Transgenic mouse models in migraine

Ven, R.C.G. van de

CHAPTER 1

ONE

General Introduction

1 Introduction

1.1 Migraine

Migraine is a neurological paroxysmal disorder, affecting up to 6% of males and 18% of females in the general population.[1] In the Netherlands, lifetime prevalence is 33% in women and 13.3% in men, with a median attack frequency of 12 per year.[2] Migraine is disabling and is a burden not only for the patient and its family but also for society due to loss of productivity.[3] Patients suffer from throbbing, often unilateral headaches lasting 4 to 72 hours that are accompanied by nausea, vomiting and/or photo- and phonophobia. The classification of the International Headache Society defines different forms of migraine: migraine with (MA) and without aura (MO) (Table 1).[4] Of all migraine patients about one-third suffers from MA[5], characterised by an aura that consists of visual disturbances, but sensory-, motor- or speech-related phenomena can occur as well.

Familial hemiplegic migraine (FHM) is an autosomal dominant subtype of MA with an aura consisting of fully reversible motor weakness (Table 1). The clinical spectrum of FHM is broad.[6,7] Apart from typical FHM, patients may present with associated symptoms like progressive ataxia, epilepsy, fever, reduced consciousness and even coma. There are several good arguments that FHM is part of the migraine spectrum and can serve a as model to study the common forms of migraine.[5] First, the aura and headache symptoms of FHM are identical to those of "non-hemiplegic" migraine and second, the majority of FHM patients also have attacks of MO or MA.

1.2 Genetics of Migraine

1.2.1 Migraine with and without aura

Migraine is a complex disorder where genetic factors play an important role.[8] Several studies of twins indicate a genetic component for migraine, suggesting a higher concordance rate for migraine in mono- than in dizygotic twins.[9-15] Family studies have found an increased genetic load in familial MA and increased relative risk of first-degree family members of MA and MO patients.[16,17]

Until now, no gene has been found for MO and MA using a linkage approach, although loci on chromosomes 4[18,19], 5[20], 6[21], 11[22], 14[23] and 15[24] have been identified. Genes with suggested association with migraine suggest involvement of the dopamine[25-27] and serotonin[28-32] pathways and homocysteine metabolism.[33] However, many association studies are underpowered or are single reports that await replication.

Table 1. International Headache Society Criteria for Migraine

Migraine without aura

- *A.* At least five attacks fulfilling criteria B-D
- *B.* Headache attacks lasting 4 to 72 hours (untreated or unsuccessfully treated)
- *C.* Headache has at least two of the following characteristics:
  1. Unilateral location
  2. Pulsating quality
  3. Moderate or severe pain intensity
  4. Aggravation by or causing avoidance of routine physical activity (e.g., walking or climbing stairs)
- *D.* During headache al least one of the following:
  1. Nausea and/or vomiting
  2. Photophobia and phonophobia
- *E.* Not attributed to another disorder

Migraine with aura

- *A.* At least two attacks fulfilling criteria B-D
- *B.* Aura consisting of at least one of the following, but no motor weakness:
  1. Fully reversible visual symptoms including positive features (e.g., flickeringlights, spots, or lines) and/or negative features (i.e., loss of vision)
  2. Fully reversible sensory symptoms including positive features (i.e., pins and needles) and/or negative features (i.e., numbness)
  3. Fully reversible dysphasic speech disturbance
- *C.* At least two of the following:
  1. Homonymous visual symptoms and/or unilateral sensory symptoms
  2. At least one aura symptom develops gradually over ≥ 5 minutes, and/or different aura symptoms occur in succession over ≥ 5 minutes
  3. Each symptom lasts ≥ 5 and ≤ 60 minutes
- *D.* Headache fulfilling criteria B-D for migraine without aura begins during the aura or follows aura within 60 minutes
- *E.* Not attributed to another disorder

Familial Hemiplegic Migraine

- *A.* At least two attacks fulfilling criteria B and C
- *B.* Aura consisting of fully reversible motor weakness and at least one of the following:
  1. Fully reversible visual symptoms including positive features (e.g., flickering lights, spots, or lines) and/or negative features (i.e., loss of vision)
  2. Fully reversible sensory symptoms including positive features (i.e., pins and needles) and/or negative features (i.e., numbness)
  3. Fully reversible dysphasic speech disturbance
- *C.* At least two of the following:
  4. At least one aura symptom develops gradually over ≥ 5 minutes, and/or different aura symptoms occur in succession over ≥ 5 minutes
  5. Each symptom lasts ≥ 5 and ≤ 24 hours
  6. Headache fulfilling criteria B-D for migraine without aura begins during the aura or follows aura within 60 minutes
- *D.* At least one first- or second-degree relative has had attacks fulfilling these criteria A-E
- *E.* Not attributed to another disorder

1.2.2 Familial hemiplegic migraine

In 1996, mutations were identified in the *CACNA1A* (FHM1) gene causing FHM, episodic ataxia type-2 (EA2)[34] and spinocerebellar ataxia type-6 (SCA-6).[35] To date, many additional mutations have been found (figure 1). The *CACNA1A* gene encodes the α_1-subunit of $Ca_v2.1$ calcium channels. Alternative splicing of *CACNA1A* yields α_1-subunits mediating either P- or Q-type Ca^{2+} currents.[36] $Ca_v2.1$ channels are widely expressed in the central nervous system, including many migraine-related brain regions.[37,38] The channels are expressed particularly high in Purkinje cells and throughout the cerebellum. In the peripheral nervous system the $Ca_v2.1$ channel is mainly expressed at the neuromuscular junction (NMJ) where it mediates acetylcholine (ACh) release, resulting in muscle contraction.[39] In cell bodies, $Ca_v2.1$ channels play a role in excitability, presumably via Ca^{2+}-dependent K^+ channels.[40] Furthermore, Ca^{2+} influx stimulates intracellular signaling pathways, mostly involving kinases, which can influence gene expression. During development, $Ca_v2.1$ channels participate in the process of neurite initiation. At mature synaptic terminals, their main function is to mediate transmitter secretion by allowing Ca^{2+} to stimulate the release-machinery complex at so-called active zones, resulting in exocytosis of synaptic vesicles.[41] The α_1-subunit of the $Ca_v2.1$ channel contains defined sites that interact with specific presynaptic proteins of the vesicle docking/fusion machinery (e.g. SNAP25, syntaxin, synaptotagmin) and structural proteins (e.g. Mint/CASK complex), required for targeted presynaptic localization and specific presynaptic function of $Ca_v2.1$ channels (Figure 1).[42,43] Other specific sites for the interaction with β-subunits and $G_{\beta\gamma}$-protein have been identified.[44]

$Ca_v2.1$ is a member of a larger family of voltage-gated calcium channels. All Ca_v channels regulate Ca^{2+} influx upon membrane depolarization, but the physiological function varies from neurotransmitter and hormone release to muscle contraction. Initially, voltage-gated calcium channels were discriminated using pharmacological and electrophysiological criteria (i.e. N-, P/Q-, L-, R- and T-types) or based on their poreforming α_1-subunit (α_{1A} to α_{1I} and α_{1S}).[45] Recently, a nomenclature was adapted based on structural relationship.[46] Table 2 gives an overview of Ca_v channels and their blockers.

Two additional FHM genes have been identified: *ATP1A2* (FHM2)[47] and *SCN1A* (FHM3).[48] FHM2 encodes the α_2-subunit of Na^+,K^+ pumps, which is expressed in neurons in early development and in astrocytes later in life.[49] The pump transports K^+-ions into the cell and at the same time exports Na^+-ions.[50] Importantly, astrocytic Na^+,K^+ pumps are also essential for the clearance of glutamate and calcium from the synaptic cleft.[51] Functional cellular studies show a deficiency of the mutated Na^+,K^+ pump and

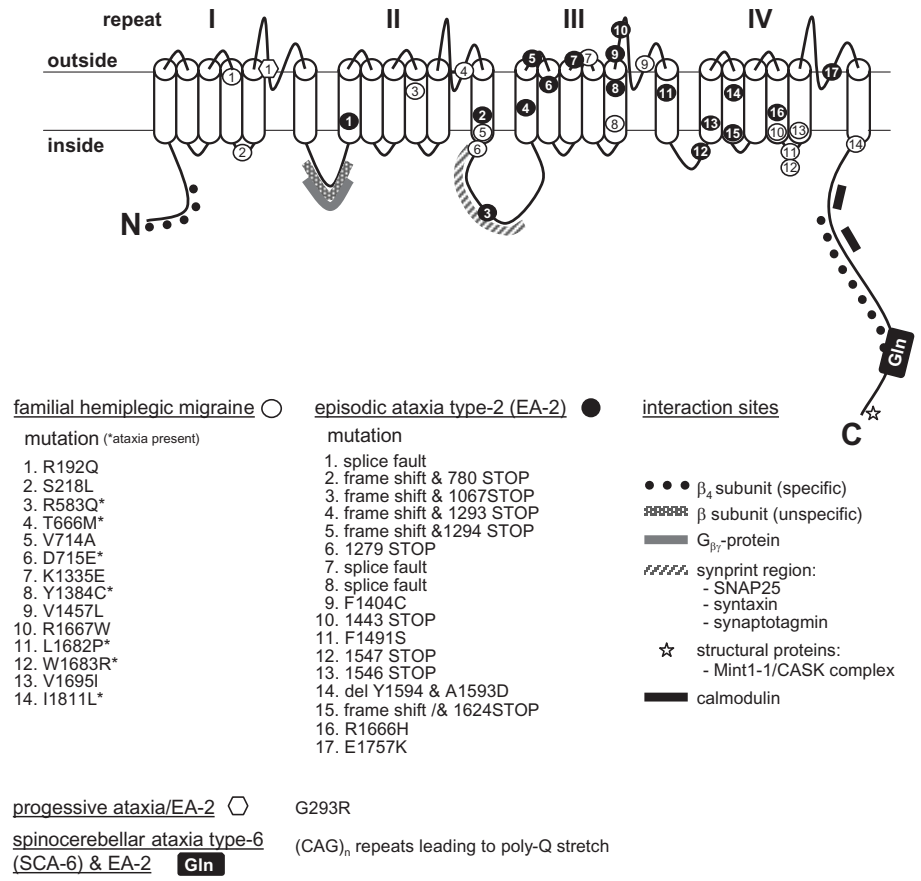

Figure 1. The α_1-subunit of $Ca_v2.1$ with human *CACNA1A* mutations and modulatory protein interaction sites. Adapted from Plomp et *al*., 2001.[40]

Table 2. Ca_v channels and their blockers

Ca_v channel	***Current***	***α_1 subunit***	***Gene***	***Blocker***
$Ca_v1.1$	L-type	α_{1S}	*CACNA1S*	
$Ca_v1.2$	L-type	α_{1C}	*CACNA1C*	Dihydropyridines, Benzothiazapines,
$Ca_v1.3$	L-type	α_{1D}	*CACNA1D*	Phenylalkylamines
$Ca_v1.4$	L-type	α_{1F}	*CACNA1F*	
$Ca_v2.1$	P/Q-type	α_{1A}	*CACNA1A*	ω-Agatoxin-IVA, ω-Conotoxin-MVIIC
$Ca_v2.2$	N-type	α_{1B}	*CACNA1B*	ω-Conotoxin-GVIA, ω-Conotoxin-MVIIC
$Ca_v2.3$	R-type	α_{1E}	*CACNA1E*	SNX-482
$Ca_v3.1$	T-type	α_{1G}	*CACNA1G*	
$Ca_v3.2$	T-type	α_{1H}	*CACNA1H*	Mibefradil, Kurtoxin, Amiloride
$Ca_v3.3$	T-type	α_{1I}	*CACNA1I*	

thus suggest a *loss-of-function* effect of FHM2 mutations.[47,52]

FHM3 encodes the pore-forming α_1-subunit of voltage-gated neuronal $Na_v1.1$ sodium channels.[48] $Na_v1.1$ sodium channels are expressed in cortical neurons where they are crucial for the generation and propagation of action potentials. One FHM3 mutation has been identified resulting in a more rapid recovery from fast inactivation of sodium channels, a physiological phenomenon that follows the depolarization-induced channel activation and inactivation. The effect of the mutation can lead to a higher frequency of neuronal firing, possibly leading to enhanced neuronal excitability and neurotransmitter release.

