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Review

Sodium Channels Involved in the Initiation of Action Potentials in Invertebrate and Mammalian Neurons

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Abstract: Living organisms react to external stimuli to adapt their activity to the environment for survival. Acquired information is encoded by neurons by action potentials (APs) in a series of discrete electrical events. Rapid initiation of the AP is critical for fast reactions and strongly relies on voltage-activated Na^+-selective channels (NaVs), which are widely expressed by both invertebrate and vertebrate neurons. Intuitively, NaVs of higher mammals should be activated faster than those of any other species. In addition to improved NaV channel structure, central mammalian neurons also demonstrate a patterned distribution of specific types of NaV1 channels at and near the site of AP initiation within the axonal initial segment (AIS). The AIS has different types of fast Nav1 channels and is thought to provide the biological basis for efficient frequency coding of information. In the present work, we review data related to the channels underlying fast initiation of action potentials in vertebrates and invertebrates, along with their evolution, distribution, and known specific roles. Current research has established that all mammalian NaV1 (1.1–1.9) channels share a similar structure, with 4 conservative transmembrane D-domains with a highly homologous sequence, but significant differences in the length of the functional cytoplasmic linkers. Similarly, the structure of NaV1 channels in invertebrates is generally similar to that of mammals, but it shows high variability across the evolutionary tree in the length of the linkers. AP initiation in mammalian cortical neurons is mediated by NaV1.2 and NaV1.6 channels, whereas interneurons mostly rely on NaV1.1 channels in their firing. Although invertebrate NaV1 channels normally display relatively slow kinetics, their activation is fast enough to produce APs, even in simple animals such as Placozoa. Remarkably, fast sodium-based excitability is not limited to animals. Recently, a photosynthetic prokaryote has been found to show rapidly activated sodium currents provided by their independently evolved single D-domain EuKatB sodium channels.

Keywords: neuron; action potential; sodium channel; excitability

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1. Theoretical Role of NaV Channels in AP Initiation

The classic Hodgkin-Huxley model (1952) [1] postulates the universal crucial role of NaV channels in AP initiation in neurons of both vertebrates and invertebrates. The model describes the transmembrane ionic currents underlying action potential initiation in the giant axons of squids. The universal model for the generation of electrical signals in nerve cells describes the electrical mechanisms underlying the initiation and development of action potentials. The Hodgkin-Huxley theory was originally based on mollusk experiments, while subsequent studies demonstrated that it applies to mammalian central neurons as well [2,3]. In the resting state, the membrane potential of a neuron is negative, ranging within −40 to −80 mV. Once the membrane potential reaches a threshold value, voltage-dependent Na^+ currents activate and evoke escalating membrane depolarization (Figure 1). This results in the inactivation and closure of Na^+ channels accompanied by the activation of voltage-dependent K^+ currents aimed at bringing the membrane potential back to the polarized state. Normally, in neurons there are several types of voltage-gated K^+ (KV) and Na^+ (NaV) channels, expressed by the same cell, which are involved in AP generation.

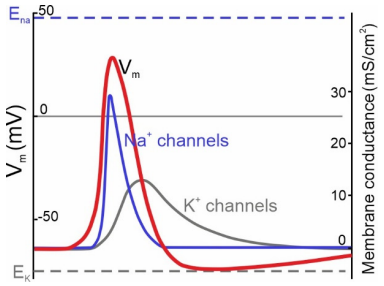

Figure 1. Ionic channels underlying the generation of APs in neurons described by the classic Hodgkin & Huxley model of the ionic mechanism of AP generation in a squid giant axon. Membrane potential (Vm) changes over time are accompanied by channel opening (modified from [1]). Na^+ channels (blue curve) activate during the rise phase and initiate the AP (red curve). K^+ channels (dark gray curve) provide membrane repolarization.

2. Normal Structure of Human NaV Channels and Their Pathological Mutations

Nine human NaV (1.1–1.9) channel genes encode a pore-forming α-subunit composed of four homologous domains (DI–DIV; Figure 2A). Each α-subunit contains six transmembrane α-helical segments (S1–S6) connected by intracellular linkers [4]. The structure of the transmembrane domains is highly conserved (Figure 2A). The sequentially linked transmembrane segments S1−S4 form a voltage-sensing domain, and segments S5–S6 form the pore-forming domain [5]. The DI-DIV domains are connected with intracellular cytoplasmic linkers (Figure 2A,B). The DIIIS6-DIVS1 linker is involved in closing the pore during fast inactivation (Figure 2A,B). Moreover, extracellular domains are involved in interactions with auxiliary β-subunits.

