T-type calcium channels in burst-firing, network synchrony, and epilepsy

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A B S T R A C T

Low voltage-activated (LVA) T-type calcium channels are well regarded as a key mechanism underlying the generation of neuronal burst-firing. Their low threshold for activation combined with a rapid and transient calcium conductance generates low-threshold calcium potentials (LTCPs), upon the crest of which high frequency action potentials can fire for a brief period. Experiments using simultaneous electroencephalography (EEG) and intracellular recordings demonstrate that neuronal burst-firing is a likely causative component in the generation of normal sleep patterns as well as some pathophysiological conditions, such as epileptic seizures. However, less is known as to how these neuronal bursts impact brain behavior, in particular network synchronization. In this review we summarize recent findings concerning the role of T-type calcium channels in burst-firing and discuss how they likely contribute to the generation of network synchrony. We further outline the function of burst-firing and network synchrony in terms of epileptic seizures. This article is part of a Special Issue entitled: Calcium channels. © 2012 Elsevier B.V. All rights reserved.

1. T-type calcium channels and burst-firing

Calcium ion entry through voltage-activated calcium channels both depolarizes the cell membrane and regulates numerous intracellular signaling pathways. T-type calcium channels are known as low voltage-activated (LVA) channels as they first open at more hyperpolarized membrane potentials compared to high-voltage activated (HVA) calcium channels, such as the L-, P/Q-, N- and R-type channels [1]. The distinct properties of T-type calcium channels allow the conduction of inward current in response to smaller membrane depolarizations than those required to activate HVA calcium or even sodium channels [2]. In this regard, T-type calcium channels are first-responders to small depolarizations and their activity further depolarizes the cell to the point where HVA and sodium channels activate allowing both large scale calcium entry and the generation of action potentials [3,4]. Despite their lower threshold for activation, T-type channels inactivate at a fast rate, resulting in a transient surge in calcium entry. Under conditions in which there is sufficient density of T-type channels, a cascade depolarization can occur generating a LTCP upon the crest of which sodium and potassium channel-mediated action potentials fire [5]. Firing continues until the T-type channels inactivate in parallel with the activation of small conductance calcium-activated potassium channels,
which repolarize the membrane back below the range of T-type channel activation [6,7]. Overall, the entire burst process takes approximately 100 to 200 ms limiting the frequency at which bursting can occur to around 5 to 10 Hz [3]. Of note, if multiple bursts occur out-of-phase with each other, burst-firing in distinct local areas can generate higher frequency output for an entire regional population of neurons [8]. The burst-firing mode is predicted to promote the synchronization of bursting and oscillations not only within particular neural nuclei of the brain, but also between interconnected loci in central nervous system (CNS) networks and to contribute to normal non-REM sleep and also to some types of epileptic seizures.

Three T-type calcium channel genes are expressed in mammalian tissues; CaV3.1, CaV3.2 and CaV3.3. The three isoforms are distinctly expressed throughout the central and peripheral nervous systems as well as in non-neuronal tissues and are predicted to play crucial roles in numerous physiological processes [2]. T-type calcium channels are most well known for their part in controlling neuronal excitability [3,9] and have been implicated in several pathophysiological disorders [10,11]. In both endogenous and exogenous systems the T-type calcium channel subtypes exhibit distinct biophysical properties concerning their rates and voltage dependences of activation and inactivation, recovery from inactivation (de-inactivation) and deactivation, allowing a wide array of cell-specific responses to be generated depending upon the expression of specific subtypes [3,12,13]. The spectrum of T-type calcium channel types is made more complex by the occurrence of multiple alternatively spliced isoforms, generating a large number of functional variants and potentially allowing for highly tailored excitability profiles. Overall, the LTCPs and the bursts that T-type calcium channels generate are predicted to vary in their voltage dependences and duration as well as rates of depolarization and repolarization [3]. The strength of burst-firing also varies in its sensitivity to depolarizing stimuli, with some bursts generating more action potentials in response to stronger stimuli and others displaying an all or nothing response, where maximal action potential numbers are seen with either threshold or supra-threshold stimuli [8]. Along these lines, it is predicted that depending upon the relative expression of particular T-type calcium channel isoforms burst-firing itself can vary, with specific burst types occurring in different neurons and in different pathophysiological states. As such, while many types of neurons exhibit burst-firing, it is important to understand the complement of T-type calcium channels expressed within particular neurons.

