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The many faces of T-type calcium channels

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Key words : T-type channels, Cav3.1, Cav3.2, Cav3.3, burst firing, tonic firing, neuronal excitability, synaptic transmission, dendritic integration.

Abstract

Since the discovery of low-voltage activated T-type calcium channels in sensory neurons and the initial characterization of their physiological function mainly in inferior olive and thalamic neurons, studies on neuronal T-type currents have predominantly focused on the generation of low-threshold spike (and associated action potential burst firing) which is strictly conditioned by a preceding hyperpolarization. This T-type current mediated activity has become an archetype of the function of these channels, constraining our view of the potential physiological and pathological roles that they may play in controlling the excitability of single cells and neural networks. However, greatly helped by the recent availability of the first potent and selective antagonists for this class of calcium channels, novel T-type current functions are rapidly being uncovered, including their surprising involvement in neuronal excitability at depolarized membrane potentials and their complex control of dendritic integration and neurotransmitter release. These and other data summarized in this short review clearly indicate a much wider physiological involvement of T-type channels in neuronal activity than previously expected.

Introduction

Low-voltage activated (LVA) Ca^{2+} currents (T-type) attracted the attention of the neuroscience community at the beginning of the eighties with the identification in inferior olive neurons of a Ca^{2+} -mediated rebound depolarization that followed a transient hyperpolarization [51]. Similar findings were described at the same time in thalamic neurons [39, 40, 50], where at hyperpolarized membrane potentials a subthreshold Ca^{2+} current gives rise to a transient slow depolarization, the so-called low-threshold spike (LTS), which in turns evokes a prototypical high frequency burst of action potentials (Figure 1A & B). The functional importance of these discoveries was quickly established by work in anesthetized and naturally sleeping animals, which linked LTSs and high-frequency burst firing to the different rhythmic activities displayed by neurons of the thalamocortical circuit during anesthesia and different stages of sleep [21, 25].

In parallel, the canonical biophysical properties of neuronal T-type currents were established in primary sensory neurons [10, 11, 31, 57]. Briefly, T-type channels are activated around -60mV and generate a transient inward Ca^{2+} current that is fully inactivated after a few tens of milliseconds (Figure 1C). Holding the neurons at a membrane potential above -60mV induces a nearly complete inactivation of the T-type channel population (Figure 1D): thus, a hyperpolarization that allows some channels to recover from inactivation is required before a substantial T-type current can be evoked. Later, different subtypes of T-type currents were

described in native tissue (see [38] for example), and 3 genes, displaying multiple alternative splicing, are now known to code for the Cav3.1, 3.2 and 3.3 T-type Ca^{2+} channels [14, 60]. The different T-type currents generated by these three subtypes share the same basic properties, i.e. low-threshold of activation, transient activation and a nearly complete inactivation at voltages close to the resting membrane potential of many neurons. However, the time courses of activation, inactivation, deactivation, and recovery from inactivation, and the precise activation and inactivation voltage-dependence slightly vary according to the specific Cav3 channel subtype [33, 42, 44, 64]. Importantly, these small biophysical differences have drastic physiological consequences, as shown for example, by a recent study investigating their impact on synaptically evoked LTSs in thalamic neurons [66].

In line with their biophysical properties and following the pioneering physiological works of Llinas and Steriade's groups [21, 39, 40, 50, 62], the vast majority of studies on the role of T-type channels have focused on the generation of LTSs (and associated burst firing), which, as mentioned earlier, is strictly conditioned by a preceding transient or tonic hyperpolarization. This, together with the extensive analysis of the role of these currents in rhythmic bursts across the thalamocortical circuit during sleep-related activity, has made the LTS the archetype of T-type channel function and constrained our view of the potential physiological (and pathological) roles that these neuronal channels may play. Of course, the lack of suitable T-type channel antagonists has hampered the discovery of more subtle functional roles for neuronal T-type currents. Thus, the commonly used divalent cation Ni^{2+} at concentrations that substantially block the 3 T-type channel isoforms also affect high-voltage activated Ca^{2+} currents [72], while the hypertensive drug, mibefradil, inhibits high-voltage Ca^{2+} and Na^+ currents as well as voltage-dependent and ATP-sensitive K^+ channels [8, 32, 48, 55, 56, 67]. Undoubtedly, the recent synthesis of piperidine-derivatives, such as 3,5-dichloro-N-[1-(2,2-dimethyl-tetrahydro-pyran-4-ylmethyl)-4-fluoro-piperidin-4-ylmethyl]-benzamide (TTA-P2, [63]), which potently and selectively block T-type currents without affecting other voltage-dependent and synaptic currents [26], will further contribute to unravel more subtle (than the LTS), but undoubtedly as important, physiological roles of neuronal T-type currents. In this review we will present some of these new T-type current functions, including the complex control exerted by this current in dendritic integration and neurotransmitter release, and its surprising involvement in neuronal excitability at depolarized membrane potentials.