From the function of the FHM genes and the effect of FHM mutations, one can hypothesize that increased susceptibility to FHM, and perhaps common migraine, arises from disturbed ionic balance and concomitantly increased release of excitatory neurotransmitter glutamate.[53] In case of FHM1 and FHM3 this may be because of an enhanced release of glutamate due to increased synaptic vesicle release and neuronal firing rate, respectively, and in case of FHM2 due to a decreased clearance of glutamate and extracellular K^+ by astrocytes.

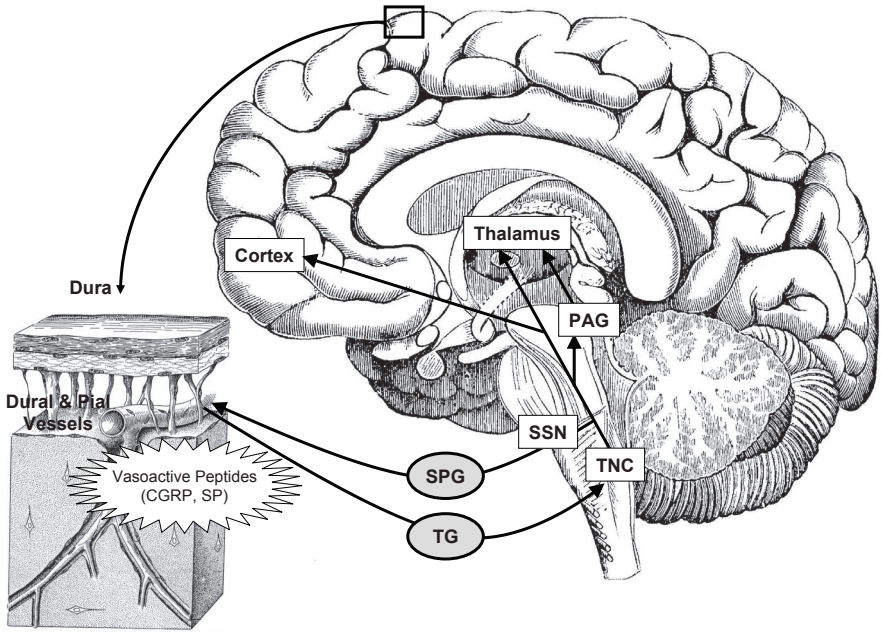

Figure 2. The trigeminovascular system (TGVS) and its projection to higher order pain centers. The main event underlying the migraine headache is the activation of the TGVS. Upon stimulation of pial and dural nerve endings, the trigeminal nucleus caudalis (TNC) and the superior salivatory nucleus (SSN) in the brainstem become activated via the trigeminal ganglion (TG) and sphenopalatine ganglion (SPG), respectively. From the TNC and SSN the pain information is projected further to brain regions involved in the modulation and perception of pain, such as the periaqueductal grey (PAG) and higher order pain centres like the thalamus and cortex.

1.3 Pathophysiology of Migraine

1.3.1 The migraine headache

The main event underlying the migraine headache is the activation of the trigeminovascular system (TGVS).[54] The TGVS consists of nerve endings originating from the ophthalmic branch of the trigeminal nerve that innervate the blood vessels of the meninges, making it - in contrast to the brain itself – pain sensitive. The trigeminal nerve projects via the trigeminal ganglion to the trigeminal nucleus caudalis (TNC) in the brainstem. Meningeal blood vessels are also innervated by neurons that project to the superior salivatory nucleus (SSN) in the brainstem via the sphenopalatine ganglion. From the TNC and SSN the pain information is projected further to brain regions involved in the modulation and perception of pain, such as the periaqueductal grey (PAG) and the thalamus (Figure 2).

The cause of TGVS activation in migraine is unknown. Experimental activation of the TGVS in animals leads to release of vasoactive neuropeptides from meningeal trigeminal nerve endings, like calcitonin gene-related peptide (CGRP) and substance P.[55] These neuropeptides cause vasodilatation of the meningeal vessels (mainly due to CGRP), plasma extravasation and mast cell degranulation with secretion of proinflammatory substances in the dura (neurogenic inflammation).[55] It has been proposed that stimulation of meningeal afferent nociceptors (e.g. by inflammatory substances) leads to central and peripheral sensitization, resulting in symptoms of cutaneous allodynia (i.e. pain resulting from a non-noxious stimulus to normal skin) and intracranial hypersensitivity (e.g. throbbing headache) during a migraine attack.[56]

1.3.2 The migraine aura

A typical migraine aura is a scintillation-scotoma consisting of characteristic fortification figures.[57] Usually, the disturbance starts at the center of the visual field, propagates to the peripheral parts within 15 minutes and diminishes within another 15 minutes. Imaging studies show that during the migraine aura a wave of spreading hyperaemia lasting ~4 minutes progresses along the cortex at a rate of approximately 3.5 mm min^{-1} and is followed by mild hypoperfusion lasting 1–2 hours.[58,59] These features are strikingly similar to those of cortical spreading depression (CSD), a self-propagating wave of neuronal depolarisation that progresses over the cortex at the same rate of ~2-3 mm min^{-1} and is followed by a long-lasting transient depression of neural activity.[60] It is now generally accepted that CSD is the underlying mechanism of the migraine aura.

It is unclear whether CSD can activate the TGVS in humans and is the initiating event

of the migraine headache. Recently, it was shown in rats that CSD induces an increase in blood flow in the middle meningeal artery, spreading cortical hyperaemia, dural plasma protein extravasation and neuronal activation of the caudal TNC.[61] In addition, CSD induces blood-brain-barrier (BBB) permeability by activation and up-regulation of the matrix metalloproteinase MMP-9, which belongs to the class of metalloproteinases that are capable of degrading matrix components.[62] At this moment it is unknown if oedema formation that has been observed in imaging studies during an FHM attack[63-65] is the equivalent of plasma protein extravasation as seen in animal migraine models after TGVS activation.[55] These results support the hypothesis that CSD, also in humans, is able to activate the TGVS, possibly inducing headache. On the other hand, one study did not show plasma extravasation and rostral TNC activation upon CSD.[66] In addition, it was shown that anti-migraine drugs have no effect on CSD propagation and initiation.[67] The fact that most migraine patients do not experience a migraine aura argues against CSD as the initiating event of migraine headache. It may however be possible that CSD occurs in these patients in brain regions other than the visual cortex.

1.3.3 Involvement of the brainstem

During MA, increased blood flow was identified in the brainstem by positron emission tomography (PET) and fMRI studies.[68-70] This increase persisted after headache relief with the serotonin receptor agonist sumatriptan, suggesting involvement of the dorsal raphe nucleus, locus coeruleus and PAG.[69] An involvement of the brainstem is further supported by the fact that a) lesions in the brainstem can cause migraine[71-73], b) electrical stimulation of the brainstem can cause headache[74,75], and c) migraineurs have an increased iron deposition in the brainstem PAG, possibly due to a high metabolic activity in migraine.[76]

1.3.4 $Ca_v2.1$ channels in migraine pathophysiology

The functional consequences of at least 12 FHM1 mutations have been investigated with patch clamp techniques (details discussed below), in heterologous expression systems (*Xenopus* oocytes and HEK293 cells) and in neurons isolated from $Ca_v2.1$-α_1 knockout mice in which human or mutant *CACNA1A* and auxilliary subunit cDNA's are overexpressed (for review see Plomp et al, 2001 and Pietrobon, 2005).[40,77] Eight FHM1 mutants showed an enhanced single-channel Ca^{2+} influx over a broad voltage range, reflecting an increased channel open probability, mainly due to a shift in channel activation to lower voltages.[78-80] Also consistent shifts to lower voltages of the current activation of whole-cell currents were found in heterologous expression systems and

transfected neurons.[78-81] 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. Moreover, Ca^{2+} influx through mutant channels can already occur in response to small depolarizations that are insufficient to open wild-type channels, suggesting that FHM1 mutations have a *gain-of-function* effect.

The role of *CACNA1A* mutations in FHM has initiated studies on the function of $Ca_v2.1$ channels in migraine pathophysiology. It is now clear that $Ca_v2.1$ channels play a role in the TGVS: inhibition of $Ca_v2.1$ channels attenuates neurogenic inflammation, plasma protein extravasation and dural dilation after electrical stimulation.[82,83] In addition, blockade of $Ca_v2.1$ channels in the TNC results in increased spontaneous firing of TNC neurons, but reduced responses to chemical and cold stimuli on the dura and cornea.[84] Pre-treatment of the brainstem with GABA prevented neuronal activity increase by application of $Ca_v2.1$ blocker. Blockade of $Ca_v2.1$ channels in the PAG facilitates both spontaneous and meningeal/dural stimulation-evoked neuronal activity in the TNC.[85] Pre-treatment of the PAG with GABA receptor antagonists prevented neuronal activity, suggesting that $Ca_v2.1$ channels mediate both inhibitory and excitatory neurotransmission in the brainstem and are important mediators in pain transmission.[86]

Present data clearly indicates involvement of $Ca_v2.1$ channels in CSD. Blockade of $Ca_v2.1$ channels inhibits spreading depression in hippocampal cultures.[87] Upon application of the $Ca_v2.1$ channel blocker ω-Agatoxin[88] to the cortical surface, CSD could still be elicited, but repetitive CSD was prevented.[89] The role of $Ca_v2.1$ channels in CSD is further supported by the fact that *tottering* and *leaner* mice have a reduced threshold for CSD (discussed below).[90]

1.4 Mouse Mutants of *Cacna1a*

1.4.1 *Tottering*

The *tottering* mouse is a spontaneous mutant with normal viability and intermittent seizures that begin in the second postnatal week.[91] It exhibits behavioural absence seizures with accompanying spike-wave discharges, episodes of dyskinesia and ataxia that becomes apparent after 4 weeks of age.[92] Seizures in *tottering* mice begin at about 3 weeks of age and are triggered by stressful stimuli, such as restraint, handling or startle.[93] The underlying *Cacna1a* gene mutation is a Proline-to-Leucine amino acid substitution at position 601 (P601L) close to the P-domain of $Ca_v2.1$-α_1 (Figure 3).[94]