2. T-type calcium channels and network synchrony

It might be predicted that a high frequency burst of action potentials in a neuronal cell body would produce a similar effect at axon terminals, generating a powerful release of neurotransmitter onto the postsynaptic cell and therefore, a more pronounced effect than LTCPs in a neuronal cell body would produce a similar effect at axon terminals, generating a powerful release of neurotransmitter onto the postsynaptic cell and thereby preventing other activities contributing to the neuronal output, at least until the LTCP abates or another burst occurs. In theory, this notion fits well with the bi-modal role of TCR neurons which act as relay cells for sensory information during wakefulness, but become locked in an oscillating thalamocortical loop in some non-REM sleep states and absence seizures. In the relay state TCRs primarily fire in a tonic manner and can modulate firing frequency in response to incoming sensory or cortical activities [16–18]. The cell membrane potential does not reach the hyperpolarized potentials necessary to de-inactivate T-type calcium channels and therefore bursts do not occur. However, during sleep or epileptic seizures TCRs become locked in a self-propagating oscillatory loop along with corticothalamic pyramidal and reticular thalamic neurons, during which they primarily burst-fire due to a long lasting hyperpolarization that promotes T-type channel de-inactivation [5]. It is in this burst-firing state that TCRs do not conduct sensory information, predictably because incoming sensory activity is filtered out by the burst frequency-locked oscillation. Together, a neuronal burst may play a dual role, with high frequency action potentials creating a powerful output signal to the postsynaptic cell to propagate the network oscillation, whilst back-propagating LTCPs filter out incoming signals that occur outside the frequency of the membrane oscillation.

The network synchronization that is thought to occur as a result of burst-firing likely develops from diffuse projections to reciprocally connected CNS nuclei [19–22]. Bursts that start in one area of the initiating nucleus are predicted to generate bursts in multiple postsynaptic neurons in the projected nucleus through axonal arborization to multiple synapses. These bursting neurons will in turn recruit additional neurons in the reciprocally connected initiating nucleus and this amplification will progress on each cycle of the oscillation. Another possibility for the development of network synchronization may occur as a result of dendrite–dendrite interconnection. As a single burst in one dendrite is capable of generating an LTCP throughout the dendritic tree [14,15] and also given that T-type dependent dendrite-dendrite synaptic neurotransmission has been reported [23–25], it is feasible that dendrites are capable of transmitting burst activity to other dendrites. If this process indeed occurs, it would provide a regional mechanism of generating synchrony in a local area without the need for conventional axonic synapses in reciprocally connected neurons. In this regard, it is of note that gap junctions have been implicated in synchronizing TCR firing, at least at alpha and theta frequencies associated with wakeful relaxation and drowsy or light sleeping states, respectively [26].

