olenych S, Bonifacino JS, Davidson MW, Lippincott-Schwartz J, and Hess HF. *Science* 313: 1642–1645, 2006.

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Question: How can imaging of intracellular fluorescence-tagged proteins be achieved at nanometer (nm) spatial resolution?

Background: Fluorescent probes that are linked to molecules inside cells can be switched on or off by exposing them to light, revealing their spatial organization at the molecular level. However, conventional fluorescent microscopes have a resolution limit of a few hundred nanometers, which means that, when tagged proteins are <200 nm apart, they are indistinguishable from one another.

Observations: Betzig, Hess, and colleagues describe a super-resolution microscopy technique called photoactivatable localization microscopy (PALM) that can localize individual fluorescent molecules between 2 and 25 nm apart. Molecules are labeled with a fluorescent probe and then exposed to a flash of light. In a small number of the molecules, the fluorescence is activated by the light, and an image of the illuminated molecules is captured by the microscope. This process is repeated approximately 10,000 times over several hours, each time recording the position of a different subset of molecules. When the frames of individual snapshots are compiled, the resulting image has a resolution previously only achievable with an electron microscope.

Significance: Although a time-consuming process, PALM provides superb spatial resolution, similar to that of electron microscopy. An advantage of this approach over electron microscopy, however, is that this technique allows for more flexibility in labeling molecules of interest. In fact, it is conceivable that this approach may one day be used to study protein-protein interactions.

Mechanisms of action of acetazolamide in the prophylaxis and treatment of acute mountain sickness: a review. Leaf DE, and Goldfarb DS. *J Appl Physiol* (October 5, 2006); doi:10.1152/japplphysiol.01572.2005.

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Question: What are the underlying mechanisms by which acetazolamide ameliorates the symptoms of acute mountain sickness (AMS)?

Background: AMS, also known as altitude sickness, is the most common pathological condition caused by acute exposure to high altitudes. Under normal conditions, CO_2 diffuses from tissues into erythrocytes where it combines with water in a reaction catalyzed by carbonic anhydrase (CA) to form carbonic acid. The carbonic acid, in turn, dissociates into HCO_3^-, which diffuses into the plasma, and H^+, which is buffered by hemoglobin. In the lungs, H^+ and HCO_3^- reassociate to form H_2O and CO_2, which is expelled during exhalation. At high altitudes, the partial pressure of O_2 in the atmosphere declines, resulting in hypoxemia and an increase in the volume of air normally inhaled with each breath (the tidal volume), which leads to respiratory alkalosis (a decrease in H^+ concentration). Respiratory alkalosis limits the adaptive increase in ventilation and contributes to the symptoms of AMS. Acetazolamide is a potent inhibitor of CA and the most common treatment and prophylactic for AMS; however, the mechanism(s) by which this effect occurs is not clear.

Observations: Leaf and Goldfarb do a thorough examination of the literature in an attempt to better understand the complex, and sometimes conflicting, mechanisms of acetazolamide action in reducing AMS. First, they conclude that an increase in tidal volume induced by acetazolamide results from central chemoreceptor (CCR) and not peripheral chemoreceptor (PCR) output. The increase in CCR output results from metabolic acidosis (an increase in H^+ concentration), which is caused by inhibition of CA in the kidneys, and tissue respiratory acidosis, which results from inhibition of CA in tissues. Furthermore, they conclude that acetazolamide-induced inhibition of PCRs reduces sleep periodic breathing (clusters of breaths separated by intervals of apnea), the effect of which may also be therapeutic. Finally, by inhibiting CA in the kidneys, acetazolamide induces diuresis and natriuresis, which also potentially contribute to the amelioration of AMS.

Significance: The collective findings of this review lead the authors to propose an alternative model to the conventional explanation for acetazolamide's mechanism of action in reducing AMS. The proposed model more completely describes the complex relationship between acetazolamide-induced CA inhibition and reduction of AMS symptoms. This model not only encompasses the conventional explanation of metabolic acidosis offsetting the effects of hypoxia-induced alkalosis but also includes the effects of tissue respiratory acidosis, sleep periodic breathing, and diuresis.

Modulation of the control of muscle sympathetic nerve activity during severe orthostatic stress. Ichinose M, Saito M, Fujii N, Kondo N, and Nishiyasu T. *J Physiol* 576: 947–958, 2006.

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Question: What prevents us from fainting when we stand up?

Background: When one assumes an orthostatic (upright) position, blood pressure must be maintained by homeostatic mechanisms to prevent syncope (fainting). This mechanism involves baroreceptors in the carotid sinuses and the aortic arch that perceive the change in blood pressure caused by standing up, compare it to a set point, and induce a central reflex [arterial baroreflex (ABR)] to increase heart rate and cause peripheral vasoconstriction via the sympathetic nervous system. As the severity of orthostatic stress increases, peripheral vascular resistance and muscle sympathetic nerve activity (MSNA), a direct measure of sympathetic nervous outflow, progressively increase. However, whether ABR-mediated control over the occurrence and strength of MSNA is progressively modulated as orthostatic stress is increased until syncope is induced is unknown.

Observations: Ichinose et al. measured

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MSNA of subjects enclosed in a lower body negative pressure (LBNP) device, which is a tool designed to apply a lower than ambient pressure (suction) to a subject's body from the hips down and cause blood to be sequestered from the thorax into regions of the pelvis and legs, effectively decreasing central blood volume. The authors found that the linear relationship between diastolic blood pressure (DAP) and burst strength of MSNA and between DAP and total MSNA were shifted progressively upward as LBNP increased until the level at which syncope occurred. However, the relationship between DAP and burst incidence of MSNA shifted upward only to a certain extent, at which point there was no further upward shift at higher levels of suction.