Electrophysiological measurement of transfected cells and isolated Purkinje cells

indicate that there is an increased current density not associated with changes in single channel conductance, although these results are inconsistent.[95,96] Neurotransmission becomes largely dependent on N-type channels, although other studies indicate that L-type channels compensate for reduced $Ca_v2.1$ function and contribute to dystonic episodes.[97-99] Altered cerebellar neurotransmission - underlying seizure generation and ataxia - and an increased threshold for CSD was found in *tottering* mice.[90,100-102] Possibly, this is due to an increased expression of GABA-A receptors and/or reduced glutamatergic transmission such as was found in thalamic slices.[103,104]

Morphological measurements including forebrain and hindbrain weight, Purkinje cell dimensions, and the thickness of the molecular layer in the paramedian lobule of the cerebellum were reduced after the onset of behavioural symptoms.[105,106] In addition, intra- and supragranular mossy fibres were more prevalent in the dentate gyrus.[107] *Tottering* mutants exhibit an increase in the number of noradrenergic axons in regions innervated by the locus coeruleus, including hippocampus, cerebellum, and dorsal lateral geniculate.[108] This observation is further supported by the fact that selective lesions of the central noradrenergic axons by systemic injection of 6-hydroxydopamine early in development obliterate seizure expression and the reduced sensitivity to noradrenalin in cortical slices.[109,110]

There is growing evidence that abnormal gene expression is widespread in *tottering* brain. In normal mice, tyrosine hydroxylase (TH) is expressed transiently between P21 to P35. In Purkinje cells of *tottering* mice however, TH expression persists throughout adulthood. Also decreased cerebellar gene expression of calretinin and ryanodine receptor type 1 (regulation of calcium homeostasis) was found.[111] Increased levels of methionine-enkephalin and preproenkaphalin mRNA in the striatum, cortex, pons and medulla, lateral caudate and the core of the nucleus accumbens, suggest an alteration of opiodergic pathways.[112,113] Decreased levels of muscarinic acetylcholine receptors, glutathione and cAMP and sodium channels in *tottering* CNS are also reported.[114-117]

1.4.2 *Leaner*

Leaner is a severely affected natural mutant that remains small and develops progressive ataxia, absence seizures and dystonia starting at P10.[118] If left unaided, *leaner* mice will die between P20 and P28. The phenotype results from exon-skipping and the inclusion of intronic sequences in the aberrantly spliced C-terminus, resulting in a short and longer transcript (Figure 3).[94] Measurements of Ca^{2+} channel activities in Purkinje cells from *leaner* mice showed a distinctive change in the voltage dependence of activation and inactivation of P-type currents and a reduction in channel open-probability.[95,96,119] Like *tottering*, *leaner* has a 10-fold decrease in threshold for CSD.[90] In addition, propagation

speed is reduced and the regenerative spread of the depolarizing wave fails to sustain.[90]

Cerebellar granule cell loss begins at P10, but marked Purkinje and Golgi cell loss is not detected until P40.[120] Comparison of the profile of cell death in *leaner* mutant mice with the expression of the zebrin epitope shows that the surviving Purkinje cells are positive for the zebrin marker and that they retain the ectopic pattern of TH expression.[121]

1.4.3 *Rolling Nagoya*

Rolling Nagoya is an ataxic natural mutant, characterized by a severe incoordination of the hind limbs and disturbance of gait.[122,123] The underlying *Cacna1a* mutation is an Arginine-to-Glycine substitution (R1262G) in the voltage sensor at repeat 3, transmembrane domain 4 (Figure 3).[124] A reduced voltage sensitivity and Ca^{2+} influx in whole-cell recordings in a non-neuronal transfection system as well as in cultured cerebellar Purkinje cells from *rolling Nagoya* mouse brains significantly impair integrative properties of Purkinje neurons.[124] Together with dysfunction of the basal ganglia and cerebellar parallel and climbing fiber systems this seems to underlie the ataxic phenotype of *rolling Nagoya* mice.[102,125,126]

Morphologically, *rolling Nagoya* mice have cerebellar weight loss, a decreased number of granule cells and Purkinje cell abnormalities, such as multiple dendritic spines synapsing with single parallel fibre varicosities and axonal swelling.[127-129] A reduced concentration of glutamate and an increased concentration of glycine and taurine have

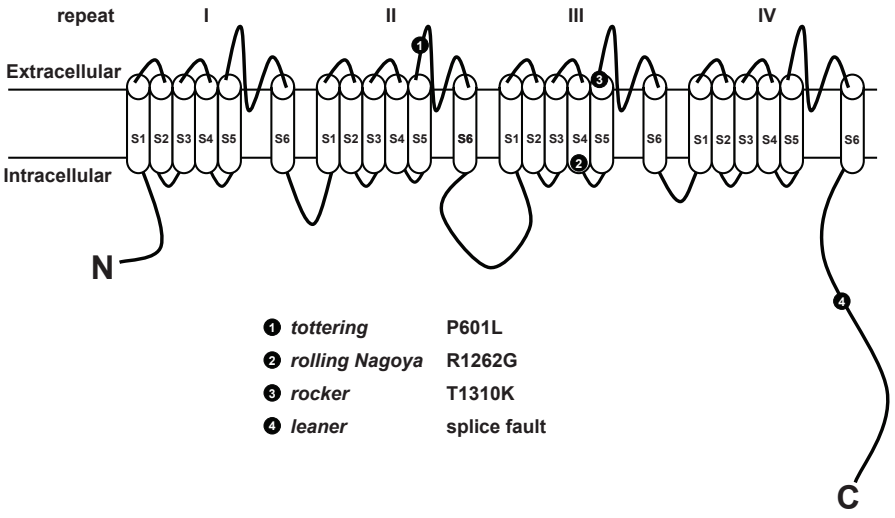

Figure 3. The α_1-subunit of $Ca_v2.1$ channels with the position of mouse *Cacna1a* mutations indicated.

been reported in the cerebellum.[130] In addition, *rolling Nagoya* has an increased activity and expression of TH.[130,131] Altered expression of many other genes has been shown, like corticotropin-releasing factor, neuronal nitric oxide synthase and glutamate and GABA receptors.[132-136] Interestingly, an increased expression of $Ca_v2.1$ channels in deep cerebellar nuclei has also been described.[137]

1.4.4 *Rocker*

Rocker is a recently identified natural mouse mutant that displays absence seizures in addition to ataxia. It has a Threonine-to-Lysine mutation (T1310K) in the *Cacna1a* gene (Figure 3).[138] Currently, no electrophysiological data from *rocker* $Ca_v2.1$ channels is available. Abnormal Purkinje cell morphology without actual Purkinje cell loss has been reported, possibly underlying the abnormal ocular motor function and abnormal cerebellar function.[138,139]

1.4.5 $Ca_v2.1$-α_1 Knockout

By gene-targeting of the *Cacna1a* gene two independent transgenic mouse models were generated that lack functional $Ca_v2.1$ channels ($Ca_v2.1$-α_1 knockout (KO)).[140,141] Like *leaner* mice, these mice appear healthy until 10 days after birth, but then start developing progressive ataxia, such that by P20 the animals were unable to walk. Mice remain small and display dystonia. If left unaided, $Ca_v2.1$-α_1 KO mice die in the fourth week of life. With considerable aid, mice can survive to adulthood, when they develop cerebellar degeneration.[140] Pathological abnormalities were mainly found in the cerebellum.[140-142] Loss of P/Q-type channels results in compensatory increased L- and N-type currents, resulting in disturbances of short-term plasticity.[140,141,143] Interestingly, $Ca_v2.1$-α_1 KO mice have a decreased sensitivity to nociceptive stimuli, supporting the importance of P/Q-type channels in pain processing.[144]

1.4.6 Genetic mouse models of migraine

With the discovery of *CACNA1A*, *ATP1A2* and *SCN1A* as causative genes for FHM, mouse models with mutations in these genes are being considered as models of migraine. However, KO mice of *Cacna1a*, *Atp1a2* and *Scn1a* die within 4 weeks after birth, making long term experiments impossible.[49,140,141,167] The natural mouse mutants of *Cacna1a* (*tottering*, *leaner*, *rolling Nagoya*, *rocker*) appear to be the best alternative mouse models. As described, these mice have ataxia and/or epilepsy. However, since none of the existing natural mouse mutants has a described human FHM1 mutation. In

addition, in contrast to $Ca_v2.1$ channels with human FHM1 mutations, $Ca_v2.1$ channels of natural mouse mutants have reduced function.[40] This argues against the use of these mice as models for migraine. Therefore, the main focus of the work described in this thesis is to provide mice with human FHM1 mutations and thus provide well defined genetic mouse models of migraine.

1.5 Generation and analysis of transgenic mouse models of migraine

1.5.1 Transgenesis

The possibility to manipulate the mouse genome and generate mouse models for human genetic disease has facilitated research on pathogenesis and screening of therapeutic agents.[145,146] In this thesis transgenesis was used to manipulate the endogenous mouse *Cacna1a* gene. Two often-used methods to manipulate the mouse genome and generate genetic mouse models are conventional transgenesis and gene-targeting. With conventional transgenesis a foreign gene or DNA sequence is introduced into the mouse germ line by injection of picoliters of DNA solution into the male pronucleus of fertilized oocytes. This technique is mostly used to study genetically dominant traits because the endogenous genome remains intact. In contrast, with gene-targeting the endogenous mouse genome is altered. In such models both dominant and recessive consequences of genome alterations is possible. Here I will focus on the three technically similar gene-targeting methods that were used in this thesis, namely knockout (KO), knockin (KI) and conditional gene targeting.

Gene-targeting is based on homologous recombination, which is the exchange from DNA of a targeting vector with the corresponding part of the endogenous genome. The design of the targeting vector is the main difference between the three approaches (Figure 4). The targeting vector is built from various blocks of DNA sequence using standard molecular cloning techniques. The relevant part of the mouse genome from a genomic library (in this thesis generated from isogenic 129/Ola genomic DNA) that contains the exon to be mutated is isolated and inserted into a cloning vector. A bacterial neomycin-resistance gene controlled by the phosphoglycerate kinase promoter (PGK-*neo*) that makes future selection of correctly targeted cells possible is inserted into the fragment and thereby - in case of a KO strategy – disrupts the exon structure of the gene (Figure

4). For the KI strategy, a missense mutation is introduced in the exon of interest (Figure 4). For the conditional strategy, the sequence-to-be-deleted *in vivo* is flanked by loxP sites (also called 'floxed'). The loxP site is a 34 basepair palindromic sequence. When Cre-recombinase – an enzyme of the P1 bacteriophage – is expressed in the cell, the two loxP sites recombine, enabling excision of the floxed sequence (for detailed review see Nagy, 2000).[147] Also PGK-*neo* is often floxed, allowing excision after embryonic stem (ES) cell selection (Figure 4).

