NEUROBIOLOGY OF MIGRAINE

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Migraine — an episodic headache — affects more than 10% of the general population. Despite recent progress, drug therapy for preventing and treating migraine remains unsatisfactory for many patients. One problem that slows the development of new therapeutic approaches is our limited understanding of migraine neurobiology. Activation of the trigeminovascular system is a central step in the development of migraine. However, two main issues remain incompletely understood: the primary cause of migraine, leading to activation of the trigeminovascular system, and the mechanisms of pain generation after its activation.

NEUROLOGICAL DISEASES

POLYGENIC
A characteristic controlled by different genes, each of which has only a small role in the phenotype.

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Migraine is a public health problem of great impact on both the patient and society. The overall migraine prevalence in western countries is 6–8% in men and 15–25% in women. It has been calculated that about 5% of the general population have at least 18 days of migraine per year, and that at least 1% — that is, more than 2.5 million people in North America — have at least one day of migraine per week. Severe migraine is rated as one of the most disabling chronic disorders. The annual cost of migraine-related lost productivity is enormous.

Migraine attacks are typically characterized by unilateral and pulsating severe headache, lasting 4–72 hours, and are often accompanied by nausea, phono- and photophobia (migraine without aura; MO). In at least 20% of patients, the attacks are preceded by transient (usually less than 60 min duration) neurological symptoms (migraine with aura; MA). Auras are most frequently visual, but can involve other senses, or occasionally cause motor or speech deficits.

Migraine has a strong (up to 50%) genetic component, which is higher in MA than MO, with a probable multifactorial POLYGENIC inheritance. Genetic load can be seen as determining an inherent migraine threshold that is modulated by external and internal factors (migraine triggers). Although several susceptibility loci have been reported in chromosomes 1q, 4q24, Xq24-28 and 19p13 (REF. 1), causative genes have not yet been identified, except for familial hemiplegic migraine (FHM) — a rare, autosomal dominant subtype of MA.

Here we review recent experimental evidence mainly from brain imaging and neurophysiological studies that, despite leaving many open questions, have advanced our understanding of migraine towards a unifying pathophysiological hypothesis to explain this disease. Convincing mechanistic explanations for some of the migraine symptoms have been discovered. So, activation of the trigeminovascular system (TGVS) is thought to be responsible for the pain itself, and cortical spreading depression (CSD) seems to underlie the aura symptoms. Important questions that remain include the primary cause of migraine, leading to activation of the TGVS, and the mechanisms of pain generation after its activation. We will discuss these questions in the context of the discovery that $Ca_v2.1$ Ca^{2+} channel dysfunction causes FHM.

Neurobiology of migraine headache

We will discuss recent advances in the neurobiology of migraine headache in the framework of the established mechanisms that are briefly summarized here and illustrated in FIG. 1 (see REFS 2–4 for reviews).

Within the skull, pain sensitivity is primarily restricted to the meningeal blood vessels, which are densely innervated by nociceptive sensory afferent fibres of the ophthalmic division of the trigeminal nerve. It is generally recognized that the development of migraine headache depends on the activation of these afferents.

In different animal models, including non-human primates, activation of the meningeal trigeminovascular

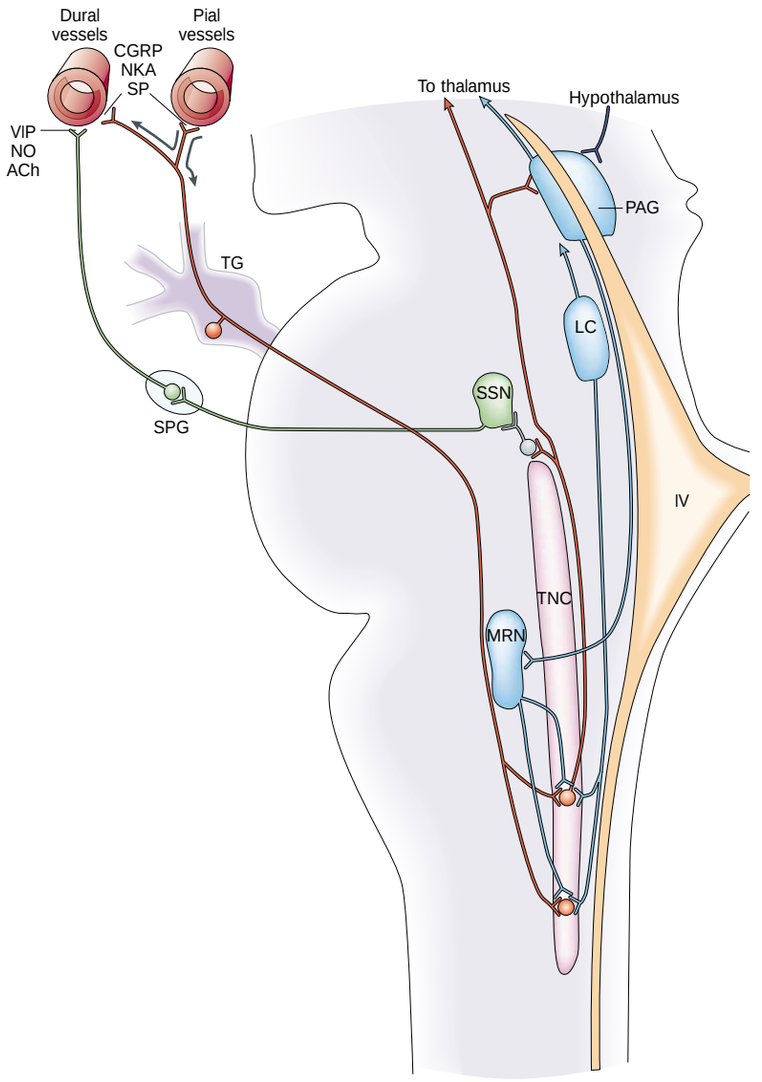

Figure 1 | **Neuronal pathways involved in trigeminovascular activation and pain processing.** IV, fourth ventricle; ACh, acetylcholine; CGRP, calcitonin gene-related peptide; LC, locus coeruleus; PAG, periaqueductal grey region; MRN, magnus raphe nucleus; NKA, neurokinin A; NO, nitric oxide; SP, substance P; SPG, superior sphenopalatine ganglion; SSN, superior salivatory nucleus; TG, trigeminal ganglion; TNC, trigeminal nucleus pars caudalis; VIP, vasoactive intestinal peptide.

afferents leads to activation of second-order dorsal horn neurons in the trigeminal nucleus pars caudalis (TNC) and the two uppermost divisions of the cervical spinal cord. Impulses are then carried rostrally to brain structures that are involved in the perception of pain, including several thalamic nuclei and the ventrolateral area of the caudal periaqueductal grey region (PAG). The PAG is involved in craniovascular pain not only through ascending projections to the thalamus, but also through descending modulation (mainly inhibitory) of nociceptive afferent information[5]. Activation of the TGVS also leads to release of vasoactive neuropeptides contained in their peripheral nerve endings, especially the calcitonin gene-related peptide (CGRP). In animal studies, the neuropeptides that are released by trigeminal ganglion stimulation produce vasodilation of the meningeal vessels (mainly due to CGRP), plasma EXTRAVASATION and mast cell degranulation with secretion of other proinflammatory substances in the dura (neurogenic inflammation). Trigeminal nerve activation also leads to vasodilation of meningeal blood vessels through activation of a parasympathetic reflex at the level of the superior salivatory nucleus (FIG. 1).

Evidence that activation of the TGVS occurs in humans during migraine is provided by the increased level of CGRP that is found in both the external and internal jugular blood during migraine attacks[6,7], and its return to normal levels after treatment with sumatriptan and subsequent headache relief[8].

The two main open issues in the neurobiology of migraine headache are, first, the primary cause of the migraine headache — that is, the mechanism of activation of the TGVS — and, second, the mechanism of pain generation after activation of the TGVS.

