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## **Diseases of the nervous system associated with calcium channelopathies**

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### **Citation**

Todorov, B. B. (2010, June 2). *Diseases of the nervous system associated with calcium channelopathies*. Retrieved from <https://hdl.handle.net/1887/15580>

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GENERAL  
INTRODUCTION



## PREFACE

Neurological brain disorders form a major burden on modern society, because they often have devastating effects on the social life of patients and their family members, and because they are associated with high economic costs<sup>[1]</sup>. Still, for most of them, the pathological mechanism is largely unknown. Dedicated research aimed at elucidating these mechanisms is dearly needed to better understand the etiology of the diseases, and may ultimately lead to the development of better treatments for patients. Often, the clinical presentation of a brain disease is not isolated to the brain itself, but it also involves other organs. Examples include disorders such as Parkinson's disease or cerebellar ataxia, in which total body motor coordination is severely disturbed.

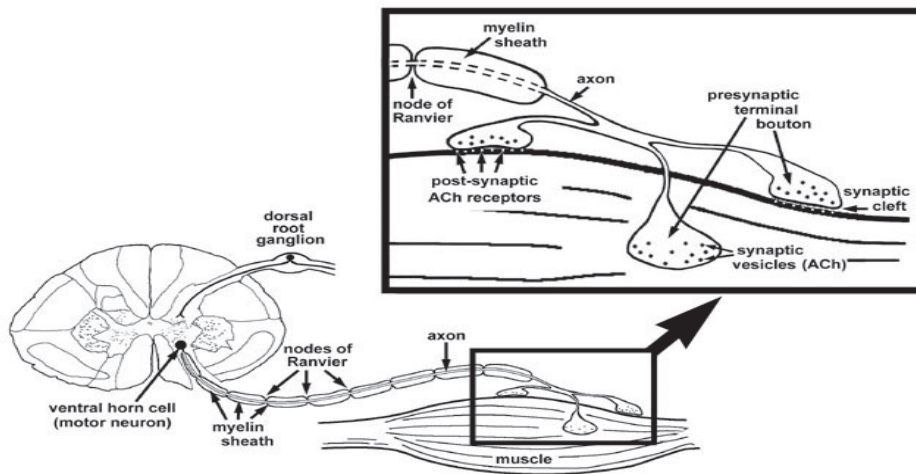
Movement is an essential function of virtually all organisms. Whether voluntary or reflex, movement requires complex patterns of muscular activity that is controlled by motor neurons. Located in the ventral horn of the spinal cord, somatic motor neurons reach the muscles in the periphery via their motor axons. In a functional sense, motor neurons execute commands from higher centers of the nervous system, such as motor centers in the brain stem and the cerebral cortex. Integration of sensory information occurs in the brain stem, which, among other things, is involved in the control of body posture, and eye and head motion. At the top of motor control hierarchy is the cerebral cortex that is involved in the selection of movement planning and the programming of sequences of movements (for review see *Calton & Taube, 2009*). Motor activity is also modulated by the action of basal ganglia and the cerebellum. While the basal ganglia are involved in the planning and initiation of movements, the cerebellum aims to coordinate movement by bringing intention to move in line with motor performance (for review see *Ito, 2002*).

This functional hierarchy in motor control is evolutionarily conserved across species, and is, for instance, very similar between humans and mice. This similarity holds the promise that knowledge obtained from experiments in mice will help to understand the pathophysiology in humans with the same disease. An increasingly important tool to study movement and associated disorders is the use of genetically sensitized animals that either are naturally occurring or are transgenic mouse models with mutations in genes known to cause disease in humans. Such models will further our knowledge of pathophysiological mechanisms of, for instance, cerebellar ataxia (one of the main topics of this thesis) and thus enable the development of novel treatment strategies for patients.