ES cells are pluripotent cells derived from the inner mass of the mouse blastocyst. In culture, these cells retain their normal karyotype and pluripotency. The targeting vector is introduced into ES cells by transfection. In most cases, the mutated gene of the targeting vector will randomly integrate into the ES cell genome, but in some cells it will replace one copy of the normal ES cell gene by homologous recombination. Because of the presence of the PGK-*neo* cassette, the ES cell will be resistant to the antibiotic neomycin and will survive selection. Correctly targeted ES cells can be selected by Southern blot analysis. Finally, cells of the right clone are microinjected into blastocysts where they eventually populate all tissues of the developing embryo. After microinjection, the blastocyst is transferred into a pseudopregnant female. The resultant offspring is chimeric, meaning that they are derived from two different cell populations (targeted ES cells and host blastocyst cells). The targeted ES cells used to generate the mice in this thesis are derived from male mice of the 129/Ola mouse strain, which typically has a beige/agouti coat colour. In contrast, the host blastocysts described in this thesis are derived from the C57Bl/6J mouse strain, which has a black coat colour. Thus, the percentage of chimerism of offspring is visible from the coat colour and reflects the relative contribution of targeted ES cells: the coat colour varies from black-agouti (weak chimera) to agouti (moderate chimera) to beige (strong chimera). In addition, the potency of the male ES cells is indicated by sex-conversion resulting in a frequency of male chimeras higher than 50%. The strongest male chimera mice are crossed with a C57Bl/6 mouse to achieve germ line transmission. The ES cell-derived offspring are easily recognisable by their agouti coat colour (heterozygous for C57Bl/6 and 129/Ola).

Cre-recombination can be obtained *in vivo* by crossing mice expressing Cre-recombinase driven by the adenovirus EIIa promoter.[148] This way, floxed DNA sequences are efficiently deleted in the very early mouse embryo, leaving only one loxP sequence. This approach can be used to delete PGK-*neo* in the KI and conditional KO mouse, leaving only one and two loxP sites, respectively (Figure 4). Specifically for conditional KO strategies, transgenic mice expressing Cre-recombinase under the control specific promoters are available that enable temporal and/or spatial excision of the floxed sequence.

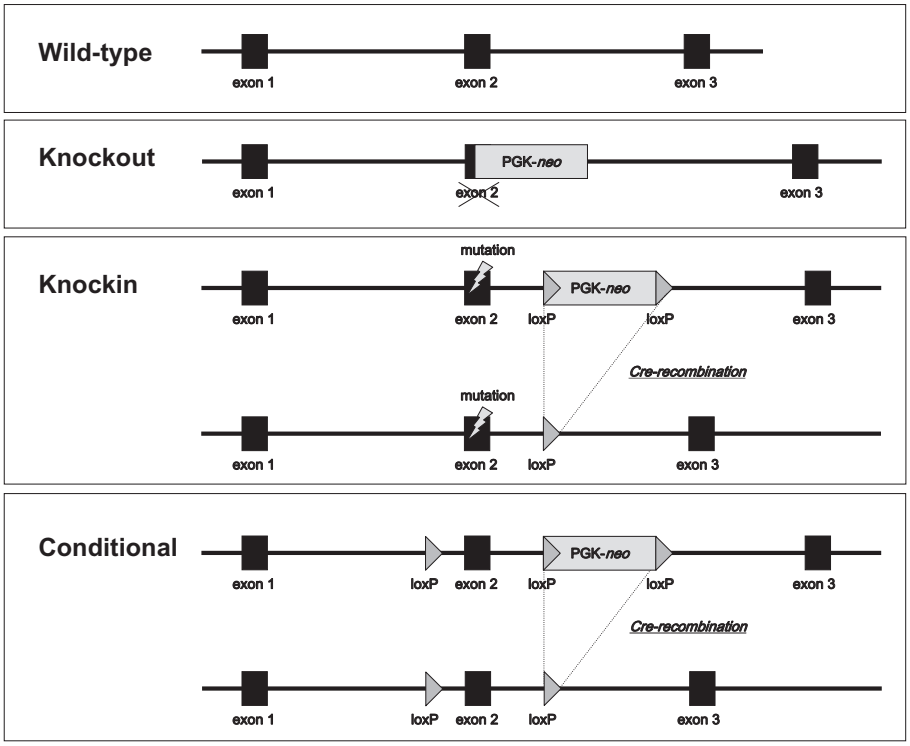

Figure 4. Knockout, knockin and conditional targeting strategies. The knockout targeting strategy is based on disruption of the exon structure by deleting one or more exons and replacing them with a PGK-*neo* sequence. With the knockin targeting strategy a specific mutation is introduced into the exon of interest. A floxed PGK-*neo* selection cassette is inserted into the non-coding intron region, allowing excision of the PGK-*neo* by Cre-recombination. The conditional strategy enables conditional excision of a coding sequence - in this case exon 2 - by flanking it with loxP sites, after prior deletion of the PGK-*neo* cassette.

1.5.2 Electrophysiological methods to study migraine models

1.5.2.1 Whole cell and single channel recording

FHM1 mutations in *CACNA1A* may affect different properties of $Ca_v2.1$ Ca^{2+} channel function, depending on the site and nature of amino acid changes in the $Ca_v2.1$-α_1 protein. Firstly, channel expression and/or localization on the cell membrane might be altered, due to a deficient interaction with factors involved in channel trafficking. This might be particularly important in the synaptic function of $Ca_v2.1$ Ca^{2+} channels, since presynaptic localization at release sites and specific interactions with presynaptic proteins are critical for their role in the process of neurotransmitter release. Secondly, the mutation may change the conductance, i.e. the 'ease' by which Ca^{2+} ions flow through

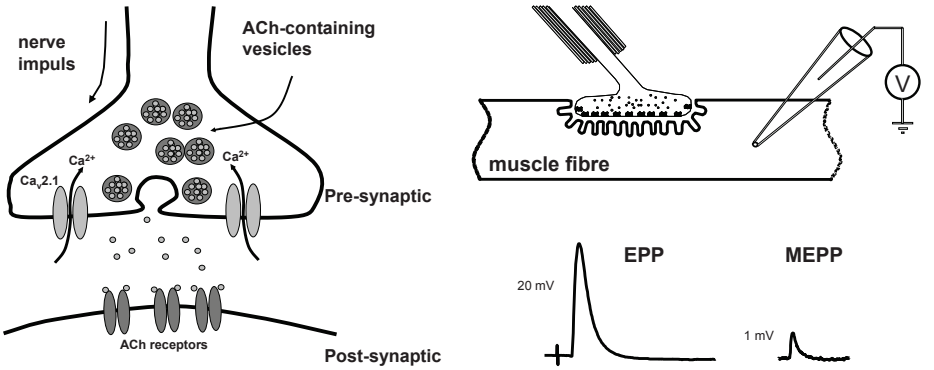

Figure 5. The Neuromuscular Junction. (**A**) Influx of Ca^{2+} through $Ca_v2.1$ channels results in release of ACh-containing vesicles. ACh binds to post-synaptic ACh receptors, resulting in depolarization of the muscle membrane. (**B**) A microelectrode is placed in the muscle fibre near the endplate region for NMJ recordings. (**C**) Recordings of endplate potentials (the depolarization resulting from nerve action potential-evoked ACh release (EPP)) and miniature endplate potentials (the spontaneous depolarizing events due to uniquantal ACh release (MEPP)).

an open channel. Thirdly, the voltage dependence of opening, closing and re-opening (activation, inactivation and recovery from inactivation, respectively) of the channel might be altered. Lastly, the duration of the open state of the channel upon depolarization may change. In principle, single mutations may lead to a combination of consequences.

Examination of the biophysical properties of calcium channels requires voltage and patch clamp technology. These techniques rely on the use of a fine-tipped glass capillary to make contact with a patch of a cell membrane in order to form a giga-ohm seal. Variants of this technique make the application of solution on the exterior and interior of whole cells and on membrane patches torn from the cell possible (outside-out *vs*. inside-out patch). The patch clamp technique has been used extensively to study the functional effects of ion channel mutations in cells isolated from mouse mutants.

By combining the patch clamp with molecular cloning techniques, the function and significance of potentially important amino acid residues are rapidly being elucidated. The gene of interest is cloned and then a mutation is inserted into the clone by mutagenesis. Next, a heterologous expression system (a cell line of a different tissue which does not endogenously express the gene of interest, like HEK293 cells) is used to express the gene. This expression can either be transient, as in RNA injected *Xenopus* oocytes, or stable, as in transfected cells. Finally, the patch clamp technique is used to characterise the function of a channel ensemble, single protein molecule or perform whole cell recordings.

1.5.2.2 The neuromuscular junction

The NMJ is an excellent model to study the effects of FHM1 mutations in single synapses, because here neurotransmitter release is largely dependent on $Ca_v2.1$ channels.[39] The NMJ is the synaptic connection formed between the motoneuron axon and a muscle fibre. The motoneuron axon terminates at the endplate, a highly excitable region that forms the muscle action potential that is necessary for muscle contraction. Neuromuscular transmission is mediated by release of packets of acetylcholine (ACh), called quanta (Figure 5A). Quantal release can be measured by placing a micropipette into the muscle fibre, near the endplate (Figure 5B). The depolarization resulting from nerve action potential-evoked ACh release is the endplate potential (EPP) (Figure 5C). Spontaneous release of an ACh quantum results in a small depolarizing event called a miniature endplate potential (MEPP) (Figure 5C). While EPPs generate a muscle action potential, MEPPs are too small for this. Since a MEPP is caused by release of one quantum and EPPs are the result of release of a number of quanta, the number of quanta that are released upon a single action potential, i.e. the quantal content, can be calculated by dividing normalized and corrected mean EPP amplitude by the normalized mean MEPP amplitude.

1.5.2.3 Cortical spreading depression

As described in section 1.3.2, CSD is a wave of neuronal depolarization, followed by neuronal inactivity. It is believed to be the underlying cause of the migraine aura. CSD can be elicited by application of a mechanical (e.g. pinprick), chemical (e.g. K^+) or electrical stimulus to the cortical surface (Figure 6A). The steady (DC) potential and

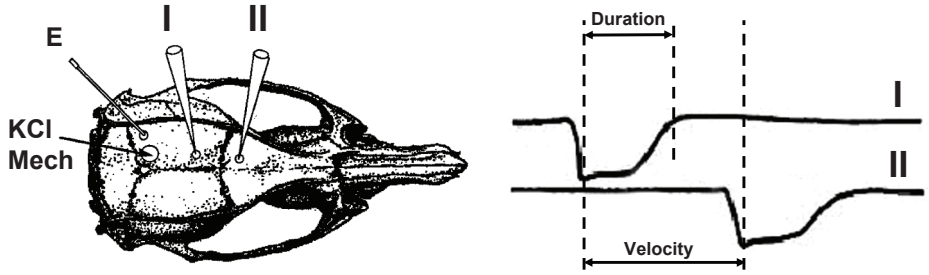

Figure 6. Induction and measurement of CSD. (**A**) CSD can be elicited by application of an electrical (E), mechanical (Mech) or chemical (KCl) stimulation to the cortex. CSD is recorded by micropipettes (I and II). (**B**) Characteristics of the CSD wave is determined from the electrocorticograms of microelectrodes I and II. Duration is calculated from the length of the wave and propagation velocity is calculated from the time between arrival of the CSD wave at micropipette I and II and the distance between the pipettes. Adapted from Ayata et *al*., 2000.[90]

electrocorticogram are recorded with glass micropipettes that are placed below the dural surface. The threshold for CSD is determined by increasing the stimulus strength to the cortex. From the electrocorticogram also the duration and propagation velocity of the CSD wave can be calculated (Figure 6B).