The T-type current is a major dendritic conductance

In the nineties, in addition to their well-accepted role as “burst firing generator”, dendritic T-type currents were shown to participate to subthreshold excitatory post-synaptic potentials (EPSPs) in neocortical and hippocampal pyramidal neurons [52, 53]. Since then, electrophysiological recordings, modeling studies and advanced optical techniques to monitor local Ca^{2+} dynamics have identified the presence of a high-density of T-type channels in the dendritic shaft and/or spines of many neuronal cell types, including thalamocortical [23, 30, 68] and nucleus reticularis thalami neurons [13, 17, 22, 41], Purkinje [34] and unipolar brush cells [24] of the cerebellum, medium spiny neurons of the striatum [12], and granule cells of the olfactory bulb [27]. Dendritic localization of the T-type channels has been further confirmed by immunofluorescence [16, 49, 54] and electron microscopy [43, 49, 59] in the neocortex, hippocampus, thalamus, and cerebellum. However, because electrophysiological recordings from distal dendritic compartments are highly challenging, the impact of the T-type current on the local dendritic membrane potential dynamics is still poorly documented.

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Nevertheless, due to their preferential dendritic localization and low-threshold of activation, T-type currents are highly suited to boost EPSP propagation. In a recent paper, Crandall et al., [17]) demonstrated using two-photon Ca^{2+} imaging in neurons of the nucleus reticularis thalami that stimulation of distal dendrites locally recruits T-type channels and generates a large distal Ca^{2+} response, even when the LTS and associated burst firing generation was precluded by holding the cell soma at -60mV . These data clearly suggest that distally located T-type currents amplify afferent inputs and allow the generation of a somatic subthreshold EPSP, thus presumably compensating for the EPSP attenuation that would normally occur along the dendrites due to their passive cable properties. Similar effects should be present in thalamocortical neurons where a large T-type channel dependent Ca^{2+} entry was recorded in distal dendrites upon focal glutamate application or synaptic stimulation [30].

In thalamic neurons, due to the extremely high-density of T-type channels, synaptic inputs appear to locally trigger a massive T-type channel activation outshining any effect beside EPSP amplification. However, in neurons where the synaptically evoked T-type current is smaller, its effect on the EPSP shape and propagation may be more complex, as local T-type dependent Ca^{2+} entry can also modulate the activity of other closely located Ca^{2+} -sensitive conductances. Indeed, it was recently shown that in homogenates of rat cerebellum Cav3.2 T-type channels co-immunoprecipitate with the intermediate conductance Ca^{2+} -activated K^+ channels, KCa3.1 [29]. These data suggest a functional channel interaction at the nanodomain level that was further confirmed by the recording in outside-out patches from Purkinje cell of a Ca^{2+} -sensitive K^+ current that was blocked by either T-type or KCa3.1 channel antagonists. As a consequence, during parallel fiber activation, the T-type channel mediated Ca^{2+} entry opens KCa3.1 K^+ channels that in turn accelerate the subthreshold EPSP decay and create a marked after-hyperpolarization, therefore limiting the summation of EPSPs [29]. Along the same line, a growing number of data points to the existence of direct functional interactions between T-type current and either Ca^{2+} -activated or KV4 A-type K^+ currents [1, 2, 19, 70], which may also have a drastic impact on dendritic integration. Finally, it should be noted that the Cav3.2–KCa3.1 complex was also shown to shape the spontaneous tonic firing of Purkinje cells at depolarized membrane potentials [29]. Therefore, in this cell type, the T-type channel-dependent increase in the EPSP rate of decay does not appear to require a strong preceding hyperpolarization, the condition that is generally assumed to be necessary to any function of the T-type current.

T-type currents participate to both dendritic and axonal synaptic release

Because of their relatively fast activation and slow deactivation, T-type Ca^{2+} channels are ideally suited to mediate large Ca^{2+} entry during brief depolarizing events such as EPSP, or action potential. These short depolarizations strongly activate the channels while inducing little inactivation and the slowly closing channels mediate a strong current upon repolarization [15, 44]. In addition, although well-known for their prominent inactivation, all three T-type Ca^{2+} channel isoforms show an overlap of their steady-state inactivation and activation curves that give rise to a window current. This current results from a small fraction of T-type channels that remain open in a voltage range close to the resting membrane potentials of CNS neurons [26, 60]. Although the amplitude and voltage-dependency of the window current are difficult to measure experimentally (but see figure 2 in [26]) and are often estimated from the fit of the steady state inactivation and activation curves, the window current is likely present in many neuronal populations where it leads to a stationary Ca^{2+} influx and a persistent enhancement of intracellular Ca^{2+} (see below and Figure 1D). Therefore, when localized at synaptic release sites, T-type channels will supply both a tonic and

