NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH Public Access
Author Manuscript
Neuron. Author manuscript; available in PMC 2012 December 22.

Published in final edited form as:
Neuron. 2011 December 22; 72(6): 899–911. doi:10.1016/j.neuron.2011.12.007.

Sodium Leak Channels in Neuronal Excitability and Rhythmic Behaviors

Dejian Ren†
Department of Biology, University of Pennsylvania, Philadelphia, PA 19104, U.S.A

Abstract

Extracellular K^+, Na^+, and Ca^{2+} ions all influence the resting membrane potential of the neuron. However, the mechanisms by which extracellular Na^+ and Ca^{2+} regulate basal neuronal excitability are not well understood. Recent findings suggest that NALCN, in association with UNC79 and UNC80, contributes a basal Na^+ leak conductance in neurons. Mutations in *Nalcn, Unc79*, or *Unc80* lead to severe phenotypes that include neonatal lethality and disruption in rhythmic behaviors. This review discusses the properties of the NALCN complex, its regulation, and its contribution to neuronal function and animal behavior.

Introduction

The membrane potential of a neuron is determined by the concentrations of ions and inside the cell as well as the permeability of the membrane to each ion. In the presence of K^+, Na^+ and Cl^-, for example, the relationship between the resting membrane potential (RMP), the concentration of each ion, as well as their relative permeability can be quantitatively determined by the Goldman-Hodgkin-Katz (GHK) voltage equation (Hodgkin and Katz, 1949b). At rest, most neurons are primarily permeable to K^+, resulting in an RMP closer to the equilibrium (Nernst) potential of K^+ (E_K ~ −90 mV) than to that of Na^+ (E_{Na}, ~ +60 mV). The influence of Cl^- can be complex because of large variation in intracellular Cl^- conentrations ($[Cl]_i$), thus E_{Cl}, due to variation in the expression of Cl^- transporters. For example, $[Cl]_i$ starts high in the immature hippocampal neurons but decreases during maturation because of increases in the expression of KCC2 K^+/Cl^- cotransporter and the increase in Cl^- exclusion, resulting E_{Cl} switching from being depolarized to RMP to one that's hyperpolarized to RMP (Rivera et al., 1999). As a consequence, the same neurotransmitter GABA acting through the Cl^- channel $GABA_A$ receptor can be excitatory in an immature neuron but inhibitory in adult (Ben-Ari et al., 1989). In some neurons without much active Cl^- transporter activity, Cl^- is generally believed to have less direct effect on RMP because the ion distributes across the membrane passively (i.e., $i_{Cl} = 0$), resulting a simplified GHK equation where RMP is mainly determined by the cell's relative permeability to Na^+ and K^+ (P_{Na}/P_K) (Hodgkin, 1958). Many Cl^- conductances have been molecularly identified (Jentsch et al., 2002). Similarly, numerous K^+ channels contribute resting K^+ conductances. In addition to some voltage-gated K^+ channels (K_V) that are open at RMP, there are K^+ conductances that are voltage-independent and are constitutively open at RMP; these contribute the "leak" K^+ current. In mammals, the two pore-domain family of

© 2011 Elsevier Inc. All rights reserved.

†***To whom correspondence should be addressed:*** dren@sas.upenn.edu, Phone: (215) 898-9271, Fax: (215) 898-8780.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

K^+ leak channels (K2P) has 16 members (Goldstein et al., 2005). K2P channels can be regulated by a wide variety of physiological stimuli such as pH, anesthetics, and mechanical force. The regulation of these channels provides a powerful mechanism by which the neuron can control its excitability (Honoré, 2007).

Despite the dominant contribution of K^+ channels to the resting conductance of neurons, the RMP of most mammalian neurons is in the range of −50 to −80 mV (as far as 40 mV depolarized to E_K), suggesting existence of other resting conductances. Indeed, each of the three cations (Na^+, K^+ and Ca^{2+}) in the Ringer's solution used in early heart-beat studies has been shown to influence neuronal excitability (Frankenhaeuser and Hodgkin, 1955; Hodgkin and Katz, 1949a; Hodgkin and Katz, 1949b; Ringer, 1883). However, the means by which Na^+ and Ca^{2+} influence basal excitability are not well elucidated. Data accumulated in the past several years suggest that NALCN, a Na^+ -permeable, non-selective cation channel widely expressed in the nervous system, contributes a TTX-resistant Na^+ leak conductance (Lu et al., 2007). In addition, the channel also plays a major role in determining the sensitivity to extracellular Ca^{2+} of neuronal excitability. The current review focuses on the molecular mechanisms, function, and regulation of the NALCN channel complex.

Na^+ Leak in Neurons

The existence of basal Na^+ conductance was first reported more than 50 years ago. In the squid giant axon, Hodgkin and Katz estimated that the resting relative permeability of Na^+ and K^+ (P_{Na}/P_K) was 0.04 (4%) (Hodgkin and Katz, 1949b). Several cellular mechanisms contribute to the resting background Na^+ conductance. First, Na^+-dependent co-transporters and some of the electrogenic exchangers allow Na^+ into neurons. Second, in some neurons, the hyperpolarization-activated cation channels (HCN, I_f/I_h current), which conduct Na^+, are open at rest (Robinson and Siegelbaum, 2003). HCN channels are not present in some animals, such as the nematode *C. elegans*. Third, persistent Na^+ currents (I_{NaP}) at RMPs can be generated by voltage-gated Na^+ (Na_V) channels through the "window" current, or by non-inactivating ion channels (Crill, 1996). The generation of I_{NaP} through Na_Vs is influenced by the voltage-dependence of the channel's activation and inactivation, which can also be regulated by modulators such as G protein βγ subunits (Ma et al., 1997). During interspike intervals, Na_Vs in some neurons can also generate "resurgent" current upon repolarization because of channel's recovery from inactivation/block during depolarization (Grieco et al., 2005; Raman and Bean, 1997). These subthreshold, Na_V-dependent conductances are highly sensitive to voltage and are mostly blocked by tetrodotoxin (TTX) in the central nervous system. Finally, many neurons also exhibit a TTX-resistant, voltage-independent, "true" background Na^+ conductance (Na^+ leak current, $I_{L\text{-}Na}$) (Atherton and Bevan, 2005; Eggermann et al., 2003; Jackson et al., 2004; Jones, 1989; Khaliq and Bean, 2010; LeSauter et al., 2011; Pena and Ramirez, 2004; Raman et al., 2000; Russo et al., 2007).

The most obvious function of the tonically active background Na^+ conductance is perhaps to balance the K^+ leak to set the RMP, which would be at ~ −90 mV (E_K) in all the neurons if there were only basal K^+ conductance. A tonic leak of other ions such as Ca^{2+}, Mg^{2+} and H^+ can hypothetically achieve the same goal, but excessive leak of these ions into neurons can be damaging to the cells because of the cellular metabolism's high sensitivity to the intracellular concentrations of the ions. By varying the basal P_{Na}/P_K, the nervous system can have a wide range of RMPs among different neurons, a heterogeneity in neuronal intrinsic properties known to exist in the brain (Kandel et al., 2000; Llinas, 1988).

Another function of the Na^+ conductance is to provide a regulation of the membrane potential by environmental stimuli. One such example was demonstrated in the excitation of

NIH-PA Author Manuscript

sympathetic ganglion neurons in the frog (Jan and Jan, 1982; Kuffler and Sejnowski, 1983). In addition to small-molecule neurotransmitters such as acetylcholine, the spinal nerves also release peptide neurotransmitters such as LHRH- and substance P (SP)-like peptides to excite the sympathetic ganglion neurons. These peptides elicit "late slow" depolarization that lasts minutes (Jan and Jan, 1982; Kuffler and Sejnowski, 1983). In addition to suppressing K^+ currents such as the M-current, the neurotransmitters can also excite the neurons through activation of a Na^+-dependent basal cation current that is apparently carried by the basal Na^+ leak conductance (Brown and Adams, 1980; Jones, 1985; Kuba and Koketsu, 1978). Such a mechanism of excitation through the activation of Na^+-leak-like basal conductances has also been found in the excitation of serotonin neurons in the dorsal raphe nucleus by orexin (Liu et al., 2002), VTA dopaminergic neurons by SP and neurotensin (Farkas et al., 1996), locus coeruleus neurons by SP and muscarine (Shen and North, 1992a; Shen and North, 1992b), and pre-Bötzinger complex neurons by serotonin and SP (Pena and Ramirez, 2004; Ptak et al., 2009). Similarly, suppression of a Na^+ leak-like current can lead to hyperpolarization by driving the RMP toward E_K, as suggested in the gastrin-releasing peptide containing retinorecipient neurons in the suprachiasmatic nucleus (SCN). In these neurons, a one-hour light exposure causes a large reduction (> 100 pA) of what appears to be a Na^+-leak current and a hyperpolarization of membrane potential by 15 mV (LeSauter et al., 2011).

Background Na^+-leak conductances are also implicated in the generation and/or maintenance of spontaneous firing of neurons. Neurons with autonomous firing have been found in many regions in the nervous systems (Hausser et al., 2004; Llinas, 1988). The ability to generate rhythmic firing in some neurons is clearly the cell's intrinsic property as it persists in dissociated neurons in culture and in slices when synaptic transmission is blocked. Subthreshold conductances such as the TTX-sensitive persistent Na^+ conductance, resurgent Na^+ conductance, voltage-activated Ca^{2+} channels and I_h have been shown to be the major determinants in the autorhythmicity in many neurons such as cerebellar Purkinje neurons (Raman and Bean, 1997; Raman et al., 2000) and substantia nigra pars compacta neurons (Chan et al., 2007; Guzman et al., 2009; Puopolo et al., 2007). In some neurons such as the cerebellar nuclei neurons (Raman et al., 2000), cerebellar unipolar brush cells (Russo et al., 2007), SCN neurons (Jackson et al., 2004), dopaminergic VTA neurons (Khaliq and Bean, 2010) and substantia nigra pars reticulata neurons (Atherton and Bevan, 2005), the autonomous firing also involves conductances similar to the TTX-insensitive background Na^+-leak conductance. The presence of such a conductance is proposed to set the "resting" membrane potential close to the threshold (for example −50 mV) above which voltage-sensitive channels are activated, or to depolarize the cells to the threshold potential during inter-spike interval (Khaliq and Bean, 2010). In several cases where the background conductances were measured at multiple voltages (Jackson et al., 2004; Raman et al., 2000), they were shown to be voltage-independent and relatively non-selective as the reversal potentials of the extrapolated current (I) −voltage (V) relationships were close to 0 mV. These properties of the currents are similar to those of NALCN, although whether they are indeed carried by NALCN remains to be tested (Lu et al., 2007).

NALCN Channel Protein

NALCN is a member of the 24-transmembrane domain (24-TM) ion channel super-family, which also includes the ten voltage-gated Ca^{2+} channels (the L-type $Ca_V1.1–1.4$, P/Q type $Ca_V2.1$, N-type $Ca_V2.2$, R-type $Ca_V2.3$, and T-type $Ca_V3.1–3.3$ channels) and ten Na^+ channels ($Na_V1.1–1.9$ voltage-gated channels and the non-voltage gated Na_X) (Lu et al., 2007; Snutch and Monteil, 2007). The pore-forming α subunits of these channels have four homologous domains (I–IV), each of which has six transmembrane segments (S1–S6) (Catterall, 2000).

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

Several other channel families having a 6-TM structure also share high sequence similarity with the NALCN/Na_V/Ca_V family (Fig. 1). These include the bacterial voltage-gated Na^+ channels (Na_VBac) (Ren et al., 2001b), the bacterial putative voltage-gated Ca^{2+} channels (Ca_VBac), and the pH-gated Ca^{2+} channels (CatSpers1-4) that are found in both vertebrates and invertebrates and, in mammals, are localized to sperm and required for male fertility (Ren and Xia, 2010). CavBac has not been electrophysiologically characterized but, because of its TFEDWTD sequence in the pore region (Fig. 2), is predicted to be Ca^{2+}-selective based on mutagenesis studies done to convert the NaChBac Na^+ channel to a Ca^{2+} channel CaChBac (Yue et al., 2002). NALCN, Ca_V, Na_V, Na_VBac, Ca_VBac, and CatSper channels contain highly homologous sequences in the S1–S4 voltage-sensing domains (VSDs) and, in particular, the channel pore regions; thus, the 24-TM channels likely evolved through duplication from 6-TM bacteria channels. Consistent with this idea, a transitional state can be observed in the two-pore segment channels (TPC), which have two six-transmembrane domain segments (12-TM) (Ishibashi et al., 2000). Mammals have two TPC channels, TPC1 and TPC2. Their overall sequences have similarity with those of the 24-TM channels, although not as high as that found among the other families. TPCs are intracellular cation channels localized on lysosomes and endosomes (Calcraft et al., 2009).