Primary cause of the migraine headache

According to the once widely accepted 'vascular theory of migraine', the symptoms of migraine aura are caused by transient ischaemia that is induced by vasoconstriction, and the headache arises from rebound abnormal vasodilation of intracranial arteries and consequent mechanical activation of perivascular sensory fibres. However, functional brain imaging during MA attacks shows spreading cortical hyperaemia that is followed by oligaemia, which outlasts the aura symptoms and extends into the headache phase[9]. Moreover, there is no clear evidence for a significant increase in the diameter of the middle cerebral artery during migraine attacks[10], and a recent study clearly shows that migraine can be induced without dilation of this artery[11]. These findings make the vascular theory untenable for most migraine patients[4]. It is now generally recognized that the primary cause of the migraine headache lies in the brain, but its cellular and molecular mechanisms remain largely unknown. Recent findings point to two main mechanisms: CSD and a brainstem generator.

Neurobiology of migraine aura and CSD. In 1941, the neuropsychologist Karl Lashley analysed the progression of his own visual aura, consisting of a SCOTOMA with a scintillating border drifting slowly across the visual field (FIG. 2a). He postulated that the scotoma resulted from a region of depressed neural activity in the visual cerebral cortex, and that the scintillations resulted from a bordering region of intense cortical excitation. He calculated that the neural disturbance propagated slowly across the cortex (at about 3 mm min^{-1}). A few years later, an electrophysiological correlate was reported by Leao in the rabbit cerebral cortex[12] and termed CSD. In animals, CSD can be triggered by focal stimulation (electrical, mechanical or with high K^+) of the cerebral cortex, more readily in the occipital region than other regions. It is characterized by a slowly propagating wave (2–6 mm min^{-1}) of sustained strong neuronal depolarization that generates a transient (in the order of seconds), intense

EXTRAVASATION
The exit of fluid from a blood vessel.

SCOTOMA
An area of lost vision that is surrounded by an area of less depressed or normal vision.

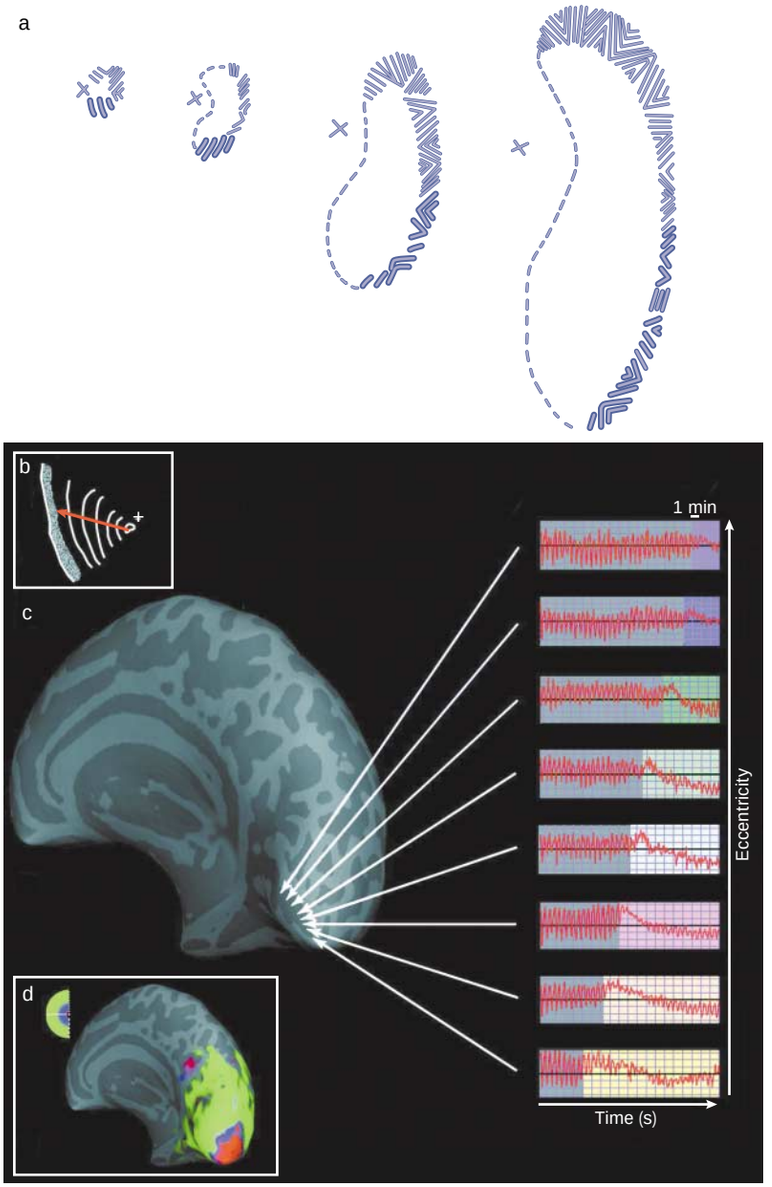

Figure 2 | **Spreading suppression of cortical activation during migraine aura. a** | Original drawing by Lashley illustrating the progression of his visual aura over time, consisting of a scotoma (within dashed line) and a scintillating border with typical fortifications. The cross indicates the fixation point. **b** | Visual field defect of a patient studied with brain imaging. The fixation point appears as a small white cross. The red arrow shows the overall direction of progression of the visual percept. **c** | Reconstruction of the patient's brain on the basis of anatomical data. The posterior medial aspect of the occipital lobe is shown in an inflated cortex format. In this format, the cortical sulci and gyri appear in darker and lighter grey, respectively, on a computationally inflated surface. Signal changes over time are shown to the right. Each time course was recorded from one in a sequence of voxels that were sampled along the primary visual cortex, from the posterior pole to a more anterior location, as indicated by arrowheads. A similar blood oxygenation level-dependent response was found within all of the extrastriate areas, differing only in the time of onset of the magnetic resonance perturbations. The perturbations developed earlier in the foveal representation, compared with more eccentric representations of retinotopic visual cortex. This finding was consistent with the progression of the aura from central to peripheral eccentricities in the corresponding visual field (**b** and **d**). **d** | The maps of retinotopic eccentricity from the same subject as in **b** and **c**, acquired during interictal scans. As shown in the logo in the upper left, voxels that show retinotopically specific activation in the fovea are coded in red (centred at 1.5° eccentricity). Parafoveal eccentricities are shown in blue, and more peripheral eccentricities are shown in green (centred at 3.8° and 10.3°, respectively). Modified, with permission, from REF. 17 © (2001) The National Academy of Sciences.

spike activity as it progresses into the tissue, followed by neural suppression that can last for minutes[13]. The depolarization phase is associated with an increase in regional cerebral blood flow (rCBF), whereas the phase of reduced neural activity is associated with a reduction in rCBF[13]. The similarities between migraine visual aura and CSD led to the hypothesis that CSD was responsible for the aura[9,13]. This hypothesis was questioned because electroencephalographic recordings during surgery did not show CSD in humans. Whereas it is clear that CSD is more difficult to elicit in human than in rodent cortex, changes in several parameters that are similar to those typical of CSD in animals were measured in the brain of a patient with head trauma[14]. Moreover, transient electrocorticogram suppressions that are consistent with CSD were recently measured in the injured neocortex of several patients[15]. CSD was induced and direct current (DC) shifts were also measured in non-human primates, in which, however, no prolonged hypoperfusion was observed after the focal hyperaemia[16].

Recently, blood oxygenation level-dependent functional magnetic resonance imaging (BOLD fMRI) showed CSD-typical cerebrovascular changes in the cortex of migraineurs while experiencing a visual aura[17,18]. A clear temporal correlation was established between the initial features of the aura percept (scintillations beginning in the paracentral left visual field) and the initial increase in the mean BOLD signal, reflecting cortical hyperaemia[17]. The subsequent decrease in BOLD was temporally correlated with the scotoma that followed the scintillations. The BOLD signal changes developed first in the extrastriate cortex (area V3A), contralateral to the visual changes. It then slowly migrated (3.5 mm min^{-1}) towards more anterior regions of the visual cortex, representing peripheral visual fields, in agreement with the progressive movement of the scintillations and scotoma from the centre of vision towards the periphery (FIG. 2b).