transient Ca^{2+} current generating a component of spontaneous or evoked neurotransmitter exocytosis whose voltage-dependence is close to the resting membrane potential. As already mentioned, T-type channels are highly expressed in dendrites and therefore are good candidates to trigger neurotransmitter release at dendro-dendritic synapses. Although this type of interaction has attracted little attention so far, it has been studied in details at the reciprocal synapse between granule and mitral cells of the olfactory bulb: here, back-propagating action potentials [27] or strong synaptic stimulation [28] induce GABA release triggered by a large Ca^{2+} entry, predominantly through T-type channels. T-type currents have also been involved in synaptic transmission of the retinal bipolar cells. In these specialized neurons, which release neurotransmitters in response to graded depolarization, Ca^{2+} imaging and capacitance measurements have shown that neurotransmitter vesicle fusion depends on both T and L-type Ca^{2+} channel activation [58].

More recently, two papers have greatly strengthened the hypothesis of a T-type channel involvement in axonal synaptic release [35, 65]. In layer III presynaptic terminals of the entorhinal cortex, electron microscopy reveals that hyperpolarization-activated cyclic nucleotide-gated channels (HCN) are colocalized with Cav3.2 channels. Block of the HCN current increased the frequency of miniature excitatory postsynaptic events in pyramidal neurons, an effect that was blocked by selective T-type channel antagonists [35]. These data suggest that the presence of presynaptic HCN channels depolarizes the synaptic terminal, thereby decreasing Ca^{2+} entry via presynaptic T-type Ca^{2+} channel activity and preventing spontaneous glutamate release (see also [3] for another example of T-type current involvement in miniature synaptic potentials). The full role of the T-type current in cortical synaptic transmission remains to be determined, but the work of Tang et al [65] analyzing GABA release from perisomatic-targeting interneurons in the hippocampal CA1 region may suggest an interesting hypothesis. These authors demonstrated the presence of Cav3.1 channels in preterminals of parvalbumin positive axons near, although not in, the active zones. Activation of presynaptic acetylcholine receptors triggered an asynchronous release of GABAergic quanta that transiently decreased pyramidal cell excitability: this effect was drastically reduced by specific T-type current antagonists. Therefore, it appears that the presence of presynaptic T-type channels is a more general feature of excitatory and inhibitory terminals than initially expected. Furthermore, by contrast to the well known $\text{G}\beta$ -dependent inhibition of the high-voltage activated Ca^{2+} channels [71], T-type channel activity has seldom been reported to be inhibited by metabotropic receptors [34, 36, 45, 61]. Therefore, T-type channel dependent asynchronous neurotransmitter release may be a privileged mechanism of synaptic transmission when neurons are receiving strong neuromodulatory input.

T-type currents directly contribute to tonic firing and neuronal excitability at depolarized potentials

The data reviewed so far demonstrate that T-type currents do not only condition burst firing generation via an LTS, but also control both synaptic release and the dynamics of subthreshold EPSPs. Furthermore, a growing number of data clearly indicate that T-type currents also participate to the generation of tonic firing. Thus, in addition to their localization in the dendritic tree and in presynaptic terminals, two-photon imaging has recently shown that T-type channels colocalize with Na^+ channels in the axon initial segment (AIS) of dorsal cochlear nucleus interneurons, cerebellar Purkinje cells and cortical layer 5 pyramidal neurons [5]. Moreover, restricted applications of T-type channel antagonists to the AIS not only increase action potential threshold and interspike intervals during burst firing, but also decrease the probability of suprathreshold EPSPs to generate a single action potential. Therefore, the T-type current in the AIS contributes both to burst and tonic firing in dorsal cochlear nucleus interneurons. Furthermore, this T-type channel-dependent

1 modulation of neuronal firing is under the control of dopamine receptors that, acting via protein kinase C,
2 downregulates the T-type current [4, 6]. Interestingly, dopamine acts specifically on T-type channels located
3 in the AIS without affecting somatodendritic channels. Since AIS T-type channels represent a small fraction
4 of the whole neuronal T-type channel population, no effect of dopamine agonists is observed when recording
5 the whole-cell T-type current [4]. Interestingly, the majority of studies dealing with the spatial cellular location
6 of T-type channels have so far mainly focus on differences in channel subtype or density [22, 23, 41].
7 However, by highlighting that the modulation of T-type channels can be restricted to a spatial location in
8 addition to a channel subtype, this study on AIS T-type channels stresses the fact that a number of localized
9 T-type current regulations with an important physiological impact may have so far gone undetected in studies
10 that only recorded whole-cell T-type currents.