## MOTOR CONTROL IN THE PERIPHERAL NERVOUS SYSTEM

The axon of each motor neuron innervates up to several hundred muscle fibers, forming a *motor unit*. The combined action of motor units allows contraction of muscles through a complex cascade of electrical and biochemical events. Much of the

action is centered around important structures at the distal end of motor neurons: the neuromuscular junctions (NMJs). NMJs are highly specialized peripheral synapses, at which neuronal activity is transduced upon muscle fibers. An NMJ consists of a presynaptic motor nerve terminal, a synaptic cleft, and a postsynaptic muscle fiber membrane that is enclosed by a Schwann cell (*Fig. 1*).



**Figure 1. Schematic drawing of a neuromuscular junction (NMJ).** An NMJ is characterized by a presynaptic motor nerve terminal, a synaptic cleft, and a postsynaptic muscle fiber membrane that is enclosed by a Schwann cell. The axon of a motor neuron enters the muscle and is split into many unmyelinated branches. These terminal fibers run along the myocytes and end at NMJs. At an NMJ, an action potential is transduced upon the muscle. Each motor neuron innervates a few to several hundred muscle fibers, but a particular muscle fiber receives input from only a single neuron. Adapted from *Plomp et al., 2003*.

The structure and function of NMJs have been well characterized and thus making NMJs a popular target to study the main characteristics of a single synapse (for review, see *Sanes & Lichtman, 1999*). The sequence of events is as follows. Electrical stimulation of the motor neuron will result in an action potential. When the action potential arrives at the motor nerve terminal,  $\text{Ca}^{2+}$  enters the neuron through specialized structures called voltage-gated  $\text{Ca}^{2+}$  channels. At the terminal, presynaptic active zones are present that contain synaptic vesicles filled with neurotransmitter; in the case of an NMJ the neurotransmitter is acetylcholine (ACh). Synaptic vesicles contain about equal amounts of ACh, also called quanta. As a result of  $\text{Ca}^{2+}$  entry, synaptic vesicles will fuse with the plasma membrane and ACh is released into the synaptic cleft; an approximately 50-nm-wide space between the nerve terminal and the muscle fiber. Massive release of quanta occurs as a result of an action potential (i.e., *evoked release*). Release of single quanta into the synaptic cleft is also possible (i.e., *spontaneous release*), but does not require an action potential. At the postsynaptic, i.e. muscle, side of the junction, the muscle fiber membrane is organized in postsynaptic junction folds

that contain acetylcholine receptors (AChRs) and voltage-gated Na<sup>+</sup> channels. When ACh crosses the synaptic cleft and binds AChRs, the receptors open and an inward sodium current is generated. As a consequence, the postsynaptic muscle membrane becomes depolarized and a postsynaptic action potential is generated. This action potential will be propagated along the muscle fiber (i.e., *all-or-nothing response*), and will ultimately cause contraction of the muscle. Normally, more ACh will be released than is required to surpass the depolarization threshold for the generation of an action potential (i.e., the *safety factor of the NMJ*). This ensures that the contraction of muscles occurs with high fidelity, which is important for any organism (for review, see *Wood & Slater, 2001*). Finally, access acetylcholine in the synaptic cleft is broken down by acetylcholinesterase to choline.

Experimentally, the characteristics of NMJs can be studied with relative ease in diaphragm preparations that contain muscle and an innervating phrenic nerve. A microelectrode is placed in the muscle to measure postsynaptic voltage changes that are the result of depolarizations of the plasma membrane after the binding of ACh to AChRs. In the case of *spontaneous* release, single quanta cause small depolarizations or miniature end-plate potentials (MEPPs). MEPPs normally occur at a rate of about 0.5 - 1.5 per second. *Evoked* release after a single action potential causes proportionally larger depolarizations or end-plate potentials (EPPs). The total number of vesicles released, can also be determined and is called quantal content. Analysis of the parameters of spontaneous and evoked release provides important information on the functioning of synapses in the peripheral motor system<sup>[4, 7]</sup>.

## MOTOR CONTROL BY THE CENTRAL NERVOUS SYSTEM

Various structures in the central nervous system (CNS) contribute to motor control, including the cerebral (motor) cortex, the basal ganglia, the cerebellum, the brain stem, and the spinal cord. Most relevant for this thesis is the role of the cerebellum in the fine-tuning of ongoing movement. Therefore its structure and function will be discussed below.