More direct evidence that CSD underlies visual aura was obtained with MAGNETOENCEPHALOGRAPHY (MEG). Slow changes of the cortical magnetic field, corresponding to the DC potential changes that are produced by the neuronal depolarization in CSD, were measured in patients during a spontaneous or visually triggered visual aura[19]. The DC MEG field shifts resembled those previously measured during CSD moving across a sulcus in animal models[20].

The demonstration of cerebrovascular and magnetic field correlates of CSD in migraineurs supports the conclusion that visual aura arises from CSD. Auras with motor or other sensory symptoms probably also result from CSD-like events within primary motor or sensory cortices.

CSD produces substantial changes in the composition of the extracellular fluid in the rat cortex[21]. Many substances such as K^+ ions, protons, nitric oxide, arachidonic acid and prostanglandins, the concentrations of which increase during CSD, can activate and/or sensitize the meningeal trigeminovascular afferents[22,23]. Recently, direct evidence was obtained that CSD, induced by either a pinprick or electrical stimulation,

can activate these afferents[24]. In the rat, transient, cortically spreading hyperaemia during CSD was followed by both sustained cortical oligaemia and dilation of the middle meningeal artery. This dilation was abolished after ipsilateral trigeminal or parasympathetic denervation. CSD also produced plasma protein extravasation from dural blood vessels (but see REF. 25) and increased *FOS* expression in caudal TNC neurons. Both effects were abolished by trigeminal nerve section. However, enhanced firing of TNC or upper cervical spinal cord neurons after CSD has not been directly demonstrated yet[25,26]. Given that neurons activated by CSD might represent a relatively small proportion of cells, possible explanations are intrinsic sample size limitations and/or incorrect localization of single-cell electrophysiological recordings. Despite the lack of direct electrophysiological evidence, the animal studies strongly support the conclusion that CSD can activate the meningeal afferents, and are consistent with the idea that CSD is the primary event that induces a migraine attack.

As CSD underlies the aura, the fact that most migraineurs do not experience an aura apparently conflicts with the idea of CSD as a primary event. The possibility that CSD also occurs in MO patients and causes headache without giving rise to preceding aura symptoms, possibly because it originates in a clinically silent area of the cerebral cortex, is neither proven nor excluded by current evidence. A bilateral decrease in blood flow, starting in visual association areas and spreading anteriorly, was measured in a patient with MO who was having an attack during a positron emission tomography (PET) scan[27]. Measurements of rCBF in MO patients after several hours from the onset of headache during spontaneous attacks gave conflicting data: an average reduced global rCBF was measured with PET[28], whereas no change in rCBF in the visual cortex was measured with fMRI PERFUSION-WEIGHTED IMAGING[29]. In patients with MA, the hypoperfusion slowly returns towards baseline during the aura phase and the initial part of the headache[29], and patchy regions of increased rCBF sometimes develop after several hours[13]. So, an unchanged average rCBF in the visual cortex of patients with MO several hours after onset of headache does not rule out development of CSD in silent areas of the cortex. Note that the studies mentioned above could all miss the initial phase of hyperaemia during CSD because the time of measurements fall well into the headache phase or might fall in the time between PET scans. However, a recent study that analysed visually triggered attacks in both MA and MO patients showed hyperaemia in the occipital cortex, independently of whether the headache was preceded by visual symptoms[30]. Moreover, bilateral hyperaemia in the occipital cortex, more widespread than expected from the localized nature of the visual symptoms, was measured in a patient with MA during a spontaneous attack[18].

In most patients with MA, the unilateral visual aura is contralateral to the pain and precedes the headache, as expected if CSD activates the TGVS. However, the presence of a small number of MA patients with ipsilateral visual aura and pain, or with aura after the headache, challenges the idea that CSD induces migraine. Imaging studies have also identified a few MA patients that develop headache contralaterally to documented changes in rCBF or preceding rCBF changes[29,30].

Brainstem generator. An alternative view considers migraine aura and headache as parallel rather than sequential processes, and proposes that the primary cause of migraine headache is an episodic dysfunction in brainstem nuclei that are involved in the central control of nociception[3,31]. Two findings have been considered to provide indirect support for this idea. First, placement of electrodes in PAG for the treatment of chronic pain can produce migraine-like headaches in non-migraineurs[32]. Second, rCBF increases in several areas of the dorsal rostral brainstem during migraine attacks[30,33,34]. Although the spatial resolution of the imaging techniques does not allow the distinction of most brainstem nuclei, the foci of maximum rCBF increase, as measured by PET, coincided with the dorsal raphe nucleus and locus coeruleus in patients with MO[33], and with the red nucleus and substantia nigra in a patient with MA during a spontaneous attack[18]. The red nucleus and the substantia nigra were also the sites of maximum rCBF increase in MA and MO patients studied with BOLD fMRI during visually triggered migraine episodes[30]. Animal studies indicate that these brainstem centres might be involved in the central control of nociception and, in particular, in descending mechanisms of pain inhibition[30,35,36]. Several observations have been considered to argue against the interpretation that increased rCBF in the brainstem during migraine attacks results just from pain perception or from increased activity of the endogenous antinociceptive system in response to pain. Increased rCBF in the dorsal rostral brainstem persisted even 30 min after pain relief following treatment with sumatriptan[33,34]. In addition, the pattern of rCBF increases in the brain differs in migraine headache, cluster headache and head pain induced by subcutaneous capsaicin in the forehead, even though all of these headaches involve activation of the first division of the trigeminal nerve[37,38]. On the basis of these observations, it has been proposed that the increased brainstem rCBF during migraine attacks might indicate defective activity that would either trigger the migraine headache (brainstem generator of migraine) or contribute to central hyperexcitability of trigeminal pathways[3,4].

As the brainstem generator hypothesis provides no clear answer to the crucial question of how the trigeminovascular afferents become activated as a consequence of brainstem dysfunction, a permissive role of dysfunctional brainstem nuclei seems more likely[4]. A defect of descending antinociceptive activity could result in decreased inhibition of TNC neurons, making them more susceptible to activation by the TGVS and to sensitization. However, activation in brainstem nuclei that are involved in the central control of nociception was not observed in all patients, at least in visually triggered migraine[30]. Moreover, activation in the red nucleus and substantia nigra was short lived and subsided before the end of aura symptoms in a spontaneous attack of MA[18].

MAGNETOENCEPHALOGRAPHY
A non-invasive technique that allows the detection of the changing magnetic fields that are associated with brain activity. As the magnetic fields of the brain are very weak, extremely sensitive magnetic detectors known as superconducting quantum interference devices, which work at very low, superconducting temperatures (–269 °C), are used to pick up the signal.

FOS
An immediate early gene that is rapidly turned on when many types of neuron increase their activity. It can therefore be used to identify responsive neurons.

PERFUSION-WEIGHTED IMAGING
Imaging technique in which the magnetic resonance signal is intrinsically sensitive to the presence and rate of blood perfusion. It commonly involves the intravenous injection of a bolus of a contrast agent, and the subsequent imaging of the changes in signal intensity as the contrast agent first passes through the brain.

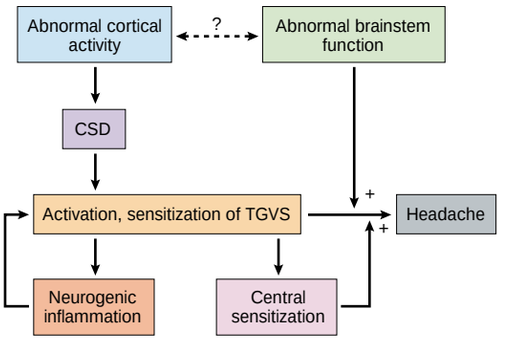

Figure 3 | **Proposed pathophysiological mechanisms in the generation of migraine headache.** Current evidence indicates that cortical spreading depression (CSD) is the most probable primary event in trigeminovascular system (TGVS) activation in migraine with aura and, perhaps, also migraine without aura. Dysfunctional brainstem nuclei involved in the central control of pain might exert a permissive role by favouring central trigeminal hyperexcitability. Abnormal cortical activity might lead to CSD when enhanced activation coincides with other triggering factors. The relationship between abnormal cortical activity and abnormal brainstem function remains hypothetical and unclear.

