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The adenosine story goes ionic: $Ca_V2.1$-type Ca^{2+} channels identified as effectors of adenosine's somnogenic actions. Commentary on Deboer *et al*. Reduced sleep and low adenosinergic sensitivity in Cacna1a R192Q mutant mice (SP-00101-12).

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Attenuating the actions of the neurochemical adenosine through a cup of coffee is part of the daily wake-up procedure of many amongst us. A large body of accumulating literature, coined as the "adenosine story"[1], indicates that adenosine is a primary sleep factor and that caffeine, coffee's active ingredient, combats adenosinergic receptor activation[2]. Adenosine results from the degradation of the energy-rich molecule ATP that is consumed in the brain during electrical and synaptic activity[2,3]. Being awake and attentive is energetically costly for the brain and markedly increases adenosinergic "tone"[2,4,5]. By binding to receptor subtypes A_1 and A_{2A}, adenosine exerts diverse neuromodulatory actions throughout the brain[6]. A_1 receptors are inhibitory, G_i-coupled receptors widely expressed in wake-active areas and central to adenosine's sleep promotion[2]. But many questions about how adenosine relates to sleep homeostasis remain. Which of the cellular signaling pathways engaged by A_1 receptors are responsible for sleep induction? The new study by Deboer and colleagues[7] in this issue of SLEEP is the first to specify the molecular basis of an ionic pathway for the somnogenic actions of A_1 receptor activation: an inhibition of $Ca_V2.1$-type Ca^{2+} channels, members of the family of voltage-gated Ca^{2+} channels. $Ca_V2.1$ channels are involved in controlling vesicular release from presynaptic terminals, therefore, attenuation of neurotransmission at $Ca_V2.1$-expressing synapses is an important pathway for sleep induction through adenosine.

Voltage-gated, Ca^{2+}-permeable ion channels are found at both presynaptic and postsynaptic sites and are key determinants of neuronal excitability and synaptic transmission. The three members of the Ca_V2 channel group, $Ca_V2.1$ - $Ca_V2.3$, are concentrated at presynaptic terminals of excitatory and inhibitory synapses, where they typically co-operate to control synaptic release. $Ca_V2.1$ channels often mediate highly reliable and temporally precise release[8] and are found throughout the brain, including in subcortical and cortical areas involved in the regulation of vigilance states[9-11]. Moreover, these channels are susceptible to inhibition by G_i-protein-coupled neurotransmitters, such as adenosinergic A_1 receptors[12]. Adenosine dampens glutamate release at several excitatory terminals in the brain[13], including through A_1-receptor-mediated Ca_V2 channel

inhibition[14,15], thereby controlling synaptic plasticity[16,17] and protecting neurons from insult[18]. $Ca_V2.1$ channels are thus particularly favorable candidates to contribute to adenosine's promotion of sleep.

However, testing an ion channel's involvement in adenosinergic regulation of synaptic transmission in the intact brain is tricky, since, if modified, network excitability and hence release of neurotransmitter will be altered, notably that of adenosine itself. Moreover, loss-of-function of $Ca_V2.1$ channels causes major motor disorders[19] and compensatory upregulation of other voltage-gated Ca^{2+} channels[20]. Therefore, assessing sleep-wake behavior in animals with dysfunctional channels is unlikely to provide conclusive insights about adenosine's molecular targets.

Deboer et al.[7] found an elegant solution when choosing a knock-in mouse carrying the R192Q mutation in the *Cacna1a* gene encoding the pore-forming subunit of $Ca_V2.1$ channels. This amino acid substitution in the S4 transmembrane domain decreases the sensitivity of the $Ca_V2.1$ channel to G_i-proteins, while leaving intact the maximal extent of inhibition[21]. The mutation also results in a shift in the voltage dependence of Cav2.1 channels[21]. Nevertheless, this mouse permitted study of the role of a functionally responsive Ca^{2+} channel with preserved expression levels, but compromised primarily in G-protein-mediated inhibition[22]. The hypothesis to be tested by Deboer et al.[7] was clear: if $Ca_V2.1$ channels mediate some of adenosinergic actions on sleep, then these animals should show attenuated sleep behavior when adenosine concentrations are elevated, but not when they are exceedingly low or high. Indeed, under natural sleeping conditions, R192Q knock-in mice showed less NREM sleep and substantially prolonged wake periods during the dark, active phase, whereas sleep-wake behavior in the light was unaltered. Additionally, mice responded normally to sleep deprivation and showed preserved rebound sleep and elevated slow-wave activity, consistent with the idea that high adenosine levels may inhibit $Ca_V2.1$ channels close-to-maximally. Again, however, animals were more active in the ensuing dark period, during which adenosine levels are lowered. Intriguingly, when the mutated animals were injected with either caffeine or the specific A_1 receptor agonist cyclopentyladenosine, they showed a more rapid reversal to the pre-drug sleep-wake behavior. $Ca_V2.1$ channels are thus effectors acting in proportion to both increases and decreases in adenosine,

indicating that they are on-going bidirectional monitors of adenosinergic "tone" and mediate the duration of its actions on sleep-wake behavior. In agreement with this, R192Q mice live under a constantly elevated sleep pressure, as adenosine levels are no longer funneled through Cav2.1 channels to promote sleep.

In conclusion, the work presented by Deboer and colleagues[7] significantly advances understanding of the signaling pathways recruited by adenosine that are important for somnogenesis. One should certainly keep in mind that in the R192Q mouse, G_i-mediated channel inhibition is compromised throughout the brain, hampering many important presynaptic modulators and altering the voltage-dependence of the Cav2.1 channels. In humans, the R192Q substitution occurs from a spontaneous missense mutation causing familial hemiplegic migraine[22,23]. Nevertheless, the choice of this mouse is particularly fortuitous, since it reveals much of adenosine's actions. So far, the sleep-inducing properties of adenosine have been dominantly associated to adenosine's direct hyperpolarization of wake-promoting neurons, to the promotion of bursting activity in thalamus[24], and to concomitant direct excitation of sleep-promoting neurons by A_{2A} receptors[6]. These postsynaptic actions are undoubtedly important, but Deboer et al.[7] now bring to the forefront that adenosine steadily suppresses an on-going presynaptic drive, notably at terminals expressing $Ca_V2.1$ channels. How exactly in the brain weakening of this drive leads to sleep remains to be discovered, but a potential site for such inhibition is the ascending cholinergic system, a primary site of adenosine action[25]. Indeed, *in vitro* studies show that glutamate release onto basal forebrain cholinergic neurons is dominated by $Ca_V2.1$ channels[10] and, in pontine tegmentum, inhibited by adenosine[26]. Moreover, adenosine inhibits glutamatergic afferents onto hypocretin/orexin neurons via inhibition of presynaptic voltage-gated Ca^{2+} channels[11]. Conversely, A_1-mediated presynaptic effects in thalamus promote desynchronization of thalamic sleep-related network activity[27]. Pioneering work of the kind presented by Deboer et al.[7] will help to balance the relative importance of adenosine's multiple sites of action, which is critical for understanding the

mechanisms of sleep regulation, and for future endeavours in specific drug development and medical treatment for sleep disorders.

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