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$Ca_V2.1$ voltage activated calcium channels and synaptic transmission in familial hemiplegic migraine pathogenesis

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Abstract

Studies on the genetic forms of epilepsy, chronic pain, and migraine caused by mutations in ion channels have given crucial insights into the molecular mechanisms, pathogenesis, and therapeutic approaches to complex neurological disorders. In this review we focus on the role of mutated Cav2.1 (i.e., P/Q-type) voltage-activated Ca^{2+} channels, and on the ultimate consequences that mutations causing familial hemiplegic migraine type-1 (FHM1) have in neurotransmitter release. Transgenic mice harbouring the human pathogenic FHM1 mutation R192Q or S218L (KI) have been used as models to study neurotransmission at several central and peripheral synapses. FHM1 KI mice are a powerful tool to explore presynaptic regulation associated with expression of Cav2.1 channels. Mutated $Ca_V2.1$ channels activate at more hyperpolarizing potentials and lead to a gain-of-function in synaptic transmission. This gain-of-function might underlie alterations in the excitatory/ inhibitory balance of synaptic transmission, favoring a persistent state of hyperexcitability in cortical neurons that would increase the susceptibility for cortical spreading depression (CSD), a mechanism believed to initiate the attacks of migraine with aura.

Keywords: Cav2.1 (P/Q-type) Ca^{2+} channels, familial hemiplegic migraine, cortical spreading depression, R192Q and S218L knock in mice, synaptic transmission.

Index

Migraine and Familial Hemiplegic Migraine (FHM)

Migraine is a common, chronic neurovascular disorder, typically characterized by recurrent attacks (1-3 days) of disabling headaches with associated autonomic symptoms. Twelve percent of the general population has on average one to two migraine attacks per month and treatments are frequently unsatisfactory. The etiology of migraine is multifactorial (for reviews, see Marmura and Silberstein 2011, Goadsby et al. 2002 and Pietrobon and Striessnig 2003). The migraine pain is likely to be triggered by activation of the trigeminovascular system, which primarily modulate sensory signal transmission trough the activation of trigeminal afferents to meningeal blood vessels, the trigeminal nerve, and brainstem nuclei.

In 20% of cases, the migraine headache is preceded by a visual hallucination/illusion known as *aura*. There is growing evidence from animal models suggesting that CSD is the electrophysiological event underlying migraine aura (Haerter et al. 2005, Lauritzen 1994). In experimental animals, CSD is an intense and steady depolarization of neuronal and glial cell membranes that last for less than one minute, which can spread to contiguous cortical areas of the brain at a rate of 3 to 5 mm/min, regardless of functional cortical divisions or arterial territories. Evoked when local extracellular K^+ concentrations exceed a critical threshold, CSD is associated with disruption of membrane ionic gradients, massive influxes of Ca^{2+} and Na^+, and massive K^+ efflux with concomitant glutamate release. All such ionic unbalance is believed to depolarize adjacent neurons and glia, thereby facilitating its spread. An advancing wave of brief excitation would then be followed by a longer-lasting inhibition of spontaneous and evoked neuronal activity that traverses the cortex (Somjen 2001, Goadsby 2007, Pietrobon 2005, Lauritzen 1994, Cutrer et al. 1998, Hadjikhani et al. 2001 and Bowyer et al. 2001). In humans, evidence for a causal role of CSD in the aura comes from functional magnetic resonance imaging (fMRI) performed during migraine attacks with aura (Hadjikhani et al 2001)

The nature and mechanisms of the primary brain dysfunction leading to the activation of the meningeal trigeminal nociceptors remain incompletely understood (for reviews see Charles 2009, Goadsby et al 2009a, Levy et al 2009) and it is controversial whether CSD can initiate the migraine headache cascade by itself. Animal studies support the idea that CSD may also initiate the headache mechanisms, but the connection between CSD and headache in patients (particularly those with migraine without aura) remains an open question (Ayata 2009, Pietrobon and Striessnig 2003). On the other hand, there is experimental animal evidence that CSD might activate the trigeminal sensory system, presumably by depolarizing perivascular trigeminal terminals at meningeal and dural blood vessels (Haerter et al. 2005, Ayata et al. 2006, Bolay et al. 2002). Zhang et al (2010, 2011) demonstrated, for the first time, that the induction of CSD by focal stimulation *in vivo* of the rat visual

cortex can lead to long-lasting activation of the nociceptors innervating the meninges in the trigeminal ganglion.

Current views of migraine describe it primarily as a multi--factorial brain disorder with an estimated heritability as high as 50% (Mulder *et al.* 2003). Genetic research in the field of migraines has mainly focused on the identification of genes involved in familial hemiplegic migraine (FHM), a rare monogenic subtype of migraines with aura. Genes for three monogenic subtypes of migraine have been identified so far (van den Maagdenberg et al 2007): *CACNA1A* (FHM1, encoding the pore-forming $_1$ subunit of $Ca_V2.1$ calcium channels; Ophoff et al. 1996), *ATP1A2* (FHM2, encoding the $_2$-subunit of sodium-potassium (Na^+, K^+) pumps present in glial cell; De Fusco et al. 2003), and *SCN1A* (FHM3, encoding the pore-forming $_1$-subunit of neuronal $Na_V1.1$ voltage gated sodium channels, Dichgans et al. 2005).

More recently, in some patients suffering from migraine an homozygous mutation in the SLC4A4 gene (NBCe1, encoding the electrogenic Na(+)-HCO(3)(-) co-transporter was found; Suzuki et al. 2010). The immunohistological and functional analyses of these mutants demonstrate that the nearly total loss of NBCe1 activity in astrocytes can cause migraine potentially through dysregulation of synaptic pH.

A link between a common form of migraine and a genetic mutation of the TWIK-related spinal cord (TRESK) two-pore domain potassium channel (K2P), encoded by KCNK18, was recently reported (Lafreniere et al 2010). By screening the KCNK18 gene in a large multigenerational family with typical migraine with aura, inherited in a dominant fashion, Lafreniere et al (2010) identified a mutation in TRESK (F139WfsX24). They also identified prominent TRESK expression in migraine salient areas such as the trigeminal ganglion. The F139WfsX24 mutation produces a frame shift that prematurely truncates the channel in the second transmembrane domain and causes a complete loss of TRESK function. In addition, the mutant subunit suppresses wild-type channel function through a dominant negative effect, thus, explaining the dominant penetrance of this allele. It is expected that the TRESK mutation would make the trigeminal neurons more easily excitable. Increasing TRESK activity might help to diminish the excitability of these neurons, and may lessen migraine severity or incidence, whereas decreasing activity (through *KCNK18* mutations) may increase the risk of migraine.