These observations seem inconsistent with a necessary dysfunction of the brainstem. Moreover, the involvement in descending inhibition of trigeminal activity evoked by dural stimulation has only been shown for the ventrolateral PAG[5,39], and analgesia induced by central stimulation was obtained in humans only from the thalamus and PAG[35].

In summary, in our view, the available experimental evidence points to CSD as the key event for episodic activation of the TGVS, resulting in migraine headache. Dysfunction of brainstem nuclei that are involved in the central control of pain might exert a permissive role by favouring central trigeminal hyperexcitability (FIG. 3).

If we accept the importance of CSD, then the central question becomes: what makes the cortex of migraineurs susceptible to CSD?

Altered cortical excitability in migraineurs. As transient synchronized neuronal excitation precedes CSD[21], changes in cortical excitability must underlie the migraine attack. Independent evidence for altered neuronal excitability in migraineurs emerges from TRANSCRANIAL MAGNETIC STIMULATION (TMS), recordings of cortical potentials and psychophysics.

MO and MA patients show more visual discomfort and illusions than control subjects when shown appropriate visual stimuli, and such stimuli can induce migraine attacks. These abnormalities probably involve visual cortex dysfunction compatible with hyperexcitability, especially in MA patients[40]. When applying TMS to the visual cortex, most[41–43], but not all[44] authors have found either a decreased threshold to produce PHOSPHENES or a higher fraction of people reporting phosphenes in MA and MO. This INTERICTAL visual cortex hyperexcitability is probably due to reduced intracortical inhibition in MA, as determined by repetitive TMS[41,43]. Controversial results regarding cortical hyperexcitability have been obtained after TMS motor cortex stimulation[45].

Recordings of evoked potentials or EVENT-RELATED POTENTIALS also indicate altered cortical processing in migraineurs. Interictal changes of evoked potentials elicited by visual, auditory and somatosensory stimulation have been reported in MA and MO, although with some inconsistencies concerning changes in amplitude and latency (see REF. 43 for review). An elevated negativity of the initial component of the so-called CONTINGENT NEGATIVE VARIATION (iCNV) was found in a large proportion of adult and juvenile MO patients[46–49]. Interestingly, such a negativity is already elevated at early ages in children with migraine[49], indicating that it is probably not a consequence of the disease. It is under debate whether the enhanced negativity of evoked potentials averaged from a series of repetitive measurements is, at least in part, the result of decreased habituation[50]. Habituation of evoked potentials — whereby their amplitudes decrease and their latencies increase with repetitive stimulation — can be shown in healthy subjects. By contrast, habituation to repeated stimuli can be decreased or absent in patients with MO or MA. For example, a habituation deficit has been consistently shown for visual, auditory and somatosensory-evoked potentials, and for the P300 POTENTIAL and the iCNV[43,51,52]. Habituation of responses to olfactory and auditory stimuli occurs more rapidly in cortical neurons than in first- or second-order neurons[53]. It is therefore possible that the observed habituation deficits reflect cortical dysfunction and are consistent with cortical hyperexcitability. Lack of habituation could contribute to the enhanced susceptibility of many migraineurs to sensory stimuli. Another consistent finding in migraineurs is a higher intensity dependence of auditory cortical evoked potentials[43,54,55], which might reflect hyperexcitability of the auditory cortex.

Most interestingly, many of the described abnormalities of evoked potentials and their habituation can return to normal during migraine attacks. Normalization of P300 latency habituation during MO attacks was preceded by an increased habituation deficit until the last measurement (about four days before the attack)[56]. Increased iCNV negativity abruptly decreased and the habituation deficit disappeared during an attack with a tendency for iCNV negativity and habituation deficit to reach a maximum the day before the attack[43,57]. Parallel changes in the electroencephalographic (EEG) power spectrum were observed[58]. These observations support a phenomenon of 'neurophysiological periodicity'[58] — periodic changes of cortical excitability that might lead to an attack when enhanced activation coincides with other precipitating stimuli. This phenomenon might also contribute to premonitory symptoms such as changes in mood, vigilance and appetite up to 24 hours before the attacks.

A reduced preactivation level[59] of sensory cortices such that sensory stimuli do not reach the level for activation of habituation as a protective mechanism has been proposed as an alternative explanation for the habituation deficit in migraineurs[59]. Consistent with this

TRANSCRANIAL MAGNETIC STIMULATION
A technique that is used to induce a transient interruption of normal activity in a relatively restricted area of the brain. It is based on the generation of a strong magnetic field near the area of interest, which, if changed rapidly enough, will induce an electric field that is sufficient to stimulate neurons.

PHOSPHENES
Luminous perceptions that are elicited by excitation of the retina by means other than light itself, as when the eyeballs are pressed through closed lids.

INTERICTAL
Refers to events that occur between attacks or paroxysms.

EVENT-RELATED POTENTIALS
Electrical potentials that are generated in the brain as a consequence of the synchronized activation of neuronal networks by external stimuli. These evoked potentials are recorded at the scalp and consist of precisely timed sequences of waves or 'components'.

CONTINGENT NEGATIVE VARIATION
A small electroencephalographic potential that is often recorded as subjects perform expectation- or attention-dependent tasks. It is also known as the expectation or E wave.

P300 POTENTIAL
A positive waveform in the electroencephalogram that occurs about 300 ms after the onset of a stimulus, and is related to the attentional and working memory demands of a task.

hypothesis is the normalization of habituation in migraineurs by high-frequency repetitive TMS stimulation, which is known to excite the cortex and could normalize preactivation levels[59]. Sensory cortices are under the control of noradrenergic, cholinergic and serotonergic (5-hydroxytryptamine- or 5-HT-mediated) inputs[60]. Noradrenergic (from the locus coeruleus) and cholinergic (from the nucleus basalis) inputs enhance arousal and attention, and lead to EEG activation in the neocortex. 5-HT-containing projections from the dorsal raphe can decrease cortical excitability[61]. This raises the important question of whether migraine-associated abnormalities in evoked potentials and cortical excitability are related to altered control by subcortical modulatory systems.

Alterations of 5-HT, noradrenaline, adrenaline and dopamine levels in plasma or cerebrospinal fluid, and of sympathetic function parameters in migraineurs have been reported, but the data are often conflicting and their pathophysiological relevance remains unclear. A more consistent finding is a reduction of platelet 5-HT during attacks in MO patients[56,62]. However, no correlation was found between platelet or plasma 5-HT content and the interictally abnormal habituation of P300 latency, which became normal in the attack[56].

The literature on cortical excitability in migraineurs is controversial, prone to several interpretations, and muddied by both methodological problems and confounding variables of the disease itself (such as age, disease duration, attack frequency and neurophysiological periodicity). However, most of the consistent findings point to hyperexcitability and enhanced responsiveness of the cerebral cortex to external stimuli in both MA and MO patients. The cyclic changes in cortical activity might render the cortex vulnerable to attacks and lead to initiation of CSD when enhanced activation coincides with other triggering factors (FIG. 3).

The mechanisms that underlie the cortical hyperexcitability and its periodicity remain unknown and might be multifactorial. Excessive excitation due to abnormal release of excitatory neurotransmitters is a possibility that is supported by the higher plasma concentration of glutamate in migraineurs[63] and by the alterations in Ca^{2+} channel function produced by FHM mutations (see later). Alternatively, hyperexcitability might be due to reduced intracortical inhibition. Although controversial[64,65], low brain Mg^{2+} and altered brain energy metabolism would also favour CSD. It remains unclear to what extent and how monoaminergic projections from brainstem nuclei contribute to the altered cortical excitability. The extent to which some of the cortical and/or subcortical alterations are affected by repetitive CSD is also not clear, as CSD produces long-lasting changes in gene expression[66] and might affect subcortical structures[67]. Moreover, a reduction of PAIRED-PULSE DEPRESSION in cortical slices after repetitive CSD has been reported[68].