Unlike some channels, such as Na_V and CatSper, NALCN is found in all animals studied, from humans to *D. melanogaster, C. elegans*, snails, sea urchins, and the placozoan *Trichoplax adhaerens*. Most species appear to have only one *Nalcn* gene; however, *C. elegans*, which does not have Na_V channel, has two *Nalcn* genes. Similar to the other 24-TM channels, NALCN is not found in plants such as *Arabidopsis*. Intriguingly, *Saccharomyces cerevisiae* (budding yeast) has only one 24-TM channel (CCH1), whose nearest homolog is NALCN, suggesting an early appearance of 24-TM channels in eukaryotes during evolution (Hong et al., 2010).

The existence of NALCN as the third branch of ion channels in the 24-TM channel family was first evident when large amount of genomic and cDNA sequences became available in the late 1990s. By searching databases with template sequences from Na_vs and high-voltage-activated Ca_Vs (the only known 24-TM channels at that time), partial sequences encoding novel channels with sequences similar to those of Na_vs and Ca_vs, especially in the pore region, were found. These include the T-type Ca_Vs (Perez-Reyes et al., 1998), TPCs (Ishibashi et al., 2000), CatSpers (Ren et al., 2001a), and NALCN from rats (named as Rb21)(Lee et al., 1999), humans (VGCNL1), *C. elegans* (NCA) and Drosophila (Dma1U for unique α1 subunit) (Littleton and Ganetzky, 2000). The *in vivo* importance of NALCN was first revealed by the findings that several alleles of an existing Drosophila mutant (*na* for *n*arrow *a*bdomen, and *har* for *ha*lothane *r*esistance) have a 9 nt deletion (in the *na* allele) predicted to lead to a deletion/alteration of four amino acids (*na*) or a point mutation predicted to alter RNA splicing (*har*) in the *Dma1U* gene (Nash et al., 2002). These hypomorphic mutant flies have severely reduced expression of the NALCN ortholog. They are viable but have disruptions in circadian rhythm and sensitivity to halothane. *Nalcn* mutations leading to significant phenotypes were later reported *C. elegans* (Humphrey et al., 2007; Jospin et al., 2007; Pierce-Shimomura et al., 2008; Yeh et al., 2008) and the mouse (Lu et al., 2007) (see more discussions in later sections.)

NALCN has several unique biophysical properties (Lu et al., 2007). For example, it is voltage-independent, with a linear current (I) −voltage (V) relationship over the tested range of −100 mV to +100 mV (Fig. 2). In addition, NALCN does not inactivate. Interestingly, NALCN is the only nonselective channel found in the 24-TM channel family and is equally permeable to Na^+, K^+, and Cs^+. At RMPs, which are normally close to E_K, the major charge-carrying ion for NALCN is Na^+.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Consistent with its unique functional properties, the NALCN protein also has two quite unusual structural features that separate itself from the other 20 members of the 24-TM family: these are the S4 segments and the pore region sequences. In K_Vs, Ca_Vs, and Na_Vs, charged residues (lysine, arginine: K/R) are present in every third position along the S4 segments. In NALCN, the S4 segments have fewer charged residues (13 vs. the 21 found in Na_V1.1 or Ca_V1.1). The S4 of domain IV (IVS4) of NALCN has only two charged residues, while Ca_Vs and Na_Vs have more than four, and these two residues are not evenly spaced in an every third position manner (Fig. 3A). In Na_vs, all the S4s contribute to channel activation, although unequally (Bezanilla, 2000; Kontis et al., 1997). MTSET accessibility studies also show that, in Na_v1.1, two of the charged residues in IVS4 (missing at NALCN's corresponding positions) move from internally accessible positions to extracellularly accessible ones (Yang et al., 1996). In addition, the charged residues in the S4s, especially of domains III and IV, are also important for Na_v inactivation (Cha et al., 1999). However, difference in the S4s alone may not explain why NALCN is voltage insensitive and doesn't have inactivation. Indeed, a mutant tetrameric K^+ channel can still be voltage-gated even when artificially engineered to have only one 6-TM subunit (equivalent to one of the four domains in the 24-TM channels) with an intact S4 but the other three without any charged residues in their S4s (Gagnon and Bezanilla, 2009). On the other hand, cyclic-nucleotide-gated (CNG) channels made of tetramers of 6-TM proteins are only weakly voltage-sensitive despite having charged residues in their S4s. Indeed, when the S4 of CNGA2 is used to replace that of the EAG (KCNH2) K_v channel, it is fully functional in sensing voltage changes and in supporting a voltage-gated K^+ channel (Tang and Papazian, 1997). It therefore remains possible that NALCN's voltage insensitivity lies in regions besides the S4s, such as the C-terminal part of S3 and the S3–S4 linker that together with S4 form the voltage-sensor paddle as shown in the crystal structure of K_v channels (Jiang et al., 2003). Alternatively, NALCN's VSDs may be functional but there is "defect" in the coupling between voltage-sensing and channel gating. The functionality of NALCN's four VSDs can be tested by transferring each of them into homotetrameric K_v channels (Bosmans et al., 2008; Xu et al., 2010).

The second unique feature of NALCN is its pore filter (Fig. 3B). The selectivity filter in Ca_V, $Na_{V,}$ and K_V is surrounded by the VSDs and is formed by the S5–S6 pore (P) loops that are contributed by each 6-TM domain (Doyle et al., 1998; Jiang et al., 2003; MacKinnon, 1995; Miller, 1995; Payandeh et al., 2011). In Ca_Vs, the Ca^{2+} selectivity requires one glutamate (E) or aspartate (D) residue contributed from each of the four homologous repeats (EEEE motif) in the pore filter (Heinemann et al., 1992; Yang et al., 1993). Na_Vs have a DEKA motif in the analogous position (Fig. 3B). NALCN has an EEKE motif, a combination of the EEEE (Ca_V) and DEKA (Na_V) motifs. The EEKE motif is conserved in NALCN homologs in mammals, *D. melanogaster* and *C. elegans*. NALCN from the fresh water snail *Lymnaea stagnalis* has an EKEE motif (Lu and Feng, 2011). In Na_v, mutating the DEKA motif into DEKE converts the Na^+ selective channel into a channel conducting primarily Na^+ but also some K^+ and Ca^{2+} (Schlief et al., 1996). Likewise, mutating the EEEE motif of Ca_Vs into EEKE enables the otherwise highly Ca^{2+}-selective channels permeable to monovalent ions (Parent and Gopalakrishnan, 1995; Tang et al., 1993; Yang et al., 1993). Therefore, NALCN's EEKE motif, and perhaps together with sequences surrounding it, is likely responsible for the channel's relative lack of selectivity among monovalent cations (Williamson and Sather, 1999; Yue et al., 2002). The *in vivo* significance of NALCN's EEKE motif has been demonstrated by the finding that a mutant cDNA encoding an EEEE motif, when transgenically expressed in the Drosophila *na* mutant, is much less capable of rescuing the phenotypes than the wild-type cDNA (Lear et al., 2005). This rescue experiment with pore mutants also provided the *in vivo* evidence confirming that NALCN is indeed an ion channel.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Currently, the only available high-resolution structure in the NALCN/Ca_V/Na_V/CatSper/Na_VBac superfamily is that of a bacterial voltage-gated Na^+ channel isolated from *Arcobacter butzleri* (Na_VAb) (Payandeh et al., 2011). Given the overall sequence similarity, especially in the pore regions, between Na_VAb and other channels in these families, the structure of the Na_VAb homotetramer likely has many of the key signatures of Na_Vs, Ca_Vs, and NALCN. The overall structure of Na_VAb is similar to that of the K_Vs and is composed of an S1–S4 VSD and a channel pore formed by S5–S6. Unique to Na_VAb is a large fenestration on the side of the pore. Na_VAb also has an additional pore helix (P2) in addition to the helix (P) also found in K_V channels. This P2 helix is C-terminal to the P helix and contains the tryptophan residue (W) of the T/SxE/DxW signature found in all the 24-TM channels (Payandeh et al., 2011) (Fig. 3B). In addition, C-terminal to the tryptophan residue in the P2 helix are several amino acids that have been shown to influence channel selectivity, as demonstrated for the bacterial voltage-gated Na^+ channel NaChBac (Ren et al., 2001b; Yue et al., 2002). In the homotetrameric Na_VAb channel, the four glutamate (E) residues in the T/SxE/DxW pore signature form the narrowest ring in the pore filter. In the NALCN protein, one of the glutamate residues in repeat III is replaced by a lysine.

NALCN Channel Complex

Many of the Ca^{2+}/Na^+ channels consist of multiple subunits. For example, the Na_V complex is composed of a pore-forming α subunit and two transmembrane auxiliary subunits, β1 and β2 (Catterall et al., 2002). Similarly, high voltage-gated Ca_Vs contain the pore-forming α1 subunit, an intracellular β subunit, an α2/δ subunit and, in some cells such as skeletal muscle cells, a transmembrane γ subunit (Catterall, 2011). Likewise, CatSper channels contain four pore-forming subunits (CatSper1-4) and at least three membrane-spanning auxiliary subunits (β, γ, and δ) (Chung et al., 2011; Ren and Xia, 2010). The subunit composition of the low-voltage gated Ca_Vs (T-type) is not known. Many of the non pore-forming, auxiliary subunits are essential for various aspects of basic channel function (Arikkath and Campbell, 2003).

The elucidation of the NALCN complex has been greatly facilitated by genetic studies in Drosophila, *C. elegans*, and mice. Several lines of evidence suggest that the NALCN complex contains the pore-forming subunit, NALCN itself, and two additional proteins, UNC79 and UNC80 (ortholog of *C. elegans* Unc-79 and Unc-80, respectively). First, mutations in *Unc79* and *Unc80* in Drosophila, *C. elegans*, and the mouse have essentially identical phenotypes to those of *Nalcn* mutants. Second, mutation in one of these genes also affects the protein levels and/or localization of the others (Humphrey et al., 2007; Jospin et al., 2007; Nakayama et al., 2006; Pierce-Shimomura et al., 2008; Yeh et al., 2008). Defining evidence that UNC79 and UNC80 form a physical complex with NALCN came from co-immunoprecipitation experiments in mouse brain showing that antibody against any one of the three could bring down the others (Lu et al., 2009; Lu et al., 2010). Whether there are other core subunits in this complex awaits the purification of the protein complex and a determination of the subunit composition.

UNC79 and UNC80 are well conserved among animals but share no obvious sequence similarity with any other protein with known function (Humphrey et al., 2007; Jospin et al., 2007; Lu et al., 2009; Lu et al., 2010). Both are large proteins (2654 aa and 3326 aa, respectively, in humans), larger than any known auxiliary subunit in the 24-TM channel family. Despite their size, there are no obviously identifiable protein domains in UNC79 and UNC80. The lack of sequence similarity among the auxiliary subunits of the NALCN, Na_Vs, Ca_Vs, and CatSper suggest that, unlike the pore-forming subunits, the auxiliary subunits evolved independently.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

In heterologous expression systems, UNC80 and NALCN have been shown to interact (Lu et al., 2009; Lu et al., 2010). UNC79 and UNC80 also associate with each other, and UNC79 requires the presence of UNC80 to associate with NALCN. These data suggest a NALCN complex model whereby UNC80 serves as a bridge between UNC79 and NALCN (Fig. 4).

In *Unc79* knockout mouse brain, UNC80 protein is also undetectable, but NALCN is present. *Unc79* mutant neurons also retain the NALCN-dependent basal Na^+ leak current (Lu et al., 2010). Unlike in wild-type neurons, the current in the mutant is not regulated by G protein-coupled receptors (GPCRs; see below). However, the GPCR regulation of NALCN can be restored by over-expression of UNC80 in the *Unc79* knockout, suggesting that UNC80 is not required for the basal function of NALCN, but is required for the regulation of the channel (Lu et al., 2010). Consistent with this idea, NALCN alone forms a leak channel when transfected into HEK293 cells, but its regulation by several GPCRs require the co-transfection of UNC80 (Lu et al., 2010). The function of UNC79 is less clear. The whole-cell basal Na^+ leak is of a similar size in neurons cultured from the wild-type and in neurons from the *Unc79* knockout mouse, and over-expression of UNC80 in an *Unc79* null background restores the regulation of NALCN, suggesting that this channel's biophysical properties in mouse brain, as they are currently understood, do not require UNC79 (Lu et al., 2010). In agreement with this concept, the co-transfection of UNC79 with UNC80 and NALCN does not appear to affect the NALCN current in HEK293 cells. Therefore, the function of UNC79 in mammalian brain may perhaps be to control the stability and trafficking of UNC80, and to determine the localization of the NALCN complex with its various isoforms, thereby indirectly affecting NALCN's function in various neuronal compartments.