A recent genome-wide association study in a large clinic-based sample of European individuals with migraine identified a genetic variant on chromosome 8q22.1 associated with migraine (Anttila et al 2011). Furthermore, in a new study including 5,122 migraineurs and 18,108 non-migraineurs, several single nucleotide polymorphism associations (SPNs) specific for migraine were identified (Chasman et al 2011) establishing a link of migraine with LRP1 (modulator of glutamate signaling) and TRPM8 receptors (related to neuropathic pain models).

a. FHM type 2: Glial cells have a central role in migraine

Mutations in the *ATP1A2* gene are responsible for at least 20 percent of FHM cases. The FHM2 ATP1A2 gene encodes the α_2 subunit of a Na^+,K^+ pump ATPase (De Fusco et al. 2003, Marconi et al. 2003). This catalytic subunit binds Na^+, K^+, and ATP, and utilizes ATP hydrolysis to extrude Na^+ ions out of the cell while moving K^+ ions in. Na^+ pumping provides the steep Na^+ gradient essential for the transport of glutamate and Ca^{2+}. The gene is predominantly expressed in neurons at the neonatal stage

and in glial cells at the adult (De Fusco *et a*l. 2003, Vanmolkot et al. 2003). An important function of this specific ATPase in adults is to modulate the key reuptake of potassium and glutamate from the synaptic cleft into the glia (Moskowitz et al 2004). When this mechanism fails, it leads to elevated extracellular levels of glutamate and potassium, and accordingly, to an increased susceptibility to CSD (Pietrobon 2007, Koenderink et al 2005). All FHM2 mutations studied in heterologous expression systems result in a "loss-of-function" or a kinetically altered Na^+, K^+ pump (Pietrobon 2007, Tavraz et al 2008, Vanmolkot et al. 2006, De Fusco et al. 2003, Segall et al. 2005).

Leo et al (2011) have reported the generation of the first mouse model of FHM2, a knock-in mutant harboring the human W887R-ATP1A2 mutation which decreases induction threshold and increases the velocity of propagation of CSD. The authors suggested a relatively minor role of the glial $_2$ Na,K pump in K^+ clearance meaning that the reduced CSD threshold in FHM2 knockin mice would not be primarily due to impaired K^+ reuptake. Instead their evidence point to an inefficient astrocyte clearance of glutamate and a consequent increased cortical excitatory neurotransmission.

b. ***FHM type 3: Sodium channels and migraine***

FHM type 3 is caused by missense mutations in SCN1A, the genes encoding the pore-forming subunits of the neuronal voltage-gated Na^+ channel $Na_V1.1$ (Dichgans et al. 2005).

Kahlig et al (2008) investigated the functional consequences of two mutations linked to FHM3: Q1489K and L263V. Q1489K mutation, when compared to WT-$Na_V1.1$, causes a predominantly loss-of-function phenotype, as deduced from a more rapid onset of slow inactivation, delayed recovery from fast and slow inactivation and a greater loss of channel availability during repetitive stimulation. However, opposite effects of the same mutation on tsA-201 cells and rat cultured neurons transfected with human Nav1.1 were observed by Cestele et al (2008). L263V exhibited biophysical abnormalities compatible with a gain-of-function mutation: increased persistent (not inactivating) current, delayed entry into fast and slow inactivation, depolarizing shifts in the steady-state voltage dependence of both fast and slow inactivation, accelerated recovery from fast inactivation, and greater channel availability during repetitive stimulation Kahlig et al (2008). Notably, greater channel availability during repetitive stimulation, as seen for L263V mutation, has also been observed for various epilepsy-associated SCN1A mutations (Rhodes et al 2005) and might correlate with the unusually high prevalence of epilepsy observed in L263V mutation carriers. The coincidence of migraine with epilepsy suggests that these two neurological disorders may share common mechanisms in L263V mutation carriers.

Nav1.1 is the major target for epileptogenic mutations and different mutations in this gene are known to be associated with epilepsy and febrile seizures (Meisler and Kearney 2005, Avanzini et al. 2007). However, interestingly, just few cases of seizures have been reported in FHM patients (Dichgans et al. 2005, Vanmolkot et al. 2007). Thus, FHM mutations could be modifying Nav1.1 differently compared to purely epileptogenic mutations.

Studies using heterologous expression and functional analysis of recombinant Nav1.1 channels suggest that epilepsy mutations in Nav1.1 may cause either gain-of-function or loss-of-function effects that are consistent with either increased or decreased neuronal excitability (Ragsdale 2008, Cestele et al 2008). However, most Nav1.1 mutations have their ultimate epileptogenic effects by reducing Nav1.1-mediated whole cell sodium currents in GABAergic neurons, resulting in widespread loss of brain inhibition, an ideal background for the genesis of epileptic seizures (for review: Catterall et al 2010, Ragsdale 2008).

GABAergic interneurons are particularly sensitive to the loss of Nav1.1 in gene-targeted mice (Yu et al. 2006, Ogiwara et al. 2007), probably because Nav1.1 is expressed at higher levels in GABAergic interneurons than in glutamatergic pyramidal neurons. Hence, epileptogenic mutations may cause seizures because of decreased inhibition in neuronal circuits.