1.5.3 Magnetic resonance imaging

1.5.3.1 Background of magnetic resonance imaging

Magnetic resonance imaging (MRI) and spectroscopy (MRS) are safe, non-invasive methods that are used extensively in clinical and research settings. The most common form of MRI is proton MRI, which generates images based on the distribution of protons (^{1}H atoms) of mainly water and lipids. The technique is based on the fact that protons possess a nuclear magnetic moment called 'spin' (Figure 7A). When these magnetic moments are placed in an external magnetic field (B_0), they orientate anti-parallel or parallel to the magnetic field, with a slight preference for the parallel orientation (Figure 7B). This results in a net magnetization (M_0) in the direction of the magnetic field. The magnitude of M_0 is proportional to B_0. When a second magnetic field is applied by giving a radiofrequency pulse (90^o pulse), this causes a shift of the 'spins' to a different plane, perpendicular to the external magnetic field, and phase coherency between all 'spins' (Figure 7C). The result is a sample magnetization M_1. Magnetization M_1 will return to its original distribution M_0 through T_1 (also called spin-lattice or longitudinal) relaxation. Loss of phase coherency is called T_2 (also called spin-spin or transverse) relaxation. Both relaxation processes occur simultaneously. Detection of magnetization M_1 with a receiver coil leads, together with application of localization gradients, to the MRI image (Figure 7D). (For more detail see Hornak, http://www.cis.rit.edu/htbooks/mri/).

T_1 and T_2 relaxation times differ for each tissue and this can be used to manipulate image contrast. Very simply put, 'free' protons (e.g. in CSF) have a long T_1 and T_2 relaxation time. 'Bound' protons (e.g. in macromolecules) have relatively short T_1 and T_2 relaxation times. T_1-weighted imaging emphasizes the differences in T_1 relaxation times between different tissues and is mainly used for anatomical information. It also provides a high sensitivity for paramagnetic contrast media, fat, fluids with high protein content and subacute hemorrhage. T_2-weighted imaging emphasizes the differences in T_2 relaxation times between different tissues. Because T_2 is increased in many pathologies it offers a high sensitivity to e.g. edema, infarction, demyelination, infection and neoplasm. Proton density images emphasize neither T_1 nor T_2 relaxation times, but produce contrast based primarily on the amount of protons present in the tissue. In a number of

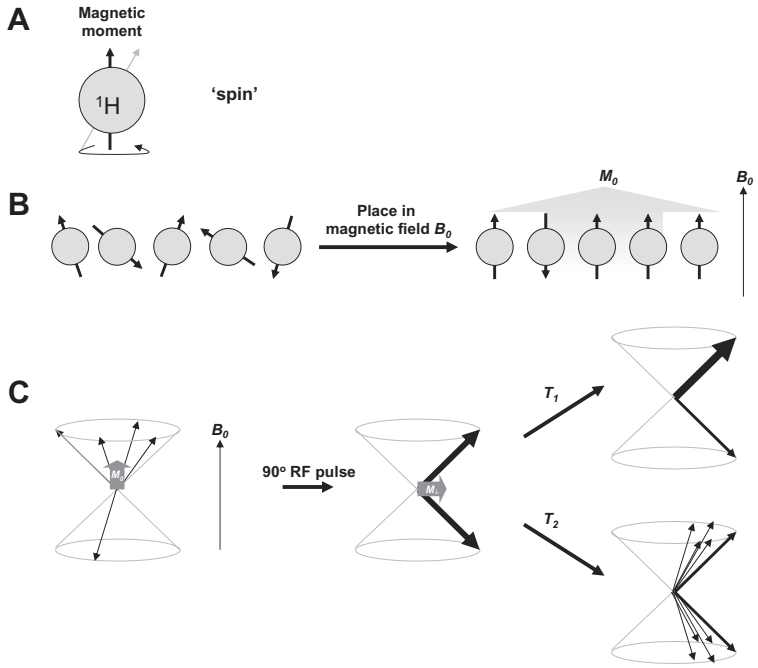

Figure 7. The basis of MRI. (**A**) Protons (^{1}H) possess a magnetic moment called 'spin'. (**B**) When placed in an external magnetic field B_0, most spins align parallel to B_0, resulting in magnetization vector M_0. This is schematically represented in (**C**). Application of a 90° radiofrequency (RF) pulse, flips M_0 perpendicular to B_0, resulting in magnetization vector $M_\perp$ and phase coherency between spins. Magnetization $M_\perp$ will return to its original distribution M_0 through T_1 relaxation. Loss of phase coherency is called T_2 relaxation.

pathological situations, proton density increases in e.g. edema, infection, inflammation, acute demyelination, hemorrhage, tumors and cysts.

1.5.3.2 Magnetic resonance imaging in migraine

Using anatomical MRI techniques, sub-clinical white matter lesions have been found in brainstem and cerebellar regions of migraine patients.[149,150] MRI of FHM patients often shows cerebellar atrophy and edema during a migraine attack.[63,64,151,152]

Functional MRI techniques have been developed that enable imaging of the physiological characteristics of the (diseased) brain, such as diffusion, perfusion, blood oxygen level-dependend (BOLD) MRI and contrast agent-enhanced MRI (for review see Galanaud, 2003 and Dijkhuizen, 2003).[153,154] These techniques have already been used to study migraine patients, and will play an important role in the unraveling of

migraine pathophysiology.[155] Functional MRI findings support the hypothesis that CSD is the underlying cause of the migraine aura and that brainstem areas are involved in migraine pathogenesis.[58,59,156]

MRS, which measures *in vivo* metabolite concentrations, of migraine patients shows metabolic abnormalities that suggest mitochondrial involvement and alteration of glutamate levels.[157-160]

1.5.3.3 Magnetic resonance imaging in mice

The possibility to image neurovascular events like CSD[161], the activation of brain regions[162] and blood-brain barrier integrity in mice, makes MRI a promising technique to study migraine pathophysiology in mouse models of migraine. However, even though they are routinely used in patients, implementation of MR techniques to image small animals is not straightforward. MRI is a relatively insensitive technique. Therefore, the small size of the mouse brain has considerable implications for obtaining a spatial resolution comparable to that obtained with MRI of humans; the small voxel size used in mouse brain imaging results in a very low signal-to-noise ratio at normal, clinical field strengths (≤ 3 T). To overcome this problem, increasingly high magnetic field strengths (up to 17.6 T) are used to increase signal-to-noise ratio.[163] In addition, higher field strengths may also have positive effects on contrast-to-noise, e.g. for magnetization transfer experiments, MRS and the BOLD effect used in functional MRI.[164,165]

Implementation of functional MRI techniques requires knowledge of tissue-specific intrinsic parameters, like T_1 and T_2 relaxation times, which differ with the magnetic field strength that is used. It is therefore important to determine tissue relaxation times of mouse brain at the magnetic field strength that will be used for imaging.

1.6 Aim of the Thesis

At the start of the thesis, various animal models were used in migraine research, but all were wild-type animals. The aim of the studies described in this thesis was to generate and analyse novel genetically predisposed mouse models for migraine by introducing mutations into the mouse genome that cause FHM in patients. Ultimately, these models will help to elucidate pathophysiological mechanisms and develop novel strategies for treatment of the migraine patient.

With the discovery of the first gene for FHM, *CACNA1A*, the opportunity to generate genetically predisposed mouse models of migraine presented itself. In ***Chapter 2*** the generation of the first genetically sensitised mouse model harbouring an FHM1 mutation, the R192Q KI mouse, is described. Because brain lesions have been described in migraine, we examined the possible presence of neuroanatomical abnormalities in these mice using high resolution MRI. Electrophysiological studies indicate that there is an increased Ca^{2+} influx through FHM1 $Ca_v2.1$ channels. It is further hypothesised that this causes an increased neurotransmitter release and subsequent increased susceptibility to CSD.[53] To test this hypothesis, neurotransmission and CSD were studied in the R192Q KI mouse.

In ***Chapter 3*** a more detailed study of morphology and neurotransmission at the R192Q KI mouse NMJ and possible effects of altered neurotransmission on muscle function are presented. Because FHM1 mutations are genetically dominant mutations, we hypothesised a possible gene-dosage effect on neurotransmission. Because chronically elevated presynaptic Ca^{2+} influx may cause damage and eventually lead to synaptic apoptosis[166], also NMJ function of aged mice was examined.

Chapter 4 describes the generation of the second transgenic FHM1 mouse model harbouring the phenotypically more severe S218L mutation. In patients, the S218L mutation causes FHM, ataxia and mild head trauma-triggered brain oedema associated with fatal coma.[65] Not only is this mouse model useful to study migraine pathophysiology, it may also serve as a model to study brain trauma. Because of the clinical severity of the S218L mutation, we speculate that the possible effects on neuropathology, neurotransmitter release and CSD are more pronounced in the S218L KI mouse as compared with the R192Q KI mouse described in chapter 2 and 3. We therefore performed histological analysis, NMJ electrophysiology and CSD measurements.

In ***Chapter 5*** an electrophysiological analysis of neuromuscular neurotransmission of the $Ca_v2.1$-α_1 KO mouse model and the natural mouse mutant *leaner* is presented. Both mouse models display a very similar behavioural phenotype of severe dyskinesia and ataxia and die 3 to 4 weeks after birth. However, in $Ca_v2.1$-α_1 KO mice no $Ca_v2.1$ channels are present, while *leaner* mice do express channels, albeit truncated. In this chapter we aimed to elucidate the molecular mechanisms of neurotransmission. Compensatory mechanisms by other voltage-gated calcium channels are compared between both mice.

In ***Chapter 6*** we aimed to develop a tool to pinpoint the most relevant cell-types and time windows contributing to $Ca_v2.1$ related neurological disorders. To achieve this, we generated a conditional $Ca_v2.1$-α_1 KO mouse by gene targeting. The strategy is aimed to delete the floxed exon 4 of the *Cacna1a* gene upon cell- or time-specific expression of cre-recombinase. The conditional $Ca_v2.1$-α_1 KO mouse was tested by crossing it with EIIA-Cre deleter mice.[148]

Because it provides the possibility to perform non-lethal, non-invasive *in vivo* studies, MRI is becoming an important tool in the study of mouse and rat models of neurodegenerative disorders. Especially for migraine and brain trauma research, where drug intervention studies, imaging of ischemia and oedema and analysis of neuronal activation are important, (functional) MRI is an excellent tool. In ***Chapter 7*** the important tissue parameter T_1 relaxation time was determined at high field. With this knowledge, (functional) brain-imaging studies of (transgenic) migraine models can be conducted.

Chapters 8 and 9 provide a general discussion. The results are reviewed and future possibilities of transgenic mouse models of migraine are discussed.