PAIRED-PULSE DEPRESSION
When two depolarizing stimuli are delivered in close succession to a group of axons, their average response to the second one is sometimes smaller than to the first. This form of short-term plasticity is more common at inhibitory than at excitatory synapses.

Pain mechanisms

Two main pain mechanisms have been considered: neurogenic inflammation of the meninges, and peripheral and central trigeminal sensitization.

Neurogenic inflammation. In animal models, the highly effective triptan antimigraine drugs (5-$HT_{1B/1D/1F}$ receptor agonists) inhibit release of vasoactive neuropeptides from trigeminovascular nerve endings, and both neurogenic plasma extravasation and vasodilation in the meninges. They also inhibit transmission of nociceptive impulses to second-order neurons of the trigeminocervical complex[69]. So, their pharmacology is of limited use in trying to discriminate between the different pain mechanisms. On the other hand, effective inhibitors of plasma extravasation in animal models (such as neurokinin-1 (NK-1), endothelin ET-A/B receptor antagonists or conformationally restricted triptan analogues) with no effect on transmission in the trigeminocervical complex lack clinical efficacy in the treatment of migraine[69]. Substance P was not found to be increased in the cranial venous circulation during migraine attacks[6], and it is not even clear whether plasma extravasation occurs in humans during migraine[70].

CGRP, the main neuropeptide that is released by activated trigeminovascular afferents during migraine[6,7], is a potent vasodilator. Neurogenic vasodilation produced by CGRP might further stimulate the nociceptive afferents and contribute to pain. In rats, vasodilation of the middle meningeal artery produced by CGRP infusion does not activate the second order neurons in the TNC, but does cause the sensitization and the facilitation of non-nociceptive central sensory transmission that is mediated by these cells[71]. CGRP infused intravenously in migraineurs dilates the middle cerebral artery (MCA) and generates a delayed headache with most of the characteristics of migraine[72]. However, sildenafil induces migraine without dilation of the MCA[11], and there is no clear evidence for significant dilation of the MCA during migraine attacks[10]. Unlike the 5-$HT_{1B/1D/1F}$ agonists, the 5-HT_{1F} selective agonist LY334370 lacks vasoconstrictive effects and does not inhibit CGRP-mediated neurogenic dural vasodilation in guinea pigs[73], but shows clinical efficacy in phase 2 clinical trials[74]. The ability of LY334370 to inhibit TNC neurons that respond to dural stimulation[75] indicates that inhibition of nociceptive transmission to second order neurons might be the key mechanism for the antimigraine effect of triptans.

In summary, the current evidence indicates that, if present in humans, neurogenic inflammation might not be sufficient to produce pain in migraine.

Sensitization. It has been indicated that the typical throbbing pain of migraine, and its worsening after coughing or other normally innocuous activities that increase intracranial pressure, might be due to increased responsiveness (sensitization) of trigeminovascular afferents to mechanical stimuli. Indeed, chemical stimuli such as K^+, protons or inflammatory agents applied to the rat dura activate the trigeminovascular afferents and sensitize them to mechanical stimuli[22]. Interestingly, CSD leads to an increased extracellular concentration of many of these sensitizing substances[66].

Evidence for sensitization of second-order trigeminal neurons during migraine attacks comes from recordings

of nociception-specific blink reflex responses[76], and from the presence in most migraine patients of cutaneous ALLODYNIA within and outside the referred pain area in the periorbital region[77]. Periorbital allodynia was interpreted as a reflection of the sensitization of trigeminal dorsal horn neurons receiving convergent input from the meninges and the periorbital skin; allodynia outside the referred pain area was interpreted as a reflection of sensitization of third-order thalamic trigeminal neurons. After chemical stimulation of the rat dura, TNC neurons receiving convergent input from dura and skin showed long-lasting (up to 10 h) increases in cutaneous mechano- and thermosensitivity, and sensitivity to dura indentation[78]. The gradual spatial and temporal spread of allodynia and its expression are consistent with the idea that initiation of central sensitization depends on the incoming impulses from trigeminovascular nociceptors[79]. Instead, maintenance of central sensitization seems to be independent of the afferent input from sensitized nociceptors, as indicated by the fact that anaesthetic block of the primary dural afferent after chemical stimulation of the dura did not inhibit the long-lasting cutaneous hypersensitivity in rats[78].

Activity-dependent plasticity in dorsal horn neurons[80] and/or alterations of endogenous central pain modulatory pathways, including the PAG[5], are plausible hypothetical mechanisms for the maintenance of central sensitization. Trigeminal hyperexcitability might persist between migraine attacks, as indicated by measurements of nociceptive corneal reflex[81] and trigeminal event-related potentials elicited by selective stimulation of nasal nociceptors[82].

An important role for nitric oxide in migraine has been indicated by the finding that intravenous infusions of glyceryl trinitrate (an exogenous nitric oxide donor) produced a delayed headache in migraineurs that was indistinguishable from a spontaneous migraine attack. Moreover, nitric oxide synthase (NOS) inhibitors improve headache pain scores in spontaneous attacks of migraine[83]. Animal experiments with either an exogenous nitric oxide donor or NOS inhibitors provide evidence for a role of nitric oxide in mediating activation and/or sensitization of the TGVS after dural stimulation[23,84,85], and in mediating central sensitization of TNC neurons receiving dural input[86–88]. The specificity of the effect of systemically applied nitric oxide donors on TNC neurons that receive dural input indicates a possible indirect effect through nitric oxide stimulation of TGVS that leads to prolonged activation of the neuronal NOS in TNC and initiates central hyperexcitability.

Familial hemiplegic migraine

The main symptoms of headache and aura (as well as the accompanying symptoms of nausea, photophobia and phonophobia) of FHM attacks are very similar to those of MA, and both types of attack might alternate in patients and co-occur within families. FHM is characterized by obligatory motor aura symptoms that consist of motor weakness or paralysis, which is often, but not always, unilateral. Three or four aura symptoms are nearly always present in FHM attacks, usually in the order: visual, sensory, motor and aphasic symptoms, and they last longer than in MA[89]. By contrast to other types of migraine, some FHM patients can have atypical severe attacks with impairment of consciousness (coma) and/or prolonged hemiplegia that lasts several days. Moreover, about 20% of FHM families show permanent cerebellar symptoms of progressive cerebellar ATAXIA and/or NYSTAGMUS[90].

FHM is genetically heterogeneous. Mutations in *CACNA1A* (chromosome 19p13), the gene coding for the pore-forming α_1-subunit of $Ca_V2.1$ — the voltage-gated P/Q-type Ca^{2+} channel — are associated with the so-called type 1 FHM (FHM1), and are found in about 50% of families tested, comprising all the families with cerebellar symptoms and most of those with coma[91–93]. Recently, MISSENSE MUTATIONS in *ATP1A2* (chromosome 1q23), the gene encoding the α_2-subunit of the Na^+/K^+ ATPase, were found in two FHM families[94], defining the so-called type 2 FHM (FHM2). The role of *CACNA1A* in common forms of migraine is under debate. LINKAGE and SIB-PAIRS ANALYSES are consistent with the involvement of the *CACNA1A*-encompassing region of chromosome 19, especially in MA[95,96], but a gene other than *CACNA1A* is probably involved[1,97].

$Ca_V2.1$ channels: localization and function. $Ca_V2.1$ channel expression is almost exclusively restricted to neuronal and neuroendocrine (such as pituitary and pancreatic β) cells. $Ca_V2.1$ channels are located in presynaptic terminals and somatodendritic membranes throughout the brain[98], and have a prominent role in controlling neurotransmitter release. In many central synapses, they are preferentially located at the release sites and are more effectively coupled to neurotransmitter release than are other Ca^{2+} channel types[99–101]. Binding of $Ca_V2.1$ channels to SNARE PROTEINS contributes to this preferential localization[100]. The somatodendritic localization of $Ca_V2.1$ channels points to additional postsynaptic roles in, for example, neural excitability[102–104] and gene expression[105].