Because $Na_V1.1$ channels are crucial for the generation and propagation of action potentials, the overall effects of FHM3 mutations are most likely an increased frequency of neuronal firing plus an enhanced neuronal excitability and neurotransmitter release.

c. FHM type 1: A central role of voltage activated calcium channels in migraine

Voltage-gated calcium channels can be classified based on their biophysical characteristics, into high-voltage-activated (HVA) and low-voltage-activated (LVA) channels. LVA Ca^{2+} channels encompass the family of T-type channels with three members (Cav3.1 through Cav3.3). They activate at a relatively negative threshold of around –60 mV, have a small conductance, and inactivate rapidly. They are inhibited by drugs like mibefradil (Ro 40-5967) or 2-octanol (Llinas 1988; Perez-Reyes 2003; Urbano et al., 2009; Bisagno et al., 2010). HVA Ca^{2+} channels include seven members ($Ca_V1.1$ to 1.4, $Ca_V2.1$ to 2.3) encoded by distinct genes each with multiple splice variants (Catteral 2000). HVA channels typically have an activation threshold of around –30 mV, with the exception of $Ca_V1.3$, which activates at around -50 mV (Xu *and* Lipscombe 2001). $Ca_V1.1$ to 1.4 channels, also referred to as L-type channels, are inhibited by three classes of drugs, dihydropyridines, phenylalkylamines, and benzothiazepines (Striessnig et al. 1998). Selective inhibitors for specific channels have also been described: $Ca_V2.1$ (P/Q-type) channels are selectively blocked by -agatoxin IVA (Mintz et al. 1992), while $Ca_V2.2$ (N-type) channels are inhibited by -conotoxin GVIA (Plummer et al. 1989, Aosaki and Kasai 1989) and $Ca_V2.3$ (R-type) channels are blocked by SNX-482 (Newcomb et al. 1998, Tottene et al. 2000).

HVA Ca^{2+} channels are heteromultimers of a pore forming α1 subunit that co-assembles with ancillary β, α2δ (and in some cases a γ subunit) into a functional channel complex (Baumgart and Perez-Reyes 2009, Catterall et al 2005). In contrast, LVA Ca^{2+} channels are α1 subunit monomers. The α1 subunit defines the channel subtype, whereas the ancillary subunits modulate α1 subunit function and surface expression. Vertebrates express four different types of calcium channel β subunits (β1 through β4),

four different types of α2δ subunits (α2δ1 through α2δ4), and as many as eight different γ subunits (Baumgart and Perez-Reyes 2009, Black 2003).

P/Q-type, N-type and, to some extent, R-type channels are highly expressed at presynaptic nerve terminals where their activities evoke neurotransmitter release (Katz et al. 1996, Iwasaki et al. 2000, Nudler et al. 2003, Trimmer and Rhodes 2004).

c.1. Cav2.1 (P/Q-type) calcium channels

Cav2.1 channel contains a pore-forming α_{1A} subunit and several regulatory subunits, including intracellular β subunits (Cavβ1–4) that bind to the intracellular loop between transmembrane domains I and II of α1A. The effect of the regulatory subunits is essential for increasing the expression levels and modulating the voltage-dependent activation and inactivation of Cav2.1 channels (Baumgart and Perez-Reyes 2009, Birnbaumer et al 1998Catterall 2000, Di Guilmi et al., 2011, Jones 2002, Stotz et al 2004, Uchitel et al., 2010) as well as targeting the channels to the plasma membrane. Cav2.1 channels are located throughout the mammalian brain and spinal cord (Westenbroek et al. 1995) at presynaptic terminals (Catterall 1998, Wu et al. 1999, Mintz et al. 1995) and at somatodendritic membranes. The CACNA1A gene encoding Cav2.1 channels undergoes alternative splicing at multiple loci in an age-, gender-, and species-dependent manner (Bourinet et al 1999, Chang et al 2007, Chaudhuri et al 2005, Kanumilli et al 2006, Soong et al 2002). This mechanism resultes in multiple Cav2.1 splice variants with different outcomes in neuronal distribution and subcellular localization, biophysical properties, and sensitivity to ω-AgaIVA. Further functional diversity of $Ca_V2.1$ channels is generated by the combination of Cav2.1 α1 subunits with various auxiliary β and α2δ subunits (Luvisetto et al 2004). Indeed, evidence exists for large functional and pharmacological diversity of native Cav2.1 channels (Mermelstein et al 1999, Mintz et al 1992, Randall and Tsien 1995, Tottene et al 1996, Tringham et al 2007, Usowicz et al 1992). They play a prominent role in initiating action-potential-evoked neurotransmitter release both at peripheral neuromuscular junctions and central synapses, mainly within the cerebellum, brainstem, and cerebral cortex (Katz et al. 1996, Catterall 1998, Iwasaki et al. 2000). Even when pharmacological and electrophysiological studies in brain slices from rodents have revealed that P/Q-, N- and R-type channels cooperate in controlling release at many central excitatory synapses, P/Q-type channels have a dominant role, partly because of their more efficient coupling to the exocytotic machinery (González Inchauspe et al. 2004, Li et al 2007, Matsushita et al. 2002, Qian and Noebels 2001, 2000, Wu et al 1999, Mintz et al. 1995).

At many central synapses, including the inhibitory synapses between Purkinje cells and deep cerebellar nuclei neurons, there is a developmental change in the Ca^{2+} channel types mediating synaptic transmission, whereby the relative contribution of Cav2.1 channels increases with post-natal age, until it becomes exclusively dependent on P/Q-type channels (Iwasaki et al 2000). At certain synapses, N- or R-type Ca^{2+} channels fail to fully compensate the lack of Cav2.1 channels in Cav2.1-/- and other

loss-of-function Cav2.1 mouse mutants (González Inchauspe et al 2004, 2007, Giugovaz et al 2011, Pietrobon 2005). Among the presynaptic Ca^{2+} channels, Cav2.1 channels are also unique in their capacity for interacting and being modulated in a complex manner by a number of Ca^{2+}-binding proteins (Catterall and Few 2008). As a result, Cav2.1 channels may exhibit either Ca^{2+}-dependent inactivation (CDI) or Ca^{2+}-dependent facilitation (CDF). Moreover, Ca^{2+}-dependent regulation of presynaptic Cav2.1 channels may play a crucial role in short-term synaptic plasticity during trains of action potentials (Adams et al 2010, González Inchauspe et al 2004, Mochida et al 2008, Muller *et al*. 2008, Takago *et al*. 2005, Xu and Wu 2005, González Inchauspe et al 2004, Tsujimoto et al 2002, Cuttle et al 1998, Takahashi *et al*. 1996).