Cellular Functions of the NALCN Channel Complex

In mice and humans, NALCN is expressed in the brain, spinal cord, heart, and pancreas, with the highest mRNA expression levels detected in the brain. In the brain and spinal cord, NALCN mRNA is widely expressed, and found in essentially all the neurons (Lu et al., 2007). The expression pattern in the nervous system suggests some fundamental roles for NALCN, and three basic cellular functions are discussed here.

NALCN's contribution to the basal Na^+ leak and the sensitivity to extracellular Na^+ of resting membrane potentials

The basal Na^+ leak current ($I_{L\text{-}Na}$) is small in most neurons, representing about 10–20 pA of whole cell current at −70 mV in cultured mouse hippocampal neurons (Lu et al., 2007). Because of its small size, $I_{L\text{-}Na}$ is perhaps best measured as the change of holding currents when extracellular Na^+ concentration ($[Na^+]_e$) is lowered from high (140 mM) to low (14 mM) concentrations under voltage clamping (Raman and Bean, 1997). In the cultured mouse hippocampal neurons, $I_{L\text{-}Na}$ can be partially blocked by TTX (~18%, presumably contributed by the window current through Na_V), and by 2 mM Cs (~10%, likely through HCN channels). The remaining ~72% current can be almost completely blocked by genetic deletion of *Nalcn* or by applying the non-specific NALCN blocker, Gd^{3+} (10 μM) (Lu et al., 2007). The complete elimination of $I_{L\text{-}Na}$ by blocking Na_Vs, HCNs, and NALCN suggests that, in these neurons, these three channels make the major contributions to the resting Na^+ leak current, with NALCN having the largest (~70%) contribution. This is somewhat surprising given that some of the 26 mammalian TRP channels are also found in neurons and, when expressed heterologously, they are open at RMPs (Ramsey et al., 2006). Many of the TRP channels are used for sensory detection and it's not clear whether they contribute basal Na^+ conductance.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

The RMP of the *Nalcn* knockout hippocampal neurons is approximately 10 mV more hyperpolarized than that of wild-type neurons, and is less sensitive to change in $[Na^+]_e$. Conversely, overexpression of NALCN leads to a depolarization of ~20 mV of the RMP (Lu et al., 2007). In the snail *Lymnaea stagnalis*, knocking down NALCN in a pacemaker neuron (RPeD1) also leads to a ~15 mV hyperpolarization of the RMP (Lu and Feng, 2011). These studies suggest that NALCN is a major player in determining the influence of extracellular Na^+ on a neuron's basal excitability.

NALCN's contribution to the regulation of neuronal excitability by extracellular Ca^{2+}

Like Na^+ and K^+, extracellular Ca^{2+} also influences the basal neuronal excitability in many brain regions. The systemic $[Ca^{2+}]$ of the body (~1.2 mM) is usually well controlled through the coordinated actions of hormones, such as parathyroid hormone (PTH), on absorption in the gastrointestinal tract, excretion/reabsorption in the kidney, and bone resorption. $[Ca^{2+}]_e$, however, can drop acutely in brain regions such as the hippocampus, neocortex, and cerebellum. For example, repetitive electrical or chemical stimulation in areas where the extracellular space is limited can cause $[Ca^{2+}]_e$ to decrease from approximately 1.3 to 0.1 mM, presumably as a result of the movement of extracellular Ca^{2+} into cells (Benninger et al., 1980; Heinemann and Pumain, 1980; Krnjevic et al., 1982; Nicholson et al., 1977; Pumain et al., 1985). Single stimuli are also believed to lead to Ca^{2+} depletion in microdomains such as the synaptic cleft (Borst and Sakmann, 1999; Rusakov and Fine, 2003; Stanley, 2000). During slow wave sleep, $[Ca^{2+}]_e$ levels have been reported to oscillate between 1.18 and 0.85 mM in the cerebral cortex of the cat. $[Ca^{2+}]_e$ changes in phase with membrane potential oscillation in this region, and $[Ca^{2+}]_e$ can drop further, below 0.5 mM, if such cortical oscillation evolves into a spike-wave seizure (Amzica et al., 2002). Drastic changes in $[Ca^{2+}]_e$ are more often found during pathophysiological conditions such as hypocalcemia and seizure. In a variety of models of seizure, hypoxia, ischemia, and trauma, large drops in $[Ca^{2+}]_e$ are observed (Heinemann et al., 1986; Morris and Trippenbach, 1993; Nilsson et al., 1993; Silver and Erecinska, 1990).

While neurons are hyperpolarized upon decreases in the extracellular concentration of K^+ or Na^+, a drop of $[Ca^{2+}]_e$ usually leads to excitation. For example, lowering $[Ca^{2+}]_e$ from 1.2 to 0.1 mM in cultured hippocampal neurons leads to ~15 mV of depolarization, comparable to the change imposed by a ten-fold increase of $[Na^+]_e$ (14 to 140 mM) (Lu et al., 2010). Artificially lowering $[Ca^{2+}]_e$ can also induce seizure in intact animals and seizure-like activities in brain slices and single neurons (Feng and Durand, 2003; Kaczmarek and Adey, 1975). These findings suggest that the effect of $Ca^{2+}{}_e$ on neuronal excitation is unlikely to be mediated directly by Ca^{2+} entry via basal permeability. Unlike the Na^+ and K^+ leak, which can be tens of picoamps, the Ca^{2+} leak current at RMPs is likely very low in neurons. A large non-inactivating basal Ca^{2+} leak would likely have detrimental effects such as cell death on the neuron, as this ion is used as a second messenger to regulate many processes and the steady state $[Ca^{2+}]_i$ needs to be kept below 1 μM (Clapham, 2007). Thus, an indirect mechanism by which $Ca^{2+}{}_e$ impacts neuronal excitability must exist.

Several mechanisms have been proposed for the negative regulation of neuronal excitability by $Ca^{2+}{}_e$. First, Ca^{2+} neutralizes negative charges on the cell membrane. Reductions in this charge-screening effect may shift the voltage dependence of biophysical properties (activation and inactivation, for example) of ion channels in the direction of hyperpolarization, thus decreasing the activation threshold, as first studied by Frankenhaeuser and Hodgkin (Frankenhaeuser and Hodgkin, 1957; Hille, 1968). Since this effect affects both Na_Vs and the "opposing" K_Vs, the net effects on neuronal excitability due to charge-screening of Ca^{2+} can be complex. Second, a reduction in $[Ca^{2+}]_e$ may influence ion channel selectivity, as best illustrated for Ca_V. Ca_Vs are highly selective for Ca^{2+} (P_{Ca}/P_{Na} >1,000), but became non-selective and conduct monovalent ions such as Na^+ and K^+

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

when $[Ca^{2+}]_e$ is dropped to μM range (Almers and McCleskey, 1984; Hess et al., 1986; Yang et al., 1993). As the IC_{50} for the Ca^{2+}-mediated blockade of monovalent ion in Ca_V's is ~ 1 μM (for $Ca_V1.2$), the effect of $[Ca^{2+}]_e$ on the Ca_V pore is unlikely to be responsible for the influence of sub-millimolar $Ca^{2+}{}_e$ on neuronal excitability. $Ca^{2+}{}_e$ also affects other channels that may be present in the neuronal membrane, such as the transient receptor potential (TRP) channel family (Owsianik et al., 2006; Wei et al., 2007).

A moderate reduction in $[Ca^{2+}]_e$, to submillimolar levels, for example, can also depolarize some types of neurons. This excitation is unlikely to be explained by the charge screening effect because it is present even when the extracellular divalent cation concentration is kept constant. One potential mechanism may be via the activation of depolarizing, nonselective cation currents by lowering $[Ca^{2+}]_e$, as found in several types of neurons (Formenti et al., 2001; Hablitz et al., 1986; Smith et al., 2004; Xiong et al., 1997). The molecular identities of the channels responsible for these currents, the mechanisms by which $[Ca^{2+}]_e$ change is coupled to channel opening, and the role of these channels in the regulation of neuronal excitability by $[Ca^{2+}]_e$ remain largely unknown.

Recent findings suggest that $Ca^{2+}{}_e$ tightly controls the size of the basal Na^+ leak current, $I_{L\text{-}Na}$ (Lu et al., 2010). In cultured mouse hippocampal neurons, $I_{L\text{-}Na}$ is highly sensitive to $[Ca^{2+}]_e$ at the physiological range. Decreasing $[Ca^{2+}]_e$, with $[Mg^{2+}]_e$ kept constant, increases $I_{L\text{-}Na}$, with an apparent IC_{50} of ~ 0.1 mM. For example, $I_{L\text{-}Na}$ increases from ~10 pA at a normal $[Ca^{2+}]_e$ of 1.5 mM to ~100 pA when $[Ca^{2+}]_e$ is lowered to 10 μM. Several findings suggest that this increase in $I_{L\text{-}Na}$ occurs by an increase of current through NALCN channels (I_{NALCN}). First, both the low $[Ca^{2+}]_e$-induced current (I_{LCa}) and I_{NALCN} are blocked by 10 μM Gd^{3+}. Second, both currents have a linear I/V relationship passing through 0 mV. Third, I_{LCa} is missing in *Nalcn* knockout neurons and can be restored upon transfection with NALCN cDNA. Finally, *Nalcn* knockout hippocampal neurons are not excited when $[Ca^{2+}]_e$ is reduced to 10 μM, suggesting that NALCN is the major mechanism by which $[Ca^{2+}]_e$ at this range controls neuronal excitability (Lu et al., 2010). Under other conditions such as further reductions in $[Ca^{2+}]_e$ and $[Mg^{2+}]_e$, neuronal excitation can perhaps be mainly achieved via the charge screening effects and/or through the actions of Ca_Vs and TRP channels.

The mechanism by which $Ca^{2+}{}_e$ modulates NALCN function is unique. For example, extracellular Ca^{2+} regulates Ca_Vs and TRPs through a pore-blocking mechanism in which Ca^{2+} ions bind to the channel pore, with affinities of μM range, and block the permeation of monovalent ions (Yang et al., 1993). The pore of NALCN, however, is insensitive to extracellular Ca^{2+} blockade; I_{NALCN} from NALCN alone expressed in HEK293 cells, unlike that in neurons, is not inhibited by Ca^{2+}. In neurons, the sensitivity of neuronal I_{NALCN} to Ca^{2+} requires the presence of UNC80. I_{NALCN} is insensitive to Ca^{2+} in the *Unc79* knockout neurons, which also lack UNC80 protein, but the Ca^{2+} sensitivity can be restored by UNC80 transfection (Lu et al., 2010). In addition, I_{NALCN}'s Ca^{2+} sensitivity in neurons requires several amino acids at the end of NALCN's intracellular C-terminus.

These properties point to an intracellular mechanism that mediates control of I_{NALCN} by $Ca^{2+}{}_e$, in contrast to the extracellular pore-block mechanism used in other channels. Indeed, the activation of NALCN by a reduction in $[Ca^{2+}]_e$ requires G-proteins, as the inclusion of non-hydrolyzable GTP (GTPγS) and GDP (GDPγS) analogs prevents I_{LCA}. The calcium-sensing receptor (CaSR), a Gq-coupled GPCR activated by extracellular cations and other ligands such as amino acids, is able to detect changes in $[Ca^{2+}]_e$ and couple them to NALCN (Lu et al., 2010). In cultured neurons, CaSR ligands inhibit I_{NALCN}. In HEK293T cells, CaSR can reconstitute I_{NALCN}'s $Ca^{2+}{}_e$ sensitivity (Lu et al., 2010). CaSR is a member of the "family C" GPCRs, which also include the mGluRs, $GABA_B$ receptors, and the T1R taste

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

receptors (Brown and MacLeod, 2001). CaSR is best known for its function in the thyroid gland where it detects serum Ca^{2+} level and controls the secretion of PTH to regulate systemic $[Ca^{2+}]_e$. Like NALCN, CaSR is also widely expressed in the brain, and is highly expressed in the hippocampus and cerebellum (Ruat et al., 1995). The neuronal function of CaSR is largely unknown. Recent studies indicate that activated CaSR stimulates dendritic growth in neurons (Vizard et al., 2008) and suppresses synaptic transmission (Chen et al., 2010; Phillips et al., 2008; Smith et al., 2004). Several CaSR mutations in patients have been found to associate with seizure. CaSR-mediated NALCN activity could thus occur indirectly via alterations in serum $[Ca^{2+}]$ in the brain due to a disruption of PTH levels. Intriguingly, several CaSR mutations have been found that do not result in altered PTH or serum Ca^{2+} levels and yet are associated with seizure in affected individuals (Kapoor et al., 2008). This systemic $[Ca^{2+}]$-independent effect of CaSR presumably reflects a non-traditional role for CaSR in the regulation of ion channels such as NALCN.