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13 More surprisingly, the T-type current has been shown not only to participate but also to promote tonic firing in
14 dopaminergic neurons of the substantia nigra. These neurons display a pacemaker tonic activity in vitro [70],
15 and the frequency and precision of this pacemaker spiking rely on small Ca^{2+} activated K^+ channels (SK)
16 that generate a large after-hyperpolarization following each action potential [69]. Pharmacological and
17 biophysical data, obtained using perforated patches to preserve the physiological intracellular Ca^{2+}
18 dynamics, clearly demonstrated that, during pacemaker tonic firing, Ca^{2+} entry through T-type channels was
19 responsible for the activation of SK channels. Moreover, application of T-type channel antagonists drastically
20 disrupts the firing regularity and reveals a burst-like pattern in some neurons [70]. Therefore, at least in an in
21 vitro preparation, the T-type current in dopaminergic neurons of the substantia nigra prevents intrinsic burst
22 firing via its functional coupling with SK channels.

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25 Finally, our recent data in thalamic neurons demonstrate that T-type current effects on cellular excitability do
26 not always require a preceding transient or tonic hyperpolarization. The density of T-type channels
27 expressed in thalamic neurons is especially high resulting in a significant number of deinactivated T-type
28 channels at depolarized potentials (Figure 2A & B). Moreover, in thalamocortical neurons relaying sensory
29 information, the amplitude of the current generated by this population of deinactivated T-type channels is
30 enhanced by a voltage-dependent phosphorylation process that specifically occurs at these potentials [7, 46]
31 (Figure 2C). Hence, the combined effect of the phosphorylation-dependent potentiation and high channel
32 density allows the generation of T-type currents at depolarized potentials with two major physiological
33 consequences. First, the window T-type current is large enough (few tens of pA) to set the resting membrane
34 potential of thalamocortical neurons (Figure 3A) [26]. Moreover, it determines the occurrence, and controls
35 the duration of the UP states of slow (<1 Hz) oscillations [9, 37] (Figure 3B) (for a detailed summary of this
36 mechanism see Fig. 3 in [18]). Second, the significant fraction of T-type channels that is available at
37 depolarized potential participates to EPSP amplification and has a drastic effect on spike probability during
38 tonic firing. Indeed, using TTA-P2 and the dynamic clamp technique, we demonstrated that the activation of
39 T-type channels during wake-like states is a major determinant for single spike occurrence (Figure 4; [20]).
40 Since the T-type current activation and its subsequent boosting of the EPSPs become gradually more
41 prominent with slight hyperpolarization, the range of depolarized membrane potentials where synaptic inputs
42 can reach the spike threshold increases. The key physiological significance of these results is that the
43 recruitment of T-type channels during tonic firing confers a remarkable robustness to the output of
44 thalamocortical neurons [20] and secures information transfer across thalamic networks.

60 **Conclusions and future directions**

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The data reviewed above firmly establish that the roles of the T-type Ca^{2+} current go far beyond their classical function as “burst generators”. These novel functions include a key involvement in dendritic physiology, in particular synaptic integration and plasticity as well as dendro-dendritic synaptic release. In addition, in many brain areas, T-type channel have been found to be located in unexpected cellular compartments, such as the AIS and the axonal presynaptic terminals, where the roles of the T-type currents are just beginning to be understood. The discovery of the first potent and selective T-type current antagonists and the availability of knock-out mice for the different isoforms of these channels will greatly help to further decipher new roles in physiological functions and in pathological conditions. At the same time, structure-functions studies are revealing that T-type currents can be modulated by a number of intracellular pathways, whose physiological impacts on single cell and neural network excitability remain to be investigated in details. Furthermore, based on these molecular data and tools, direct interactions between T-type channels and other proteins, including ionic channels, have been established, suggesting that a diversity of localized T-type current functions and regulations may still need to be elucidated. Finally, from the time of their discovery it has been assumed that in the vast majority of neuronal types the generation of an LTS (and associated burst firing) was the only physiological role of T-type Ca^{2+} channels, since their nearly complete inactivation around the resting membrane potential should preclude their involvement in single action potential firing. However, it is now clearly established that, at least in thalamic neurons, the high density of T-type channels, and/or their regulatory mechanisms, ensure that a component of the T-type Ca^{2+} current is available at depolarized membrane potentials where it may play a major role in controlling neuronal excitability during activities related to the awake state.

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Figure legends

Figure 1: Generation of LTS and voltage-dependence of the T-type calcium current in thalamocortical neurons.

A. Voltage-dependence of the T-type current mediated low frequency oscillations in a thalamocortical neuron. Note that rhythmic LTSs crowned by high-frequency bursts of action potentials occur in a narrow range of membrane potential around -65 mV. Modified with permission from [47].