### Anatomy and morphology of the cerebellum

The cerebellum is a symmetric, highly foliated structure of the hind brain. In brief, deep horizontal fissures separate the anterior, the large posterior, and the smaller flocculo-nodular lobes of the cerebellum. Two longitudinal grooves running through the posterior lobes demarcate a mid-line vermis and two hemispheres, each containing ten smaller lobules (for review, see *Voogd & Glickstein, 1998*).

The morphology of the cerebellum is well described and has two main components: the cerebellar cortex and the various nuclei embedded in the underlying white matter. In humans, the cerebellum has four deep cerebellar nuclei (DCN). From lateral to

medial, these nuclei are known as dentate, emboliform, globose, and fastigial nuclei. Mice do not have distinct emboliform and globose nuclei, but instead have a single, fused interposed nucleus. In addition, closely related to the cerebellum, there are the hind brain structures: inferior olivary nuclei, consisting of neurons part of medulla oblongata, and the vestibular nuclei, formed by the neurons of the vestibular nerve. There are three sources that provide input to the cerebellum: (1) the pontine nuclei, which carry information from the contralateral side of the cerebral cortex; (2) the spinocerebellar tract, delivering information from the ipsilateral side of the spinal cord; and (3) the inferior olivary nuclei, providing input from the contralateral side of the body.

The cerebellar cortex consists of three layers: the *Purkinje cell layer*, the inner *granular cell layer*, and the outer *molecular layer*.

The Purkinje cell layer consists of a monolayer of Purkinje cells (PCs) that is located between the molecular and granule cell layers. PCs are large neurons with a characteristic beet-shaped morphology<sup>[9]</sup>. In addition, PCs have very characteristic extensive and heavily branched dendrite trees with an extremely flat structure (average PC dimensions are  $250\ \mu\text{m} \times 250\ \mu\text{m} \times 6\ \mu\text{m}$ ). Notably, PCs form the sole output of the cerebellar cortex with their axons projecting to cerebellar and vestibular nuclei.

The granule cell layer contains the cell bodies of small granule cells and several types of large neurons. The latter include the Golgi cells and less known neurons, such as the neuron of Lugaro, and the unipolar brush neuron (for reviews, see *Ambrosi et al., 2007*; *Simat et al., 2007*). Granule cells are excitatory, glutamatergic, neurons and are by far the most abundant type of neurons in this layer. Axons of granule cells are unmyelinated and ascend towards the molecular layer<sup>[11]</sup>, where they bifurcate into so-called parallel fibers<sup>[8]</sup>.

Finally, the molecular layer contains the dendritic trees of PCs (and Golgi cells), the parallel fiber tracts originating from the granule cells, and climbing fiber endings originating from the inferior olive nucleus in the brainstem. Although not cell dense, the molecular layer also contains two types of inhibitory interneurons: the stellate and the basket cells. These interneurons form GABAergic synapses onto PC dendrites<sup>[13]</sup>.

In mammals, the cerebellar cortex is functionally organized in a pattern of parallel longitudinal zones<sup>[14]</sup>. PCs of a specific zone receive input only from a particular region of the inferior olive, and in turn send output to a particular region of the DCN, thereby forming discrete olivo-cerebellar complexes (for review, see *Voogd & Glickstein, 1998*).

### Function of the cerebellum

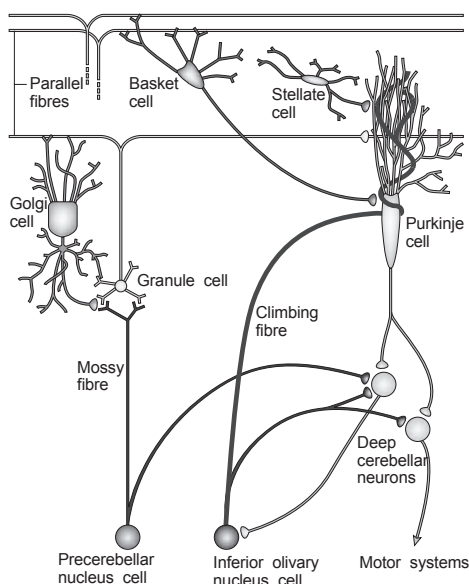
The cerebellum coordinates movement by integrating afferent information into electrical signals guiding the precise execution and timing of motor tasks. To this end, the cerebellum continuously compares objectives (i.e., motor cortex input) and outcomes (i.e., proprioceptive feedback) (for review, see *Ito, 1984*). In addition