G protein-independent activation of NALCN by G protein-coupled receptors

Perhaps the most unusual mechanism for NALCN regulation is that by GPCRs in a G protein-independent manner. GPCR regulation of channel targets is generally believed to be via the trimeric G-proteins Gα or Gβγ, either directly through channel binding or indirectly through protein phosphorylation and second messenger generation. Many cloned ion channels have been shown to be regulated by GPCRs in this fashion (Hille, 2001). However, earlier patch clamp recordings with the inclusion of GTPγS or GDPβS in the pipette to “lock” G proteins in active or inactive states, respectively, suggest that there are channel currents activated by GPCRs without the active involvement of G proteins. One such current was recorded in cardiac myocytes, in which muscarine activated a Na^+-dependent and TTX-insensitive inward current in the presence of GTPγS or GDPβS (Shirayama et al., 1993). Similar “atypical” G-protein independent GPCR-activated currents have also been recorded from pancreatic β cells and from neurons in several brain areas (Heuss and Gerber, 2000; Rolland et al., 2002). The ion channels and the mechanisms underlying this activity are largely unknown; to date, NALCN is one of the best-characterized channels activated in this GPCR-dependent, G protein-independent fashion.

In several types of neurons, such as ventral tegmental area (VTA) dopaminergic neurons and hippocampal pyramidal neurons, NALCN can be activated by neuropeptides as substance P (SP) and neurotensin (NT) (Lu et al., 2009). The receptors for these peptides GPCRs. However, the inclusion of GTPγS or GDPβS in the recording pipette does not prevent NALCN activation, suggesting G- protein independence. The mechanisms underlying this G protein–independent NALCN activation are not fully understood but involve Src kinases, as the application of Src family kinase inhibitors, such as PP1, abolishes NALCN activation by the neuropeptides. Likewise, activation of Src kinases by including a Src-activating compound in the recording pipette can bypass GPCR activation, resulting in NALCN-mediated currents (Lu et al., 2009). The activation of NALCN by SP also requires UNC80, which binds Src and helps scaffold Src into the NALCN complex (Wang and Ren, 2009). A similar G protein-independent, Src-dependent activation of NALCN is also found in pancreatic β-cells upon stimulation with acetylcholine (Swayne et al., 2009). Since the activation of Src kinases lies downstream of many physiological stimuli such as neurotransmitters, growth factors, cytokines, cell adhesion molecules and mechanical stretch, these stimuli may regulate neuronal excitability via their action on NALCN.

Synergism between the G protein-dependent and -independent GPCR regulations of NALCN

Both of the G protein-dependent and -independent regulation of NALCN via GPCR signaling converge onto UNC80 and NALCN but require different intracellular signaling

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

molecules (G proteins vs. Src family kinases) (Fig. 4). In addition, these mechanisms rely on different structural components of NALCN. Unlike the G protein-dependent regulation by $Ca^{2+}{}_e$, the G protein-independent pathway activated by SP does not require the last C-terminal amino acids of NALCN, as truncated NALCN lacking the last C-terminal 100 amino acids, though insensitive to $[Ca^{2+}]_e$, can still be activated by SP (Lu et al., 2010). The two pathways can also act synergistically on NALCN. For example, the reduction of $[Ca^{2+}]_e$ to 0.1 mM, or the application of SP, alone elicits ~40 pA inward current. Simultaneous application of these stimuli induces a ~400 pA current that is much larger than the sum of the two currents (Fig. 5). NALCN channels that lack the C-terminal amino acids do not display this synergism (Lu et al., 2009). A similar synergistic effect of $[Ca^{2+}]_e$ reduction and the activation of Src kinase was also observed in the excitation of neurons, in which $[Ca^{2+}]_i$ is “paradoxically” increased by decreasing $[Ca^{2+}]_e$ (Burgo et al., 2003). Whether these two pathways influence distinct parameters such as the number of available channels (N) and the channel opening probability (P_o) remains unknown. The *in vivo* significance of this synergism is also not clear. In a mouse model of epilepsy, an increase of SP expression is believed to help induce and maintain the epileptic status (Liu et al., 1999). Similarly, increases in Src kinase activity are accompanied by the induction of epileptiform activity in rat brain slices and inhibition of Src kinase can reduce epileptiform discharge (Sanna et al., 2000). Since a reduction in $[Ca^{2+}]_e$ is associated with epilepsy, and can itself induce epileptiform activity, the synergistic effect of low $[Ca^{2+}]_e$, together with excitatory neuropeptides and/or the activation of Src kinases, on NALCN-mediated currents may provide a powerful excitatory signal to the neurons (Lu et al., 2010).

NALCN Function at the Whole-Organism Level

Mutational analyses of *Nalcn, Unc79*, and *Unc80* in mice, *D. melanogaster,* and *C. elegans* have clearly established NALCN as an essential ion channel. Mice without functional *Nalcn* or *Unc79* are neonatal lethal (Lu et al., 2007; Nakayama et al., 2006; Speca et al., 2010). In *D. melanogaster,* and *C. elegans*, mutating any of the three components of the NALCN complex results in severe behavior phenotypes (Humphrey et al., 2007; Jospin et al., 2007; Nash et al., 2002).

Perhaps the most common phenotype resulting from mutations in any one of the three NALCN complex components is the disruption in rhythmic behaviors. In mammals, the rhythmic contraction of the diaphragm muscle used for breathing is directly controlled by electrical signals from the nerves. The respiratory rhythms are generated in regions such as the pre-Bötzinger complex (PBC) in the brain stem through network mechanisms and/or together with pacemaking mechanisms (Feldman et al., 2003; Ramirez et al., 2004). *Nalcn* mutant mouse pups have severely disrupted respiratory rhythm. Wild-type newborn pups have a rhythmic breathing at a frequency of about one breath per second. In the *Nalcn* mutant, the breathing is characterized by 5 seconds of apnea followed by 5 seconds of breath. This disrupted breathing rhythm represents an “electrical defect,” as the rhythmic electrical discharges recorded from wild-type C4 nerves are essentially absent in the *Nalcn* mutant (Lu et al., 2007). NALCN is also expressed in the spinal cord. Whether it is involved in the central pattern generators used for rhythmic locomotion such as walking and running requires studies using conditional knockouts with NALCN disrupted in the spinal cord.

Defects in rhythmic behaviors are also obvious in the *Drosophila melanogaster,* and *C. elegans* mutants. In the fly, hypomorphic alleles of the *Nalcn* ortholog (*Na*), though viable, display altered circadian locomotor rhythms (Nash et al., 2002). Under diurnal light/dark (LD) cycle, the mutant flies have more activity in the darkness but suppressed activity in the light cycles, a pattern “inverted” to what’s seen in the wild-type. When released to free-running condition in constant darkness (DD) from the entrainment of diurnal LD cycles,

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

many mutant flies quickly become arrhythmic. In addition, the mutant flies do not seem to have the light-on response characterized by a marked increase in locomotor activities in the wild-type when light is turned on. Indeed, the mutant's activities quickly decrease (Nash et al., 2002).

In both *Unc79* and *Nalcn* mutants, flies also have a "fainter" locomotion phenotype characterized by "hesitant" walking and frequent disruptions of the rhythmic, smooth movements shown in wild-type flies climbing up vial walls after being tapped to the bottom (Humphrey et al., 2007; Nash et al., 2002). Similarly disrupted locomotion rhythms are found in C. elegans *Nalcn* (*NCa*), *unc-79,* and *unc-80* mutants (Jospin et al., 2007; Pierce-Shimomura et al., 2008; Yeh et al., 2008). In the worm, at least two rhythmic locomotion patterns with quite distinct kinematics are used: the animal crawls while in solid food but switches to swimming when dropped into liquid (Pierce-Shimomura et al., 2008). A smooth switch between the two behaviors requires sensory neurons. In a forward genetic screening, *unc-79* and *unc-80* mutants were identified to have relatively normal crawling (though with the "fainter" phenotype) in solid but unable to switch to a normal swimming pattern. Indeed, the mutant worms become paralyzed in the liquid instead of expressing a smooth swimming locomotion pattern as seen in the wild-type. A similar swimming phenotype also exists in the *C. elegans NCa* mutant (Pierce-Shimomura et al., 2008). In the snail *Lymnaea stagnalis*, the rhythmic bursting of action potentials in the pacemaker RPeD1 neurons are abolished and the respiratory behaviors of the animal are disrupted when NALCN is knocked down with siRNA (Lu and Feng, 2011).

The reason for the apparent conservation of NALCN's role in rhythmic behaviors is a matter of speculation. In Drosophila, the expression of NA (NALCN ortholog) itself doesn't seem to have a circadian oscillation. In addition, the oscillation of key circadian proteins in the "central clock" such as PERIOD appears normal in the fly *na* mutant. These findings suggest that NA is not part of the core circadian oscillator but rather, the defect in circadian rhythms of locomotor activities in the mutant is a result of disruption in the coupling between the central clock and the neuronal networks controlling locomotion (Lear et al., 2005; Nash et al., 2002). Consistent with this prediction, the pacemaker neurons in the mutant have higher level of PDF (pigment-dispersing factor, a neuropeptide released by the pacemaker neurons to coordinate circadian behaviors in the flies), suggesting a potential decrease in the release of PDF (and/or an increase in production) by these neurons in the mutant (Lear et al., 2005). Transgenic expression of NA in circadian pacemaker neurons in the *na* mutant using the Gal4-UAS system restores the circadian phenotypes (Lear et al., 2005). Remarkably, NA expression in a small subset of the neurons (~ 20 DN1 dorsal neurons) is sufficient to rescue some of the phenotypes, including the acute light-on locomotor activity response (Zhang et al., 2010). It's not clear whether any residual function of the NA protein in the hypomorphic mutant used for the rescue experiment, if present, plays a supporting role in the other neurons. How NA contributes the fly circadian responses remains further investigated, but it's interesting to note that mammalian NALCN is activated by neuropeptides in hippocampal, VTA and pre-Bötzinger complex pacemaking neurons (Lu et al., 2009; Pena and Ramirez, 2004; Ptak et al., 2009), and the channel appears to be controlled by light input in the SCN (LeSauter et al., 2011).

In autonomously firing neurons and pacemaking neurons within a local circuitry, NALCN as a channel that leaks Na^+-mediated current may provide a constant, non-inactivating, depolarizing force used to generate or modulate the rhythmic electrical activities for the control of behaviors (Atherton and Bevan, 2005; Jackson et al., 2004; Khaliq and Bean, 2010; Ptak et al., 2009; Raman et al., 2000; Russo et al., 2007). Oscillation of membrane potential is not restricted to neurons in the brain and spinal cord, but rather can be found throughout the body and is perhaps best characterized in the SA node and conduction system

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

cells of heart. The depolarizing force during the diastole cycle in the heart is a result of interplay of several ion channels, but HCNs (I_h) are generally believed to be the major contributor (DiFrancesco, 2006; Vassalle, 1995). However, HCN knockout adult mice have roughly normal (Herrmann et al., 2007) or reduced heartbeat rates (Baruscotti et al., 2011), and the rate acceleration by sympathetic stimulation is intact, suggesting additional important player in heart rate regulation. NALCN is also highly expressed in the heart (Lee et al., 1999). The use of conditional *Nalcn* knockout mice should clarify whether NALCN plays a role in heartbeat control. Likewise, NALCN is expressed in pancreatic β cells, where the rhythmic oscillation of E_m is coupled to cell glucose metabolism and the secretion of insulin. Thus, NALCN channel may also play a role in the regulation of insulin secretion (Swayne et al., 2009).

Given the wide expression of *Nalcn* in the nervous system, it is not surprising that NALCN is involved in a diffuse array of additional functions. Both worm and fly *Nalcn* mutants have an altered sensitivity to anesthetics such as halothane (Humphrey et al., 2007; Nash et al., 2002). It's not clear whether the NALCN complex is a direct target of the drugs, or if the altered sensitivity is a result of a disruption in the balance between the hyperpolarizing activity of K^+ channels and the depolarization provided by NALCN. Finally, heterozygous *Unc79* mutant mice display a hypersensitivity to alcohol and an increased voluntary alcohol consumption (Speca et al., 2010). SP-regulated, I_{NALCN}-like current has been detected in VTA dopaminergic neurons and spinal cord sensory neurons, implicating NALCN in a wide array of animal responses such as pain sensation and reward-seeking behavior. The future use of conditional knockout mice carrying *Nalcn* deletion in specific brain and spinal cord regions during selected time windows should be useful in determining whether regulation of NALCN is important for important animal behaviors such as pain sensation and substance addiction.