The expression of $Ca_V2.1$ channels is particularly high in the mammalian cerebellum[98,106], where P/Q channels account for most of the Ca^{2+} current in Purkinje cells and a large fraction of the current in granule cells[107–109], and where they have a predominant role in both excitatory and inhibitory neurotransmission[99,110–112]. Moreover, in the cerebellum, this type of channel is postsynaptically involved in spike generation, signal processing, neural excitability, plasticity and survival[104,113,114]. Mice with a null mutation in the *Cacna1a* gene show severe cerebellar ataxia and DYSTONIA, together with selective progressive cerebellar degeneration[114,115]. Different mouse strains with spontaneous $Ca_V2.1\alpha_1$ mutations suffer from ataxia and show reduced P/Q-type currents in Purkinje cells (see REF. 93 for review). About half of the $Ca_V2.1\alpha_1$ mutations that are linked to FHM also cause progressive cerebellar symptoms. In humans, other neurological disorders with cerebellar dysfunction, such as episodic ataxia type 2 and spinocerebellar ataxia type 6 (REFS 91,116), are caused by $Ca_V2.1\alpha_1$ mutations.

ALLODYNIA
The perception of a stimulus as painful when previously the same stimulus was reported to be non-painful.

ATAXIA
Lack of movement coordination that is commonly associated with cerebellar damage.

NYSTAGMUS
An involuntary, rapid and rhythmic movement of the eyeball.

MISSENSE MUTATIONS
Mutations that result in the substitution of an amino acid in a protein.

LINKAGE ANALYSIS
An analysis of the frequency of co-inheritance of a pair of genetic markers to obtain an index of their physical proximity on a chromosome.

SIB-PAIRS ANALYSIS
A means to establish whether affected siblings have the same allele at a particular locus.

SNARE PROTEINS
A family of membrane-tethered coiled-coil proteins that are required for membrane fusion in exocytosis (such as during neurotransmitter release) and other membrane transport events. When trans-SNARE complexes are formed between vesicle SNAREs and target-membrane SNAREs, they pull the two membranes close together, presumably causing them to fuse.

DYSTONIA
The occurrence of dyskinetic movements due to alterations of muscle tone.

$Ca_V2.1$ channels are expressed in all structures that are known to have an important role in the pathogenesis of migraine and/or the expression of the migraine pain. In the cerebral cortex, $Ca_v2.1$ channels are located in the soma, dendrites and synaptic terminals of most neurons[98,117]. They account for about one third of the Ca^{2+} current in dissociated cortical neurons[107,118] and for the largest fraction of the action potential-evoked Ca^{2+} influx in single boutons of layer 2–3 pyramidal cells, where they also mediate almost 40% of the action potential-evoked Ca^{2+} influx in dendritic spines and shafts[119]. P/Q channels contribute to the regulation of the firing behaviour (in particular SPIKE-FREQUENCY ADAPTATION) of cortical neurons through activation of Ca^{2+}-activated K^+ channels and the generation of afterhyperpolarization[103]. Release of glutamate from cortical neurons depends predominantly on P/Q-type channels[101,120], whereas the release of GABA (γ-aminobutyric acid) depends mostly on the N-type, with a secondary role for the P/Q-type at some synapses[117]. In *LEANER* MICE, with a $Ca_V2.1$ mutation that reduces the channel open probability and that shifts its activation curve to more depolarized voltages[121,122], a strong decrease in glutamate and almost no change in GABA release was measured in the neocortex by *in vivo* microdialysis[123]. Interestingly, this mouse also showed a striking elevation in the threshold for initiating CSD, and a slower velocity and frequent failure of propagation of CSD[123]. These data show the importance of P/Q channels in the initiation and spread of CSD, and support the conclusion that reduced Ca^{2+} entry through $Ca_v2.1$ channels reduces neuronal cortical network excitability and makes the cortex more resistant to CSD (see also REF 124).

There is evidence for the localization of $Ca_V2.1$ channels in brainstem nuclei that are involved in the central control of nociception, including the PAG, dorsal raphe and raphe magnus[125,126]. P/Q-type channels account for 30–40% of the Ca^{2+} current in PAG[127,128], dorsal raphe[129], caudal raphae[102], locus coeruleus[130] and substantia nigra[131], and contribute to the generation of AHP and to the regulation of firing in caudal raphe neurons[102]. In the rat model of TGVS activation, blockade of P/Q-type channels in the ventrolateral PAG facilitates trigeminal nociception (as inferred from the firing rate of nociceptive TNC neurons that receive inhibitory projections from PAG), pointing to a role of $Ca_V2.1$ channels in the descending inhibitory system that regulates the perception of pain[132].

P/Q-type channels account for a large proportion (40%) of the Ca^{2+} current of dissociated trigeminal ganglion neurons[133] and, together with N-type channels, they control CGRP release from capsaicin-sensitive trigeminovascular afferents[134]. The sequiterpene α-eudesmol — a slightly selective P/Q-type blocker — inhibits neurogenic vasodilation in facial skin and plasma extravasation in the dura after electrical stimulation of the trigeminal ganglion *in vivo*[135]. It remains unknown whether P/Q channels are involved in neurotransmitter release from trigeminovascular afferent terminals in the TNC, but there is evidence for localization of $Ca_V2.1$ channels in a small number of cells in the spinal trigeminal nucleus[126]. In the dorsal horn of the spinal cord, $Ca_V2.1$ channels are primarily located in nerve terminals, but show little, if any, co-localization with substance P (REF. 136). In animal models of persistent pain, selective blockade of spinal P/Q channels has disclosed a role for these channels in the initiation (but not the maintenance) of central sensitization, possibly through their control of glutamate release from excitatory interneurons[137]. P/Q channels account for 50% of the Ca^{2+} current in dissociated spinal interneurons[107], and have an important role in controlling release from inhibitory spinal interneurons[137,138]. *Leaner* mice show enhanced acute thermal nociceptive responses, proposed to be due to impaired presynaptic inhibition by GABA interneurons but reduced acute mechanical nociceptive responses[139].

Functional consequences of FHM mutations. At least fourteen different missense mutations in CACNA1A have been reported to be associated with FHM1 (30 families, 4 sporadic cases). All of these mutations result in substitutions of conserved amino acids in important functional regions of the channel, including the pore-lining segments and the voltage sensors (FIG. 4a)[92,93]. Symptom variability between subjects with the same mutation indicate that other genetic or environmental factors also influence the phenotype. Pure FHM1, and FHM1 with cerebellar symptoms (FHM1+PCA), are associated with distinct mutations[90]. A strong correlation between the frequent T666M mutation and the FHM1+PCA phenotype was found. T666M also showed the highest PENETRANCE of FHM (98%) and of incidence of severe attacks with coma (50%)[90]. The S218L mutation was found in patients from two families with severe cerebral oedema and coma triggered by minor head trauma[140]. Other variable neurological symptoms were present, including typical FHM attacks and progressive ataxia, which perhaps place these patients at the far end of the migraine spectrum[92].

The functional consequences of FHM1 mutations on recombinant $Ca_V2.1$ channels have been investigated in heterologous expression systems[141–144] and, more recently, in neurons from *Cacna1a*$^{-/-}$ mice expressing human $Ca_V2.1\alpha_1$ subunits[144]. The seven FHM1 mutations that have been analysed (FIG. 4a) alter both the single-channel biophysical properties and the density of functional channels in the membrane. A common functional effect of FHM1 mutations is to shift the activation curve of $Ca_V2.1$ channels to more hyperpolarized voltages, therefore increasing their open probability, over a broad voltage range. The increase in open probability is more than enough to compensate for the reduction in unitary current and conductance that is produced by some mutations. So, a common functional effect of the FHM1 mutations is to increase Ca^{2+} influx through single human $Ca_V2.1$ channels over a large voltage range[144]. Moreover, Ca^{2+} influx through mutant channels can occur in response to small depolarizations that are insufficient to open wild-type channels. The FHM1 mutations also affect the kinetics of inactivation of $Ca_V2.1$ channels, but the effects are variable and result in increased, decreased or unaffected inactivation during a

SPIKE-FREQUENCY ADAPTATION
A decrease in the rate of action potentials fired by a neuron under prolonged depolarization.