Significance: This study was the first to quantify total MSNA, which is dependent on both the burst frequency and the burst strength, and is thus a better index for quantifying the level of MSNA. The orthostatic-induced increase in burst incidence, burst strength, and total MSNA was mediated by the modulation of ABR function, but ABR control over MSNA was impaired immediately before the severe hypotension associated with orthostatic syncope.

Preparatory activity in premotor and motor cortex reflects the speed of the upcoming reach. Churchland MM, Santhanam G, and Shenoy KV. *J Neurophysiol* (July 19, 2006). doi:10.1152/jn.00307.2006.

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Question: Is a non-spatial aspect of a cued reach, speed, represented by preparatory activity in discrete brain regions?

Background: Before a voluntary movement is executed, there are preparatory changes in neuronal firing in discrete brain areas, such as the dorsal premotor cortex (PMd) and primary motor cortex (M1), which occur during the temporal delay that separates the instruction stimulus from the "go" stimulus. Although this preparatory activity has most often been associated with reference to spatial aspects of the upcoming reach to a target, there is accumulating evidence that activity in the PMd/M1 may also reflect non-spatial aspects. In other words, activity in these specific brain areas may also depend on factors other than the location of the target in space.

Observations: Chruchland et al. set out to determine whether preparatory activity in the PMd and M1 was reflective of the speed of the upcoming cued reach, a non-spatial aspect. Expectedly, they found that preparatory activity was strongly influenced by the direction and distance of the cued reach into space. In addition and in support of their hypothesis, they also found that the instructed speed of the reach (as relayed by target color) strongly influenced the delay-period activity.

Significance: These intriguing findings may indicate that the speed of a cued reach is represented directly during delay period activity. However, it is noted that the observed tuning for speed could also be related to factors that correlate with reach velocity, such as the different activation patterns of muscles. Finally, it is suggested that preparatory activity may be best understood in terms of its causal role in generating movement, rather than in terms of "coded" parameters. Regardless of the exact interpretation, this research indicates that even non-spatial aspects of an upcoming reach are represented and prepared for during the delay period.

Phosphoinositide 3-kinase binds to TRPV1 and mediates NGF-stimulated TRPV1 trafficking to the plasma membrane. Stein AT, Ufret-Vincenty CA, Hua L, Santana LF, and Gordon SE. *J Gen Physiol* 128: 509–522, 2006.

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Question: Is phospholipase C (PLC) activation involved in the nerve growth factor (NGF)-induced sensitization of the transient receptor potential vanilloid 1 (TRPV1) ion channel, which leads to hyperalgesia?

Background: TRPV1 is responsible for detecting painful thermal and chemical stimuli. It has generally been thought that sensitization of TRPV1, or hyperalgesia, by NGF involves activation of PLC, which leads to the metabolism of phosphatidyl inositol 4,5-bisphosphate (PIP_2) and disinhibition of TRPV1. However, more recent studies suggest that NGF may sensitize TRPV1 by interacting with PI3 kinase, which leads to Src-induced trafficking of TRPV1 to the plasma membrane.

Observations: Stein et al., perhaps unexpectedly, found that direct application of PIP_2 potentiated, rather than inhibited, the activation of TRPV1. In contrast, removal of PIP_2 inhibited, rather than potentiated, the activation of TRPV1. In four separate experiments, they go on to demonstrate that PI3 kinase is both physically and functionally coupled to TRPV1. Finally, they provide evidence that the mechanism by which NGF causes potentiation of TRPV1 is by increasing the number of channels expressed in the membrane.

Significance: These results suggest that the role PIP_2 plays in TRPV1 sensitization is completely opposite of what was previously thought. Moreover, the authors propose a new model of NGF-mediated hyperalgesia, which involves PI3 kinase-dependent trafficking of TRPV1 to the plasma membrane leading to increased TRPV1 activity.

Waking experience affects sleep need in *Drosophila*. Ganguly-Fitzgerald I, Donlea J, and Shaw PJ. *Science* 313: 1775–1781, 2006.

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Question: How does sleep influence memory consolidation of social experiences?

Background: Although sleep is an essential phenomenon that occurs in the animal kingdom, it is not clear why we sleep. Several studies that involve sleep deprivation support a role for sleep in the consolidation of memories. In this study, Ganguly-Fitzgerald et al. provide a molecular connection between sleep, plasticity, and memory formation.

Observations: *Drosophila* fruit flies were exposed to an environment enriched with other flies or an impoverished environment, in which flies were singly housed. The flies that were housed in groups slept significantly more then their isolated siblings. Interestingly, the amount of sleep in the group-housed flies was proportional to the size of the group in which they were housed. These differences were not due to differences in activity levels, mating experience, or access to space and food, and were not affected by mutations in the genes that control circadian rhythms. In contrast, the

amount of sleep required was dependent on the most recent social experience, likely gleaned via visual and olfactory input during prior waking. Moreover, the authors determined that the socially deprived flies had a third less of dopamine and that, when endogenous dopamine levels were disrupted, so were the sleep patterns. Furthermore, mutations in both long- and short-term memory genes affected experience-dependent plasticity in sleep need. Finally, when male flies were trained on a courtship conditioning task, which leads to the formation of long-term memories, they subsequently needed more sleep. If this sleep was disrupted, so was the formation of memory.

Significance: Collectively, these findings suggest that new experiences increase the need for sleep, which is necessary for new connections to be made in the brain. By using *Drosophila* as a model for the study of sleep and memory, it provides a segue into our understanding of this relationship from a molecular perspective. Although far from complete, this work at least provides some insight into the fundamental process of sleep. Future research efforts will undoubtedly be focused on analogous effects in humans. ■