B. Synaptically evoked LTSs. In a thalamocortical neuron maintained at -70 mV, an EPSP evoked by stimulation of the sensory afferents activates T-type channels leading to the generation of a LTS crowned by a high frequency burst of action potentials (left trace). In the same neuron at a holding potential where T-type channels are inactivated (-60 mV), a rebound LTS is induced following a GABA mediated IPSP that allows some T-type channels to recover from inactivation (right trace). An enlargement of the LTS is presented in inset. Modified with permission from [26].

C. T-type currents (bottom traces) evoked from a holding potential of -100 mV in response to depolarizing current steps of increasing intensity (first step: -70 mV; step: 2.5 mV; top traces).

D. Activation and steady-state inactivation curves. The activation curve (filled circles) is constructed from the

1 peak current amplitude of the currents presented in C. For the steady-state inactivation curve (empty circles),
2 the channel availability is estimated from the current amplitude measured at -50 mV as a function of a 2 s
3 conditioning prepulse from -100 mV to -60 mV. Note the low voltage of activation of the T-type channels and
4 their nearly complete inactivation at around -60 mV. Note also the overlap of the activation and inactivation
5 curves that allow the presence of a window current. The voltage-dependence of the window current,
6 centered around -60 mV, estimated by multiplying the fits of the steady-state inactivation and activation
7 curves, is illustrated in inset.
8
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10 Figure 2: The high channel density and a phosphorylation-dependent potentiation facilitate the generation of
11 T-type currents at depolarized membrane potentials in thalamocortical neurons

12 A. In the presence of TTX, rebound LTSs were evoked in a thalamocortical neuron on the offset of variable
13 transient hyperpolarizations to -80 mV that induced partial recovery of the T-type channels from inactivation.
14 Note that maximal LTSs were already evoked with 300 ms-long hyperpolarization at -80 mV although the
15 recovery from inactivation of the whole T-type channel population would have required at least a 1 s
16 hyperpolarization at -100 mV. Modified with permission from [7].
17

18 B. In a thalamocortical neuron maintained at -60 mV, rebound LTSs were evoked by stimulations of
19 GABAergic afferents in control condition and when 50% of the T-channels population is blocked using 20 nM
20 TTA-P2 (see ref [25] for more details). This massive reduction in T-type channel availability did not have any
21 effect on the amplitude of the LTS or the number of action potentials in the burst, but only delayed the start
22 of the LTS. Modified with permission from [26].
23

24 C. Schematic representation of the mechanisms underlying T-type current potentiation. *Left panel:* At
25 depolarized membrane potential, inactivated T-type channels are phosphorylated. During a short
26 hyperpolarization the channels that recover from inactivation and enter the closed state are still
27 phosphorylated. Activation of these phosphorylated channels generates a large amplitude T-type current.
28 *Right panel:* After prolonged hyperpolarization, the closed T-type channels are de-phosphorylated and their
29 activation generates a current of smaller amplitude.
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39 Figure 3: Window T-type current contributes to neuronal excitability of thalamocortical neurons.

40 A. Contribution of the window T-type current to the resting membrane potential. Block of T-type channels
41 with TTA-P2 induced a clear hyperpolarization in thalamocortical neurons held at -60 mV (potential
42 corresponding to the peak amplitude of the window current) but not at -70 mV where the window current is of
43 minimal amplitude (see figure 1D).
44

45 B. Progressive block of the T-type channel population induced a gradual modification of the intrinsic slow
46 oscillations in thalamocortical neuron. Starting from a slow (<1 Hz) oscillation (a), application of TTA-P2
47 progressively decreased the duration of the slow oscillation UP state (b & c) that was then abolished after a
48 few additional minutes of drug exposure. Modified with permission from [26].
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54 Figure 4: T-type current boosts stimulus-evoked firing at depolarized membrane potentials and confers
55 robustness to the neuronal response across a large range of membrane potentials.

56 A. Spike raster plots of a thalamocortical neuron injected with gAMPA of fixed amplitude following a 10 Hz
57 Poisson distribution (bottom trace) and continuously submitted to synaptic excitatory and inhibitory noise.
58 Using the dynamic clamp technique, the same sequences of gAMPA and excitatory/inhibitory noise were
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1 injected in control condition and when the T-type channels are blocked by TTA-P2. The neuron was
2 successively maintained at mean membrane potentials of -60, -65, and -70 mV. The intracellular activities
3 recorded during the time window indicated by the black line in A are illustrated in B. The consistent decrease
4 in the number of spikes in the presence of TTA-P2 compared with the control condition demonstrates the
5 contribution of T-type channels to the firing probability at depolarized potentials (-60 mV). In addition, note
6 that the firing probability was barely affected by membrane potential changes in the control condition while it
7 was decreased upon hyperpolarization in the presence of TTA-P2. Modified with permission from [20].
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Figure 1