to its role in the coordination of movements, the cerebellum is also involved in reflex adaptation, motor learning, and possibly cognition (for review, see *Glickstein & Doron, 2008*). Investigating the cerebellum is specifically interesting because this structure has an unique way of processing information that requires action and feedback from various neurons: *the cerebellar circuitry* (Fig. 2). The characteristics of the cerebellar circuitry are being unraveled rapidly and will further our understanding of cerebellar function and its role in motor coordination dysfunction (for reviews see, *Houk et al., 1997; D'Angelo & De Zeeuw, 2009*).

### The cerebellar circuitry

The cerebellum receives sensory and cortical input from the mossy fibers (for review, see *Glickstein, 1997*). Mossy fibers deliver their information to DCN neurons, but also to granule and Golgi cell neurons of the granule layer<sup>[21]</sup>. Granule cells, which form the sole output of the granule layer, deliver the information further through their ascending axons (directly and via the parallel fibers) to interneurons of the molecular layer<sup>[22]</sup> and to many tiny distal branchlets of PC dendrites<sup>[23]</sup>. While a parallel fiber forms only a few synapses with a given PC, each PC is innervated by no less than 150,000 to 200,000 parallel fibers. The efficiency of a given parallel fiber-to-PC (PF-PC) synapse is low<sup>[24]</sup>. However, when between 30 and 150 parallel fibers are simultaneously activated, an action potential, i.e. *simple spike*, can be generated in a PC. The frequency and pattern of these simple spikes is important for motor behavior, since they comprise the output of the cerebellar cortex<sup>[25, 26, 27]</sup>.

Next to the “mossy-fiber–granule-cell–parallel-fiber” pathway, PCs receive input from climbing fibers. Climbing fibers originate from the inferior olivary complex and their input is believed to signal motor error or discoordination (for review, see *Gibson et al.,*



**Figure 2. Schematic representation of the cerebellar circuitry.** The climbing fiber and mossy fiber pathways are the main types of afferents of the cerebellum. Climbing fibers originate from the inferior olivary complex and directly innervate the Purkinje cells (PCs). Mossy fibers originate from precerebellar nuclei (i.e. the pontine nuclei and the spinocerebellar tract) and project to neurons at the deep cerebellar nuclei (DCN) with axon collaterals innervating cerebellar granule cells. These granule cells project further to PCs via ascending axons (known as parallel fibers) and through inhibitory interneurons of the molecular layer. PCs form the sole output of the cerebellar cortex and project to neurons of the DCN and the vestibular nuclei. Adapted from *Wang and Zoghbi, 2001*.



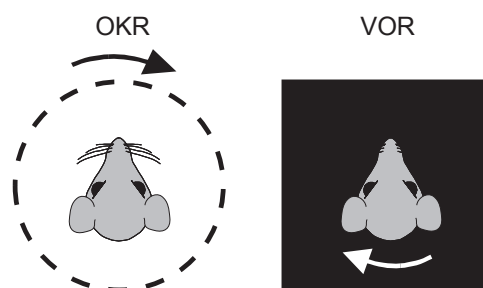
2004). The primary projections of a climbing fiber are multiple, powerful synapses that dock close to the proximal end of a PC dendrite (for review, see Sugihara, 2006). In adult brain, each climbing fiber makes contact with several PCs, but each PC receives input from only a single climbing fiber. Approximately 1,500 synaptic contacts are activated simultaneously, thus triggering extremely powerful postsynaptic responses that override all other synaptic input<sup>[30]</sup>. A PC action potential generated in response to climbing fiber activation is called a *complex spike*. A complex spike is followed by a slow after-hyperpolarization of the PC plasma membrane, interrupting the simple spike activity (i.e. climbing fiber pause). Both simple and complex spike activity determine the PC firing pattern.