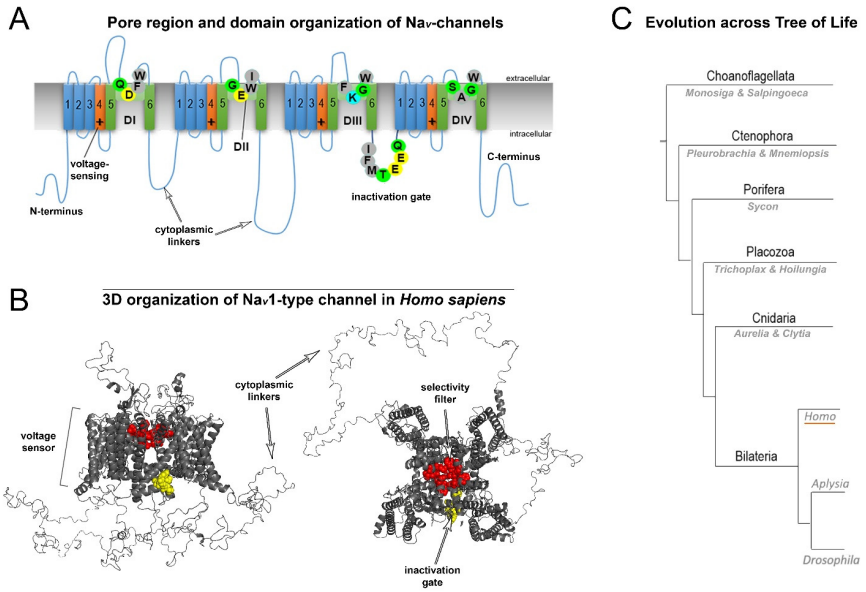

Figure 2. General structure and evolution of NaV channels. (**A**) Pore region and domain organization of NaV-channels (Homo sapiens). 2D model shows critical amino acids of NaV domains (D1-DIV), which contribute to pore selectivity motifs (marked as SF–Selectivity Filter) and inactivation gates. All species contain a critical lysine (K) in the pore region, which is responsible for sodium selectivity. Transmembrane α-helical segments are marked with numbers (1–6). Capital letters show single-letter amino acid code (**B**) 3D organization of NaV1-type channels (*H. sapiens*). The scheme includes all domains: the selectivity filter, inactivation gate and cytoplasmic linkers. (**C**) Evolution across the tree of life. Schematic phylogenetic relationships among representative animal clades of different classes of sodium channels. *Homo* (humans), *Aplysia*, and *Drosophila* represent mammals, mollusks, and insects, respectively.

Fast neuronal NaV channels (NaV1.1, NaV1.2, NaV1.3, NaV1.6) have the longest cytoplasmic linkers. In contrast, the cytoplasmic linkers of myocyte Nav1.4 channels are the shortest. NaV1.8, NaV1.9, and NaV1.5 channels display intermediate cytoplasmic linkers (Supplementary Sequence S1). Mutations of the most well studied channels, NaV1.1, NaV1.2 and NaV1.6, are found to be associated with specific functional epileptic disorders. More specifically, missense mutations in the cytoplasmic linkers and N-/C-termini of those channels are often found in patients with benign epileptic syndromes, whereas mutations in transmembrane segments and D-domains in most cases are related to severe epileptic encephalopathy [6].

3. Variability of Linkers of NaV Channels in the Evolutionary Tree

Organization of the neuronal membrane incorporates a large number of variable structural elements that are located close to one another. The structures of voltage-gated sodium channels significantly differ between evolutionarily distant branches of animals (Figure 2C). Importantly, cytoplasmic linkers are shorter in the ascidian species Sycon compared to in squids or humans. Vertebrates display a very conservative arrangement of the selectivity filter and transmembrane domains. Important differences between species arise in the length of linkers. In contrast, invertebrates show variable organization of the selectivity filter that does not include lysine in the DIII linker, which is characteristic of mammalian fast NaV channels. This suggests that the fast kinetics of the channel is not only due to the arrangement of the selectivity filter, but additional specific features of channel organization may also contribute. There is a substantial difference in the length of cytoplasmic linkers: in ascidians, the sequences are shorter compared to humans (1695 vs. 2009 aminoacids, respectively; Figure 3).