LEANER MICE
Mice with mutations in *Cacna1a*. They are characterized by marked cerebellar atrophy that is accompanied by ataxia, wobbly gait and dyskinesia.

PENETRANCE
The probability that an individual with a particular genotype will manifest a given phenotype. Complete penetrance corresponds to the situation in which every individual with the same specific genotype manifests the phenotype in question.

ACTIVE ZONE
A portion of the presynaptic membrane that faces the postsynaptic density across the synaptic cleft. It constitutes the site of synaptic vesicle clustering, docking and transmitter release.

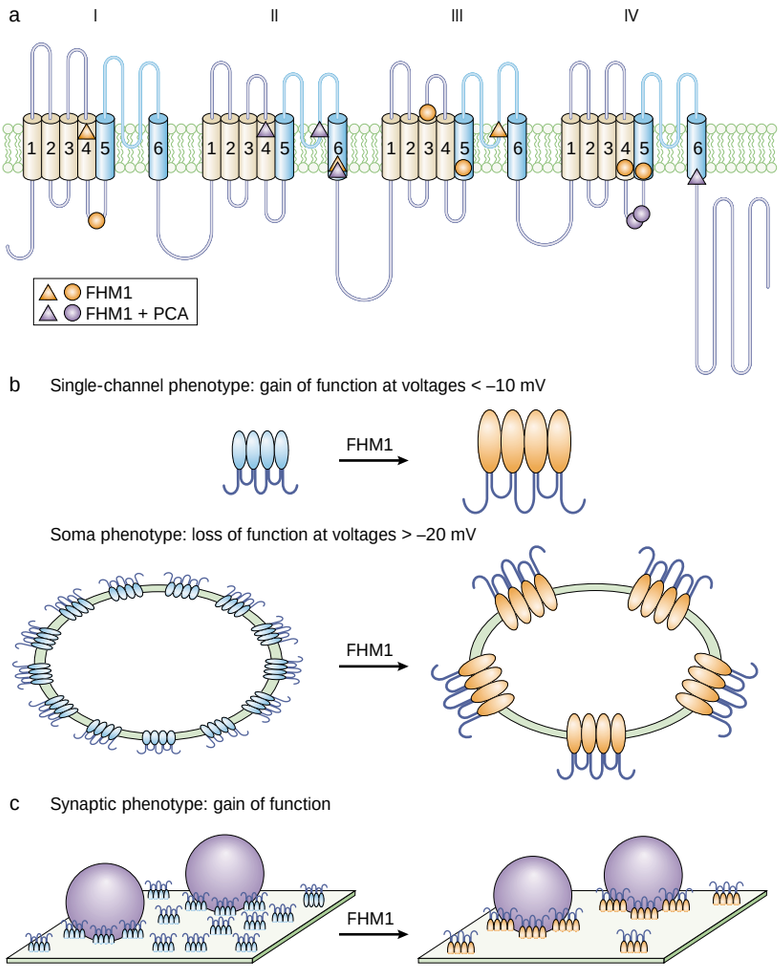

Figure 4 | **Functional effects of type 1 familial hemiplegic migraine (FHM1) mutations on neuronal $Ca_V2.1$ channels. a** | Location of FHM1 (orange) and FHM1 with cerebellar symptoms (FHM1 + PCA; purple) mutations in the secondary structure of the $Ca_V2.1$ α_1-subunit. Triangles indicate mutations, the functional consequences of which have been studied so far. **b** | Functional effects of FHM1 mutations on neuronal $Ca_V2.1$ channels. All FHM1 mutations analysed so far increase Ca^{2+} influx through single $Ca_V2.1$ channels for voltages lower than –10 mV (single-channel gain-of-function phenotype), and decrease the density of functional channels in the somatic membrane and the soma Ca^{2+} current density for voltages higher than –20 mV (soma loss-of-function phenotype). Functional channel complexes with FHM1 mutations (orange) and wild-type channels (blue) are shown. **c** | Synaptic phenotype. $Ca_V2.1$ channel clusters that are associated with synaptic vesicles contain only a few channel complexes. Decreased expression density of mutant channels might not result in a relevant decrease of channel complexes specifically targeted to synaptic vesicles. The more negative activation threshold and increased single channel Ca^{2+} influx of mutant channels might therefore lead to increased action potential-evoked Ca^{2+} influx at the active zones and increased neurotransmitter release in synapses where the Ca^{2+} sensor is not saturated (gain-of-function synaptic phenotype). Among other phenomena, this could explain the enhanced cortical network excitability and, perhaps, lower cortical spreading depression threshold in migraineurs.

KNOCK-IN
The insertion of a mutant gene at the exact site in the genome where the corresponding wild-type gene is located. This approach is used to ensure that the effect of the mutant gene is not affected by the activity of the endogenous locus.

train of pulses, depending on the mutation[141–143]. Whereas FHM1 mutant channels expressed in neurons or HEK293 cells showed similar alterations in single-channel function, the changes in the density of functional channels in the membrane were different in the two cell types[144]. In HEK293 cells, the density of functional channels in the membrane was reduced by most mutations, but was increased by two of them — R192Q and D715E (REFS 142,144 and D.P., unpublished observations). As a consequence, the whole-cell Ca^{2+} current density was either increased or decreased, depending on the mutation. In neurons, the four FHM1 mutations analysed, including R192Q, decreased the density of functional channels in the membrane, together with the maximal $Ca_V2.1$ current density. $Ca_V2.1$ current densities were similar to wild type at lower voltages because of the negatively shifted activation of the FHM1 mutants[144].

Given the two apparently contradictory functional effects that are common to all FHM1 mutations analysed so far (gain of function at the single-channel level for voltages lower than –10 mV; loss of function at the whole-cell level for voltages higher than –20 mV, with 'function' defined as the amount of Ca^{2+} influx in a certain time period), the FHM1 phenotype at the synaptic ACTIVE ZONES might be different from that at the soma[144]. Phasic neurotransmitter release at each release site is controlled by a cluster of only a few Ca^{2+} channels that are located sufficiently close to the Ca^{2+} sensor to contribute to the local Ca^{2+} increase that triggers release in response to single action potentials[145]. Given the preferential localization of $Ca_V2.1$ channels at the release sites in many central excitatory synapses, and their specific interaction with presynaptic proteins, a reasonable prediction is that the number of specialized Ca^{2+} channels at each release site will remain similar in wild-type and mutant synapses, despite a reduced number of mutant channels in the soma. The FHM1 synaptic phenotype would then be mainly determined by the mutational changes in single-channel Ca^{2+} influx, and therefore be a gain-of-function phenotype, characterized by increased action potential-evoked Ca^{2+} influx at the active zones[146] and increased neurotransmitter release in synapses where the Ca^{2+} sensor is not saturated (FIG. 4b). FHM1 mutations (with an opposite synaptic phenotype to the *leaner* mutation) are then expected to increase the release of glutamate in the cortex (leaving that of GABA relatively unchanged), increase neuronal cortical network excitability and make the cortex more susceptibile to CSD.

The gain-of-function synaptic FHM1 phenotype predicts hyperexcitability of nociceptive trigeminovascular pathways, due to enhanced release of vasoactive neuropeptides from perivascular nerve endings and, possibly, facilitation of sensitization of second-order central trigeminal neurons. It is not clear whether the PAG $Ca_V2.1$ channels that are involved in pain inhibition are postsynaptic or presynaptic. Furthermore, the projection of PAG neurons to raphe neurons can cause either excitation or inhibition of TNC second-order neurons. It is therefore difficult to predict the consequences of mutant FHM1 channels on central control of trigeminal nociception. KNOCK-IN mouse models containing human FHM1 mutations will be instrumental in elucidating how alterations of $Ca_V2.1$ channel function cause FHM and its typical episodic symptoms.