In summary, the cerebellar circuitry receives sensory and cortical information, processes this information, and provides feedback to DCN neurons by changing the firing pattern of the (inhibitory) PCs (for review, see D'Angelo & De Zeeuw, 2009). The information content is then transmitted further downstream to, for instance, the thalamus and motor centers in the brain stem and the spinal cord, ultimately resulting in adaptations of motor behavior.

### Experimental methods investigating cerebellar function

Neuronal activity in the cerebellar circuitry is constantly adapting during movement. For instance, PCs, which are tonically active at rest change their firing frequency upon sensory input. By recording PC activity, such as recording simple and complex spike frequency during various motor tasks, one can obtain information about the function of the cerebellum in relation to the timing and the coordination of movement. The analysis of *compensatory eye movements* is a popular test paradigm to study cerebellar control of movement<sup>[31]</sup>. Compensatory eye movements occur, for instance, when one looks out of a train window and tries to lock on a nearby object; to perform the task, the motor system tries to minimize the movement of the image on the retina (i.e., to minimize the retinal slip). As a consequence, the eye follows the object as long as possible until it quickly returns to its original position and the sequence restarts. What happens is that the optokinetic reflex (OKR) aims to constrain the moving image on a part of the retina. Similarly, the vestibulo-ocular reflex (VOR) moves the eye in response to head movement in an attempt to reduce retinal slip. Performance in OKR and VOR (and the combined visually-enhanced VOR, VVOR) tests is presented as *gain* and *phase values* (Fig. 3). While gain values represent the ratio between the amplitude of the eye and the stimulus velocity, phase values correspond to the time difference between eye and stimulus expressed in degrees. Both gain and phase are important indicators for the function of the cerebellum and its ability to integrate the vestibular and/or optokinetic sensory information needed for controlling movement<sup>[32]</sup>.

**Figure 3. Schematic drawing of the training paradigm used to study optokinetic reflex (OKR) and vestibulo-ocular reflex (VOR) in mice.** While the OKR allows the eye to follow objects in motion when the head remains stationary, VOR preserves the image on the center of the visual field during head movement by moving the eyes in the direction opposite to head movement. The “gain” of the VOR for example is defined as the change in the eye angle divided by the change in the head angle during the head turn. “Phase” is a parameter that describes the timing relationship between head movement and the reflexive eye response. When the head and eyes move at exactly the same velocity in opposite directions, they are said to be completely out of phase, or 180°. If the reflex eye movement leads the head movement, a phase lead is present. Likewise, if the compensatory eye movement trails the head movement, a phase lag is present.



## NEURONAL CALCIUM INFLUX

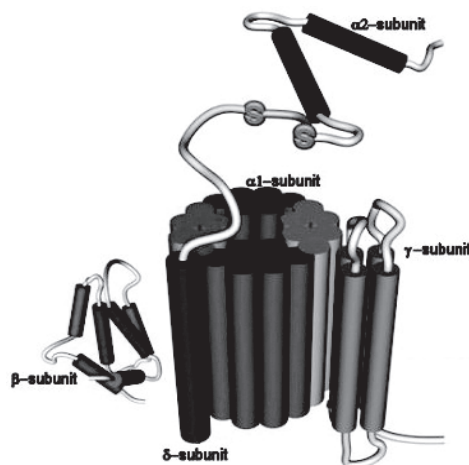
$\text{Ca}^{2+}$  is important for the functioning of the motor system, both at the periphery and in the CNS. Most relevant to this thesis is the entry of  $\text{Ca}^{2+}$  into the neurons through voltage-gated  $\text{Ca}^{2+}$  channels, which results in the release of neurotransmitters, the generation of postsynaptic currents, and, thereby, the regulation of synaptic activity<sup>[33]</sup>.

