CaV1.1 channels are organized into four homologous domains (domains I–IV), each composed of a nonpermeable voltage sensor domain (VSD) and a calcium-permeable pore domain. Mutations in the VSD may create a leak and render the VSD permeable to ions. Recording of such a leak current, known as a gating pore current, was hampered by the low expression of these channels in a heterologous expression system. In this issue, Wu et al. show for the first time the biophysical properties of this current using Stac3 to boost the expression of the CaV1.1 channel in Xenopus laevis oocytes.

**Early studies**
The first evidence of a current flowing through a mutated voltage sensor domain (VSD) was reported by Starace and Bezanilla (2004). This was a somewhat fortuitous discovery, as the authors’ studies at the time were focused on the structure and operation of the VSD and the accessibility of the S4 segment arginine residues during gating. They showed that substitution of the first positive charge (R1) of the S4 segment of the Drosophila melanogaster Shaker VSD to a histidine residue (R1H) led to the appearance of a specific proton (H+) leak. The current that the authors observed was sensitive to changes in extracellular pH, and they proposed that H+ ions pass through the channel protein using a pathway distinct from the physiological K+ ion permeation pathway. The H+ leak was not sensitive to agitoxin II, a toxin known to obstruct the pore of the channel. However, the current did pass through the VSD via a histidine factor, which can bind a proton from the extracellular medium and then release it into the intracellular medium.

The R1H substitution revealed the presence of a permeation pathway across the VSD of the Shaker K+ channel, most probably involving a proton wire, through which H+ ions could translocate into the cell in a way similar to that proposed for gramicidin channels (Starace and Bezanilla, 2004). Proton conduction through gramicidin channels has been described as occurring via a hop and turn mechanism in which H+ ions hop between water molecules, accounting for the high H+ selectivity observed.

Subsequent studies by Tombola et al. (2007) showed that the substitution of Shaker R1 with smaller uncharged amino acid residues generated a gating pore current that the authors called the “omega current.” In this case, the current was selective for cations rather than H+ ions and conducted guanidinium efficiently. To date, three pathologies have been associated with the creation of a gating pore: periodic paralysis, peripheral nerve hyperexcitability, and cardiac arrhythmias associated with dilated cardiomyopathy.

**Periodic paralysis**
Periodic paralysis manifests in two forms: hypokalemic periodic paralysis (HypoPP) and normokalemic periodic paralysis (NormoPP). HypoPP is characterized by paralytic attacks that occur in the context of low serum K+ concentrations (<3 meq/liter). These paralytic episodes are triggered by various factors including exercise, emotional stress, cold, fever, and high-fat meals (Cannon, 2006). HypoPP has been associated with mutations in CaV1.1, the skeletal muscle Ca2+ channel encoded by the CACNA1S gene (60% of cases), and NaV1.4, the skeletal muscle Na+ channel encoded by the SCN4A gene (10% of cases). However, initial studies reported only minor impairments of the biophysical properties of mutant channels. In addition, divergent biophysical properties have been reported for some mutations in the SCN4A gene, which manifest as either increased or reduced inactivation.

Paralytic attacks occur in response to the presence of two stable resting membrane potentials (VRest) of muscle myocytes. The value of the first stable VRest in pathological myocytes is approximately −75 mV, and the value of the second stable VRest in pathological myocytes is approximately −60 mV (−85 mV being the normal resting potential of a myocyte; Struyk and Cannon, 2007; Struyk et al., 2008; Jurkat-Rott et al., 2009; Cannon, 2010; Wu et al., 2011). When myocytes adopt the second stable VRest, they become nonexcitable or paralytic (Jurkat-Rott et al., 2009). However, the HypoPP biophysical defects initially reported were of different...
symmetry of the subunits (∼ current flows through the permeation pore of the channel, but instead flow through the VSD, which is composed of the S1–S4 segments of the channel (Fig. 1). The highly charged S4 segments in these complexes are surrounded by water crevices that extend deep into the membrane from both the extracellular and intracellular surfaces. Only a narrow proteinaceous region separates the inner cavity from the outer cavity. Normally, ions do not cross the voltage sensor complex. Rather, charged residues on the S4 segments (Arg or Lys) are translated from the inner crevice to the outer crevice during gating. However, several investigators have shown that mutations in the VSD of the K V7.2 channel (R207Q and R207W) could result in an overload of intracellular Na+. The pathological mechanism would thus be similar to that described for HypoPP; however, the primary effect of these mutations would still be ion leakage in the depolarized state (Sokolov et al., 2008; Fan et al., 2013; Groome et al., 2014). Given the ionic selectivity, K+ should be the most permeating physiological ion when the gating pore is open. However, the pathological consequences of such an ion leak have not been clearly elucidated.

Peripheral nerve hyperexcitability
Mutations in the VSD of the Kv7.2 channel (R207Q and R207W) have been associated with the development of peripheral nerve hyperexcitability (Dedek et al., 2001; Wuttke et al., 2007). Kv7.2 channels, which are expressed in the brain and spinal cord, oligomerize with Kv7.3 channels to generate the M current, which primarily functions to maintain the resting membrane potential. It is interesting to note that Kv7.2 channel mutations are usually located in the pore domain and are associated with the development of neonatal epilepsies, which suggests a role in maintaining the resting potential. Because of experimental difficulties with Kv7.2 channel expression, studies have focused on mutations in the Kv7.4 channel that are equivalent to R207Q and R207W (Miceli et al., 2012). Two such mutations result in the creation of a gating pore that remains open at depolarized potentials. The appearance of this new conductance would cause a slight depolarization of the membrane potential of motor neurons, facilitating the generation of action potentials and ultimately leading to cellular hyperexcitability.