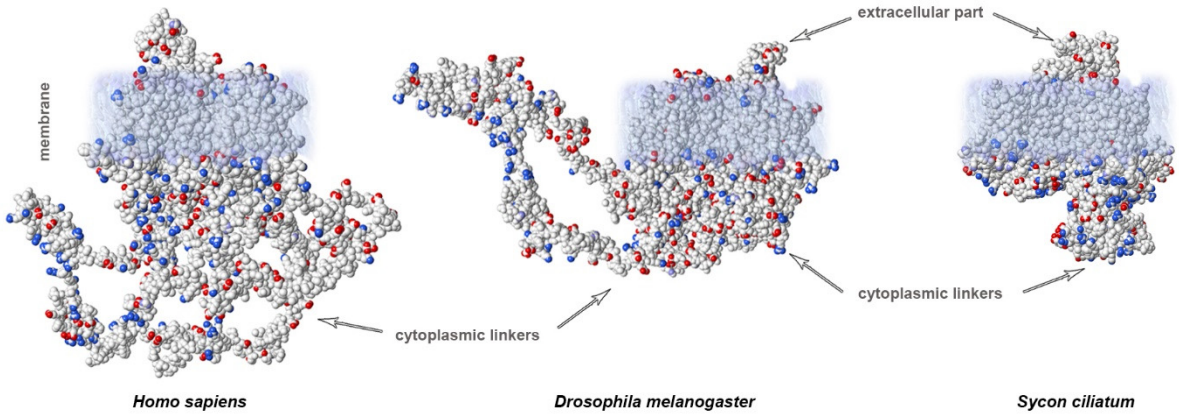

Figure 3. Calculated electrostatic potentials reveal different lengths of cytoplasmic linkers of NaV channels: mammals (H. sapiens) show the longest linkers compared to those of insects (*Drosophila*) or sponges (*Sycon*). Sequences obtained from GenBank.

4. Sites of AP Initiation in Mammalian Neurons

In mammalian pyramidal cells, the AP originates from the distal part of the non-myelinated axonal initial segment (AIS, 20–60 μm long; [7,8]. From the site of initiation, the AP propagates back to the soma and forward to axonal terminals (Figure 4). At the site of initiation at the distal AIS, AP onset displays a smooth exponential-like shape, which is indicative of its compliance with the Hodgkin-Huxley model [3,9]. By contrast, if recorded in the soma, APs display sharp non-exponential "kink"-like onset dynamics [10,11], which is thought to be due to passive AP invasion as a result of backpropagation [3]. Remarkably, APs with "kink"-like onset dynamics are also observed in mollusks, and are recorded when the AP is initiated at a distance from the recording site [12]. Apart from initiation at the AIS, APs are known to rejuvenate at the nodes of Ranvier as a result of saltatory conductance in myelinated axons. Moreover, de novo AP initiation at the proximal node of Ranvier

has been shown in Purkinje neurons [13,14], which adds to the computational abilities of those neurons.

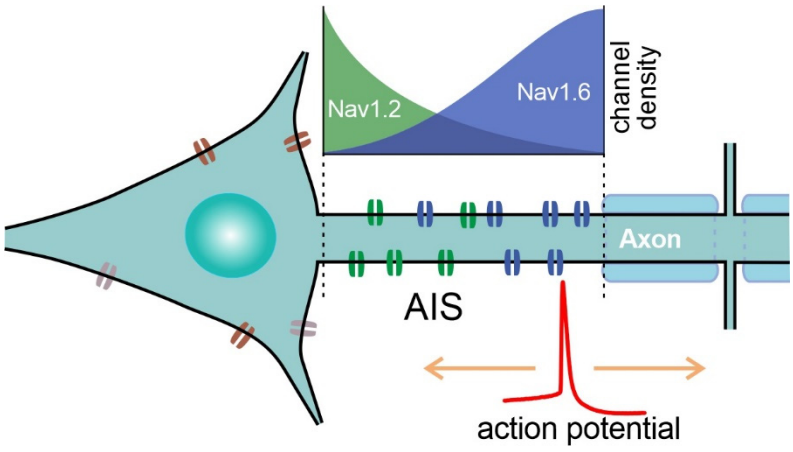

Figure 4. Schematic of sodium channel distribution at the AIS of pyramidal neurons. NaV1.6 density is higher at the distal part of the AIS, whereas NaV1.2 channels are clustered at the proximal part of the AIS. APs are initiated at the distal part of the AIS, which is enriched with low-threshold NaV1.6 channels.