Cav2.1 channels are expressed in those brain structures implicated in the pathogenesis of migraine, including the cerebral cortex, the trigeminal ganglia, and brainstem nuclei involved in the central control of nociception, their expression being particularly high in the cerebellum (Pietrobon and Striessnig 2003). In the cerebral cortex, excitatory synaptic transmission at pyramidal cell synapses in different cortical areas depends predominantly on Cav2.1 channels (Ali and Nelson 2006, Iwasaki et al 2000, Koester and Sakmann 2000, Rozov et al 2001, Tottene et al 2009, Zaitsev et al 2007). They also mediate about 40% of the AP-evoked Ca^{2+} influx in dendritic spines and shaft of layer 2/3 cortical pyramidal neurons (Koester and Sakmann 2000) and contribute to the regulation of the intrinsic firing of the same neurons via activation of different Ca^{2+}-dependent K^+ (K_{Ca}) channels (Pineda et al 1998).

However, it is still a central problem in Ca^{2+} channel neurobiology studies to be able to translate their genetic complexity to their functional role in synaptic transmission. The availability of several mouse models with mutations in Ca^{2+} channel genes have provided a valuable tool to define Ca^{2+} channel functions. It would be expected that changes in the functional properties of Cav2.1 channels alter the behavior of neuronal networks.

c.2. Familial Hemiplegic Migraine type 1.

FHM1 is an autosomal-dominant subtype of migraine with aura, caused by a spontaneous missense mutation in the *CACNA1A* gene encoding the ion-conducting, pore-forming α_{1A} subunit of Cav2.1 channels (Pietrobon 2005, Ophoff et al. 1996). FHM1 missense mutations reported so far produce substitutions of conserved amino acids in important functional regions of the Cav2.1 channel including the pore lining and the voltage sensors (Cuenca-Leon et al 2008, Pietrobon 2007, Thomsen et al 2007) and have been associated with a wide range of clinical phenotypes (Haan et al. 2005, Kors et al. 2004, Alonso et al. 2004). Aside from the characteristic transient hemiparesis, typical attacks of FHM1 are identical to those of the common forms of migraine with aura (Pietrobon and Striessnig 2003, Pietrobon 2005, Thomsen et al. 2002). Different clinical phenotypes include pure types of FHM1 (Ophoff *et a*l. 1996), combinations of FHM1 with various degrees of cerebellar ataxia (Ducros et al. 2001, Ophoff et al. 1996), or fatal coma due to excessive cerebral edema (Kors et al. 2001, 2002).

Disorders not associated with FHM are episodic ataxia type 2 (Zafeiriou et al 2009, Cuenca-Leon et al 2009, Cricchi et al 2007, Jen et al. 2004, 2007, 2008, Ophoff et al. 1996), progressive ataxia (Yue et al. 1997), spinocerebellar ataxia type 6 (Tsou et al 2011, Restituito et al 2000, Ishikawa et al 1999, Zhuchenko et al. 1997) and absence (Imbrici et al. 2004) and generalized epilepsy (Haan et al. 2005, Jouvenceau et al. 2001). In addition FHM1 mutations were also found in family members who had only "normal" no-paretic migraine but no FHM. This suggests that gene mutations for FHM may also be responsible for the common forms of migraine, probably due to different genetic and no-genetic modulating factors. Hence, FHM is considered a valid monogenic model for studying the pathogenetic mechanisms involved in the genetically more complex common forms of migraine (Ferrari *et. al*. 2008, Ducros et al 2001).

c.3. Functional consequences of FHM1 gene mutations: Studies in heterologous systems

Biophysical analysis of FHM1 Ca^{2+} channel dysfunction in heterologus systems show that the FHM1 mutations alter many biophysical properties of human Cav2.1 channels in a complex way, which is rather debateable since both loss-of-function and gain-of-function phenotypes have been reported (Cao et al. 2004, Barrett et al. 2005, Cao and Tsien 2005, Tottene et al. 2002, Kraus et al. 2000, 1998, Hans et al. 1999). The most consistent effect revealed by the analysis of single-channel properties of human $Ca_V2.1$ channels carrying FHM1 mutations was an increase in channel open probability and in single channel Ca^{2+} influx over a broad voltage range, mainly due to a shift of channel activation to more negative voltages (Tottene et al. 2002, 2005, Hans et al. 1999). Shifts to lower voltages of activation of the mutant channels were also revealed by measuring whole-cell currents in heterologous expression systems and transfected neurons (Adams et al 2009, Melliti et al 2003, Mullner et al 2004, Serra et al 2009, Tottene et al 2002, 2005, Weiss et al 2008). As a result, channels open with smaller depolarizations. However, the overall change in calcium influx at presynaptic terminals is difficult to predict since there have been contradictory results. For instance, Cao and colleagues (2004) found evidence for reduced calcium influx at the whole cell level in transfections of cultured mouse hippocampal neurons. What happens in the mutant brain will therefore be determined by the delicate interplay between the functional effects of a particular mutation, the different channel properties and density, the type of channel subunits, and the direct and indirect cellular environment. Since the biophysical effects of FHM1 mutations can be affected by a number of factors, including Cav2.1 splice-variation (Adams et al 2009), β subunit coexpression (Mullner et al. 2004), and the nature of the expression system (Kraus et al 1998), it seems pertinent to study the functional consequences of gene mutations on P/Q-type Ca^{2+} and synaptic transmission in their native neuronal environment and at their endogenous level of expression in knock-in mouse models carrying human pathogenic mutations.

c.4. Functional consequences of FHM1 gene mutations: Studies in mice models including FHM1 CACNA1A Knock-in mutations

The generation of two knock-in FHM1 mouse models carrying either the human pathogenic R192Q or S218L missense mutations allowed the first analysis of the functional consequences of FHM1 mutations on Cav2.1 channels and synaptic transmission in neurons expressing the channels at the endogenous physiological level (Maagdenberg et al 2010, 2004, Kaja et al. 2010, Tottene et al 2009, González Inchauspe et al 2010, Adams et al 2010).

The R192Q KI mice exhibit no overt clinical phenotype or structural abnormalities. This situation is very similar in humans, where the R192Q mutation causes only mild FHM attacks, very similar to the common forms of migraine, albeit with hemiparesis, but without any other neurological symptoms. The S218L mutation causes a severe migraine phenotype combined with slowly progressive cerebellar ataxia and atrophy, epileptic seizures, coma or profound stupor, and severe, sometimes fatal, cerebral edema which can be triggered by a trivial head trauma (Chan et al 2008, Kors et al 2001, van den Maagdenberg et al 2010).