Conclusions and Perspective

As is the case for K^+ leak conductances, Na^+ leak currents have been known by physiologists for over 50 years. In recent years, researchers have finally identified a Na^+ leak channel, elucidated the members of the channel complex, and revealed some of this channel's fundamental roles in neuronal function and animal behavior. Surprisingly, the NALCN channel is not completely selective, but instead is permeable to both Na^+ and K^+. The basal P_{Na}/P_K in neurons is very low (4% in the squid giant axon (Hodgkin and Katz, 1949b)). Under certain conditions, as a result of the synergistic actions between the G protein-dependent and -independent GPCR signaling pathways, the NALCN current can be increased more than 20-fold over basal levels. Given the dominant contribution of NALCN to basal P_{Na}, this synergistic action may provide a powerful non-inactivating excitation to the neurons. Future studies using NALCN blockers and conditional knockout mice will reveal the specific excitatory actions of NALCN in different types of neurons, as well as in the various compartment of a single neuron.

UNC79 and UNC80 are large and highly-conserved proteins that, as yet, have no identifiable domains. It is perhaps not likely that these two large proteins function solely in channel conduction *per se*. Future studies may reveal "non-channel" functions of these proteins and their involvement in the regulation of neuronal excitability and plasticity. NALCN-related mutations may also be found to associate with human diseases. In humans, NALCN, UNC79, and UNC80 are encoded by genes that together span a large genomic region of ~1 Mb on three chromosome locations (13q32, 14q32 and 2q34). Genetic diseases have been mapped to these regions and disease-causing mutations in NALCN, UNC79, and UNC80 may be discovered in the future. In particular, we may discover that NALCN sequence variations in the human population, particularly in the poorly conserved C-

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

terminus where single amino acid mutations drastically change the channel's calcium sensitivity, lead to variability in excitability in the nervous system.

REFERENCES

Almers W, McCleskey EW. Non-selective conductance in calcium channels of frog muscle: calcium selectivity in a single-file pore. J Physiol. 1984; 353:585–608. [PubMed: 6090646]

Amzica F, Massimini M, Manfridi A. Spatial buffering during slow and paroxysmal sleep oscillations in cortical networks of glial cells in vivo. J Neurosci. 2002; 22:1042–1053. [PubMed: 11826133]

Arikkath J, Campbell KP. Auxiliary subunits: essential components of the voltage-gated calcium channel complex. Curr Opin Neurobiol. 2003; 13:298–307. [PubMed: 12850214]

Atherton JF, Bevan MD. Ionic mechanisms underlying autonomous action potential generation in the somata and dendrites of GABAergic substantia nigra pars reticulata neurons in vitro. J Neurosci. 2005; 25:8272–8281. [PubMed: 16148235]

Baruscotti M, Bucchi A, Viscomi C, Mandelli G, Consalez G, Gnecchi-Rusconi T, Montano N, Casali KR, Micheloni S, Barbuti A, DiFrancesco D. Deep bradycardia and heart block caused by inducible cardiac-specific knockout of the pacemaker channel gene Hcn4. Proc Natl Acad Sci U S A. 2011; 108:1705–1710. [PubMed: 21220308]

Ben-Ari Y, Cherubini E, Corradetti R, Gaiarsa JL. Giant synaptic potentials in immature rat CA3 hippocampal neurons. J Physiol. 1989; 416:303–325. [PubMed: 2575165]

Benninger C, Kadis J, Prince DA. Extracellular calcium and potassium changes in hippocampal slices. Brain Res. 1980; 187:165–182. [PubMed: 7357467]

Bezanilla F. The voltage sensor in voltage-dependent ion channels. Physiol Rev. 2000; 80:555–592. [PubMed: 10747201]

Borst JG, Sakmann B. Depletion of calcium in the synaptic cleft of a calyx-type synapse in the rat brainstem. J Physiol. 1999; 521:123–133. [PubMed: 10562339]

Bosmans F, Martin-Eauclaire MF, Swartz KJ. Deconstructing voltage sensor function and pharmacology in sodium channels. Nature. 2008; 456:202–208. [PubMed: 19005548]

Brown DA, Adams PR. Muscarinic suppression of a novel voltage-sensitive K+ current in a vertebrate neurone. Nature. 1980; 283:673–676. [PubMed: 6965523]

Brown EM, MacLeod RJ. Extracellular calcium sensing and extracellular calcium signaling. Physiol Rev. 2001; 81:239–297. [PubMed: 11152759]

Burgo A, Carmignoto G, Pizzo P, Pozzan T, Fasolato C. Paradoxical Ca2+ rises induced by low external Ca2+ in rat hippocampal neurones. J Physiol. 2003; 549:537–552. [PubMed: 12692181]

Calcraft PJ, Ruas M, Pan Z, Cheng X, Arredouani A, Hao X, Tang J, Rietdorf K, Teboul L, Chuang K-T, et al. NAADP mobilizes calcium from acidic organelles through two-pore channels. Nature. 2009; 459:596–600. [PubMed: 19387438]

Catterall WA. From ionic currents to molecular mechanisms: the structure and function of voltage-gated sodium channels. Neuron. 2000; 26:13–25. [PubMed: 10798388]

Catterall WA. Voltage-gated calcium channels. Cold Spring Harb Perspect Biol. 2011; 3:a003947. [PubMed: 21746798]

Catterall WA, Goldin AL, Waxman SG. Voltage-gated sodium channels. The IUPHAR Compendium of Voltage-Gated Ion Channels. 2002:11–30.

Cha A, Ruben PC, George AL, Fujimoto E, Bezanilla F. Voltage sensors in domains III and IV, but not I and II, are immobilized by Na+ channel fast inactivation. Neuron. 1999; 22:73–87. [PubMed: 10027291]

Chan CS, Guzman JN, Ilijic E, Mercer JN, Rick C, Tkatch T, Meredith GE, Surmeier DJ. "Rejuvenation" protects neurons in mouse models of Parkinson's disease. Nature. 2007; 447:1081–1086. [PubMed: 17558391]

Chen W, Bergsman JB, Wang X, Gilkey G, Pierpoint CR, Daniel EA, Awumey EM, Dauban P, Dodd RH, Ruat M, Smith SM. Presynaptic external calcium signaling involves the calcium-sensing receptor in neocortical nerve terminals. PLoS One. 2010; 5:e8563. [PubMed: 20052292]

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

Chung J-J, Navarro B, Krapivinsky G, Krapivinsky L, Clapham DE. A novel gene required for male fertility and functional CATSPER channnel formation in spermatozoa. Nat Commun. 2011; 2:153. [PubMed: 21224844]

Clapham DE. Calcium signaling. Cell. 2007; 131:1047–1058. [PubMed: 18083096]

Crill WE. Persistent sodium current in mammalian central neurons. Annu Rev Physiol. 1996; 58:349–362. [PubMed: 8815799]

DiFrancesco D. Serious workings of the funny current. Prog Biophys Mol Biol. 2006; 90:13–25. [PubMed: 15975637]

Doyle DA, Morais Cabral J, Pfuetzner RA, Kuo A, Gulbis JM, Cohen SL, Chait BT, MacKinnon R. The structure of the potassium channel: molecular basis of K+ conduction and selectivity. Science. 1998; 280:69–77. [PubMed: 9525859]

Eggermann E, Bayer L, Serafin M, Saint-Mleux B, Bernheim L, Machard D, Jones BE, Muhlethaler M. The wake-promoting hypocretin-orexin neurons are in an intrinsic state of membrane depolarization. J Neurosci. 2003; 23:1557–1562. [PubMed: 12629156]

Farkas RH, Chien PY, Nakajima S, Nakajima Y. Properties of a slow nonselective cation conductance modulated by neurotensin and other neurotransmitters in midbrain dopaminergic neurons. J Neurophysiol. 1996; 76:1968–1981. [PubMed: 8890307]

Feldman JL, Mitchell GS, Nattie EE. Breathing: rhythmicity, plasticity, chemosensitivity. Annu Rev Neurosci. 2003; 26:239–266. [PubMed: 12598679]

Feng Z, Durand DM. Low-calcium epileptiform activity in the hippocampus in vivo. J Neurophysiol. 2003; 90:2253–2260. [PubMed: 14534265]

Formenti A, De Simoni A, Arrigoni E, Martina M. Changes in extracellular Ca2+ can affect the pattern of discharge in rat thalamic neurons. J Physiol. 2001; 535:33–45. [PubMed: 11507156]

Frankenhaeuser B, Hodgkin AL. The effect of calcium on the sodium permeability of a giant nerve fibre. J Physiol. 1955; 128:40P–41P. [PubMed: 14392634]

Frankenhaeuser B, Hodgkin AL. The action of calcium on the electrical properties of squid axons. J Physiol. 1957; 137:218–244. [PubMed: 13449874]

Gagnon DG, Bezanilla F. A single charged voltage sensor is capable of gating the Shaker K+ channel. J Gen Physiol. 2009; 133:467–483. [PubMed: 19398775]

Goldstein SA, Bayliss DA, Kim D, Lesage F, Plant LD, Rajan S. International Union of Pharmacology. LV. Nomenclature and molecular relationships of two-P potassium channels. Pharmacol Rev. 2005; 57:527–540. [PubMed: 16382106]

Grieco TM, Malhotra JD, Chen C, Isom LL, Raman IM. Open-channel block by the cytoplasmic tail of sodium channel beta4 as a mechanism for resurgent sodium current. Neuron. 2005; 45:233–244. [PubMed: 15664175]

Guzman JN, Sanchez-Padilla J, Chan CS, Surmeier DJ. Robust pacemaking in substantia nigra dopamineric neurons. J Neurosci. 2009; 29:11011–11019. [PubMed: 19726659]

Hablitz JJ, Heinemann U, Lux HD. Step reductions in extracellular Ca2+ activate a transient inward current in chick dorsal root ganglion cells. Biophys J. 1986; 50:753–757. [PubMed: 3779010]

Hausser M, Raman IM, Otis T, Smith SL, Nelson A, du Lac S, Loewenstein Y, Mahon S, Pennartz C, Cohen I, Yarom Y. The beat goes on: spontaneous firing in mammalian neuronal microcircuits. J Neurosci. 2004; 24:9215–9219. [PubMed: 15496653]

Heinemann SH, Terlau H, Stuhmer W, Imoto K, Numa S. Calcium channel characteristics conferred on the sodium channel by single mutations. Nature. 1992; 356:441–443. [PubMed: 1313551]

Heinemann U, Konnerth A, Pumain R, Wadman WJ. Extracellular calcium and potassium concentration changes in chronic epileptic brain tissue. Adv Neurol. 1986; 44:641–661. [PubMed: 3518350]

Heinemann U, Pumain R. Extracellular calcium activity changes in cat sensorimotor cortex induced by iontophoretic application of aminoacids. Exp Brain Res. 1980; 40:247–250. [PubMed: 7428880]

Herrmann S, Stieber J, Stockl G, Hofmann F, Ludwig A. HCN4 provides a "depolarization reserve" and is not required for heart rate acceleration in mice. Embo J. 2007; 26:4423–4432. [PubMed: 17914461]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Hess P, Lansman JB, Tsien RW. Calcium channel selectivity for divalent and monovalent cations. Voltage and concentration dependence of single channel current in ventricular heart cells. J Gen Physiol. 1986; 88:293–319. [PubMed: 2428919]

Heuss C, Gerber U. G-protein-independent signaling by G-protein-coupled receptors. Trends Neurosci. 2000; 23:469–475. [PubMed: 11006463]

Hille B. Charges and potentials at the nerve surface. Divalent ions and pH. J Gen Physiol. 1968; 51:221–236. [PubMed: 5641636]

Hille, B. Ion Channels of Excitable Membranes, ed. 3 edn. Sunderland, MA: 2001.