5. Specific NaV Channels Involved in AP Initiation in the CNS of Mammals

Aggregation of NaV channels at the AIS and nodes of Ranvier is critical for the initiation, waveform and propagation of APs. High NaV density at the AIS is not required for axonal AP initiation, but it is crucial for a high bandwidth of information encoding and precise AP timing [15]. To initiate the AP, voltage-gated channels are required to display sufficiently fast activation at relatively low voltage thresholds. Computational studies of the contribution of axonal channels to computational abilities suggest that coding features strongly depend on the voltage sensitivity of axonal ion channels [16]. Voltage-gated NaV channels consist of a pore-forming α-subunit with ion selectivity, conductance and voltage sensing properties, and the accessory β-subunits, which are able to modify the kinetics and voltage dependence of gating. However, expression of the α-subunit alone in a heterologous expression system is sufficient to produce a voltage-gated sodium current [17]. In the adult mammalian CNS, three tetrodotoxin-sensitive NaV channel isoforms (NaV1.1, NaV1.2 and NaV1.6) underlie AP generation, and to a large extent determine the AP dynamics in central neurons. Adding low concentrations of tetrodotoxin, a selective blocker of fast NaV channels substantially compromises AP dynamics by reducing the number of available NaV channels [10]. Likewise, more precise experiments with local application of tetrodotoxin to the AIS or lowering Na^+ concentration result in compromised AP dynamics and a decline in the frequency transfer function at higher frequency ranges [18]. Compared to invertebrates, physiologically dissected neuronal Na^+ currents carried by NaV channels display much faster dynamics of activation (Table 1). The difference might be even stronger at physiological temperatures, as these experiments are normally performed at lower temperatures to preserve the mammalian neurons from quick degradation in electrophysiological experiments in situ.

Table 1. Electrical characteristics of NaV channels cloned into heterologous expression systems in situ (blue panel) and physiologically dissected transient Na+ currents (I_{NaT}s) of molluscan neurons (green panel) obtained using the voltage-clamp technique. The voltage characteristics of activation threshold and peak current, as well as times-to-halfpeak (the length of time it takes to reach half-peak activation) are shown. Voltage values obtained in patch-clamp experiments might contain errors due to liquid junction potentials.

Channel/Current	Threshold: Approx. mV	Peak, mV	Time to $\frac{1}{2}$ Peak, ms	References
NaV1.6/AnkG	−45	−10	~0.25	[19]
NaV1.2	−40	−5	~0.3	[19]
NaV1.1	−40	0	~0.28	[20]
Ciona NaV1a	−30	5	~0.5	[21]
Insect DmNaV1/tipE	−45	−5	~1.1–1.2	[22,23]
Plant EukCatB	−90	−40	~0.6	[24]
Lymnaea I_{NaT}	−55	−35	~2.5	[25,26]
Loligo I_{NaT}	−35	−5	~0.6	[27]
Aplysia I_{NaT}	−30	0	~0.9	[27]

6. Crucial Role of Nav1.6 in AP Initiation by Principal Neurons of the Cortex

Among the tetrodotoxin-sensitive NaV channels, the low threshold Nav1.6 channels are thought to be the most important for the initiation of APs in the majority of neurons. In pyramidal cells, Nav1.6 channels are distributed along the AIS and are preferentially clustered at the distal part of the AIS (Figure 4, [28]), which makes them critical for AP initiation [29–33]. Knockout of NaV1.6 channels does not prevent pyramidal neurons from generating APs but increases the voltage threshold of AP initiation [32,34]. Using a specific blocker, NaV1.6 channels were demonstrated to greatly affect the threshold of AP initiation, its dynamics and coding abilities in neocortical pyramidal cells [35]. Moreover, NaV1.6 knockout results in a compensatory increase in the density of NaV1.2 channels spread along the whole AIS from its distal part, where they are normally expressed in wild type cells (Figure 4, [31,32]). Thus, NaV1.2 channels are able to initiate APs themselves without NaV1.6 channels. Adding to that, NaV1.2 but not NaV1.6 are clustered in the axons and nodes of Ranvier of the developing nervous system, whereas NaV1.6 is the main axonal sodium channel isoform in adults [36]. Inward currents carried by NaV1.2 and NaV1.6 channels experimentally cloned and expressed in TsA201 cells in situ display similar thresholds and dynamics of activation [19]. However, NaV1.6 channels co-expressed with Ankyrin G, an AIS clustering protein display a lower threshold of activation [19], which allows them to activate earlier than any other channels and initiate the AP.