As noted before, CSD is implicated in the pathophysiology of FHM1. In both R192Q and S218L KI mice, a lower threshold for CSD induction and an increased velocity of CSD propagation was reported (van denMaagdenberg *et al.* 2004, 2010, Tottene *et al.* 2009, Eikermann-Haerter et al. 2009 *a*). In agreement with the higher incidence of migraine in females, the velocity of propagation and the frequency of CSDs were larger in females than in males of both mutant strains (Eikermann-Haerter *et al.* 2009 *a*,*b*). The sex difference was abrogated by ovariectomy and enhanced by orchiectomy, suggesting that female and male gonadal hormones exert reciprocal effects on CSD susceptibility. A female predominance is also described for familial (5:2 ratio) and sporadic (4.25:1 ratio) hemiplegic migraine (Eriksen et al 2006, Thomsen et al 2007, 2002). All these findings underscore the complex synergistic interactions between genetic and hormonal factors determining migraine susceptibility.

Accordingly to the larger gain-of-function observed *in vitro,* the S218L KI mice shows higher CSD susceptibility, *in vivo*, compared to the R192Q mice. Experimentally induced CSD induces pure hemiplegia in R192Q mutant mice, whereas S218L mutants additionally develop coma and often fatal seizures (van den Maagdenberg et al. 2010, Eikermann-Haerter et al. 2009 Recently, Eikermann-Haerter et al (2011) have provided evidence for an enhanced subcortical SD susceptibility in both FHM1 mutant mice compared to WT. Whereas the facilitated subcortical spread appeared limited to the striatum in R192Q mutants, hippocampal and thalamic spread was detected in the S218L mutants with an allele-dosage effect. Their findings suggest a role for subcortical SD as a potential mechanism to explain hemiplegia, seizures, and coma in FHM1.

Other studies in knock in animals revealed multiple gain-of-function effects. In cerebellar granule cells and cortical pyramidal cells of R192Q and S218L KI mice, the Cav2.1 Ca^{2+} current density was larger

than in WT neurons in a wide range of relatively mild depolarizations, reflecting activation of mutant mouse Cav2.1 channels at 8–9 mV more negative voltages than the corresponding WT channels. P/Q current densities were similar in KI and WT neurons at higher voltages (that elicit maximal Cav2.1 channel open probability), indicating similar densities of functional Cav2.1 channels (van den Maagdenberg et al 2004, 2010, Tottene et al 2009). Thus, in two different FHM1 mouse models and two different types of neurons the functional consequences of the FHM1 mutations on native neuronal mouse Cav2.1 channels were quite similar to those on single recombinant human Cav2.1 channels (Tottene et al 2002, 2005). In agreement with the lower threshold of activation of human S218L Cav2.1 channels compared to that of human R192Q CaV2.1 channels (Tottene et al 2005), the gain of function of the P/Q current at low voltages was larger in S218L than R192Q KI mice (van den Maagdenberg et al 2004, 2010). Similarly, a negative shift in the activation voltage of presynaptic Cav2.1 mutated channels was confirmed at the calyx of Held synapse of KI S218L mice (Di Guilmi et al, unpublished results).

The first indication that the gain of function of Cav2.1 channels produced by FHM1 mutations could lead to enhanced evoked neurotransmitter release was obtained at the neuromuscular junction (NMJ), where evoked neurotransmission was unaltered at physiological Ca^{2+} ion concentrations but increased at 0.2 mM Ca^{2+} in KI mice (Kaja et al 2005, van den Maagdenberg et al 2004, 2010). Recently, cortical excitatory neurotransmission was investigated in neuronal microcultures and in brain slices from homozygous R192Q KI mice (Tottene et al 2009). The results show increased synaptic strength at physiological Ca^{2+} concentration, presumably, due to enhanced action potential-evoked Ca^{2+} influx through mutant Cav2.1 channels and enhanced probability of glutamate release at cortical pyramidal cell (PC) synapses. Short-term synaptic depression during trains of action potentials was enhanced. Neither amplitude nor frequency of miniature postsynaptic currents were altered, indicating the absence of homeostatic compensatory mechanisms at excitatory synapses onto pyramidal cells (Tottene et al 2009).

Characteristically, CSD arises spontaneously in response to specific triggers that somehow create in the cortex of migraineurs the conditions for initiating the positive feedback cycle that overwhelms the regulatory mechanisms controlling cortical $[K^+]_o$ and ignites CSD. Insights into how this might occur have been provided by the interesting finding that, in contrast with the enhanced glutamatergic excitatory neurotransmission at PC synapses, inhibitory GABAergic neurotransmission at fast spiking (FS) interneurons was not altered in R192Q KI mice, despite being mediated by Cav2.1 channels (Tottene et al 2009). Given the evidence that the magnitude (or even the presence) of the negative shift in activation of human Cav2.1 channels produced by FHM1 mutations may depend on the particular recombinant Cav2.1 $\alpha 1$ splice variant and/or Cav2.1 β subunit (Mullner *et al.* 2004, Adams *et al.* 2009), a possible explanation for the unaltered inhibitory transmission at the FS interneurons may be