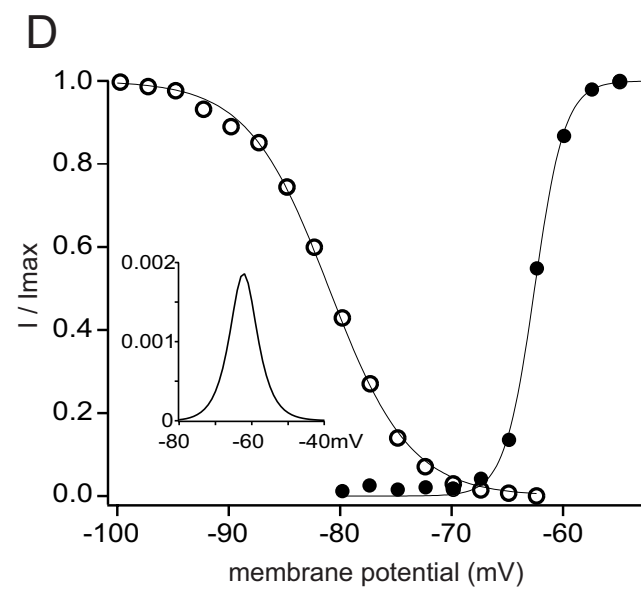
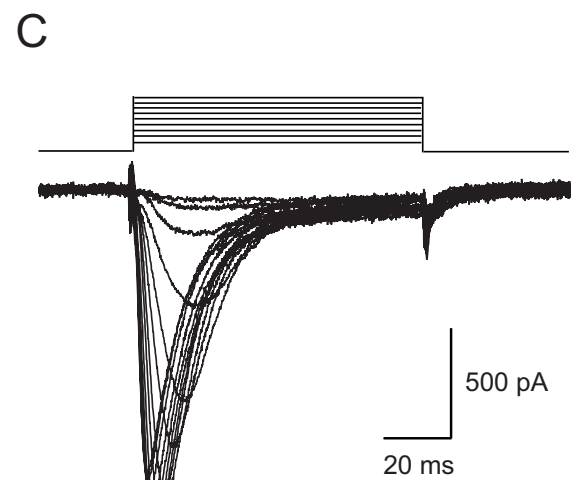
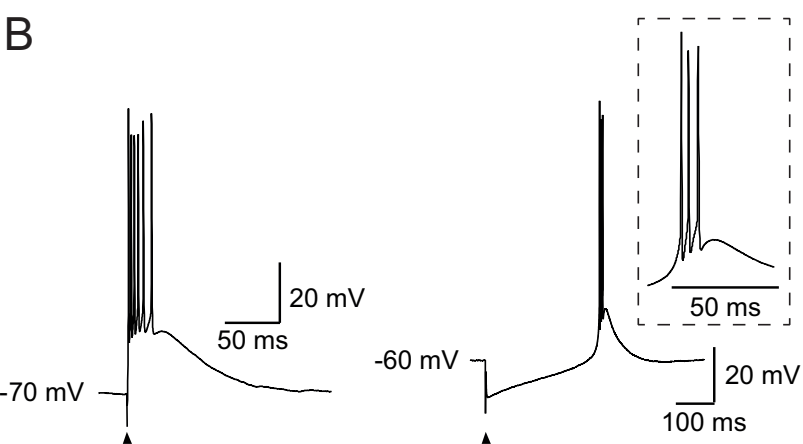
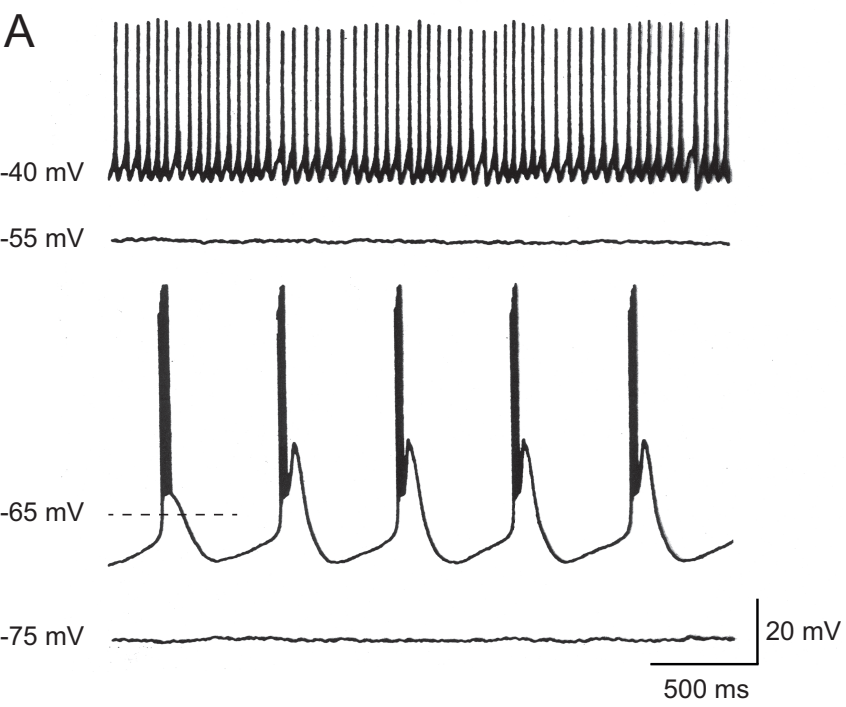