Hodgkin AL. Ionic movements and electrical activity in giant nerve fibres. Proc R Soc Lond B Biol Sci. 1958; 148:1–37. [PubMed: 13494473]

Hodgkin AL, Katz B. The effect of calcium on the axoplasm of giant nerve fibers. J Exp Biol. 1949a; 26:292–294. pl. [PubMed: 15395900]

Hodgkin AL, Katz B. The effect of sodium ions on the electrical activity of the giant axon of the squid. J Physiol. 1949b; 108:37–77. [PubMed: 18128147]

Hong M-P, Vu K, Bautos J, Gelli A. Cch1 restores intracellular Ca2+ in fungal cells during endoplasmic reticulum stress. J Biol Chem. 2010; 285:10951–10958. [PubMed: 20123986]

Honoré E. The neuronal background K2P channels: focus on TREK1. Nat Rev Neurosci. 2007; 8:251–261. [PubMed: 17375039]

Humphrey JA, Hamming KS, Thacker CM, Scott RL, Sedensky MM, Snutch TP, Morgan PG, Nash HA. A putative cation channel and its novel regulator: cross-species conservation of effects on general anesthesia. Curr Biol. 2007; 17:624–629. [PubMed: 17350263]

Ishibashi K, Suzuki M, Imai M. Molecular cloning of a novel form (two-repeat) protein related to voltage-gated sodium and calcium channels. Biochem Biophys Res Commun. 2000; 270:370–376. [PubMed: 10753632]

Jackson AC, Yao GL, Bean BP. Mechanism of spontaneous firing in dorsomedial suprachiasmatic nucleus neurons. J Neurosci. 2004; 24:7985–7998. [PubMed: 15371499]

Jan L, Jan Y. Peptidergic transmission in sympathetic ganglia of the frog. J Physiol. 1982; 327:219–246. [PubMed: 6181250]

Jentsch TJ, Stein V, Weinreich F, Zdebik AA. Molecular structure and physioogical function of chloride channels. Physiol Rev. 2002; 82:503–568. [PubMed: 11917096]

Jiang Y, Lee A, Chen J, Ruta V, Cadene M, Chait BT, MacKinnon R. X-ray structure of a voltage-dependent K+ channel. Nature. 2003; 423:33–41. [PubMed: 12721618]

Jones SW. Muscarinic and peptidergic excitation of bull-frog sympathetic neurones. J Physiol. 1985; 366:63–87. [PubMed: 2414436]

Jones SW. On the resting potential of isolated frog sympathetic neurons. Neuron. 1989; 3:153–161. [PubMed: 2560389]

Jospin M, Watanabe S, Joshi D, Young S, Hamming K, Thacker C, Snutch T, Jorgensen E, Schuske K. UNC-80 and the NCA ion channels contribute to endocytosis defects in synaptojanin mutants. Curr Biol. 2007; 17:1595–1600. [PubMed: 17825559]

Kaczmarek LK, Adey WR. Extracellular release of cerebral macromolecules during potassium- and low-calcium-induced seizures. Epilepsia. 1975; 16:91–97. [PubMed: 1122901]

Kandel, ER.; Schwartz, JH.; Jessell, TM. Principles of Neural Science, ed. 4 edn. McGraw-Hill; 2000.

Kapoor A, Satishchandra P, Ratnapriya R, Reddy R, Kadandale J, Shankar SK, Anand A. An idiopathic epilepsy syndrome linked to 3q13.3–q21 and missense mutations in the extracellular calcium sensing receptor gene. Ann Neurol. 2008; 64:158–167. [PubMed: 18756473]

Khaliq ZM, Bean BP. Pacemaking in dopaminergic ventral tegmental area neurons: depolarizing drive from background and voltage-dependent sodium conductances. J Neurosci. 2010; 30:7401–7413. [PubMed: 20505107]

Kontis KJ, Rounaghi A, Goldin AL. Sodium channel activation gating is affected by substitutions of voltage sensor positive charges in all four domains. J Gen Physiol. 1997; 110:391–401. [PubMed: 9379171]

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

Krnjevic K, Morris ME, Reiffenstein RJ, Ropert N. Depth distribution and mechanism of changes in extracellular K+ and Ca2+ concentrations in the hippocampus. Can J Physiol Pharmacol. 1982; 60:1658–1671. [PubMed: 7165861]

Kuba K, Koketsu K. Synaptic events in sympathetic ganglia. Prog Neurobiol. 1978; 11:77–169. [PubMed: 33420]

Kuffler S, Sejnowski T. Muscarinic and peptidergic excitation of bull-frog sympathetic neurons. J Physiol. 1983; 341:257–278. [PubMed: 6137560]

Lear BC, Lin JM, Keath JR, McGill JJ, Raman IM, Allada R. The ion channel narrow abdomen is critical for neural output of the Drosophila circadian pacemaker. Neuron. 2005; 48:965–976. [PubMed: 16364900]

Lee JH, Cribbs LL, Perez-Reyes E. Cloning of a novel four repeat protein related to voltage-gated sodium and calcium channels. FEBS Lett. 1999; 445:231–236. [PubMed: 10094463]

LeSauter J, Silver R, Cloues R, Witkovsky P. Light exposure induces short- and long-term changes in the excitability of retinorecipient neurons in suprachiasmatic nucleus. J Neurophysiol. 2011; 106:576–588. [PubMed: 21593396]

Littleton JT, Ganetzky B. Ion channels and synaptic organization: analysis of the Drosophila genome. Neuron. 2000; 26:35–43. [PubMed: 10798390]

Liu H, Mazarati AM, Katsumori H, Sankar R, Wasterlain CG. Substance P is expressed in hippocampal principal neurons during status epilepticus and plays a critical role in the maintenance of status epilepticus. Proc Natl Acad Sci U S A. 1999; 96:5286–5291. [PubMed: 10220458]

Liu RJ, van den Pol AN, Aghajanian GK. Hypocretins (orexins) regulate serotonin neurons in the dorsal raphe nucleus by excitatory direct and inhibitory indirect actions. J Neurosci. 2002; 22:9453–9464. [PubMed: 12417670]

Llinas R. The intrinsic electrophysiological properties of mammalian neurons: insights into central nervous system function. Science. 1988; 242:1654–1664. [PubMed: 3059497]

Lu B, Su Y, Das S, Liu J, Xia J, Ren D. The neuronal NALCN channel contributes resting sodium permeability and is required for normal respiratory rhythm. Cell. 2007; 129:371–383. [PubMed: 17448995]

Lu B, Su Y, Das S, Wang H, Wang Y, Liu J, Ren D. Peptide neurotransmitters activate a cation channel complex of NALCN and UNC-80. Nature. 2009; 457:741–744. [PubMed: 19092807]

Lu B, Zhang Q, Wang H, Wang Y, Nakayama M, Ren D. Extracellular calcium controls background current and neuronal excitability via an UNC79-UNC80-NALCN cation channel complex. Neuron. 2010; 68:488–499. [PubMed: 21040849]

Lu TZ, Feng Z-P. A Sodium Leak Current Regulates Pacemaker Activity of Adult Central Pattern Generator Neurons in*Lymnaea Stagnalis*. Plos One. 2011; 6:e18745. [PubMed: 21526173]

Ma JY, Catterall WA, Scheuer T. Persistent sodium currents through brain sodium channels induced by G protein betagamma subunits. Neuron. 1997; 19:443–452. [PubMed: 9292732]

MacKinnon R. Pore loops: an emerging theme in ion channel structure. Neuron. 1995; 14:889–892. [PubMed: 7538310]

Miller C. The charybdotoxin family of K+ channel-blocking peptides. Neuron. 1995; 15:5–10. [PubMed: 7542463]

Morris ME, Trippenbach T. Changes in extracellular [K+] and [Ca2+] induced by anoxia in neonatal rabbit medulla. Am J Physiol. 1993; 264:R761–R769. [PubMed: 8476118]

Nakayama M, Iida M, Koseki H, Ohara O. A gene-targeting approach for functional characterization of KIAA genes encoding extremely large proteins. Febs J. 2006; 20:1718–1720.

Nash HA, Scott RL, Lear BC, Allada R. An unusual cation channel mediates photic control of locomotion in Drosophila. Curr Biol. 2002; 12:2152–2158. [PubMed: 12498692]

Nicholson C, Bruggencate GT, Steinberg R, Stockle H. Calcium modulation in brain extracellular microenvironment demonstrated with ion-selective micropipette. Proc Natl Acad Sci U S A. 1977; 74:1287–1290. [PubMed: 265573]

Nilsson P, Hillered L, Olsson Y, Sheardown MJ, Hansen AJ. Regional changes in interstitial K+ and Ca2+ levels following cortical compression contusion trauma in rats. J Cereb Blood Flow Metab. 1993; 13:183–192. [PubMed: 8436609]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Owsianik G, Talavera K, Voets T, Nilius B. Permeation and selectivity of TRP channels. Annu Rev Physiol. 2006; 68:685–717. [PubMed: 16460288]

Parent L, Gopalakrishnan M. Glutamate substitution in repeat IV alters divalent and monovalent cation permeation in the heart Ca2+ channel. Biophys J. 1995; 69:1801–1813. [PubMed: 8580323]

Payandeh J, Scheuer T, Zheng N, Catterall WA. The chrystal structure of a voltage-gated sodium channel. Nature. 2011; 475:353–358. [PubMed: 21743477]

Pena F, Ramirez JM. Substance P-mediated modulation of pacemaker properties in the mammalian respiratory network. J Neurosci. 2004; 24:7549–7556. [PubMed: 15329402]

Perez-Reyes E, Cribbs LL, Daud A, Lacerda AE, Barclay J, Williamson MP, Fox M, Rees M, Lee JH. Molecular characterization of a neuronal low-voltage-activated T-type calcium channel. Nature. 1998; 391:896–900. [PubMed: 9495342]

Phillips CG, Harnett MT, Chen W, Smith SM. Calcium-sensing receptor activation depresses synaptic transmission. J Neurosci. 2008; 28:12062–12070. [PubMed: 19005071]

Pierce-Shimomura JT, Chen BL, Mun JJ, Ho R, Sarkis R, McIntyre SL. Genetic analysis of crawling and swimming locomotry pattern in C. elegans. Proc Natl Acad Sci U S A. 2008; 105:20982–20987. [PubMed: 19074276]

Ptak K, Yamanishi T, Aungst J, Milescu LS, Zhang R, Richerson GB, Smith JC. Raphe´ Neurons Stimulate Respiratory Circuit Activity by Multiple Mechanisms via Endogenously Released Serotonin and Substance. P. J Neurosci. 2009; 29:3720–3737. [PubMed: 19321769]

Pumain R, Menini C, Heinemann U, Louvel J, Silva-Barrat C. Chemical synaptic transmission is not necessary for epileptic seizures to persist in the baboon Papio papio. Exp Neurol. 1985; 89:250–258. [PubMed: 2988992]

Puopolo M, Raviola E, Bean BP. Role of subthreshold calcium current and sodium current in spontaneous firing of mouse midbrain dopamine neurons. J Neurosci. 2007; 27:645–656. [PubMed: 17234596]

Raman IM, Bean BP. Resurgent sodium current and action potential formation in dissociated cerebellar Purkinje neurons. J Neurosci. 1997; 17:4517–4526. [PubMed: 9169512]

Raman IM, Gustafson AE, Padgett D. Ionic currents and spontaneous firing in neurons isolated from the cerebellar nuclei. J Neurosci. 2000; 20:9004–9016. [PubMed: 11124976]

Ramirez JM, Tryba AK, Pena F. Pacemaker neurons and neuronal networks: an integrative view. Curr Opin Neurobiol. 2004; 14:665–674. [PubMed: 15582367]

Ramsey IS, Delling M, Clapham DE. An introduction to TRP channels. Annu Rev Physiol. 2006; 68:619–647. [PubMed: 16460286]

Ren D, Navarro B, Perez G, Jackson AC, Hsu S, Shi Q, Tilly JL, Clapham DE. A sperm ion channel required for sperm motility and male fertility. Nature. 2001a; 413:603–609. [PubMed: 11595941]

Ren D, Navarro B, Xu H, Yue L, Shi Q, Clapham DE. A prokaryotic voltage-gated sodium channel. Science. 2001b; 294:2372–2375. [PubMed: 11743207]

Ren D, Xia J. Calcium signaling through CatSper channels in mammalian fertilization. Physiology (Bethesda). 2010; 25:165–175. [PubMed: 20551230]

Ringer S. A further Contribution regarding the influence of the different Constituents of the Blood on the Contraction of the Heart. J Physiol. 1883; 4:29–42.