3. T-type calcium channels and epilepsy

3.1. Human epilepsy mutations in T-type calcium channels

A number of single base changes have been identified in the genes encoding for the CaV3.1 and CaV3.2 T-type calcium channels in some patients with generalized epilepsies [10,11,27–31]. Of 13 CACNA1G (encoding CaV3.1) variants identified in one study of genetic generalized epilepsy patients, five resulted in amino acid substitutions, with one occurring in the domain I–II intracellular linker and the rest found in the domain II–III linker [27]. No statistically significant differences were identified in CaV3.1 functional properties when evaluated exogenously using heterologous expression systems, although moderately faster inactivation decay rates were noted with some mutations. A large number of base changes have been identified in the CACNA1H gene in patients with childhood absence [28,30,32] as well as juvenile absence, juvenile myoclonic, myoclonic atatic, febrile and temporal lobe epilepsies [29], many of which result in amino acid substitutions. Childhood absence epilepsy mutations occur primarily in the CaV3.2 domain I–II linker with most of the remaining mutations found in the voltage-sensing, pore and carboxyl-terminal regions [10,11]. The mutations associated with other epilepsies display a fairly diverse distribution in their structural location [29]. Upon exogenous evaluation of CaV3.2 calcium channel biophysical properties many of the alterations were found to induce changes considered to be a gain-of-function (i.e., hyperpolarized shift in the voltage-dependence of activation),
although some had no apparent effect and others displayed an apparent loss-of-function (such as reduced current density) [32–37]. While these varied effects may reflect the polygenic nature of epilepsy, some genetic variability may also represent single nucleotide polymorphisms (SNPs) unrelated to the epileptic condition. In addition, recent studies have shown that some genetic mutations in calcium channel isoforms exhibit splice variant-specific functional effects [38–40]. Therefore, it is important to consider when studying calcium channelopathies using cloned channels that the contextually relevant variants for a particular CNS region or even specific neuron type are likely crucial towards determining biophysical alterations in channel properties. The testing of splice variants insensitive to a particular mutation/SNP may exhibit a false negative result thus alterations that lack effects exogenously should not necessarily be discounted as irrelevant towards epileptogenesis.

3.2. T-type calcium channels and burst-firing in limbic epilepsy

Some of the most convincing data linking T-type calcium channels and burst firing to seizures come from rodent models of epilepsy wherein increased T-type calcium currents are often observed in parallel with enhanced burst-firing properties [41]. Systemic administration of pilocarpine is used in rats and mice as a model of complex-partial and temporal-lobe epilepsy, in the short- and long-term, respectively [42,43]. Animals that develop chronic seizures several days after pilocarpine treatment display up-regulated CaV3.2 expression and increased T-type calcium current density that correlates with increased burst-firing in dendrites of hippocampal CA1 pyramidal neurons [44–46]. Genetic ablation of CaV3.2 prevents the seizure-induced increase in T-type currents, as well as burst-firing, and also attenuates the neuronal damage observed in the CA1 and CA3 regions following initial status epilepticus [47]. In the same model, enhanced burst-firing is also observed in subiculum neurons [48], as well as in midline thalamic neurons that project to the hippocampal CA1 and entorhinal cortex in parallel with increased CaV3.2-mediated T-type calcium currents [49]. How this increased burst-firing activity affects network synchrony in the hippocampus and related structures is unknown. However, it could be postulated that bursting would increase cycling oscillatory activity within the limbic network as increasing numbers of neurons are recruited and as firing becomes synchronized in response to frequency filtering.

3.3. T-type calcium channels and burst-firing in absence epilepsy

Further evidence implicating burst-firing in seizures is observed in rodents with either genetically-induced or spontaneously-acquired epileptic phenotypes [41,50]. Genetically modified mice with HVA calcium channel dysfunction display absence seizures accompanied by enhanced T-type (likely CaV3.1-mediated) calcium currents in TCR neurons [51]. In support, the genetic enhancement of CaV3.1 currents in mouse TCR neurons, either directly via CaV3.3 channel over-expression or indirectly by knockout of phospholipase Cδ4, induces absence seizure-like phenotypes [52–54]. Furthermore, genetic ablation of CaV3.1 reduces sensitivity to some types of pharmacologically-induced absence seizures [55]. Interestingly, a recent study proposed that different splice variants of the mouse Cacna1g gene act as candidate modifiers for the severity of the epileptic phenotype induced by mutations in voltage-gated sodium channels [56].

In the Wistar Albino Glaxo Rats from Rijswijk (WAG/Rij) model of absence epilepsy that display spontaneously-acquired seizures [57,58], enhanced CaV3.1 and CaV3.3 currents have been observed in lateral geniculate and central lateral TCR neurons [59]. Computer modeling suggests that the enhanced T-type current reduces the stimulus required to induce burst-firing in these neurons. In addition, a modest increase in CaV3.2 currents is observed in WAG/Rij reticular thalamic nucleus (RTN) neurons. Combined simultaneous multunit, EEG and intracellular recordings show that TCR neurons from several TCR (e.g. central lateral; Fig. 1D) areas as well as RTN neurons burst-firing during seizures and correlate with the spikes in spike-and-wave discharges (SWDs), a hallmark of absence seizures on surface EEG recordings [60–62]. It should be noted that not all thalamic regions exhibit recurrent burst-firing, as some only fire one or two action potentials, or infrequent bursts per EEG spike. However, spike frequency-locked membrane oscillations resembling low-level LTCPs are present in recordings from neurons that do not display burst-firing. In addition, while burst-firing in RTN neurons occurs at the same time as spikes in SWDs, action potential firing in other TCR (e.g., central lateral, paracentral; Fig. 1D and E) areas is observed to lag EEG spikes.

