Crossing the channel: Surprising new findings in the neurology of sleep and vigilance

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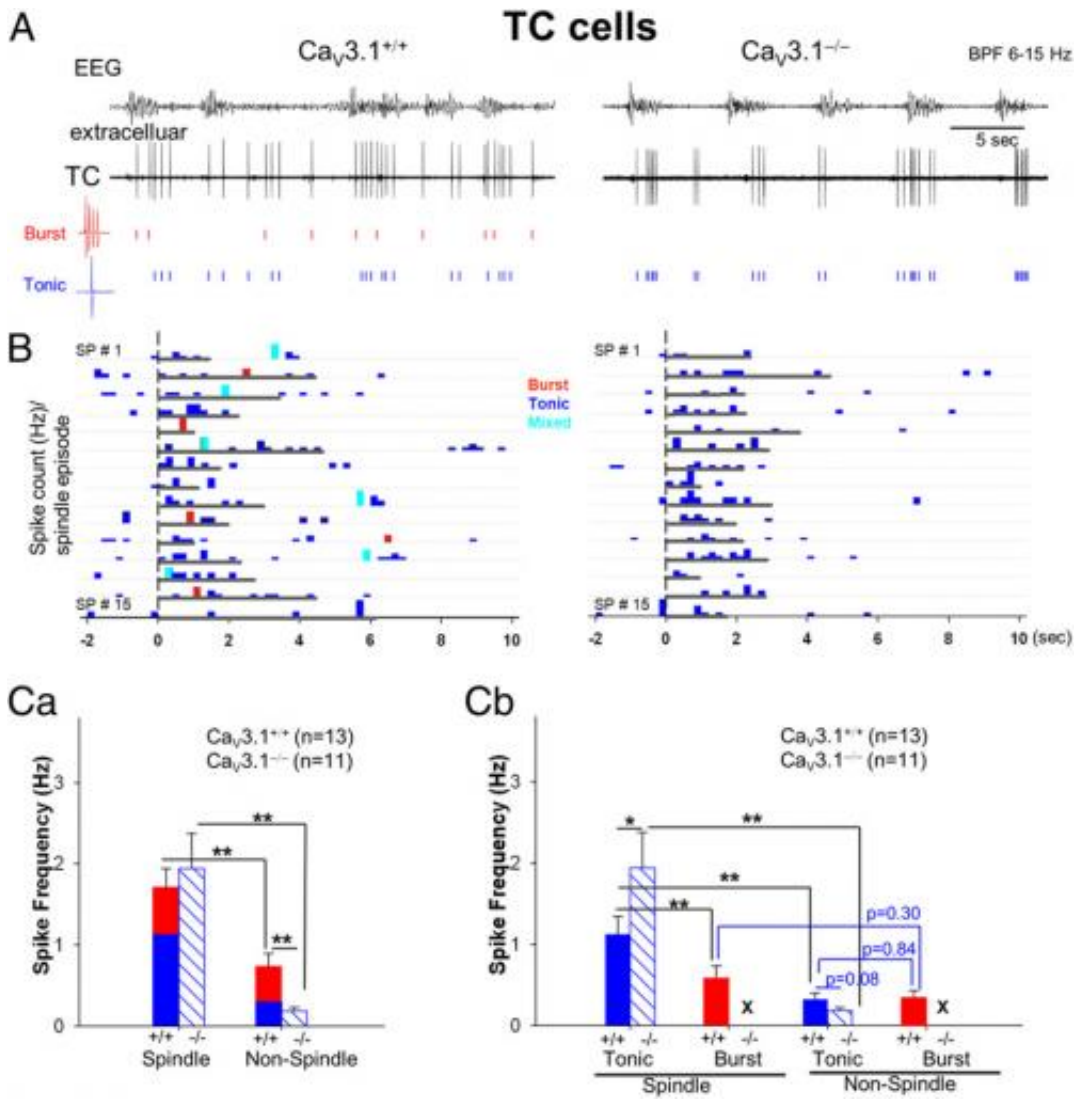

Sleep spindles during NREM sleep in *$Ca_V3.1^{+/+}$* (WT) and *$Ca_V3.1^{-/-}$* (KO) mice. (*A*) Sample traces show the raw (upper trace) and filtered (lower trace) EEG

signals recorded during NREM sleep. Bandpass-filtered (6–15 Hz) EEG signals clearly show spindle events (arrowheads) in both genotypes. (*B*) There were no differences in the mean length of each spindle episode, number of episodes, mean peak-to-peak amplitude, and peak frequency between $Ca_V3.1^{+/+}$ (WT) and $Ca_V3.1^{-/-}$ mice. , doi:10.1073/pnas.1320572110

(Medical Xpress)—A recent neurological addressing one of the most fundamental issues in sleep rhythm generation study underscores an inconvenient truth—namely, that established scientific facts have and will continue to change. Researchers at Institute for Basic Science (Daejeon), Korea Institute of Science and Technology (Seoul) and Yonsei University (Seoul) have demonstrated significant exceptions to the theory, long accepted as dogma, that low-threshold burst firing mediated by T-type Ca^{2+} channels in thalamocortical neurons is the key component for sleep spindles. (A *T-type Ca^{2+} channel* is a type of voltage-gated ion channel that displays selective permeability to calcium ions with a transient length of activation. *Burst firing* refers to periods of rapid neural spiking followed by quiescent, silent, periods. *Sleep spindles* are bursts of oscillatory brain activity visible on an EEG that occurs during non-rapid eye movement stage 2, or NREM-2, sleep, during which no eye movement occurs, and dreaming is very rare.) The scientists presented both *in vivo* and *in vitro* evidence that sleep spindles are generated normally in the *absence* of T-type channels and burst firing (periods of rapid neural spiking followed by quiescent, silent, periods) in thalamocortical neurons. Moreover, their results show what they describe as a potentially important role of tonic (constant) firing in this rhythm generation. They conclude that future studies should be aimed at investigating the detailed mechanism through which each type of thalamocortical oscillation is generated.