### Voltage-gated $\text{Ca}^{2+}$ channels

Voltage-gated  $\text{Ca}^{2+}$  channels (VGCC) are responsible for  $\text{Ca}^{2+}$  influx into many excitable cells<sup>[34]</sup>. It has been known since the 1980's<sup>[35]</sup> that  $\text{Ca}^{2+}$  channels are multimeric protein complexes, with a pore-forming  $\alpha_1$  subunit and auxiliary subunits  $\beta$  and  $\alpha_2\delta$ . In some cases, a  $\gamma$  subunit makes up part of the protein complex (Fig. 4).

VGCCs differ with respect to protein composition, location of expression, as well as pharmacological and electrophysiological properties (for review, see *Doering & Zamponi, 2003*) (Table 1). The nomenclature of VGCCs is based on the presence of a specific  $\alpha_1$  subunit<sup>[37]</sup>.

At the genetic level, four genes encoding  $\beta$  subunits (i.e.,  $\beta_1$  to  $\beta_4$ ),



**Figure 4. Schematic structure of voltage-gated  $\text{Ca}^{2+}$  channels.** A voltage-gated  $\text{Ca}^{2+}$  channel consists of a pore-forming  $\alpha_1$  subunit, a dimeric  $\alpha_2\delta$  subunit, an intracellular  $\beta$  subunit, and in some cases a  $\gamma$  subunit.

**Table 1. Nomenclature according to Ertel et al., 2000 and certain properties of voltage-gated calcium channels**

Channel Type	$\alpha$ 1 Subunit	$\alpha$ 1 Gene	Ca <sup>2+</sup> current	Specific blocker(s)	Primary localization of channel
Ca <sub>v</sub> 1.1	$\alpha_{1S}$	CACNA1S	L-type		Skeletal muscle; transverse tubules
Ca <sub>v</sub> 1.2	$\alpha_{1C}$	CACNA1C	L-type	Dihydropyridines Phenylalkylamines Benzothiazepines	Cardiac & smooth muscle myocytes; endocrine and neuronal cells
Ca <sub>v</sub> 1.3	$\alpha_{1D}$	CACNA1D	L-type		Endocrine cells and neurons; atrial myocytes & pacemaker cells
Ca <sub>v</sub> 1.4	$\alpha_{1F}$	CACNA1F	L-type		Retina & spinal cord, glands
Ca <sub>v</sub> 2.1	$\alpha_{1A}$	CACNA1A	P/Q-type	$\omega$ -Agatoxin IVA $\omega$ -Conotoxin-MVIIC	Nerve terminals and dendrites
Ca <sub>v</sub> 2.2	$\alpha_{1B}$	CACNA1B	N-type	$\omega$ -Conotoxin-GVIA $\omega$ -Conotoxin-MVIIC	Nerve terminals and dendrites
Ca <sub>v</sub> 2.3	$\alpha_{1E}$	CACNA1E	R-type	SNX-482	Neuronal cell bodies and dendrites
Ca <sub>v</sub> 3.1	$\alpha_{1G}$	CACNA1G	T-type	Mibefradil	Neuronal cell bodies and dendrites;
Ca <sub>v</sub> 3.2	$\alpha_{1H}$	CACNA1H	T-type	Kurtoxin	cardiac and smooth muscle
Ca <sub>v</sub> 3.3	$\alpha_{1I}$	CACNA1I	T-type	Amiloride	myocytes

four genes encoding  $\alpha_2\delta$  subunits (i.e.,  $\alpha_2\delta$ -1 to  $\alpha_2\delta$ -4), and eight genes encoding  $\gamma$  subunits (i.e.,  $\gamma$ 1 to  $\gamma$ 8) have been identified (for review, see *Arikkath & Campbell, 2003*).  $\alpha_2\delta$  and  $\gamma$  subunits are membrane-associated proteins, while the  $\beta$  subunit is localized entirely in the cytoplasm. The function of auxiliary subunits is to modify the biophysical properties of their respective channel (for review, see *Dolphin et al., 2009*). These subunits can also effect the expression of Ca<sup>2+</sup> channels in the plasma membrane<sup>[39]</sup>. Since there are many possibilities of, for instance,  $\beta$  and  $\alpha_2\delta$  to associate with  $\alpha_1$  subunits, there is an enormous diversity of VGCCs that are expressed in a cell type-specific and tissue-specific manner<sup>[40]</sup>.