References

1. Russell,M.B., Rasmussen,B.K., Thorvaldsen,P. & Olesen,J. Prevalence and sex-ratio of the subtypes of migraine. *Int. J. Epidemiol.* **24**, 612-618 (1995).
2. Launer,L.J., Terwindt,G.M. & Ferrari,M.D. The prevalence and characteristics of migraine in a population-based cohort: the GEM study. *Neurology* **53**, 537-542 (1999).
3. Berg,J. & Stovner,L.J. Cost of migraine and other headaches in Europe. *Eur. J. Neurol.* **12 Suppl 1**, 59-62 (2005).
4. The International Classification of Headache Disorders: 2nd edition. *Cephalalgia* **24 Suppl 1**, 9-160 (2004).
5. Ferrari,M.D. Migraine. *Lancet* **351**, 1043-1051 (1998).
6. Ducros,A. *et al.* The clinical spectrum of familial hemiplegic migraine associated with mutations in a neuronal calcium channel. *N. Engl. J. Med.* **345**, 17-24 (2001).
7. Terwindt,G.M., Ophoff,R.A., Haan,J., Frants,R.R. & Ferrari,M.D. Familial hemiplegic migraine: a clinical comparison of families linked and unlinked to chromosome 19.DMG RG. *Cephalalgia* **16**, 153-155 (1996).
8. Russell,M.B. & Olesen,J. Increased familial risk and evidence of genetic factor in migraine. *BMJ* **311**, 541-544 (1995).
9. Gervil,M., Ulrich,V., Kyvik,K.O., Olesen,J. & Russell,M.B. Migraine without aura: a population-based twin study. *Ann. Neurol.* **46**, 606-611 (1999).
10. Honkasalo,M.L. *et al.* Migraine and concomitant symptoms among 8167 adult twin pairs. *Headache* **35**, 70-78 (1995).
11. Larsson,B., Bille,B. & Pedersen,N.L. Genetic influence in headaches: a Swedish twin study. *Headache* **35**, 513-519 (1995).
12. Svensson,D.A., Larsson,B., Waldenlind,E. & Pedersen,N.L. Shared rearing environment in migraine: results from twins reared apart and twins reared together. *Headache* **43**, 235-244 (2003).
13. Ulrich,V., Gervil,M., Kyvik,K.O., Olesen,J. & Russell,M.B. Evidence of a genetic factor in migraine with aura: a population-based Danish twin study. *Ann. Neurol.* **45**, 242-246 (1999).
14. Ziegler,D.K., Hur,Y.M., Bouchard,T.J., Jr., Hassanein,R.S. & Barter,R. Migraine in twins raised together and apart. *Headache* **38**, 417-422 (1998).
15. Mulder,E.J. *et al.* Genetic and environmental influences on migraine: a twin study across six countries. *Twin. Res.* **6**, 422-431 (2003).
16. Kors,E.E. *et al.* Recent findings in headache genetics. *Curr. Opin. Neurol.* **17**, 283-288 (2004).
17. Noble-Topham,S.E. *et al.* Genetic loading in familial migraine with aura. *J. Neurol. Neurosurg. Psychiatry* **74**, 1128-1130 (2003).
18. Bjornsson,A. *et al.* Localization of a gene for migraine without aura to chromosome 4q21. *Am. J. Hum. Genet.* **73**, 986-993 (2003).
19. Wessman,M. *et al.* A susceptibility locus for migraine with aura, on chromosome 4q24. *Am. J. Hum. Genet.* **70**, 652-662 (2002).
20. Nyholt,D.R. *et al.* Genomewide significant linkage to migrainous headache on chromosome 5q21. *Am. J. Hum. Genet.* **77**, 500-512 (2005).
21. Carlsson,A. *et al.* Identification of a susceptibility locus for migraine with and without aura on 6p12.2-p21.1. *Neurology* **59**, 1804-1807 (2002).
22. Cader,Z.M. *et al.* Significant linkage to migraine with aura on chromosome 11q24. *Hum. Mol. Genet.* **12**, 2511-2517 (2003).
23. Soragna,D. *et al.* A locus for migraine without aura maps on chromosome 14q21.2-q22.3. *Am. J. Hum. Genet.* **72**, 161-167 (2003).
24. Russo,L. *et al.* A new susceptibility locus for migraine with aura in the 15q11-q13 genomic region containing three GABA-A receptor genes. *Am. J. Hum. Genet.* **76**, 327-333 (2005).
25. Del Zompo,M. *et al.* Association between dopamine receptor genes and migraine without aura in a Sardinian sample. *Neurology* **51**, 781-786 (1998).

26. Peroutka,S.J., Wilhoit,T. & Jones,K. Clinical susceptibility to migraine with aura is modified by dopamine D2 receptor (DRD2) NcoI alleles. *Neurology* **49**, 201-206 (1997).
27. Mochi,M. *et al.* A genetic association study of migraine with dopamine receptor 4, dopamine transporter and dopamine-beta-hydroxylase genes. *Neurol. Sci.* **23**, 301-305 (2003).
28. Borroni,B. *et al.* Functional serotonin 5-HTTLPR polymorphism is a risk factor for migraine with aura. *J. Headache Pain* **6**, 182-184 (2005).
29. Kotani,K., Shimomura,T., Shimomura,F., Ikawa,S. & Nanba,E. A polymorphism in the serotonin transporter gene regulatory region and frequency of migraine attacks. *Headache* **42**, 893-895 (2002).
30. Marziniak,M., Mossner,R., Schmitt,A., Lesch,K.P. & Sommer,C. A functional serotonin transporter gene polymorphism is associated with migraine with aura. *Neurology* **64**, 157-159 (2005).
31. Ogilvie,A.D. *et al.* Altered allelic distributions of the serotonin transporter gene in migraine without aura and migraine with aura. *Cephalalgia* **18**, 23-26 (1998).
32. Yilmaz,M. *et al.* Significance of serotonin transporter gene polymorphism in migraine. *J. Neurol. Sci.* **186**, 27-30 (2001).
33. Scher,A.I. *et al.* Migraine and MTHFR C677T genotype in a population-based sample. *Ann. Neurol.* **59**, 372-375 (2006).
34. Ophoff,R.A. *et al.* Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca2+ channel gene CACNL1A4. *Cell* **87**, 543-552 (1996).
35. Zhuchenko,O. *et al.* Autosomal dominant cerebellar ataxia (SCA6) associated with small polyglutamine expansions in the alpha 1A-voltage-dependent calcium channel. *Nat. Genet.* **15**, 62-69 (1997).
36. Bourinet,E. *et al.* Splicing of alpha 1A subunit gene generates phenotypic variants of P- and Q-type calcium channels. *Nat. Neurosci.* **2**, 407-415 (1999).
37. Craig,P.J. *et al.* Distribution of the voltage-dependent calcium channel alpha(1A) subunit throughout the mature rat brain and its relationship to neurotransmitter pathways. *J. Comp Neurol.* **397**, 251-267 1998).
38. Westenbroek,R.E. *et al.* Immunochemical identification and subcellular distribution of the alpha 1A subunits of brain calcium channels. *J. Neurosci.* **15**, 6403-6418 (1995).
39. Uchitel,O.D. *et al.* P-type voltage-dependent calcium channel mediates presynaptic calcium influx and transmitter release in mammalian synapses. *Proc. Natl. Acad. Sci. U. S. A* **89**, 3330-3333 (1992).
40. Plomp,J.J., van den Maagdenberg,A.M., Molenaar,P.C., Frants,R.R. & Ferrari,M.D. Mutant P/Q-type calcium channel electrophysiology and migraine. *Curr. Opin. Investig. Drugs* **2**, 1250-1260 (2001).
41. Seagar,M. & Takahashi,M. Interactions between presynaptic calcium channels and proteins implicated in synaptic vesicle trafficking and exocytosis. *J. Bioenerg. Biomembr.* **30**, 347-356 (1998).
42. Sheng,Z.H., Westenbroek,R.E. & Catterall,W.A. Physical link and functional coupling of presynaptic calcium channels and the synaptic vesicle docking/fusion machinery. *J. Bioenerg. Biomembr.* **30**, 335-345 (1998).
43. Walker,D. & De Waard,M. Subunit interaction sites in voltage-dependent Ca2+ channels: role in channel function. *Trends Neurosci.* **21**, 148-154 (1998).
44. De Waard,M. *et al.* Direct binding of G-protein betagamma complex to voltage-dependent calcium channels. *Nature* **385**, 446-450 (1997).
45. Birnbaumer,L. *et al.* The naming of voltage-gated calcium channels. *Neuron* **13**, 505-506 (1994).
46. Ertel,E.A. *et al.* Nomenclature of voltage-gated calcium channels. *Neuron* **25**, 533-535 (2000).
47. De Fusco,M. *et al.* Haploinsufficiency of ATP1A2 encoding the Na+/K+ pump alpha2 subunit associated with familial hemiplegic migraine type 2. *Nat. Genet.* **33**, 192-196 (2003).
48. Dichgans,M. *et al.* Mutation in the neuronal voltage-gated sodium channel SCN1A in familial hemiplegic migraine. *Lancet* **366**, 371-377 (2005).
49. Moseley,A.E. *et al.* The Na,K-ATPase alpha 2 isoform is expressed in neurons, and its absence disrupts neuronal activity in newborn mice. *J. Biol. Chem.* **278**, 5317-5324 (2003).
50. Mobasheri,A. *et al.* Na+, K+-ATPase isozyme diversity; comparative biochemistry and physiological implications of novel functional interactions. *Biosci. Rep.* **20**, 51-91 (2000).
51. Volterra,A., Trotti,D., Tromba,C., Floridi,S. & Racagni,G. Glutamate uptake inhibition by oxygen free radicals in rat cortical astrocytes. *J. Neurosci.* **14**, 2924-2932 (1994).