Dr. Hee-Sup Shin and Prof. Eunji Cheong discussed the paper that they

recently published in *Proceedings of the National Academy of Sciences*. "The previous theory implicated thalamocortical TC burst firing in all sleep waves which appear in different sleep stages," Cheong tells Medical Xpress. "However, we've long questioned the extent to which thalamocortical T-type Ca^{2+} channels and the resulting burst firing contribute to the heterogeneity of thalamocortical oscillations during non-rapid eye movement sleep consisting of multiple brain waves." A *T-type* Ca^{2+}*channel* is a type of voltage-gated ion channel which displays selective permeability to calcium ions, in this case with a transient length of activation.

Shin notes that the scientists faced a number of issues in designing and interpreting the results of the *in vivo* and *in vitro* experiments to test their hypothesis. "Since we observed the quite intact sleep spindles in $Ca_V3.1$ knockout mice, we tried to figure out how the sleep spindles are generated in the absence of a thalamocortical burst." (A *gene knockout*, or KO, is a genetic technique in which one of an organism's genes is made inoperative to learn about its function from the difference between the knockout organism and normal individuals. *$Ca_V3.1$* is a T-type calcium channel found in neurons, cells that have pacemaker activity.) "The issues were if the spindles are generated within the thalamocortical circuit as previously known, and how thalamocortical neurons generate spikes during spindles in the presence or absence of a thalamocortical burst." All of the researchers' the experiments were designed to investigate these questions.

"The purpose of *in vitro* thalamocortical-thalamic reticular nucleus," or TC-TRN, "network oscillations was to show if thalamocortical oscillations observed in $Ca_V3.1$ knockout mice could be generated either within an intrathalamic network or if they were cortical driven oscillations," Cheong points out. "Another difference between *in vivo* and *in vitro* networks is that compared to *in vivo* network all the afferent inputs into TC or TRN are *not* intact in an *in vitro* TC-TRN network."

The results showed that spindle-like oscillations were generated even in the absence of cortex.

The study shows that these differences also relate to *In vivo* data suggesting that TRN neurons are spindle pacemakers. "There have been debates on the leading role of TRN versus cortex in pacing the sleep spindles. In an *in vitro* TC-TRN network, both the afferent inputs and corticothalamic inputs onto TC neurons are not intact," Shin explains. "Therefore, major inputs onto TC neurons in those experiments come from TRN neurons. The generation of intrathalamic oscillations under this condition indicates that the reciprocal connection between TRN and TC could generate the oscillations, which adds weight to the TRN neurons as spindle pacemakers. The generation of $Ca_V3.1$ knockout mice which lack T-type Ca^{2+} channels in TC neurons was the key to address this issue."

Cheong emphasizes that the study's major findings call into question the essential role of low-threshold burst firings in thalamocortical neurons. "It's noteworthy that tonic spikes were more abundant than burst spikes during spindles even in wild Type thalamocortical neurons – not only in $Ca_V3.1^{-/-}$ TC neurons – whereas no difference in tonic and burst spike frequency was seen during non-spindle periods. Moreover," he continues, "the tonic spike frequency increases significantly during cortical spindle events compared to non-spindle periods even in wild-type TC neurons. This is clearly different from that seen for burst spike frequency in wild-type TC neurons, which occurred with almost equal incidence during both the spindle and non-spindle periods." Therefore, Cheong points out, the scientists concluded that TC burst firing is not required for the generation in spindle generation.

The researchers also found that the peak frequency of sleep spindles was not different between wild and $Ca_V3.1$ KO mice, which suggested that TC spikes are not critical in determining the spindle frequency.

However, Shin notes, the question of what drives TC neurons to fire during spindles remains to be further investigated, although they think that TC firing during spindles indicates that the TC-TRN network is not as simple as previously believed.

Moving forward, Cheong tells Medical Xpress, the researchers would like to further investigate the firing pattern of TC neurons during natural NREM sleep, including spindle, delta and slow waves. and also elucidate the detailed ensemble behavior of neuron within thalamocortical network during sleep. Moreover, TC burst firing has long been implicated in both physiological thalamocortical oscillations during both sleep and pathological thalamocortical oscillations, such as spike-wave-discharges appearing in absence epilepsy. "Our current study clearly showed that TC burst are not essential for sleep spindles, which would be helpful information to develop the anti-epileptic agents," Shin concludes.

More information: Sleep spindles are generated in the absence of T-type calcium channel-mediated low-threshold burst firing of thalamocortical neurons, *PNAS* December 10, 2013 vol. 110 no. 50 20266-20271, doi:10.1073/pnas.1320572110