Calcium channels in higher-level brain function

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Nature has a habit of using relatively simple cellular mechanisms in the service of the higher-level integrative functions of multicellular organ systems. In the article by Llinás *et al.* (1), published in a recent issue of PNAS, we can see an example of how a mechanism that probably evolved for maintaining the integrity of the excitable membranes of nerve, heart, glandular, and muscle cells, when placed in the context of a highly interconnected network of neurons, is recruited as a fundamental component of brain mechanisms that underlie states of consciousness and that are also involved in those of perception and cognition.

Voltage-Gated Calcium Channels

The focus of the article by Llinás *et al.* (1) is on the P/Q type of calcium channel, a member of a family of voltage-gated calcium channels that is itself part of an even larger superfamily of transmembrane ion channels that also includes voltage-gated sodium and potassium channels. Each calcium channel is made up of four or five protein subunits encoded by different genes (2). The large $\alpha 1$ subunit, like similar subunits in other voltage-gated ion channels, consists of four homologous domains, each containing six transmembrane segments. This large protein forms the conduction pore, and different segments serve as the voltage sensor and determine ion selectivity and channel conductance. Differences in the $\alpha 1$ subunit, which depend on the expression of at least 10 known genes, determine the properties of individual channel types. Other subunits form the links to downstream intracellular processes, modulate the pharmacological properties of the ion channel, and provide an additional basis for the diversity of channel types.

P/Q channels (formally named $Ca_v2.1$ channels) belong to the Ca_v2 superfamily of voltage-gated calcium channels and are distinguished from other voltage-gated calcium channels by the presence of an α_{1A} subunit. As we see in the article by Llinás *et al.* (1), these channels are selectively blocked by a spider venom, ω-agatoxin, a useful tool for experimental studies. In the normal central nervous system, P/Q channels, when activated by strong depolarization, support Ca^{2+}-dependent synaptic transmission between neurons at many sites, including the thalamus, which is the brain region at the heart of the investigation by Llinás *et al.*

The Thalamus and Higher Brain Function

The thalamus, a part of the diencephalon, receives input from the peripheral sense organs and transmits this to the cerebral cortex as part of the pathway to perception. The traffic between thalamus and cortex is not, however, all one way, for there is a very substantial return projection from the cortex to the thalamus, and in the normal course of forebrain function, the interconnected thalamo-cortico-thalamic network is engaged as an integrated whole (3). This integrated activity is likely to extend to the basal ganglia as well.

Earlier work by Jahnsen and Llinás (4) in the same *in vitro* slice preparation used in the present study, supplemented and extended by that of Steriade and coworkers (5) in whole animals, first served to demonstrate the importance of activity in the thalamo-cortico-thalamic network in determining the state of arousal of an animal, the relationship of this activity to perception and cognition, and the fundamental role of the voltage-gated calcium channels expressed in thalamic neurons in determining the activity of the network in different states of consciousness (6).

When the thalamic neurons whose axons provide the input to the cerebral cortex are, as it were, left to themselves in states of drowsy inattentiveness when input from the sense organs is reduced, they tend to drift toward hyperpolarization, an effect that depends in the main on leakage of potassium ions through another cation channel (7). When its membrane potential approaches −70 mV, another voltage-gated calcium channel, the $Ca_v3.1$ or T-type channel, located mainly on cell bodies and proximal dendrites, becomes de-inactivated and causes the neuron, when subjected to weak depolarization, to discharge a calcium-mediated action potential that in turn causes the neuron to fire a short, high-frequency burst of regular Na^+/K^+ action potentials. At this point, a train of discharges in corticothalamic axons that are reentering the thalamus and having their greatest effect on the GABAergic, inhibitory neurons of the reticular nucleus (8) (which promote rhythmic hyperpolarizations of the relay neurons) will entrain the repetitive bursts of individual neurons into low-frequency oscillations of the whole network. These can be recorded as the high-voltage, slow waves in the electroencephalogram, waves that represent the stigmata of inattentiveness and sleep. Pathologically enhanced synchrony of bursting thalamic neurons is a feature of the condition known as absence or petit mal epilepsy and can be recorded as spike and wave discharges in an EEG. The thalamic neurons of mice null for the $Ca_v3.1$ (T) channel lack burst discharges and slow wave synchrony, and the animals display a resistance to the spike and wave discharges that can be induced in normal mice by injection of agonists of the $GABA_B$ receptor (9).