Figure 2

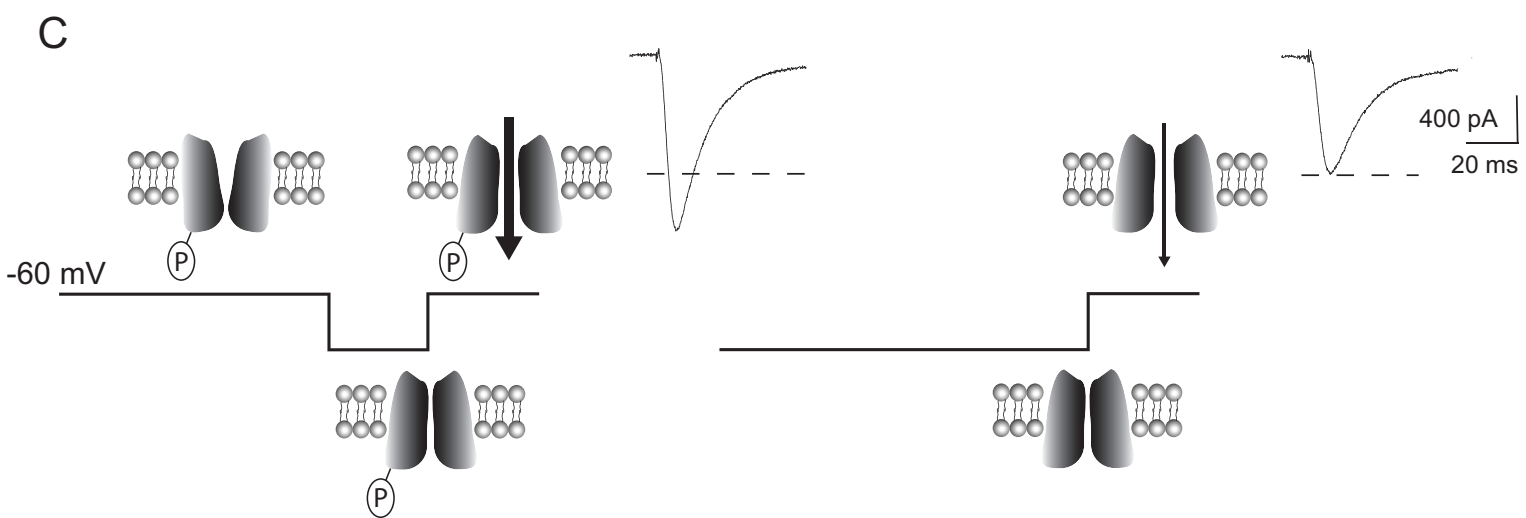
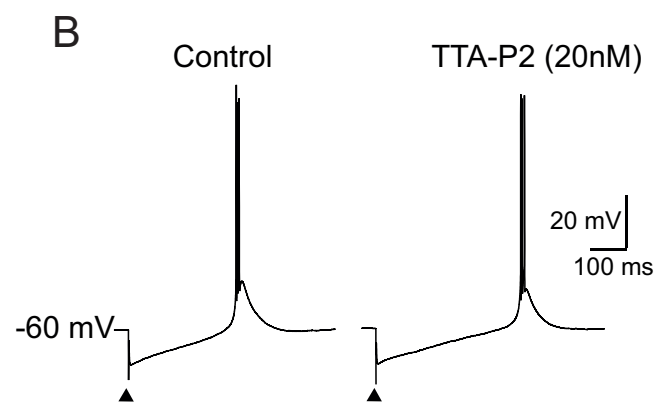
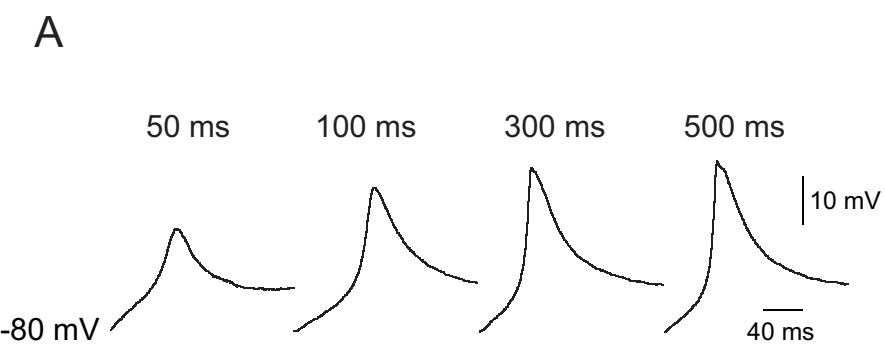