the presence of a Cav2.1 isoform that is little affected by the mutation or a near saturation of the presynaptic Ca^{2+} sensors. Evoked transmitter release was also found to be normal at the neuromuscular junction and at the calyx of Held synapse of R192Q KI mice when studied in normal Ca/Mg concentration in the external solution (González Inchauspe et al 2010). This finding was unexpected since mutated R192Q Cav2.1 channels at the calyx of Held nerve terminals are active at lower membrane potentials as predicted. Interestingly, transmitter release at the FS interneurons, at the neuromuscular junction and at the calyx of Held is triggered by a relatively brief action potential. In each of these R192Q KI preparations transmitter release is not affected. Thus, one factor that may be controlling the expression of a synaptic gain of function in the FHM1 KI mice is the duration of the action potential (AP) triggering transmitter release. To address this question we used the calyx of Held, a giant glutamatergic synapse in the mammalian auditory brainstem where, due to its size and accessibility, it is possible to make direct patch-clamp recordings from the presynaptic nerve terminal and its postsynaptic target, the principal neurons of the medial nucleus of the trapezoid body (MNTB). The large size of the calyx of Held allows it to harbor hundreds of active zones and thus a single presynaptic AP releases hundreds of quanta, generating a large excitatory postsynaptic current (EPSC) that rapidly depolarizes the MNTB neuron to threshold. A single MNTB principal neuron receives input from only one calyx of Held presynaptic terminal, and glutamatergic synaptic transmission mediated by AMPA receptors is triggered almost exclusively by Cav2.1 channels in mature mice (for a review, Schneggenburger and Forsythe 2006). The presynaptic Ca^{2+} currents (I_{pCa}) from the calyx of Held terminals recorded in brainstem slices also activate at more hyperpolarizing potentials in R192Q KI than in WT mice (González Inchauspe et al 2010). Assuming presynaptic Ca^{2+} currents through Cav2.1 channels can be modeled by Hodgkin–Huxley equations, a shift to more negative activation voltages by the FHM1 mutation theoretically should generate larger Ca^{2+} currents during APs (Borst and Sakmann 1999). However, the short duration of the calyx of Held APs (half-width: 0.45 ± 0.02 ms) elicited I_{pCa} with similar amplitudes in both WT and R192Q KI mice. Only when the AP duration was prolonged to 2 ms (typical mean half-width of pyramidal neuron APs), the shift in the activation by the R192Q mutation was sufficient to cause greater presynaptic Ca^{2+} influx (González Inchauspe et al 2010). According to the similarity in I_{pCa} amplitudes evoked by physiological calyx of Held APs, there were no differences in mean amplitudes of EPSCs between WT and R192Q KI mice. However, when synaptic transmission was triggered by long duration APs (obtained by inhibiting K^+ channels with 4-Aminopyridine and tetraethylammonium chloride), FHM1 R192Q KI mice did show a significant increase in both amplitude and area of EPSCs compared to WT mice (González Inchauspe et al., unpublished results). Thus, the expression of FHM1 mutations might vary according to the shape of the APs in charge of triggering synaptic transmission, adding to the complexity of the pathophysiology of migraine.

c.5. Effects of FHM1 mutation on synaptic plasticity

The dynamics and strength of neural circuits are essential for encoding and processing information in the CNS and rely on short and long forms of synaptic plasticity. In several synapses, repetitive stimulation causes short term depression (STD). Short-term depression of synaptic release has been traditionally accredited to depletion of the release-ready vesicle pool (von Gersdorff and Borst 2002, Wong et al. 2003, Zucker and Regehr 2002, Wang and Kaczmarek 1998), although other mechanisms like receptor desensitization (Wong et al. 2003) may be involved. At the large calyx of Held synapse, it has been shown that short-term synaptic plasticity is also achieved by a mechanism involving the regulation of presynaptic Cav2.1 Ca^{2+} currents (Cuttle et al 1998, Tsujimoto et al 2002), like calcium channel inactivation (Xu and Wu 2005, Muller *et al* 2008, Forsythe et al. 1998, Di Guilmi et al. 2011) and calcium channel inhibition by presynaptic metabotropic glutamate receptor (von Gersdorff et al. 1997, Takahashi *et al*. 1996) or AMPA receptors (Takago *et al*. 2005). Hennig et al. (2008) have developed a model of synaptic depression at the calyx of Held synaptic terminal that combines many of the mechanisms involved in short term depression and plasticity, including vesicle recycling, facilitation, activity-dependent vesicle retrieval and multiple mechanisms affecting calcium channel activity and release probability. The efficacy of synaptic transmission during repetitive stimulation is also determined by the rate of recovery from STD, owed to the replenishment of the readily releasable pool of synaptic vesicles, which is dynamically regulated by Ca^{2+} influx through voltage-gated Ca^{2+} channels in an activity dependent manner (Zucker and Regehr 2002, Wang and Kaczmarek 1998). High-frequency stimulation of presynaptic terminals significantly enhances the rate of replenishment. Therefore, mutations or neuromodulators capable of altering the activation/inactivation properties of calcium channels (Di Guilmi et al. 2011; Uchitel et al. 2010) may also alter STD and/or vesicle replenishment. Recordings in connected MNTB neurons at the calyx of Held synapses show that EPSC amplitudes depress to the same extent and with similar kinetics in WT and R192Q KI mice, but, interestingly, the rate of recovery of EPSCs amplitudes was significantly faster in R192Q KI compared to WT synapses (González Inchauspe et al., unpublished results). Ca^{2+}-dependent facilitation (CDF) of Cav2.1 channels is an important mechanism required for normal synaptic plasticity at fast synapses in the mammalian CNS. As an example, at the calyx of Held synapse of Cav2.1 KO mice, lack of pair pulse facilitation of presynaptic Ca^{2+} channels was in correlation with absence of facilitation of synaptic transmission (González Inchauspe et al 2004, 2007). In the same animal model, pair pulse facilitation of end plate potential was also impaired in the neuromuscular junction (Urbano et al 2003). Short-term facilitation of synaptic release has been attributed to enhanced vesicle release resulting from the accumulation of intracellular Ca^{2+} in presynaptic terminals during repetitive action potentials (APs), where the buildup of residual Ca^{2+} enhances binding to those sensor proteins directly mediating vesicle fusion and transmitter release