Gamma Oscillations and Cognition

In contrast, when thalamic neurons are relatively depolarized, mainly under the influence of the brainstem-activating systems, as in attentive wakefulness, the low-threshold, T-type channels are inactivated and, instead, the higher-threshold P/Q-type channels, located mainly on more peripheral dendrites, are activated. In a sense, the neurons are now ready synaptically to transmit the input from the sense organs reliably and without decrement to the cerebral cortex. At this point, the influence of the reticular nucleus is at its weakest, and a volley of corticothalamic action potentials tends to promote depolarization of the relay cells and thus their effectiveness as thalamocortical relays when exposed to inputs from the periphery. Moreover, in this relatively depolarized state, the P/Q channels promote a remarkable high-frequency (≈40 Hz) subthreshold oscillation of the neuronal membrane, an oscillation that helps entrain synaptic inputs (10). These are in turn entrained by the corticothalamic inputs so that a high-frequency oscillation then engages the whole thalamo-cortico-thalamic network. This oscillation is represented in the EEG as the typical low-amplitude, fast (30–80 Hz) waves ("gamma waves") characteristic of the aroused, attentive state.

Heightened gamma activity and associated increased functional activity of the cerebral cortex is normally found during performance of demanding cog-

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nitive tasks such as associative learning and during perception of sensory stimuli (11). It is generally agreed that widespread synchrony of forebrain gamma activity is a prerequisite for perceptual feature binding. Reduced gamma synchrony is detected in the frontal lobe of individuals such as schizophrenics in whom normal cognitive control is impaired (12).

The article by Llinás *et al.* (1) combines patch-clamp recordings of thalamic neurons and voltage-sensitive dye imaging in *in vitro* slices of the connected thalamus and cortex from normal mice and from mice null for the P/Q channel, along with EEG recordings from the same mice. The findings serve to advance our knowledge of the relationships of gamma-wave oscillations in the thalamo-cortico-thalamic network to forebrain activities underlying cognitive states.

Knockout of the gene coding for the α_{1A} subunit and thus an absence of P/Q channels is not incompatible with early life, although the mice die at ≈3 weeks of age. During the postnatal period, as shown by Llinás *et al.* (1), compensatory expression of N-type ($Ca_v2.2$) channels in thalamic and cortical neurons can support a small complement of high-threshold current in thalamic neurons, serving to maintain excitatory synaptic transmission between the thalamus and cortex and supporting activation of the cortex by the thalamus. These compensatory effects of N-type channels were insufficient, however, to maintain the subthreshold ≈40-Hz membrane oscillations observed in depolarizing thalamic neurons from normal mice. Nor could they constrain 40-Hz activation of the cortex by thalamic fibers to the narrow columns observed in slices from normal animals and thought to be essential for the shaping of activity underlying higher-level cognitive function (13). The columns normally depend on the recruitment of intracortical inhibitory neurons by a high-frequency (gamma) thalamic input, the mechanisms of which are still not fully understood. In keeping with the lack of columnarity, EEG recordings from freely moving P/Q-channel-null mice displayed little activity in the gamma range of frequencies; instead, they displayed repetitive, high-amplitude spike and wave discharges compatible with the interpretation that these mice were in an almost permanent state of absence epilepsy. Persistent absence is also a feature of *tottering* mice, a naturally occurring strain with a mutation of the $Ca_v2.1$ gene (14). The obvious interpretation of this finding is that, in the absence of P/Q channels, persistence of low-threshold, T-type channels in the thalamic neurons made bursting behavior and the low-frequency oscillations of the network that go with it preeminent over any higher-frequency oscillations. Confirming the interpretation, low-threshold, T-type currents were preeminent in thalamic neurons in the study by Llinás *et al.*, and administration of a selective T-channel blocker, l-octanol, significantly reduced the frequency of spike and wave discharges in the P/Q-channel-null mice, almost abolishing low-frequency activity in the EEG and sending the mice into a comatose state.

There is a very substantial return projection from the cortex to the thalamus.

The article by Llinás *et al.* (1) is important not only in drawing attention to the manner in which something so seemingly simple as the differential engagement of two types of voltage-gated calcium channel in individual neurons can play such a profound role in determining the difference between unconsciousness and arousal, but also in reaffirming the current view of the thalamus as part of an interconnected network whose large-scale activities are the determinants of higher levels of forebrain activity and of higher mental states such as perception and cognition. The older view of the thalamus as a synaptic station for the relay of externally and internally generated activity speedily and without decrement to the cortex is by no means abrogated by this study. However, it must now be set in the context of the activity of the network of forebrain connections, of which the thalamus forms a part and which plays a fundamental role in determining the higher integrative properties of the brain.

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