The electrical characteristics of fast NaV1 channels are finely tuned for optimal excitability of central neurons, and any inconsistencies can alter the balance of excitation and inhibition in the CNS. For example, both mutations of NaV1.2 channels that increase sodium currents as well as those decreasing sodium currents are known to produce epileptic phenotypes [37]. Paradoxically, deficiency in NaV1.2 channels is able to increase overall cortical excitability [38,39]. Similarly, both gain-of-function and loss-of-function de novo mutations of the NaV1.6 gene cause various forms of epileptic disorders [40].

Apart from axonal NaV channels, tetrodoxin-resistant NaV1.5 channels were found to preferentially express at the soma and proximal dendrites of the central neurons [31,41], which suggests their contribution to subthreshold dendritic depolarization and backpropagation of the AP but not to AP initiation.

7. NaV1.1 Function in GABA-ergic Neurons

The axonal localization of NaV1.1 channels at the proximal AIS and nodes of Ranvier [31,42] strongly suggests their possible involvement in AP initiation. NaV1.1 channels are known to aggregate at the proximal AIS of ~80% of spinal motor neurons [42]. Even more importantly, NaV1.1 channels localize to the proximal AIS in both parvalbumin-

positive and somatostatin-positive central inhibitory interneurons [43–45]. Thus, the excitability of GABA-ergic interneurons depends on NaV1.1 channel function. To the best of our knowledge, the exact location of AP initiation within the AIS of interneurons has not been correlated with specific NaV1 channel densities so far. However, the net fast Na^+ current dramatically decreases in interneurons of NaV1.1-defficient mice, and the threshold of AP initiation increases [46]. Similarly, in humans with a loss-of-function mutation of NaV1.1, pluripotent stem-cell-derived telencephalic inhibitory interneurons, but not excitatory neurons, display reduced fast Na^+ currents [47]. Loss-of-function mutations in the NaV1.1 channel gene decrease sodium currents and AP amplitudes in GABA-ergic inhibitory interneurons and Purkinje cells without a detectable effect on excitatory pyramidal neurons [48]. Therefore, the dependence of inhibitory neurons on NaV1.1 channel function is crucial for the balance of excitation and inhibition of the brain. In human patients, loss-of-function mutations of NaV1.1 may cause severe epileptic encephalopathy (Dravet syndrome) and ataxia [49]. Nav1.1-related disorders are thought to be due to compromised excitability of GABA-ergic inhibitory interneurons that rely on NaV1.1-dependent AP initiation [50].

8. Important NaV Channels of Peripheral Neurons

Outside the CNS, spinal neurons of dorsal root ganglia are rich in various NaV channel types that are relevant to their sensory function. NaV1.7, NaV1.8 and NaV1.9 regulate thresholds, AP conduction and frequency of firing of nociceptive C-fibers [51–55]. Among these, the relatively slow NaV1.8 and NaV1.9 channels are tetrodotoxin-resistant and evolutionary closer to the ancestral NaV1 channels [56]. Adding to that, the expression of NaV1.3 channels in spinal neurons can be induced by injuries, which result in altered neuronal excitability and neuropathic pain due to their relatively fast activation and slow closed-state inactivation kinetics [57,58].

In neuroendocrine cells, fast NaV1.3 and NaV1.7 channels were shown to contribute to AP generation and firing patters [59].

9. NaV Channels Underlying AP Initiation in Insects

In contrast to the numerous mammalian NaV channels, insects appear to have only a single sodium channel gene that encodes the α-subunit DmNaV1 [60] in several isoforms due to alternative splicing and RNA editing [61]. Orthologs of the mammalian β subunit have not been discovered in insects. However, a Drosophila transmembrane protein, TipE, is thought to be an auxiliary subunit of the insect sodium channel. Cloning and heterologous co-expression of TipE or its homologous genes with DmNaV1 demonstrated that co-expression shifts the voltage-dependence and increases the net sodium current [23]. The dynamics of DmNaV1 appear to be slower than that of any mammalian NaV channels involved in AP initiation by the central mammalian neurons reviewed above [22,23] (Table 1). The activation kinetics and voltage range of the physiologically dissected transient Na^+ current in isolated insect neurons are similar to that carried by DmNaV1 channels cloned into a heterologous expression system [62].

The difference between the pharmacological characteristics of insect NaV channels and those of mammals allowed the construction of specific molecules that act on insect NaVs with virtually no effect on mammalian NaVs. The best-known example is synthetic pyrethroids that are toxic to insects with very low toxicity to mammals [63].