Both FHM2 missense mutations recently found in *ATP1A2* cause loss of function of the Na^+/K^+ ATPase[94]. Impaired clearance of K^+ by astrocytes, where expression of the α_2- ATPase isoform is particularly high[147], might

make the cortex more susceptible to CSD. Moreover, the specific co-localization of the α_2-isoform with the Na^+/Ca^{2+} exchanger in microdomains that overlie subplasmalemmal endoplasmic reticulum indicates that this isoform might regulate Ca^{2+} content of this compartment[147]. Its loss of function would lead to increased local intracellular Ca^{2+} and subplasmalemmal endoplasmic reticulum Ca^{2+} content[94].

Implications for new therapeutic strategies

At present, acute migraine attacks are treated with non-steroidal anti-inflammatory drugs (such as acetylsalicylic acid), triptans (5-$HT_{1B/1D/1F}$ agonists) or intranasal dihydroegotamine[4,148]. However, 20–30% of patients do not respond to these therapies and headache recurrence is a common problem. The recommended first-line agents for migraine prevention, including β-adrenergic receptor antagonists, valproic acid, amitryptiline and flunarizine, are also unsatisfactory in many patients[4,148]. So, new therapeutic strategies are needed.

On the basis of our current understanding of the pain mechanisms involved, neither inhibition of neurogenic vasodilation nor inhibition of plasma protein extravasation seem to be the most effective therapeutic strategies. Inhibition of trigeminal nociceptive transmission to second-order neurons, associated sensitization mechanisms and CSD remain as more attractive possibilities.

Pre- and postsynaptic structures might serve as targets for the inhibition of TNC. The receptors that modulate release from central terminals of afferent fibres seem to be good presynaptic targets. GR79236 — an adenosine A1 receptor agonist— inhibits CGRP release and trigeminal nucleus activation after electrical stimulation of the superior sagittal sinus in animals, and has been reported to abort acute migraine attacks in humans[149]. A1 receptor agonists might act as presynaptic inhibitors of central pain transmission.

Presynaptic inhibition could also be achieved by presynaptic Ca^{2+} channel block. Unfortunately, there are no data about the role of different Ca^{2+} channel types for neurotransmitter release at central trigeminal synapses. It is probably controlled by $Ca_v2.2$ channels, in analogy to other segments of the spinal cord. $Ca_v2.2$ blockers had strong analgesic actions in the treatment of neuropathic pain in humans. Systemic toxic effects of such peptide blockers (for example, ziconotide) that are known from clinical studies[150] would prevent their use in migraine. Obviously, new generations of non-peptide, orally bioavailable $Ca_v2.2$ inhibitors with less systemic toxicity would have to be developed for migraine therapy.

There are also postsynaptic targets for inhibiting TNC activation. As ionotropic glutamate receptors mediate nociceptive transmission and central sensitization in the trigeminal system, glutamate receptor antagonists should also have antimigraine effects. Owing to the small therapeutic window of NMDA (*N*-methyl-D-aspartate) receptor antagonists, non-NMDA receptor antagonists have been developed. LY293558 — a non-selective AMPA/kainate receptor antagonist— is clinically well tolerated at intravenous doses that have analgesic actions on post-operative pain in humans and that reduce capsaicin-induced skin hyperalgesia[151,152]. A phase 2 clinical trial in acute migraine indicates that its clinical efficacy might be comparable to that of sumatriptan[153].

Another useful therapeutic strategy would be to reduce excitability and/or sensitization of primary trigeminal afferents. This might be an important mechanism of action of sumatriptan and other triptans if they increase a Ca^{2+}-activated K^+ current and hyperpolarize the trigeminal ganglion cells, as proposed by some authors[154,155]. The clinical efficacy of NOS inhibitors was mentioned earlier. Another idea currently pursued is the desensitization of vanilloid VR1 receptors with continuous application of VR1 agonists[156]. Non-peptide CGRP receptor antagonists will represent important pharmacological tools to show whether CGRP that is released during migraine attacks is just an epiphenomenon that is triggered by trigeminal activation or whether it has an active role in the generation of pain and sensitization.

If increased neuronal hyperexcitability and CSD are important primary events in migraine attacks, then drugs that decrease neuronal hyperexcitability and/or prevent CSD should have antimigraine actions, especially as prophylactic agents. As long as the molecular mechanisms responsible for neuronal hyperexcitability are unclear, therapies will aim at the pharmacological increase of inhibitory neurotransmission, such as with valproic acid[157], or reduction of excitatory neurotransmission.

Inhibition of CSD is not a property of known antimigraine drugs, but could represent an attractive target for new prophylactic strategies. Unfortunately, the mechanisms of CSD initiation and propagation are unclear, although it is known that NMDA receptors are involved[21]. This can explain the efficacy of ketamine in relieving aura in some patients with FHM[158]. Interestingly, tonabersat (SB-220453), a new benzopyran with anticonvulsant properties that acts on an unidentified binding site, was found to block CSD induced by KCl in the feline brain[159]. Clinical studies with this compound should provide valuable information about the role of CSD as a primary mechanism, not only in MA, but also in MO.

On the basis of the alterations in $Ca_v2.1$ channel function in FHM1 and those found in *leaner* mice with increased CSD threshold, one could speculate that drugs capable of shifting the activation range of $Ca_v2.1$ channels to more depolarized voltages might inhibit CSD.

Concluding remarks and future directions

Most of the current evidence points to CSD, the phenomenon that underlies the migraine aura, as the most probable primary cause of activation of the TGVS and consequent headache. Direct evidence that CSD can activate the TGVS has been obtained in animals. Whereas the occurrence of CSD in MA patients has been established, the evidence of its occurrence in MO patients is not so strong, and further imaging data seem to be necessary to verify the hypothesis that CSD in clinically silent areas of the cerebral cortex causes MO. The alternative view that migraine aura and headache are parallel rather than sequential processes also lacks

sufficient experimental support. It remains unclear whether brainstem nuclei that are involved in the central control of nociception are dysfunctional in migraineurs.

The mechanisms for the initiation and propagation of experimental CSD remain incompletely understood, and the molecular and cellular mechanisms that lead to CSD vulnerability in migraineurs remain unknown. The relationship between CSD vulnerability and the periodic alterations in cortical excitability measured in migraineurs is also unclear. Whether the cortex of migraineurs is hypo- or hyperexcitable is still a matter of debate, although most of the consistent findings point to hyperexcitability, and the hyperexcitability hypothesis seems better suited to explain vulnerability to CSD. The mechanisms that underlie the cortical hyperexcitability and its periodicity remain unknown and might be multifactorial. The discovery of causative genes for migraine would be crucial to direct future research trying to answer these fundamental open questions.

The identification of the gene for FHM1 has introduced a new perspective into the area of migraine research by characterizing migraine also as a channelopathy. As most channelopathies are disorders of cellular excitability, this discovery stresses the importance of alterations in neural excitability in the pathogenesis of migraine. Our understanding of the molecular basis of FHM supports the idea that migraine is a multisystem disorder of neuronal hyperexcitability. The alterations in $Ca_V2.1$ channel function that are produced by FHM1 mutations point to cortical hyperexcitability as the basis for CSD vulnerability. In *leaner* mice, loss of $Ca_V2.1$ channel function reduces glutamate release and cortical network excitability, and makes the cortex more resistant to CSD. The opposite gain-of-function single-channel phenotype of FHM1 mutants should increase glutamate release and cortical network excitability, making the cortex more susceptible to CSD. The same effect should result from loss of function of the α_2-isoform of the Na^+/K^+ ATPase that are associated with FHM2. Knock-in mice carrying FHM1 mutations are beginning to become available and will allow verification of these predictions. These mice will be invaluable, not only to understand how the alterations in channel function cause FHM and its typical episodic symptoms, but also to understand the pathophysiology of migraine in general. They will allow us to test the hypothesis that dysfunctional antinociceptive brainstem nuclei are involved in the pathogenesis of migraine headache. Current evidence supports the view that peripheral and central sensitization has a key role in the generation of migraine pain, but the cellular and molecular mechanisms of central sensitization and its maintenance remain largely unknown. The gain-of-function single action phenotype of FHM1 mutants could imply hyperexcitable trigeminal pathways, which would make FHM1 knock-in mice a good model for studying the neurobiology of migraine pain.

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