52. Segall,L. *et al.* Alterations in the alpha2 isoform of Na,K-ATPase associated with familial hemiplegic migraine type 2. *Proc. Natl. Acad. Sci. U. S. A* **102**, 11106-11111 (2005).
53. Moskowitz,M.A., Bolay,H. & Dalkara,T. Deciphering migraine mechanisms: clues from familial hemiplegic migraine genotypes. *Ann. Neurol.* **55**, 276-280 (2004).
54. Goadsby,P.J. Migraine pathophysiology. *Headache* **45 Suppl 1**, S14-S24 (2005).
55. Reuter,U., Sanchez,d.R. & Moskowitz,M.A. Experimental models of migraine. *Funct. Neurol.* **15 Suppl 3**, 9-18 (2000).
56. Burstein,R. Deconstructing migraine headache into peripheral and central sensitization. *Pain* **89**, 107-110 (2001).
57. Lauritzen,M. Cortical spreading depression in migraine. *Cephalalgia* **21**, 757-760 (2001).
58. Cao,Y., Welch,K.M., Aurora,S. & Vikingstad,E.M. Functional MRI-BOLD of visually triggered headache in patients with migraine. *Arch. Neurol.* **56**, 548-554 (1999).
59. Hadjikhani,N. *et al.* Mechanisms of migraine aura revealed by functional MRI in human visual cortex. *Proc. Natl. Acad. Sci. U. S. A* **98**, 4687-4692 (2001).
60. Leão,A.A.P. Spreading depression of activity in the cerebral [cortex]. *J. Neurophysiol.* **7**, 359-390 (1944).
61. Bolay,H. *et al.* Intrinsic brain activity triggers trigeminal meningeal afferents in a migraine model. *Nat. Med.* **8**, 136-142 (2002).
62. Gursoy-Ozdemir,Y. *et al.* Cortical spreading depression activates and upregulates MMP-9. *J. Clin. Invest* **113**, 1447-1455 (2004).
63. Dreier,J.P. *et al.* Opening of the blood-brain barrier preceding cortical edema in a severe attack of FHM type II. *Neurology* **64**, 2145-2147 (2005).
64. Butteriss,D.J., Ramesh,V. & Birchall,D. Serial MRI in a case of familial hemiplegic migraine. *Neuroradiology* **45**, 300-303 (2003).
65. Kors,E.E. *et al.* Delayed cerebral edema and fatal coma after minor head trauma: role of the CACNA1A calcium channel subunit gene and relationship with familial hemiplegic migraine. *Ann. Neurol.* **49**, 753-760 (2001).
66. Ebersberger,A., Schaible,H.G., Averbeck,B. & Richter,F. Is there a correlation between spreading depression, neurogenic inflammation, and nociception that might cause migraine headache? *Ann. Neurol.* **49**, 7-13 (2001).
67. Kaube,H. & Goadsby,P.J. Anti-migraine compounds fail to modulate the propagation of cortical spreading depression in the cat. *Eur. Neurol.* **34**, 30-35 (1994).
68. Bahra,A., Matharu,M.S., Buchel,C., Frackowiak,R.S. & Goadsby,P.J. Brainstem activation specific to migraine headache. *Lancet* **357**, 1016-1017 (2001).
69. Weiller,C. *et al.* Brain stem activation in spontaneous human migraine attacks. *Nat. Med.* **1**, 658-660 (1995).
70. Afridi,S.K. *et al.* A PET study exploring the laterality of brainstem activation in migraine using glyceryl trinitrate. *Brain* **128**, 932-939 (2005).
71. Gee,J.R., Chang,J., Dublin,A.B. & Vijayan,N. The association of brainstem lesions with migraine-like headache: an imaging study of multiple sclerosis. *Headache* **45**, 670-677 (2005).
72. Goadsby,P.J. Neurovascular headache and a midbrain vascular malformation: evidence for a role of the brainstem in chronic migraine. *Cephalalgia* **22**, 107-111 (2002).
73. Katsarava,Z., Egelhof,T., Kaube,H., Diener,H.C. & Limmroth,V. Symptomatic migraine and sensitization of trigeminal nociception associated with contralateral pontine cavernoma. *Pain* **105**, 381-384 (2003).
74. Raskin,N.H., Hosobuchi,Y. & Lamb,S. Headache may arise from perturbation of brain. *Headache* **27**, 416-420 (1987).
75. Veloso,F., Kumar,K. & Toth,C. Headache secondary to deep brain implantation. *Headache* **38**, 507-515 (1998).
76. Welch,K.M., Nagesh,V., Aurora,S.K. & Gelman,N. Periaqueductal gray matter dysfunction in migraine: cause or the burden of illness? *Headache* **41**, 629-637 (2001).
77. Pietrobon,D. Migraine: new molecular mechanisms. *Neuroscientist.* **11**, 373-386 (2005).
78. Hans,M. *et al.* Functional consequences of mutations in the human alpha1A calcium channel subunit

linked to familial hemiplegic migraine. *J. Neurosci.* **19**, 1610-1619 (1999).

79. Tottene,A. *et al.* Familial hemiplegic migraine mutations increase Ca(2+) influx through single human CaV2.1 channels and decrease maximal CaV2.1 current density in neurons. *Proc. Natl. Acad. Sci. U. S. A* **99**, 13284-13289 (2002).
80. Tottene,A. *et al.* Specific kinetic alterations of human CaV2.1 calcium channels produced by mutation S218L causing familial hemiplegic migraine and delayed cerebral edema and coma after minor head trauma. *J. Biol. Chem.* **280**, 17678-17686 (2005).
81. Mullner,C., Broos,L.A., van den Maagdenberg,A.M. & Striessnig,J. Familial hemiplegic migraine type 1 mutations K1336E, W1684R, and V1696I alter Cav2.1 Ca2+ channel gating: evidence for beta-subunit isoform-specific effects. *J. Biol. Chem.* **279**, 51844-51850 (2004).
82. Akerman,S., Williamson,D.J. & Goadsby,P.J. Voltage-dependent calcium channels are involved in neurogenic dural vasodilatation via a presynaptic transmitter release mechanism. *Br. J. Pharmacol.* **140**, 558-566 (2003).
83. Asakura,K. *et al.* alpha-eudesmol, a P/Q-type Ca(2+) channel blocker, inhibits neurogenic vasodilation and extravasation following electrical stimulation of trigeminal ganglion. *Brain Res.* **873**, 94-101 (2000).
84. Ebersberger,A., Portz,S., Meissner,W., Schaible,H.G. & Richter,F. Effects of N-, P/Q- and L-type calcium channel blockers on nociceptive neurones of the trigeminal nucleus with input from the dura. *Cephalalgia* **24**, 250-261 (2004).
85. Knight,Y.E., Bartsch,T., Kaube,H. & Goadsby,P.J. P/Q-type calcium-channel blockade in the periaqueductal gray facilitates trigeminal nociception: a functional genetic link for migraine? *J. Neurosci.* **22**, RC213 (2002).
86. Knight,Y.E., Bartsch,T. & Goadsby,P.J. Trigeminal antinociception induced by bicuculline in the periaqueductal gray (PAG) is not affected by PAG P/Q-type calcium channel blockade in rat. *Neurosci. Lett.* **336**, 113-116 (2003).
87. Kunkler,P.E. & Kraig,R.P. P/Q Ca2+ channel blockade stops spreading depression and related pyramidal neuronal Ca2+ rise in hippocampal organ culture. *Hippocampus* **14**, 356-367 (2004).
88. Mintz,I.M. *et al.* P-type calcium channels blocked by the spider toxin omega-Aga-IVA. *Nature* **355**, 827-829 (1992).
89. Richter,F., Ebersberger,A. & Schaible,H.G. Blockade of voltage-gated calcium channels in rat inhibits repetitive cortical spreading depression. *Neurosci. Lett.* **334**, 123-126 (2002).
90. Ayata,C., Shimizu-Sasamata,M., Lo,E.H., Noebels,J.L. & Moskowitz,M.A. Impaired neurotransmitter release and elevated threshold for cortical spreading depression in mice with mutations in the alpha1A subunit of P/Q type calcium channels. *Neuroscience* **95**, 639-645 (2000).
91. Green,M.C. & Sidman.R.L. Tottering--a neuromusclar mutation in the mouse. And its linkage with oligosyndacylism. *J. Hered.* **53**, 233-237 (1962).
92. Noebels,J.L. & SIDMAN,R.L. Inherited epilepsy: spike-wave and focal motor seizures in the mutant mouse tottering. *Science* **204**, 1334-1336 (1979).
93. Fureman,B.E., Jinnah,H.A. & Hess,E.J. Triggers of paroxysmal dyskinesia in the calcium channel mouse mutant tottering. *Pharmacol. Biochem. Behav.* **73**, 631-637 (2002).
94. Fletcher,C.F. *et al.* Absence epilepsy in tottering mutant mice is associated with calcium channel defects. *Cell* **87**, 607-617 (1996).
95. Dove,L.S., Abbott,L.C. & Griffith,W.H. Whole-cell and single-channel analysis of P-type calcium currents in cerebellar Purkinje cells of leaner mutant mice. *J. Neurosci.* **18**, 7687-7699 (1998).
96. Wakamori,M. *et al.* Single tottering mutations responsible for the neuropathic phenotype of the P-type calcium channel. *J. Biol. Chem.* **273**, 34857-34867 (1998).
97. Qian,J. & Noebels,J.L. Presynaptic Ca(2+) influx at a mouse central synapse with Ca(2+) channel subunit mutations. *J. Neurosci.* **20**, 163-170 (2000).
98. Campbell,D.B. & Hess,E.J. L-type calcium channels contribute to the tottering mouse dystonic episodes. *Mol. Pharmacol.* **55**, 23-31 (1999).
99. Etheredge,J.A., Murchison,D., Abbott,L.C. & Griffith,W.H. Functional compensation by other voltage-gated Ca(2+) channels in mouse basal forebrain neurons with Ca(V)2.1 mutations. *Brain Res.* (2005).

100. Abbott,L.C., Bump,M., Brandl,A. & De Laune,S. Investigation of the role of the cerebellum in the myoclonic-like movement disorder exhibited by tottering mice. *Mov Disord.* **15 Suppl 1**, 53-59 (2000).

101. Hoebeek,F.E. *et al.* Increased noise level of purkinje cell activities minimizes impact of their modulation during sensorimotor control. *Neuron* **45**, 953-965 (2005).

102. Matsushita,K. *et al.* Bidirectional alterations in cerebellar synaptic transmission of tottering and rolling Ca2+ channel mutant mice. *J. Neurosci.* **22**, 4388-4398 (2002).

103. Caddick,S.J. *et al.* Excitatory but not inhibitory synaptic transmission is reduced in lethargic (Cacnb4(lh)) and tottering (Cacna1atg) mouse thalami. *J. Neurophysiol.* **81**, 2066-2074 (1999).

104. Tehrani,M.H., Baumgartner,B.J., Liu,S.C. & Barnes,E.M., Jr. Aberrant expression of GABAA receptor subunits in the tottering mouse: an animal model for absence seizures. *Epilepsy Res.* **28**, 213-223 (1997).

105. Isaacs,K.R. & Abbott,L.C. Development of the paramedian lobule of the cerebellum in wild-type and tottering mice. *Dev. Neurosci.* **14**, 386-393 (1992).

106. Isaacs,K.R. & Abbott,L.C. Cerebellar volume decreases in the tottering mouse are specific to the molecular layer. *Brain Res. Bull.* **36**, 309-314 (1995).

107. Stanfield,B.B. Excessive intra- and supragranular mossy fibers in the dentate gyrus of tottering (tg/tg) mice. *Brain Res.* **480**, 294-299 (1989).

108. Levitt,P. & Noebels,J.L. Mutant mouse tottering: selective increase of locus ceruleus axons in a defined single-locus mutation. *Proc. Natl. Acad. Sci. U. S. A* **78**, 4630-4634 (1981).

109. Magistretti,P.J., Hof,P.R. & Celio,M.R. Noradrenergic sub-sensitivity in the cerebral cortex of the tottering mouse, a spontaneously epileptic mutant. *Brain Res.* **403**, 181-185 (1987).

110. Noebels,J.L. A single gene error of noradrenergic axon growth synchronizes central neurones. *Nature* **310**, 409-411 (1984).

111. Cicale,M. *et al.* Decreased gene expression of calretinin and ryanodine receptor type 1 in tottering mice. *Brain Res. Bull.* **59**, 53-58 (2002).

112. de Bartolomeis,A., Koprivica,V., Pickar,D., Crawley,J.N. & Abbott,L.C. Opioidergic and dopaminergic gene expression in the caudate-putamen and accumbens of the mutant mouse, tottering (tg/tg). *Brain Res. Mol. Brain Res.* **46**, 321-324 (1997).

113. Patel,V.K., Abbott,L.C., Rattan,A.K. & Tejwani,G.A. Increased methionine-enkephalin levels in genetically epileptic (tg/tg) mice. *Brain Res. Bull.* **27**, 849-852 (1991).

114. Abbott,L.C., Nejad,H.H., Bottje,W.G. & Hassan,A.S. Glutathione levels in specific brain regions of genetically epileptic (tg/tg) mice. *Brain Res. Bull.* **25**, 629-631 (1990).

115. Liles,W.C., Taylor,S., Finnell,R., Lai,H. & Nathanson,N.M. Decreased muscarinic acetylcholine receptor number in the central nervous system of the tottering (tg/tg) mouse. *J. Neurochem.* **46**, 977-982 (1986).