In the Genetic Absence Epilepsy Rats from Strasbourg (GAERS) model [58,63], burst-firing occurs in RTN neurons and correlates closely with spike-timing in cortical EEG recorded SWDs (Fig. 1A) [64,65]. In this model, RTN neurons display increased T-type currents [66] via CaV3.2 channel up-regulation [67,68]. In addition, these animals possess a missense mutation in the gene encoding for CaV3.2 which increases the rate of channel recovery from inactivation and is predicted to enhance calcium conductance to RTN neurons during burst depolarizations [38]. While RTN neurons are GABAergic, the hyperpolarization they induce in TCR neurons can play an excitatory role. T-type (primarily CaV3.1) calcium channels in the relatively depolarized TCR neurons are predominantly inactive at rest and hyperpolarization induced by GABA release from RTN axon terminals drives their de-inactivation. On cessation of hyperpolarization, TCR neurons can then burst-fire; a process known as rebound burst-firing. However, at least in GAERS it appears that GABAergic drive to TCR neurons may not actually induce rebound bursts during absence seizures, but instead induce membrane oscillations [69]. Similar to some TCR regions in the WAG/Rij model, neurons in the GAERS ventrobasal thalamic nucleus (e.g., ventroposterior lateral) appear to display sub-threshold membrane oscillations in time with SWDs, but only fire single or double action potentials (if any) upon the crest of the depolarization (Fig. 1C) [70,71]. Neurons in a nearby TCR region; the ventromedial thalamic nucleus, do appear to occasionally burst-fire on the crest of membrane oscillations that occur in time with SWDs, however bursts do not occur on each depolarization, appearing almost sporadically with sometimes no action potentials or only one or two action potentials firing [71]. In both cases, neuronal rebound burst firing can be manually induced by direct hyperpolarizing current injection, however during SWDs bursting does not occur. If TCR burst-firing does not faithfully follow seizure activity in GAERS (as well as in some WAG/Rij TCR regions) it could be questioned whether TCR burst-firing is truly required for absence seizure propagation, contrary to the studies discussed above concerning genetically modified mice with altered CaV3.1 activity in TCR neurons.

3.4. Silencing neuronal output with membrane oscillations

GAERS cortical pyramidal neurons also display robust burst-firing that precedes spikes in the SWDs recorded on cortical EEGs (Fig. 1B) [69,72]. Without a reciprocal positive feedback from TCR neurons, how does cortical burst-firing continue to propagate? Corticothalamic pyramidal neurons project to TCR neurons, but also to the RTN (Fig. 2). Burst-firing in the RTN can only be conveyed back to the cortex via burst-firing TCR neurons, since the RTN has no direct connection to the cortex. Therefore, if TCR neurons do not burst faithfully in response to burst-firing in either the RTN or the cortex then this activity will not be propagated as thalamocortical network oscillations. In addition, as discussed in Section 2, for the oscillations to spread and recruit other cortical areas, in the classic theory of absence seizures, functional and bursting connections between TCR neurons and the cortex are required. If this network theory is correct then without positive feedback from burst-firing in the TCR neurons, regions outside the cortical epileptic focus cannot be recruited. At least for GAERS, there appear to be three possible explanations for the lack of burst-firing observed in TCR neurons. In one scenario, simultaneous EEG and intracellular recordings may not yet have
Correlation between burst-firing and subthreshold membrane oscillations with SWDs. In each panel upper traces correspond to EEG/ElectroCorticoGram (ECoG) recordings while lower traces correspond to intracellular recordings from representative single neurons. Panels on the right hand side show expanded time scales from the panels on left hand side. (A) SWDs in GAERS correlate closely with burst-firing in RTN neurons (*Ai and Aiii) while non-burst action potentials do not correlate with SWDs (*Ai and Aii). (B) SWDs in GAERS also correlate with bursts in cortical layer V/VI pyramidal neurons (representative example from a layer VI neuron shown here). Note that bursts precede spikes in SWDs (Bii). (C) In GAERS VPL neurons SWDs correlate with subthreshold membrane oscillations with sporadic action potential firing but without bursting (Cii). (D) Contrastingly, in WAG/Rij CL neurons SWDs correlate with burst-firing (Dii), but also display single action potentials or subthreshold membrane oscillations (Di). (E) In WAG/Rij PC neurons SWDs correlate with subthreshold membrane oscillations with sporadic action potentials (Eii). Also note that action potential firing takes place out of synchrony with spike-timing in SWDs (Eii). VPL, CL, and PC represent different types of TCR neurons. SWD = spike–wave discharge, RTN = reticular thalamic nucleus, VPL = ventroposterior lateral, CL = centrolateral, PC = paracentral. (A) Reproduced from Reference [64], (B) Reproduced from Reference [72], (C) Reproduced from Reference [70], (D and E) Reproduced from Reference [61].