Rivera C, Voipio J, Payne JA, Ruusuvuori E, Lahtinen H, Lamsa K, Pirvola U, Saarma M, Kaila K. The K+/Cl− co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. Nature. 1999; 397:251–255. [PubMed: 9930699]

Robinson RB, Siegelbaum SA. Hyperpolarization-activated cation currents: from molecules to physiological function. Annu Rev Physiol. 2003; 65:453–480. [PubMed: 12471170]

Rolland JF, Henquin J-C, Gilon P. G protein-independent activation of an inward Na+ current by muscarinic receptors in mouse pancreatic cells. J Biol Chem. 2002; 277:38373–38380. [PubMed: 12161432]

Ruat M, Molliver ME, Snowman AM, Snyder SH. Calcium sensing receptor: molecular cloning in rat and localization to nerve terminals. Proc Natl Acad Sci U S A. 1995; 92:3161–3165. [PubMed: 7724534]

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

Rusakov DA, Fine A. Extracellular Ca2+ depletion contributes to fast activity-dependent modulation of synaptic transmission in the brain. Neuron. 2003; 37:287–297. [PubMed: 12546823]

Russo MJ, Mugnaini E, Martina M. Intrinsic perperties and mechanisms of spontaneous firing in mouse cerebellar unipolar brush cells. J Physiol. 2007; 581:709–724. [PubMed: 17379636]

Sanna PP, Berton F, Cammalleri M, Tallent MK, Siggins GR, Bloom FE, Francesconi W. A role for Src kinase in spontaneous epileptiform acivity in the CA3 region of the hippocampus. Proc Natl Acad Sci U S A. 2000; 97:8653–8657. [PubMed: 10890901]

Schlief T, Schonherr R, Imoto K, Heinemann SH. Pore properties of rat brain II sodium channels mutated in the selectivity filter domain. Eur Biophys J. 1996; 25:75–91. [PubMed: 9035373]

Shen KZ, North RA. Muscarine increases cation conductance and decreases potassium conductance in rat locus coeruleus neurones. J Physiol. 1992a; 455:471–485. [PubMed: 1484360]

Shen KZ, North RA. Substance P opens cation channels and closes potassium channels in rat locus coeruleus neurons. Neuroscience. 1992b; 50:345–353. [PubMed: 1279460]

Shirayama T, Matsumoto K, Pappando AJ. Carbachol-induced sodium current in guinea pig ventricular myocytes is not regulated by guanine nucleotides. J Pharmacol Exp Ther. 1993; 2:641–648. [PubMed: 8388456]

Silver IA, Erecinska M. Intracellular and extracellular changes of [Ca2+] in hypoxia and ischemia in rat brain in vivo. J Gen Physiol. 1990; 95:837–866. [PubMed: 2163431]

Smith SM, Bergsman JB, Harata NC, Scheller RH, Tsien RW. Recordings from single neocortical nerve terminals reveal a nonselective cation channel activated by decreases in extracellular calcium. Neuron. 2004; 41:243–256. [PubMed: 14741105]

Snutch TP, Monteil A. The sodium "leak" has finally been plugged. Neuron. 2007; 54:505–507. [PubMed: 17521564]

Speca DJ, Chihara D, Ashique AM, Bowers MS, Pierce-Shimomura JT, Lee J, Rabbee N, Speed TP, Gularte RJ, Chitwood J, et al. Conserved role of unc-79 in ethanol responses in lightweight mutant mice. PLoS Genet. 2010; 6:1–14.

Stanley EF. Presynaptic calcium channels and the depletion of synaptic cleft calcium ions. J Neurophysiol. 2000; 83:477–482. [PubMed: 10634889]

Swayne LA, Mezghrani A, Varrault A, Chemin J, Bertrand G, Dalle S, Bourinet E, Lory P, Miller RJ, Nargeot J, Monteil A. The NALCN ion channel is activated by M3 muscarinic receptors in a pancreatic beta-cell line. EMBO Rep. 2009; 10:873–880. [PubMed: 19575010]

Tang C-Y, Papazian DM. Transfer of voltage independence from a rat olfactory channel to the Drosophila either-a-go-go K+ channel. J Gen Physiol. 1997; 109:301–311. [PubMed: 9089438]

Tang S, Mikala G, Bahinski A, Yatani A, Varadi G, Schwartz A. Molecular localization of ion selectivity sites within the pore of a human L-type cardiac calcium channel. J Biol Chem. 1993; 268:13026–13029. [PubMed: 8099908]

Vassalle M. The pacemaker current (I(f)) does not play an important role in regulating SA node pacemaker activity. Cardiovasc Res. 1995; 30:309–310. [PubMed: 7585820]

Wang H, Ren D. UNC80 functions as a scaffold for Src kinases in NALCN channel function. Channels (Austin). 2009; 3:161–163. [PubMed: 19535918]

Wei WL, Sun HS, Olah ME, Sun X, Czerwinska E, Czerwinski W, Mori Y, Orser BA, Xiong ZG, Jackson MF, et al. TRPM7 channels in hippocampal neurons detect levels of extracellular divalent cations. Proc Natl Acad Sci U S A. 2007; 104:16323–16328. [PubMed: 17913893]

Williamson AV, Sather WA. Nonglutamate pore residues in ion selection and conduction in voltage-gated Ca(2+) channels. Biophys J. 1999; 77:2575–2589. [PubMed: 10545358]

Xiong Z, Lu W, MacDonald JF. Extracellular calcium sensed by a novel cation channel in hippocampal neurons. Proc Natl Acad Sci U S A. 1997; 94:7012–7017. [PubMed: 9192683]

Xu Y, Ramu Y, Lu Z. A Shaker K+ channel with a miniature engineered voltage sensor. Cell. 2010; 142:580–589. [PubMed: 20691466]

Yang J, Ellinor PT, Sather WA, Zhang JF, Tsien RW. Molecular determinants of Ca2+ selectivity and ion permeation in L-type Ca2+ channels. Nature. 1993; 366:158–161. [PubMed: 8232554]

Yang N, George AL, Horn R. Molecular basis of charge movement in voltage-gated sodium channels. Neuron. 1996; 16:113–122. [PubMed: 8562074]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Yeh E, Ng S, Zhang M, Bouhours M, Wang Y, Wang M, Hung W, Aoyagi K, Melnik-Martinez K, Li M, et al. A putative cation channel, NCA-1, and a novel protein, UNC-80, transmit neuronal activity in C. elegans. PloS Biol. 2008; 6:e55. [PubMed: 18336069]

Yue L, Navarro B, Ren D, Ramos A, Clapham DE. The cation selectivity filter of the bacterial sodium channel, NaChBac. J Gen Physiol. 2002; 120:845–853. [PubMed: 12451053]

Zhang L, Chung BY, Lear BC, Kilman VL, Liu Y, Mahesh G, Meissner R-A, Hardin PE, Allada R. DN1p circadian neurons coordinate acute light and PDF inputs to produce robust daily behavior in Drosophila. Curr Biol. 2010; 20:591–599. [PubMed: 20362452]

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

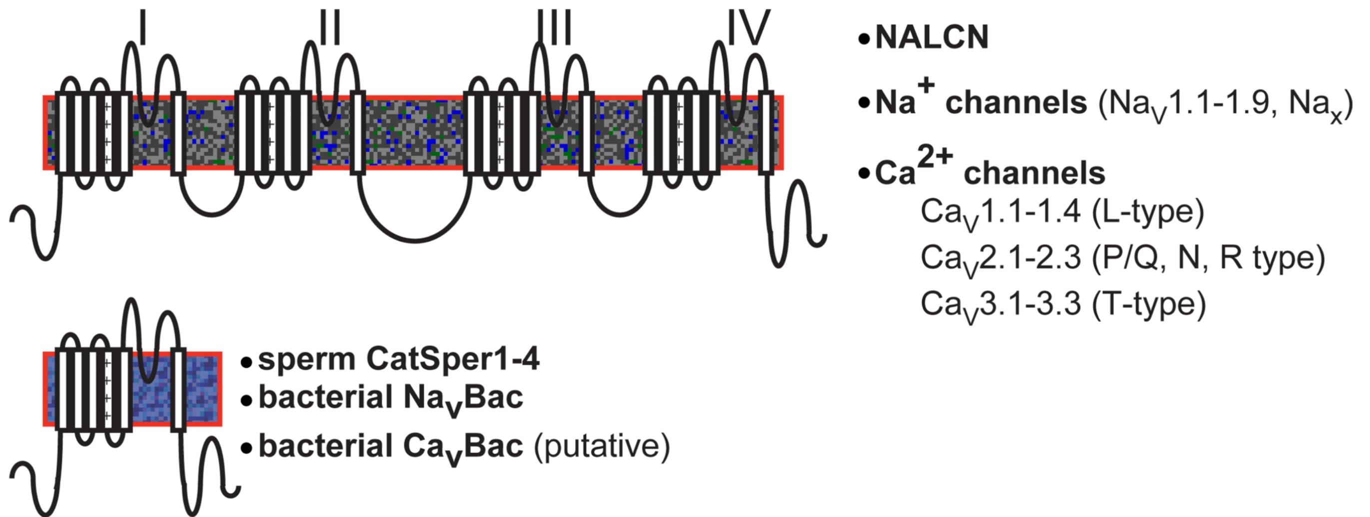

Figure 1. Ion channel families sharing high sequence similarities with Na_Vs and Ca_Vs

The 24-TM (four-repeat, I, II, III & IV) channel superfamily (**upper**) can be divided into three branches: NALCN (voltage-independent, non-selective, one member in mammals), Ca_Vs (voltage-gated, Ca^{2+} selective, 10 members) and Na^+-selective channels (9 Na_Vs and one Na_x). Three families of 6-TM channels (**lower**) also have the T/S-x-E/D-x-W pore motif (see Fig. 2) and have high sequence similarity with the 24-TM channels. They are the alkalinization-activated CatSper Ca^{2+} channels (CatSper1-4), bacterial voltage-activated Na^+ channels (Na_vBac) and putative bacterial voltage-activated Ca^{2+} channels Ca_vBac (see Fig. 2.)

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

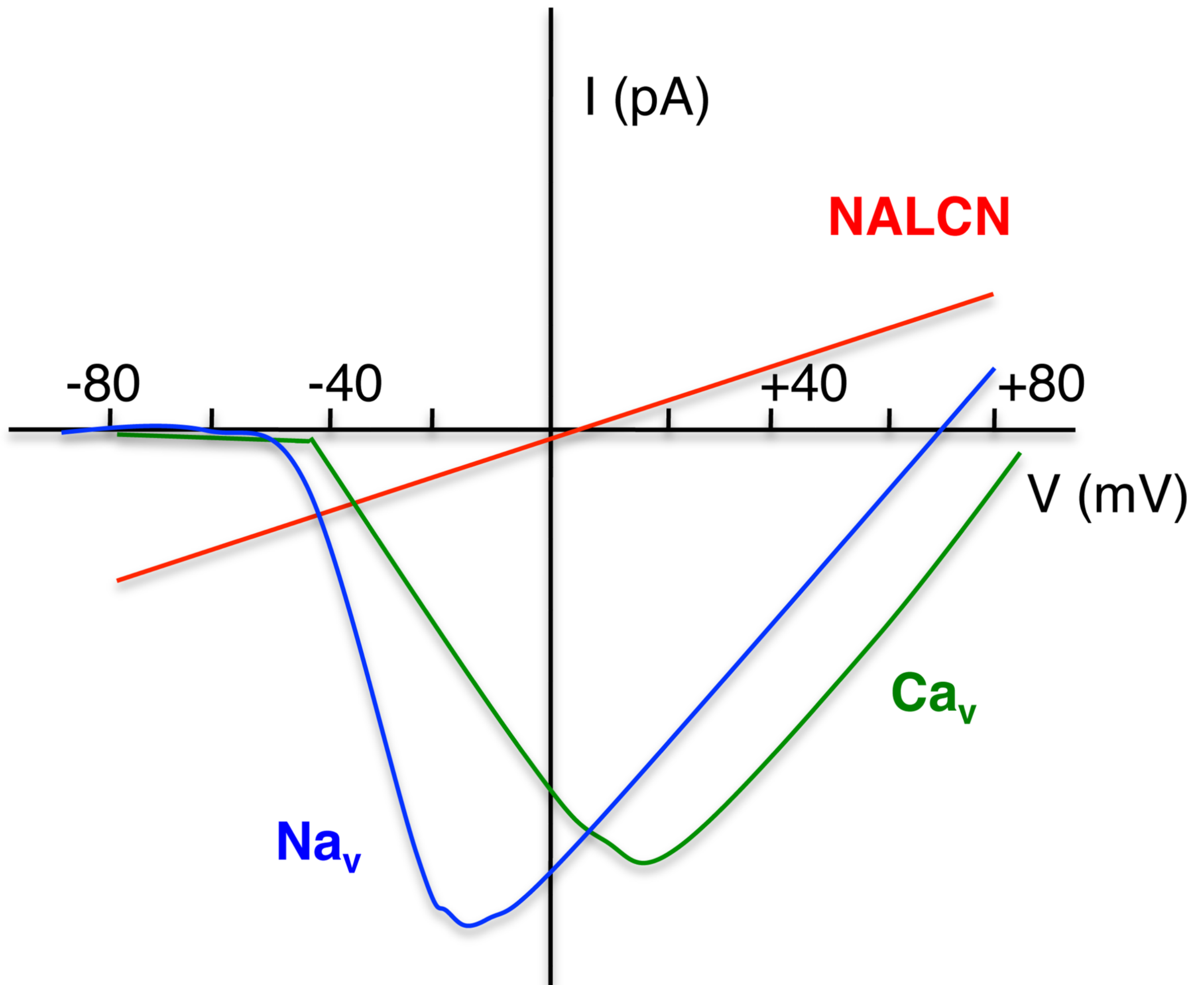

Figure 2. Illustrative current-voltage relationships of the voltage-independent NALCN channel in comparison with those of voltage-gated channels Na_Vs and Ca_Vs