116. Tehrani,M.H. & Barnes,E.M., Jr. Basal and drug-induced cAMP levels in cortical slices from the tottering mouse. *Epilepsy Res.* **7**, 205-209 (1990).

117. Willow,M., Taylor,S.M., Catterall,W.A. & Finnell,R.H. Down regulation of sodium channels in nerve terminals of spontaneously epileptic mice. *Cell Mol. Neurobiol.* **6**, 213-220 (1986).

118. Meier,H. & MacPike,A.D. Three syndromes produced by two mutant genes in the mouse. Clinical, pathological, and ultrastructural bases of tottering, leaner, and heterozygous mice. *J. Hered.* **62**, 297-302 (1971).

119. Lorenzon,N.M., Lutz,C.M., Frankel,W.N. & Beam,K.G. Altered calcium channel currents in Purkinje cells of the neurological mutant mouse leaner. *J. Neurosci.* **18**, 4482-4489 (1998).

120. Herrup,K. & Wilczynski,S.L. Cerebellar cell degeneration in the leaner mutant mouse. *Neuroscience* **7**, 2185-2196 (1982).

121. Hess,E.J. & Wilson,M.C. Tottering and leaner mutations perturb transient developmental expression of tyrosine hydroxylase in embryologically distinct Purkinje cells. *Neuron* **6**, 123-132 (1991).

122. Oda,S. [The observation of rolling mouse Nagoya (rol), a new neurological mutant, and its maintenance (author's transl)]. *Jikken Dobutsu* **22**, 281-288 (1973).

123. Tamaki,Y., Oda,S. & Kameyama,Y. Postnatal locomotion development in a neurological mutant of rolling mouse Nagoya. *Dev. Psychobiol.* **19**, 67-77 (1986).

124. Mori,Y. *et al.* Reduced voltage sensitivity of activation of P/Q-type Ca2+ channels is associated with the

ataxic mouse mutation rolling Nagoya (tg(rol)). *J. Neurosci.* **20**, 5654-5662 (2000).

125. Yamaguchi,T., Kato,M., Fukui,M. & Akazawa,K. Rolling mouse Nagoya as a mutant animal model of basal ganglia dysfunction: determination of absolute rates of local cerebral glucose utilization. *Brain Res.* **598**, 38-44 (1992).
126. Kato,M., Hosokawa,S., Tobimatsu,S. & Kuroiwa,Y. Increased local cerebral glucose utilization in the basal ganglia of the rolling mouse Nagoya. *J. Cereb. Blood Flow Metab* **2**, 385-393 (1982).
127. Muramoto,O., Ando,K. & Kanazawa,I. Central noradrenaline metabolism in cerebellar ataxic mice. *Brain Res.* **237**, 387-395 (1982).
128. Rhyu,I.J. *et al.* Morphologic investigation of rolling mouse Nagoya (tg(rol)/tg(rol)) cerebellar Purkinje cells: an ataxic mutant, revisited. *Neurosci. Lett.* **266**, 49-52 (1999).
129. Suh,Y.S., Oda,S., Kang,Y.H., Kim,H. & Rhyu,I.J. Apoptotic cell death of cerebellar granule cells in rolling mouse Nagoya. *Neurosci. Lett.* **325**, 1-4 (2002).
130. Muramoto,O., Kanazawa,I. & Ando,K. Neurotransmitter abnormality in Rolling mouse Nagoya, an ataxic mutant mouse. *Brain Res.* **215**, 295-304 (1981).
131. Sawada,K. *et al.* Abnormal expression of tyrosine hydroxylase immunoreactivity in cerebellar cortex of ataxic mutant mice. *Brain Res.* **829**, 107-112 (1999).
132. Rhyu,I.J. *et al.* Altered neuronal nitric oxide synthase expression in the cerebellum of calcium channel mutant mice. *Brain Res.* **977**, 129-140 (2003).
133. Sawada,K. *et al.* Over-expression of corticotropin-releasing factor mRNA in inferior olivary neurons of rolling mouse Nagoya. *Brain Res. Mol. Brain Res.* **117**, 190-195 (2003).
134. Yamaguchi,T., Hayashi,K., Murakami,H., Maruyama,S. & Yamaguchi,M. Distribution and characterization of the glutamate receptors in the CNS of ataxic mutant mouse. *Neurochem. Res.* **9**, 497-505 (1984).
135. Yamaguchi,T., Hayashi,K., Murakami,H., Maruyama,S. & Yamaguchi,M. Distribution and characterization of the GABA receptors in the CNS of ataxic mutant mouse. *Neurochem. Res.* **9**, 485-495 (1984).
136. Onodera,H., Takase,S., Oda,S., Sobue,I. & Kogure,K. Neurotransmitter receptors of the rolling mouse Nagoya: a quantitative autoradiographic study. *Neurosci. Res.* **5**, 316-324 (1988).
137. Sawada,K., Sakata-Haga,H., Ando,M., Takeda,N. & Fukui,Y. An increased expression of Ca(2+) channel alpha(1A) subunit immunoreactivity in deep cerebellar neurons of rolling mouse Nagoya. *Neurosci. Lett.* **316**, 87-90 (2001).
138. Zwingman,T.A., Neumann,P.E., Noebels,J.L. & Herrup,K. Rocker is a new variant of the voltage-dependent calcium channel gene Cacna1a. *J. Neurosci.* **21**, 1169-1178 (2001).
139. Stahl,J.S., James,R.A., Oommen,B.S., Hoebeek,F.E. & De Zeeuw,C.I. Eye Movements of the Murine P/Q Calcium Channel Mutant Tottering, and the Impact of Aging. *J. Neurophysiol.* (2005).
140. Fletcher,C.F. *et al.* Dystonia and cerebellar atrophy in Cacna1a null mice lacking P/Q calcium channel activity. *FASEB J.* **15**, 1288-1290 (2001).
141. Jun,K. *et al.* Ablation of P/Q-type Ca(2+) channel currents, altered synaptic transmission, and progressive ataxia in mice lacking the alpha(1A)-subunit. *Proc. Natl. Acad. Sci. U. S. A* **96**, 15245-15250 (1999).
142. Miyazaki,T., Hashimoto,K., Shin,H.S., Kano,M. & Watanabe,M. P/Q-type Ca2+ channel alpha1A regulates synaptic competition on developing cerebellar Purkinje cells. *J. Neurosci.* **24**, 1734-1743 (2004).
143. Inchauspe,C.G., Martini,F.J., Forsythe,I.D. & Uchitel,O.D. Functional compensation of P/Q by N-type channels blocks short-term plasticity at the calyx of held presynaptic terminal. *J. Neurosci.* **24**, 10379-10383 (2004).
144. Luvisetto,S. *et al.* Altered sensitivity to nociception stimuli in mice lacking the CaV2.1alpha1 subunit of P/Q-type calcium channels. FENS Abstr. 2, A016.012. 2004.
145. Heck,S., Qian,X. & Velleca,M. Genetically engineered mouse models for drug discovery: new chemical genetic approaches. *Curr. Drug Discov. Technol.* **1**, 13-26 (2004).
146. Price,D.L., Sisodia,S.S. & Borchelt,D.R. Genetic neurodegenerative diseases: the human illness and transgenic models. *Science* **282**, 1079-1083 (1998).
147. Nagy,A. Cre recombinase: the universal reagent for genome tailoring. *Genesis.* **26**, 99-109 (2000).
148. Lakso,M. *et al.* Efficient in vivo manipulation of mouse genomic sequences at the zygote stage. *Proc. Natl. Acad. Sci. U. S. A* **93**, 5860-5865 (1996).

149. Kruit,M.C. *et al.* Migraine as a risk factor for subclinical brain lesions. *JAMA* **291**, 427-434 (2004).

150. Kruit,M.C., Launer,L.J., Ferrari,M.D. & van Buchem,M.A. Brain stem and cerebellar hyperintense lesions in migraine. *Stroke* **37**, 1109-1112 (2006).

151. Chabriat,H. *et al.* Decreased hemispheric water mobility in hemiplegic migraine related to mutation of CACNA1A gene. *Neurology* **54**, 510-512 (2000).

152. Vahedi,K. *et al.* CACNA1A gene de novo mutation causing hemiplegic migraine, coma, and cerebellar atrophy. *Neurology* **55**, 1040-1042 (2000).

153. Dijkhuizen,R.M. & Nicolay,K. Magnetic resonance imaging in experimental models of brain disorders. *J. Cereb. Blood Flow Metab* **23**, 1383-1402 (2003).

154. Galanaud,D. *et al.* Multimodal magnetic resonance imaging of the central nervous system. *Biochimie* **85**, 905-914 (2003).

155. Sanchez,d.R. & Alvarez,L.J. Functional neuroimaging of headaches. *Lancet Neurol.* **3**, 645-651 (2004).

156. Cao,Y., Aurora,S.K., Nagesh,V., Patel,S.C. & Welch,K.M. Functional MRI-BOLD of brainstem structures during visually triggered migraine. *Neurology* **59**, 72-78 (2002).

157. Dichgans,M., Herzog,J., Freilinger,T., Wilke,M. & Auer,D.P. 1H-MRS alterations in the cerebellum of patients with familial hemiplegic migraine type 1. *Neurology* **64**, 608-613 (2005).

158. Sarchielli,P. *et al.* Functional 1H-MRS findings in migraine patients with and without aura assessed interictally. *Neuroimage.* **24**, 1025-1031 (2005).

159. Sparaco,M., Feleppa,M., Lipton,R.B., Rapoport,A.M. & Bigal,M.E. Mitochondrial dysfunction and migraine: evidence and hypotheses. *Cephalalgia* **26**, 361-372 (2006).

160. Uncini,A. *et al.* Abnormal brain and muscle energy metabolism shown by 31P-MRS in familial hemiplegic migraine. *J. Neurol. Sci.* **129**, 214-222 (1995).

161. Henning,E.C., Meng,X., Fisher,M. & Sotak,C.H. Visualization of cortical spreading depression using manganese-enhanced magnetic resonance imaging. *Magn Reson. Med.* **53**, 851-857 (2005).

162. Nair,G. & Duong,T.Q. Echo-planar BOLD fMRI of mice on a narrow-bore 9.4 T magnet. *Magn Reson. Med.* **52**, 430-434 (2004).

163. Hu,X. & Norris,D.G. Advances in high-field magnetic resonance imaging. *Annu. Rev. Biomed. Eng* **6**, 157-184 (2004).

164. Benveniste,H. & Blackband,S. MR microscopy and high resolution small animal MRI: applications in neuroscience research. *Prog. Neurobiol.* **67**, 393-420 (2002).

165. Hennig,J., Speck,O., Koch,M.A. & Weiller,C. Functional magnetic resonance imaging: a review of methodological aspects and clinical applications. *J Magn Reson. Imaging* **18**, 1-15 (2003).

166. Mattson,M.P., Keller,J.N. & Begley,J.G. Evidence for synaptic apoptosis. *Exp. Neurol.* **153**, 35-48 (1998).

167. Yu FH, Mantegazza M, Westenbroek RE, et al. Reduced sodium current in GABAergic interneurons in a mouse model of severe myoclonic epilepsy in infancy. Nat. Neurosci. **9**, 1142–1149 (2006).