Cardiac arrhythmias and dilated cardiomyopathy
Several similar mutations in the S4 segments of the Na V1.5 channel (the cardiac homologue of the Na V1.4 channel) have been identified. Patients with these mutations have an atypical clinical phenotype that associates complex cardiac arrhythmias with dilated cardiomyopathy. Although the clinical phenotypes
observed in patients with these mutations are similar, the first
biophysical studies of these mutations have described, surpris-
ingly, divergent biophysical alterations. The biophysical char-
acterization of the R219H mutation, identified in a patient with
cardiac arrhythmia and dilated cardiomyopathy, revealed the
existence of a proton-selective gating pore current activated by
hyperpolarization (Gosselin-Badaroudine et al., 2012). This alter-
native permeation pathway was subsequently identified in the
context of two other mutations (R222Q and R225W; Moreau et
al., 2015) that cause the appearance of a selective pore through
which cations permeate after depolarization. The creation of a
gating pore could thus be a new pathological mechanism for
causing cardiac dysfunctions. However, the cascade of events
linking the creation of such a permeation pathway and the clin-
ical phenotype is still a matter of debate.

**Gating pore currents in calcium channels**

Although gating pore currents have been reported in Na+ chan-
nels, the detection of such currents in Ca2+ channels has been
hampered by the low expression levels of Ca2+ channels in het-
erologous expression systems. Therefore, in the case of HypoPP,
although most mutations affect Ca9 channels, the majority of stud-
ies concern Na+, mutations. However, recent progress has been
made with recordings from muscle fibers from knockin mutant
mice using the three-microelectrode voltage-clamp approach (Wu
et al., 2011) and, after the overexpression of these channels by in
vivo local electroporation, with currents recorded using the sili-
cone-clamp method. However, even with these approaches, the
presence of other ion channels has made the characterization of
gating pore currents in Ca2+ channels very difficult.

In this issue of the *Journal of General Physiology*, Wu et al.
(2018) took advantage of the fact that Ca9,1.1 channels are highly
expressed when coexpressed with Stac3. Stac3 is a skeletal mus-
cle–specific protein that localizes to the triad and is a component
of the excitation–contraction coupling machinery. Mutations in
human Stac3 cause myopathy (Horstick et al., 2013). Stac3 coex-
pression has been shown to enhance the levels of Ca9,1.1 at the cell
surface (Horstick et al., 2013; Polster et al., 2015). The authors
coexpressed Stac3 in Xenopus laevis oocytes and found that the
200-fold increase in Ca2+ currents was sufficient to ascer-
tain whether HypoPP mutant Ca9,1.1 channels are leaky because of
missense mutations of arginine residues in the S4 segments
of the VSD. Using the high-resolution, cut-open oocyte volt-
age-clamp method to record currents, the authors showed that
R528H and R528G (R1H/R1G) in the S4 of domain II both support
gating pore currents. However, unlike other R/H HypoPP muta-
tions, R528H does not selectively conduct an H+ current.

This is an interesting advance in terms of recording gating pore currents from mutated Ca2+ channels. It does, however, raise
several questions that warrant further studies, such as develop-
ning a structural model to investigate why R528H displays mixed
Na+ and H+ selectivity and why it is impermeable to guanidinium
ions; determining the efficacy of the histidine in that position
as a H+ transporter; and determining the position of R528 in the
charge transfer center. Such results would indicate whether the
R528H-dependent gating pore has a size and permeation pathway
different from other R/H mutations. However, it should be
noted that the authors did not use any offline linear leak subtrac-
tion (Fig. 7 in Wu et al., 2018). Such a process is usually used to get
rid of inherent nonspecific leak and would allow an easier way to
assess the gating pore properties.

The majority of Ca9,1.1 mutations identified in HypoPP
patients are located in the S4 segment of the VSD. Recently, a
new mutation (V876E), which is located in the S3 segment of
domain III VSD rather than in the S4 segment, has been identi-
fied in patients with severe HypoPP outcomes (Ke et al., 2009).
Functional studies using electroporated muscle fibers showed that
muscle fibers expressing the V876E mutation exhibit a leak
current at negative voltages, which is increased by external acid-
ification, suggesting that the leak current is carried by H+ ions
(Fuster et al., 2017). This constitutes a highly intriguing advance
because all positive S4 charges could still prevent the permeation
of cations. Furthermore, the authors also described a voltage
dependence of the current that remains to be understood. Coex-
pressing the Ca9,1.1 channel carrying the V876E mutation with
Stac3 could be used to confirm whether such a mutation induces a
gating pore current in a heterologous expression system and
to study its biophysical properties (ion selectivity and voltage
dependence) in greater detail. Most voltage-gated K+, Na+, and
Ca2+ channels are built in a similar way; VSDs, comprising four
transmembrane segments, drive the opening of the 4 × 2 trans-
membrane pore domain. Because of previous experimental diffi-
culties, the intimate biophysical properties of Ca9 channels were
often extrapolated from the knowledge acquired from the study
of other voltage-gated ion channels. A recent study (Capes et al.,
2012) has reported that gating pores cannot be opened by single
S4 arginine mutations in domain IV of Na9 channels. In contrast,
similar mutations do create gating pores in Ca9 channels, illus-
trating profound differences between these two channels besides
structural and functional similarities.

The coexpression of Stac3 used by Wu et al. (2018) thus brings
the opportunity to access and study the specific biophysical prop-
erties of Ca9 channels.

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