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bursts that occur on membrane oscillations are sufficient to maintain oscillatory activity in the thalamocortical network? It may be that the one or two action potentials or sporadic firing only with subthreshold membrane oscillations without burst-firing in pyramidal neuron A, while also recruiting pyramidal neuron B via TCR axonal arborization. Pyramidal neuron B innervates TCR neurons 2 and 3, and also in RTN neurons II and III via axonal arborisation, in turn providing drive to TCR neurons 3 and 4. However, TCR neurons 2, 3 and 4 respond only with subthreshold membrane oscillations without burst-firing, therefore the no burst-firing signal is returned to the RTN or cortex. TCR neuron 5 receives oscillatory input from dendrite-dendrite synapses, generating burst-firing in TCR neuron 5, which recruits RTN neuron IV (continuing recruitment propagation throughout the network) and cortical neuron C. Lastly, cortical neuron C recruits cortical neuron D via intracortical axonal and/or dendrite-dendrite synaptic connections and continues recruitment propagation throughout the network. The potential mechanisms illustrated could occur as different combinations of the individual connections described. RTN = reticular thalamic nucleus, TCR = thalamocortical relay.

been undertaken in the specific TCR neuronal region[s] in which burst-firing occurs in synchrony with spikes in SWDs. This seems plausible given the TCR nucleus-specific differences in burst-firing described above in the WAG/Rij model. Indeed, absence seizures may display a more focal as opposed to typically generalized nature, affecting only particular thalamocortical regions, which would fit with the finding that only particular areas of the thalamus propagate the burst-firing SWD activity [73].

A second possibility arises if the thalamocortical circuit can sustain network oscillatory activity without the requirement of burst-firing in TCR neurons. Since burst-firing appears to occur in cortical as well as RTN neurons during seizures, this would make the lack of bursting in TCR neurons an exception to the rule. In this circumstance could non-bursting yet membrane oscillatory activity in TCR neurons be sufficient to maintain oscillatory activity in the thalamocortical network? It may be that the one or two action potentials or sporadic bursts that occur on membrane oscillations are sufficient to propagate the seizure activity and recruit other cortical areas. However, it seems unlikely that such sporadic and low level activity would be sufficient to generate the drive required to recruit and amplify thalamocortical network oscillations.

A third possible scenario is that subthreshold membrane oscillations themselves, irrespective of action potentials, may be sufficient to induce the absence seizure phenotype (i.e. sensory shutdown). This would require sustained drive to occur from the cortex, without the need for TCR feedback in order for propagation of the seizure to occur. Subthreshold membrane oscillations observed in GAERS TCR neurons during seizures are proposed to occur as a result of interplay between GABA(A) receptor-mediated GABAergic (RTN) and glutamatergic (corticothalamic) synaptic activity [70,71,74]. It is thus feasible that these sub-threshold membrane oscillations could generate T-type channel-mediated calcium potentials that propagate throughout the cell body and dendritic tree, such as that occurs with LTCPs. In doing so this may allow TCR neurons to act as a frequency filter without increasing their action potential output. These membrane oscillations typically occur in the presence of a hyperpolarization that lasts for the duration of the oscillation [70,71] thus hyperpolarization of TCR neurons could promote enhanced T-type channel de-inactivation and support a role for calcium conductance during membrane oscillations. In addition, as discussed in Section 2, dendrite-dendrite synapses or gap junctions could spread such membrane oscillations through local TCR regions.