10. Fast Sodium Currents in Mollusks

Classically, the nervous system of the mollusk has been chosen as a refence model for the theory of AP initiation in nerve cells. However, to the best of our knowledge, at present, none of the NaV channels of the mollusk have been cloned in a heterologous expression system for the characterization of electrical properties in isolation from other neuronal channels. Substitution of K^+ or Na^+ with non-permeable cations in combination with channel blockers is usually employed to physiologically dissect the transient Na^+

current (I_{NaT}) of molluscan neurons. Fast I_{NaT} dissected with blockers and low Ca^{2+} in giant cerebral cells (CGC) in the CNS of *Lymnaea*, a fresh water pulmonate mollusk, demonstrates relatively slow activation kinetics and low tetrodotoxin sensitivity [25,26] (Table 1). Thus, the physiological characteristics of *Lymnaea* I_{NaT} display some similarity to slow pain-related mammalian NaV1.8 and NaV1.9 channels. Indeed, genome sequencing data shows the best similarity between the *Lymnaea* NaV1 and mammalian NaV1.9 channels [56]. In contrast, I_{NaT} currents of neurons of the marine mollusks *Aplysia* and *Loligo* show faster activation kinetics and substantial sensitivity to tetrodotoxin [27]. Thus, there is significant variability in NaV channels within phylum Mollusca, though I_{NaT}s in all the mentioned molluscan species are slower compared to the Na^+ currents carried by the mammalian axonal NaV channels of CNS neurons (Table 1).

11. NaV Channels of Archaic Invertebrates and Photosynthetic Eucaryotes

Recently, the cells of the simplest known free-living animal *Trichoplax* (phylum Placozoa) have been demonstrated to generate APs [64]. Their recorded amplitude was ~10 mV (i.e., ~10 times smaller than that of mammalian or snail neuronal spikes). Similarly to mammalian neurons, the APs in *Trichoplax* cells were voltage-gated and sodium-dependent. At the same time, >20 placozoan NaV channels have been revealed by genomic analysis of their DNA. Molecular cloning of AP-related placozoan NaVs for characterization of their electrical properties remains to be considered.

CiNaV1, an α-subunit of the NaV channel of the simple invertebrate ascidian animal *Ciona*, (phylum Chordata), closest to mammals, have been cloned to investigate the characteristics of Na^+ current in a heterologous expression system in situ [21] (Table 1). CiNaV1a exhibits tetrodotoxin-insensitive sodium currents with relatively fast kinetics of activation and inactivation. No orthologs of the vertebrate β-subunit were found in the *Ciona* genome; however, the gene of the CiNaV1a channel contains an ankyrin-binding motif similar to that of mammalian NaV1 channels, which is employed to target them to the AIS of mammalian cortical neurons.

Apart from animals, photosynthetic plankton have been recently shown to express a tetrodotoxin-sensitive variant of NaV channels. Unlike NaV1 channels that are formed with four transmembrane D-domains, the novel EukCatB channel of *Coccolithophore* is single-domain with relatively rapid kinetics of activation in a heterologous expression system in situ (Table 1). This example clearly demonstrates that the capacity for Na^+-based fast signaling at single-cellular level in eukaryotes is not restricted to animals, and this channel type appears to have evolved multiple times independently [24].

12. Conclusions

The best studied example of AP initiation by pyramidal neurons is a complex process, which normally requires at least two different NaV1 channel isoforms located at a non-myelinated axonal initial segment. Adding to that, rapid mammalian NaV1 channels display faster kinetics than those of any studied invertebrate species. Thus, the evolutionary advanced nervous system of mammals relies on fast excitability at single-neuronal level provided by both channel properties and the spatial organization of channels at the AP-initiation sites. Most epileptogenic NaV variants are associated with NaV1.1, NaV1.2, NaV1.3, or NaV1.6, whereas genetic variants of NaV1.7, NaV1.8, or NaV1.9 are linked to neuropathic pain. However, the current lack of highly specific channel blockers/activators limits the functional characterization of each isoform.

Overall, NaV1 channels of both mammals and invertebrates share the same structure with 4 transmembrane D-domains that are strongly homologous between channels, but their sequences greatly differ in the length of the cytoplasmic linkers. Invertebrate NaV1 activation is fast enough to produce action potentials, even in simple animals such as snails and *Placozoa*.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/biophysica2030019/s1.

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