Figure 3

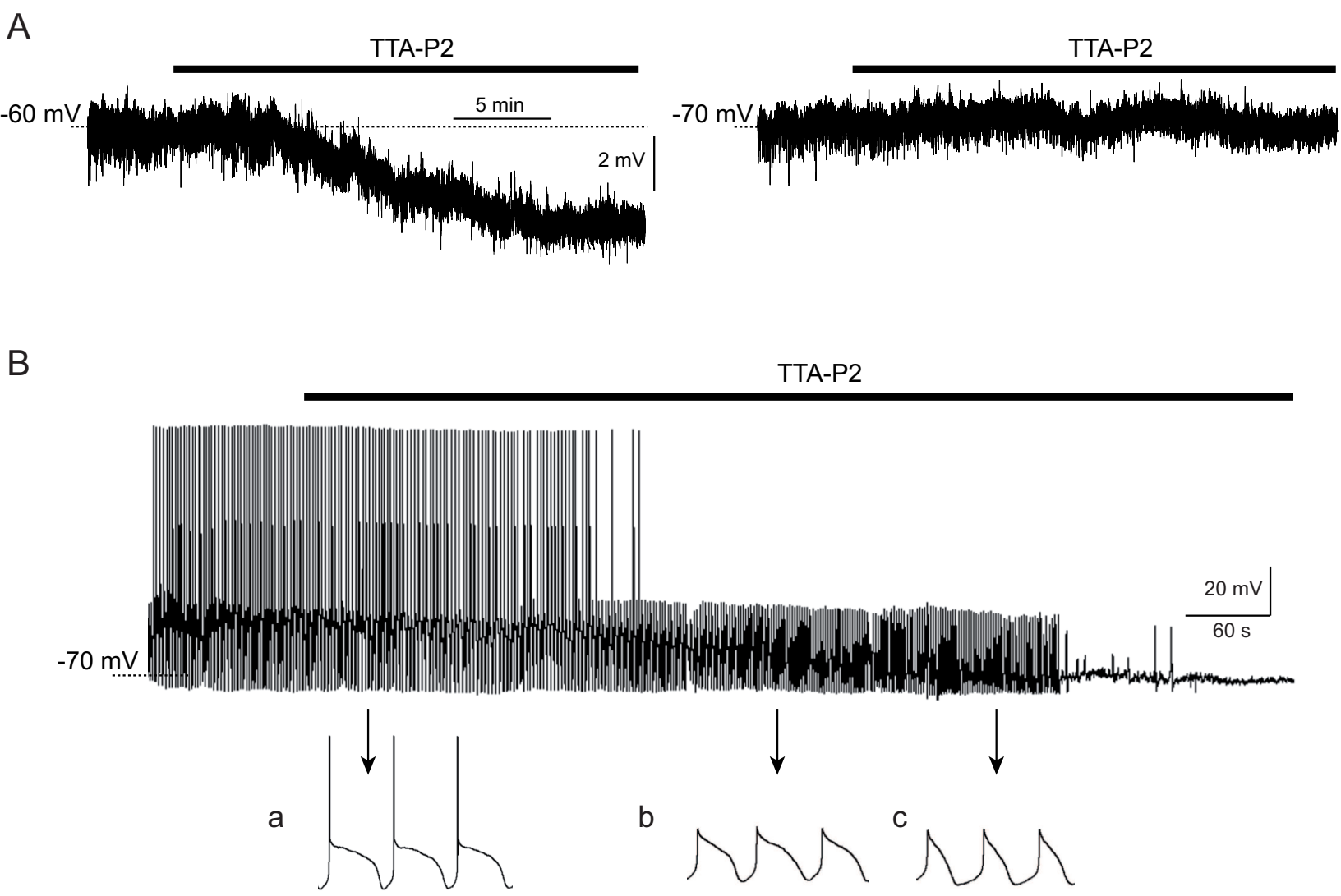


Figure 4