(Atluri et al 1996, Zucker and Regehr 2002). There may exists at least another mechanism of facilitation, driven by a Ca^{2+} sensor with high Ca^{2+} affinity that can detect modest, transient levels of Ca^{2+}, most likely near the pore of presynaptic Ca^{2+} channels (Atluri et al 1996). A recent study using recombinant Cav2.1 channels expressed in cultured superior cervical ganglion neurons demonstrated that the Ca^{2+}-dependent facilitation (CDF) and Ca^{2+}-dependent inactivation (CDI) of Cav2.1 channels are mediated through neuronal Ca^{2+} sensor proteins (CaSs) like calmodulin (CaM), which binds the Cav2.1 subunit carboxyl terminus (Mochida et al 2008). CaM-mediated CDF and CDI are robust forms of Cav2.1 channel modulation in which CaM interacts with the Cav2.1 carboxyl terminus in a bipartite regulatory processes where CDF is mediated by a local increase in Ca^{2+} and CDI through a global increase in Ca^{2+} (Tsujimoto et al 2002, Chaudhuri et al 2007). Adams et al (2010) provide supporting evidence for the hypothesis that Ca^{2+} influx at Purkinje fibers boutons induces calcium-dependent facilitation of Cav2.1 channels as a means to enhance Ca^{2+} influx during subsequent APs and achieve synaptic facilitation at the cerebellar parallel fiber (PF)-to-Purkinje cell (PC) central synapse. Interestingly, they demonstrated that mutations associated with human diseases affect this process. They observed that FHM1 gain-of-function missense mutations (R192Q and S218L) significantly occlude CDF of Cav2.1 currents in recombinant and native systems and that this alteration correlates with reduced short term synaptic facilitation at cerebellar PF-to-PC synapses. These findings suggest that CDF of Cav2.1 is an important mechanism required for normal synaptic plasticity at a fast synapse in the mammalian CNS. Additional evidence provided by two-photon imaging supported the fact that Cav2.1 FHM1 mutant presynaptic channels are in a constitutively facilitated state that basally increases presynaptic Ca^{2+} influx and transmitter release. Thus, observed changes in synaptic plasticity at the PF-to-PC synapse may result from a larger initial Ca^{2+} influx through basally facilitated mutant channels relative to unfacilitated WT channels in Purkinje fibers boutons. Overall, these results provide evidence that FHM1 mutations directly affect both Cav2.1 channel CDF and synaptic plasticity and that together they likely contribute toward the pathophysiology underlying FHM1. The findings also suggest that Cav2.1 channel CDF is an important mechanism required for normal synaptic plasticity at a fast synapse in the mammalian CNS.

d. Conclusions

Calcium channels containing the $_{1A}$ subunit ($Ca_V2.1$ or P/Q-type) are expressed throughout the human and mammalian brain with a higher concentration in the cerebellum and are localized in most presynaptic terminals (Catterall 1998, Wu et al. 1999, Mintz et al. 1995) as well as in the cell body and dendrites of many neurons (Volsen et al. 1995, Westenbroek et al. 1995). In many central synapses, $Ca_V2.1$ channels are preferentially located at the release sites and are more effectively coupled to neurotransmitter release than other Ca^{2+} channel types (Li et al 2007, Matsushita et al. 2002, Qian and

Noebels 2001, 2000, Dunlap et al. 1995, Mintz et al. 1995, Wu et al. 1999, Iwasaki et al. 2000). At these synapses, the action potential-evoked Ca^{2+} influx and the local Ca^{2+} increase that triggers neurotransmitter release are mainly determined by the kinetics of opening and closing, the open probability and the unitary conductance of $Ca_V2.1$ channels (Borst and Sakmann 1998, Sabatini and Regehr 1999, Meinrenken et al. 2002, 2003).

In addition to the types of voltage-dependent Ca^{2+} channels mediating Ca^{2+} entry at the terminal, the relationship between presynaptic Ca^{2+} influx and neurotransmitter release is a critical aspect characterizing the Ca^{2+} induced-release of neurotransmitter. Neurotransmitter release is a power function of presynaptic Ca^{2+} current, with a power number between 3 and 4 (Dodge and Rahamimoff 1967, Bollmann et al. 2000, Schneggenburger and Neher 2000, Mintz et al. 1995). So, even small changes in presynaptic Ca^{2+} influx have a large effect on the number of vesicles released by an action potential (Sabatini and Regehr 1999, Meinrenken et al. 2002). The impact of a relatively small change in the kinetics of activation of presynaptic Ca^{2+} channels on the Ca^{2+} current and neurotransmitter release evoked by an action potential at a central synapse has been shown by Borst and Sakmann (1998).The importance of the kinetics of activation of presynaptic Ca^{2+} channels in determining the fraction of channels that open during a short action potential and consequently, in Ca^{2+} current waveform and neurotransmitter release has been also described by Sabatini and Regehr (1999) and our group (González Inchauspe et al. 2010). Liu et al. (2003) have shown that different kinetics of inactivation and especially voltage- dependence of steady-state inactivation of Ca^{2+} channels have a striking impact on the amount and temporal pattern of Ca^{2+} influx in response to repetitive firing waveforms.

The specifically dominant and efficient roles of $Ca_V2.1$ channels in controlling fast neurotransmitter release from central excitatory synapses suggest that the human and mouse $Ca_V2.1$ channelopathies and their episodic neurological symptoms, ranging from migraine to absence epilepsy and ataxia, might be primarily synaptic diseases. These different disorders probably arise from a disruption of the finely tuned balance between excitation and inhibition in neuronal circuits of specific brain regions: the cortex in the case of migraine, the thalamus in the case of absence epilepsy and the cerebellum in the case of ataxia. Synapses are affected differently depending on intrinsic differences in the relative reliance on P/Q channels and shape of the action potential.

The mutations in the $Ca_V2.1$ channels linked to FHM1 affect the biophysical properties and the density of presynaptic calcium currents as well as several other properties of synaptic transmission, like short term plasticity. A gain of function was observed in R192Q and S218L KI mice, that includes an increased Ca^{2+} influx through $Ca_v2.1$ channels as a consequence of mutant channels that open at lower voltages than WT channels (González Inchauspe et al. 2010, Tottene et al. 2002, 2005, 2009, van den Maagdenberg et al. 2004, 2010). Given the three-fourth power dependence of neurotransmitter release

on intracellular Ca^{2+} concentration, small changes in amplitude or time course of Ca^{2+} influx at the release sites are expected to be very effective in modulating transmitter output at those synapses where the Ca^{2+} sensors are not saturated during an action potential (Schneggenburger and Neher 2000). This predicts that, at such synapses, $Ca_V2.1$ channels that open more readily and at lower voltages because of the R192Q mutation will lead to an increased action potential-evoked Ca^{2+} influx and a consequent increase in neurotransmitter release. At the calyx of Held in P11 and older mice, transmitter release is triggered exclusively by P/Q-type Ca^{2+} channels. The hyperpolarizing shift in both the activation and inactivation of $Ca_v2.1$ channels had little effect on presynaptic Ca^{2+} currents evoked by the short duration APs of the same calyces. In consequence, similar EPSC amplitudes, release probability and pair pulse facilitation were observed in R192Q KI mice compared to WT ones. These results contrast with the increased release probability of the glutamatergic pyramidal cell synapses recently reported by Tottene et al. (2009). Nevertheless, they agree with the normal transmitter release observed at the fast spiking interneuron inhibitory synapses and at the neuromuscular junction studied in the same animal model (Kaja et al 2005, Tottene et al 2009).