In this third hypothesis sporadic action potentials would be relatively irrelevant towards the role of TCR neurons during absence seizures. SWD activity has been shown to develop initially in the cortex via paroxysmal intrinsic oscillatory activity, which then recruits a potentially hyperexcitable RTN [72,75]. The driving force arriving from upstream cortical and RTN neurons may simply act to silence TCR output, while allowing the synchronization of their sub-threshold oscillations via LTCP-like oscillatory activity. This would have the effect of blocking afferent sensory input to the thalamus and therefore to the cortex, as well any afferent information from the cortex to the periphery. This hypothesis is likely only relevant if reciprocal feedback from TCR neurons to the cortex is not necessary to maintain and spread oscillatory activity in the thalamocortical network [17,76]. If cortical oscillations can be maintained in and spread to neighboring cortical areas from the epileptic focus then the theory becomes plausible.

Overall, we hypothesize it most likely that a combination of scenarios one and three is likely most relevant to the GAERS model and that as observed in WAG/Rij, there are region-specific roles for burst-firing and sub-threshold oscillations in TCR subregions (Fig. 2). If this is the case, primary TCR regions may burst-fire in phase with SWDs, which act to propagate the oscillatory activity and absence seizures, while secondary TCR regions generate subthreshold membrane oscillations that, although not directly participating in thalamocortical network oscillations, act to dampen and suppress the relay mode of TCR output and any sensory relay activity within secondary TCR regions.

4. Concluding remarks

While the link between T-type calcium channels and burst-firing is well established, the link between burst-firing and epilepsy is not as clearly understood. It is known that burst-firing increases network synchrony and recruitment, and that this contributes to seizure generation and propagation. In rodent models of limbic epilepsy, increased T-type activity and enhanced burst-firing has been observed in a number of hippocampal and thalamic regions, although the effect on network synchrony are less clear. In absence epilepsy, numerous studies have linked enhanced T-type activity to seizures associated with cortical, RTN and TCR regions of the thalamocortical network. While cortical areas of the epileptic focus and RTN neurons display burst firing in synchrony with spikes in SWDs, a lack of correlation is seen in several TCR subregions. It is predicted that burst-firing acts to
propagate and synchronize firing both locally and throughout the thalamocortical network, while also acting as a frequency filter preventing sensory information being relayed to the cortex. In the non-bursting regions it is possible that subthreshold oscillations are capable of performing a filtering task without increasing neuronal output. Sub-threshold oscillations alone may allow particular cortical areas to perform a silencing role during sleep and epileptic seizures, suppressing sensory relay to specific cortical areas. Contrastingly, bursting regions may spread network oscillations and recruit interconnected cortical areas, while simultaneously acting as a frequency filter.

As advances are made towards understanding the mechanisms of network synchronization, so too will our understanding of how burst-firing synchronizes both local and remote interconnected regions. Some questions can likely be approached more promptly, for example whether bursts or LTPCs can be propagated from neuron through dendrites could be established with calcium imaging studies. Similarly, why some TCR neurons burst-fire during SWDs while others do not, despite having the capability to do so may relate to the intrinsic resting membrane potential of the neurons involved [61,62] and/or to region-specific differences in T-type channel expression. With such information it may be possible to develop more specific drug treatments that target network oscillations at specific nuclei, thereby suppressing pathophysiological absence seizures without interfering with normal physiological, for example, relay activity. While ethosuximide is a front line presence epilepsy treatment without interfering with normal physiological, for example, relay activity: an in vitro study, Arch. Ital. Biol. 122 (1984) 73

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