### Ca<sub>v</sub>2.1 channels

The main focus of this thesis is on a particular type of VGCC, namely Ca<sub>v</sub>2.1 channels. The  $\alpha_1$  subunit of Ca<sub>v</sub>2.1 channel is encoded by the *CACNA1A* gene. Many Ca<sub>v</sub>2.1 channels contain  $\beta_4$  and  $\alpha_2\delta$ -2 auxiliary subunits, but combinations with other auxiliary subunits have been also found. Alternative splicing of the *CACNA1A* gene results in further diversity in the composition of Ca<sub>v</sub>2.1 channels. Depending on their composition, the Ca<sub>v</sub>2.1 channels exhibit (sometimes subtle) differences in their electrophysiological properties. For instance, alternative splicing can result in Ca<sub>v</sub>2.1  $\alpha_1$  subunits that produce either P- or Q-type Ca<sup>2+</sup> currents as can be deduced from their pharmacological profile in response to specific Ca<sub>v</sub>2.1 channel blockers<sup>[41]</sup>.

Although Ca<sub>v</sub>2.1 channels are broadly expressed throughout the central nervous system, their expression is particularly high in the cerebellum<sup>[42, 43]</sup>. Most neurons of the cerebellar circuitry are to a considerable extent dependent on Ca<sub>v</sub>2.1 channel

function: in PCs, over 90% of  $\text{Ca}^{2+}$  current density is  $\text{Ca}_v2.1$  channel-dependent<sup>[44]</sup>; and in granule cell neurons this figure is about 50%<sup>[45]</sup>. Outside the CNS,  $\text{Ca}_v2.1$  channels are crucial for synaptic transmission at NMJs<sup>[46]</sup>.

## DISEASES OF THE MOTOR SYSTEM IN WHICH $\text{Ca}_v2.1$ CHANNELS PLAY A ROLE

Normal  $\text{Ca}_v2.1$  channel function is necessary for proper motor coordination. Most relevant to this thesis, is the *dysfunction* of these calcium channels, which can result in impairment of the sensorimotor system and cerebellar ataxia. This impairment is characterized by gait disturbances, poor coordination of movement, and a wide-based, unsteady gait. During ataxic movements, motor behavior is typically characterized by abnormal timing with delayed muscle activation and/or sudden interruptions of movement, followed by exaggerated corrections<sup>[47]</sup>. Cerebellar ataxia can occur during attacks, can be present chronically, and, depending on the type of ataxia, can progress during the course of the disease (for review, see *Manto & Marmolino, 2009*).

### $\text{Ca}_v2.1$ -dependent diseases with cerebellar ataxia signs

Cerebellar ataxia associated with  $\text{Ca}_v2.1$  channel dysfunction can be either the main clinical feature as in *Episodic Ataxia type 2* (EA2) and *Spinocerebellar Ataxia type 6* (SCA6), or it can be part of a more complex clinical syndrome such as in *Familial Hemiplegic Migraine (FHM) with ataxia* (with or without additional clinical symptoms of epilepsy and/or mild head trauma-induced edema), or in the autoimmune diseases *Lambert Eaton Myasthenic Syndrome* (LEMS) and *Guillain-Barré Syndrome* (GBS).

#### - *Episodic Ataxia type 2*

EA2 manifests as recurrent attacks of cerebellar ataxia lasting from hours to days that can differ widely in severity (for review, see *Jen et al., 2004*). Clinical symptoms include gait disturbances, nystagmus, vertigo, and generalized weakness. Migraine occurs in as many as half of the patients. Interictal cerebellar symptoms are not uncommon and include gaze-holding deficits, saccadic smooth pursuit, and impaired visual suppression of the vestibulo-ocular reflex, especially downbeat nystagmus. Cerebellar atrophy, especially of the anterior vermis, has been observed in many cases (for review, see *Strupp et al., 2007*). EA2 is an autosomal dominant genetic disorder that is caused by mutations in the *CACNA1A* gene<sup>[51]</sup>. EA2 mutations include truncation and certain missense mutations that result in a severely dysfunctional or non-functional  $\text{Ca}_v2.1$ - $\alpha_1$  protein<sup>[52, 53]</sup>. EA2 mutations are loss-of-function mutations and are predicted to decrease neurotransmitter release<sup>[54]</sup>. In cellular assays, EA2 mutations were shown to exert a dominant-negative effect, thereby leading to inactivity of co-expressed normal  $\alpha_1$  protein<sup>[54, 55]</sup>.