Interestingly, R192Q KI mice did show a significant enhancement in both I_{pCa} and EPSCs at the calyx of Held synapse when currents were elicited by long duration APs. These findings strongly suggest that synapses driven by larger amplitude and short duration APs (e.g., calyx of Held and interneurons APs) are less affected by the mutation-induced hyperpolarizing shift in voltage-dependence of Ca^{2+} channel activation, than those driven by longer duration APs (e.g., pyramidal neurons APs). The differences in AP durations that trigger cortical excitatory and inhibitory synapses might explain the unaltered inhibitory neurotransmission observed by Tottene et al. (2009) at the fast spiking (FS) interneuron-pyramidal cell (PC) synapses. It may also explain the gain of function observed at the PC-FS interneuron excitatory synapses, since several types of interneurons and other neurons that display fast spiking behavior have APs with short half-widths durations (Ali et al. 2007), while PCs depicts long APs. Additional fctors like a faster recovery of vesicle recycling during high frequency transmission could also contribute to the increased excitability in FHM1 mutant mice. Although an established model explaining migraine attacks is still lacking, a favored hypothesis considers that the abnormal balance of cortical excitation-inhibition and the resulting persistent state of hyperexcitability of neurons in the cerebral cortex may be associated with the increased susceptibility for cortical spreading depression (CSD), which is believed to initiate the episodes of migraine with aura (van den Maagdenberg et al 2010, Tottene et al 2009, Haerter et al. 2005, Pietrobon 2005, Lauritzen 1994, Welch 1998, Flippen and Welch 1997). Several results also indicate that the underlying mechanism changing synaptic strength by the R192Q mutation might be related to the variability in calcium currents according to the shape of the action potentials.

Genetic migraine models are useful in unraveling the triggering mechanisms for migraine attacks and in identifying novel migraine preventive targets and therapies. The FHM data support a key role of CSD in the pathogenesis of migraine aura and possibly migraine headache. It also points to cortical hyperexcitability as the basis for vulnerability to CSD. Cortical spreading depression seems to be a common mechanism underlying different forms of FHM (Moskowitz et al. 2004, Goadsby 2007). Mutations in the FHM1 CACNA1 gene cause increased neuronal release of neurotransmitters, more specifically glutamate in the cortex (Pietrobon et al. 2010) that can induce, maintain, and propagate CSD. On the other hand mutations in the FHM2 sodium-potassium pump gene cause reduced reuptake of K^+ and glutamate from the synaptic cleft into the glial cell (Moskowitz et al 2004), whereas mutations in the FHM3 sodium channel gene result in hyperexcitabiality, excessive neuronal firing and most likely increased release of neurotransmitters in the synaptic cleft. The overall result is an increased level of glutamate and K^+ in the synaptic cleft resulting in an elevated propensity for CSD (van den Maagdenberg et al. 2004), which supports the concept that the migrainous brain is hyperexcitable (Aurora and Wilkinson 2007). This fact could easily explain the aura of FHM attacks. More controversial, however, is whether the enhanced tendency for CSD might also be responsible for triggering the headache phase. Lastly, the most important question that remains to be answered is whether the same mechanisms also are involved in the common forms of migraine with and without aura.

More than 15 years after the introduction of sumatriptan, triptans continue to be a mainstay for acute migraine therapy. Drugs selectively targeting the circuits now implicated in migraine, such as glutamate, nitric oxide, triptans, 5-hydroxytryptamine (5-Ht) 1B/1D receptor agonists (Ferrari et al 2001) and calcitonin gene-related peptide (CGRP) receptor antagonists have demonstrated efficacy in both preventing (topiramate) and aborting (triptans, CGRP receptor antagonists) acute migraine attacks. Among these, CGRP receptor antagonists, such us olcegepant (BiBn4096Bs, Olesen et al 2004) and telcagepant (Ho et al 2008, 2009, Williams et al 2006) may be the closest to availability for widespread clinical use (Ho et al 2010). Novel therapeutic strategies that consider CSD and cortical hyperexcitability as key targets of preventive migraine treatment promise to improve the lives of the large number of migraine sufferers. Notably, although drugs with migraine preventive activity belong to a wide range of pharmacological classes (e.g., anti-epileptics and blockers of calcium channels and serotinergic, beta-adrenergic, and histaminergic receptors), they all seem to share anti-CSD activity (Akerman & Goadsby 2005, Ayata et al. 2006). A particularly interesting compound is Tonabersat (SB-220453), which inhibits CSD, CSD-induced nitric oxide (NO) release and cerebral vasodilation (Smith et al. 2000). If proven effective, it would be the first migraine prophylactic agent developed on the basis of the CSD hypothesis (Goadsby et al 2009b). Other examples of potential compounds effective as preventive therapy for chronic migraine are Memantine, an activity-dependent *N*-methylD-

aspartate receptor blocker, and Topiramate (Diener et al 2007, Silberstein et al 2007, Bussone et al. 2005, Storer and Goadsby 2004, 2005), both inhibiting CSD (Goadsby et al 2009b, Akerman and Goadsby 2004). In an attempt to correlate the preventive action of a range of medications with an effect on CSD, Ayata et al. (2006) have studied topiramate, valproate, amitriptyline, propranolol and methysergide. Topiramate, valproate and amitriptyline inhibited CSD induction at doses that are typically used in rodent studies, none of which was similar to the dose that is used in migraine prevention. The dose of propranolol used was four times that reported in previous rat studies (Smits et al.1980), whereas methysergide had no effect on the speed of propagation (Ayata et al. 2006). CSD inhibition might thus be a good model for the study of migraine to help in developing preventive drugs.

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Highlights

Mutations in Cav2.1 Ca^{2+} channels cause familial hemiplegic migraine type 1 (FHM1).

$Ca_V2.1$ channels with the human FHM1 mutations activate at more hyperpolarizing potentials.

FHM1 generates a gain-of-function in synaptic transmission, affecting also synaptic plasticity.

FHM1 increases the susceptibility for cortical spreading depression (CSD).