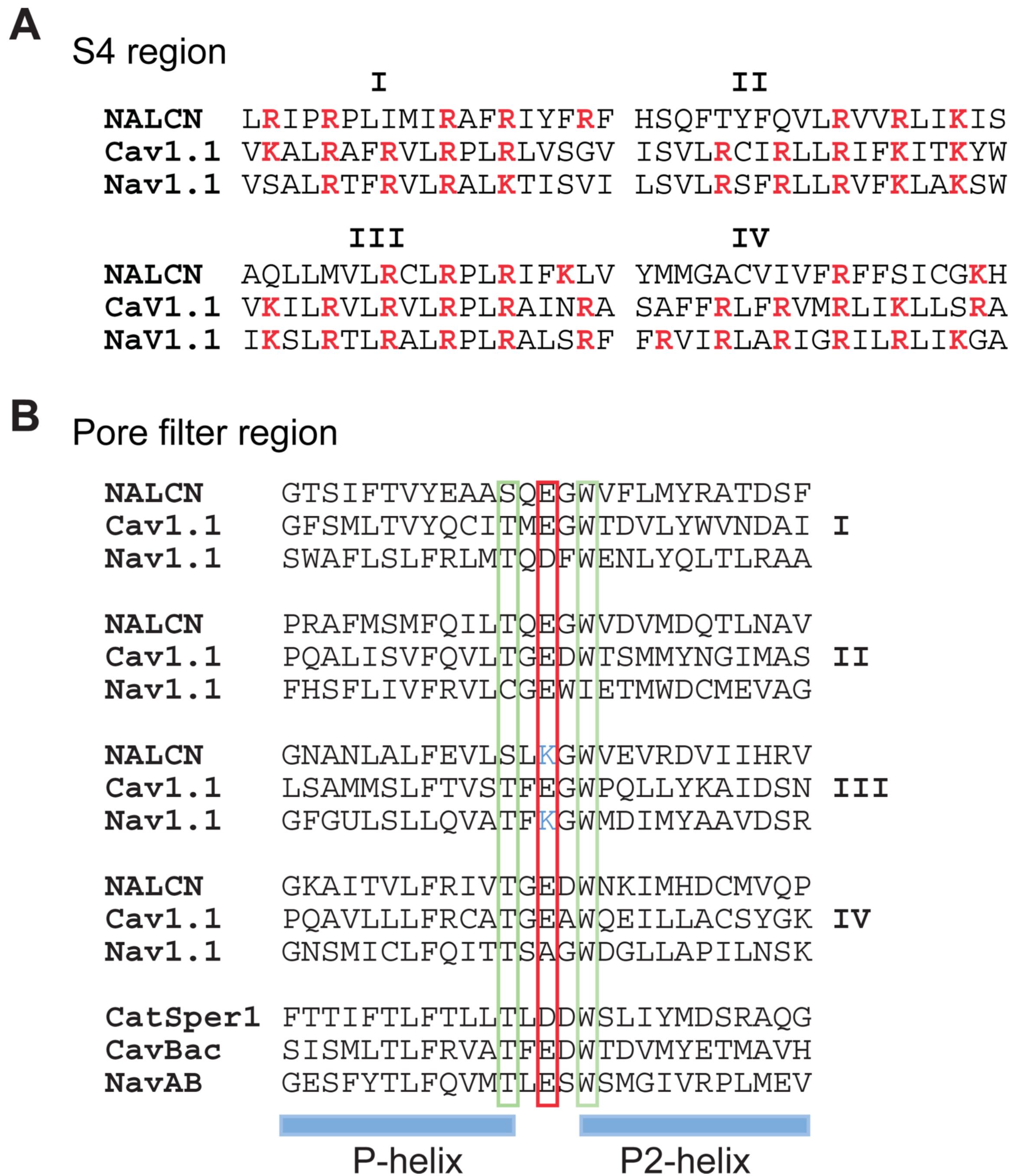

Figure 3. Unique sequences of NALCN in the S4 and the pore filter regions

(**A**) Alignment of the IVS4 regions between human NALCN, representative Ca_V (Ca_v1.1) and Na_V (Na_v1.1) channels. Charged residues (K/R) are colored. (**B**) Alignment of the two helices (P, P2) in the pore filter regions of representative 24-TM, four-repeat (I, II, III, IV) channels (NALCN, Ca_v and Na_v) and 6-TM, single repeat channels (CatSper, CavBac, NavAB). The T/SxE/DxW pore signature is boxed. NavAB is the only one whose structure has been determined at high resolution (Payandeh et al., 2011). The CavBac sequence is from psychrophilic bacteria *Colwellia psychrerythraea* (strain 34H, accession # AAR26732). Its sequences in the P2 helix region (F**ED**WT**D**) predict that it's a Ca^{2+} channel based on mutagenesis study in NaChBac (Yue et al., 2002). Many bacteria strains

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

have been found to have CavBac proteins, but they have not been electrophysiologically characterized.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

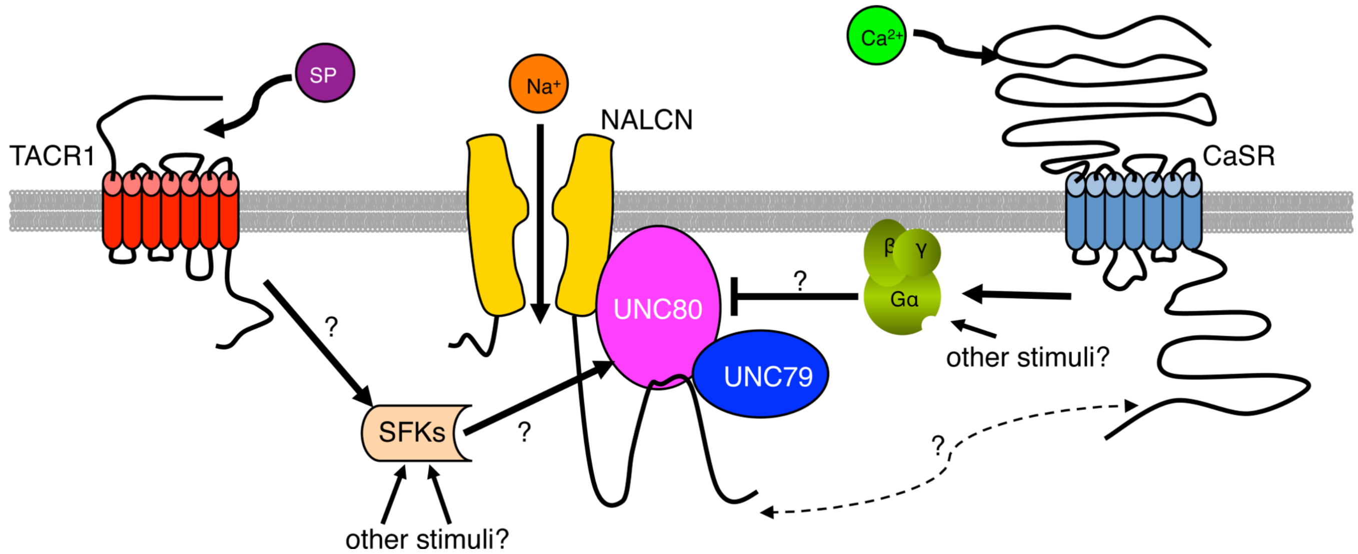

Figure 4. A Model illustrating the G protein –dependent and G protein–independent activation of NALCN by GPCRs

The NALCN complex in the brain consists of NALCN, UNC80 and UNC79. UNC80 directly associates with NALCN and UNC79 forms part of the complex by its interaction with UNC80. There is a tonic suppression of I_{NALCN} by activated CaSR at normal $[Ca^{2+}]_e$ level, which involves G-proteins, UNC80, the last amino acids of NALCN and perhaps those of CaSR. Lowering $[Ca^{2+}]_e$ releases the suppression, increases I_{NALCN}, generates a low $[Ca^{2+}]_e$-activated inward current (I_{LCA}) and excites neurons. NALCN can also be activated by substance P (SP) receptor TACR1 in a G protein-independent manner that requires the Src family of kinases (SFKs) and UNC80. The interplay between the positive regulation of NALCN through SFKs and the negative control via G proteins may determines the basal levels of Na^+ leak in neurons (modified from (Lu et al., 2010)).

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

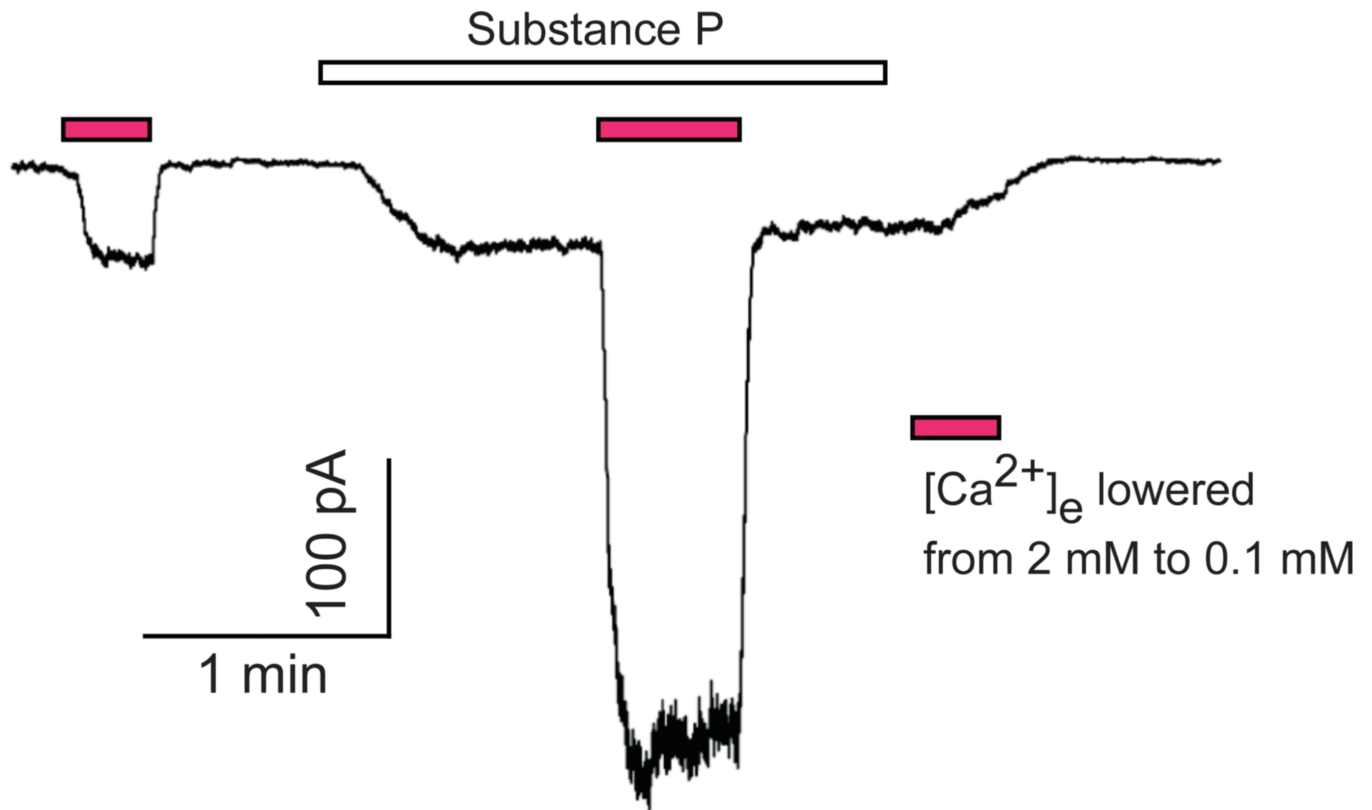

Figure 5. Synergism between the G protein –dependent and –independent activation of NALCN

A decrease in $[Ca^{2+}]_e$ from 2 mM to 0.1 mM or an application of substance P (1 μM) each activates a small NALCN-dependent inward current in a hippocampal neuron. Simultaneous application of both the stimuli generates a large synergistic excitatory action (modified from (Lu et al., 2010)).