### - Spinocerebellar ataxia type 6

SCA6 is a late-onset, permanent, slowly progressive autosomal dominant type of ataxia with nystagmus, dysarthria, and sensory loss, and exhibits considerable phenotypic overlap with EA2 (for review, see *Frontali, 2001*). SCA6 is caused by moderate expansions of a polyglutamine (CAG) stretch in the part of *CACNA1A* gene that encodes the carboxyl terminus of the  $\text{Ca}_v2.1\text{-}\alpha_1$  protein<sup>[57]</sup>. Healthy individuals have between 4 and 18 CAG-repeats, while SCA6 patients have 20 to 30 repeats. It is not entirely clear what the consequences are of the SCA6  $\text{Ca}_v2.1\text{-}\alpha_1$  protein, but evidence suggests that toxic protein aggregates can be formed<sup>[58]</sup>. Investigating the electrophysiological properties of the SCA6 protein in transfected cells yielded contradictory results: both an increased  $\text{Ca}_v2.1$  current density<sup>[59]</sup> and normal  $\text{Ca}_v2.1$  current densities accompanied by a negative shift in the voltage dependence of inactivation<sup>[60]</sup> have been reported.

### - Familial hemiplegic migraine with cerebellar ataxia

FHM is a rare subtype of migraine with aura that is characterized by hemiparesis during the aura phase. The aura precedes the headache and is caused by a phenomenon called cortical spreading depression (CSD), a wave of neuronal and glial cell depolarization that starts in the occipital cortex and slowly progresses to more frontal regions of the brain<sup>[61]</sup>. The headache is caused by dysfunction of the trigeminovascular system (for review, see *Goadsby, 2005*). Experiments in rats have suggested that CSD may activate brain stem nuclei, and thereby trigger headache mechanisms<sup>[63]</sup>, indicating the relevance of CSD in rare FHM and common migraine. Three genes have been identified in FHM (i.e., FHM1, FHM2, and FHM3), all encoding ion transporters (for review, see *van den Maagdenberg et al., 2007*). FHM1 is caused by certain heterozygous missense mutations in the *CACNA1A* gene, which encodes the  $\alpha_1$  subunit of  $\text{Ca}_v2.1$  channels<sup>[51]</sup>. In about 20% of FHM1 patients, cerebellar ataxia is observed. Depending on the mutation, epilepsy, mental retardation, and mild head trauma-triggered delayed brain edema (that can even result in the death of a patient) can also be part of the clinical spectrum<sup>[65]</sup>. Detailed electrophysiological investigation of the functional consequences of *CACNA1A* mutations has revealed that, due to a combination of increased channel open probability and/or a rather dramatic left-shift in channel activation, specifically at lower voltages, all eight studied FHM1 mutations (including mutations R192Q, S218L, and T666M that are specifically relevant for this thesis) show enhanced single-channel  $\text{Ca}^{2+}$  influx over a broad voltage range<sup>[66, 67, 68]</sup>. Consequently, FHM1 mutations are portrayed as *gain-of-function* mutations. Notably, whole-cell experiments pointed to a *loss-of-function* effect of FHM1 mutations<sup>[66, 67, 69]</sup>, and resulted in considerable debate on the consequences of FHM1 mutations. Clearly, FHM seems to be the result of a disturbed ionic (and neurotransmitter) balance in the brain, with single-channel data pointing to an increased release of the excitatory neurotransmitter glutamate (for review, see *Moskowitz et al., 2004*).

### - Lambert-Eaton Myasthenic Syndrome and Guillain-Barré Syndrome

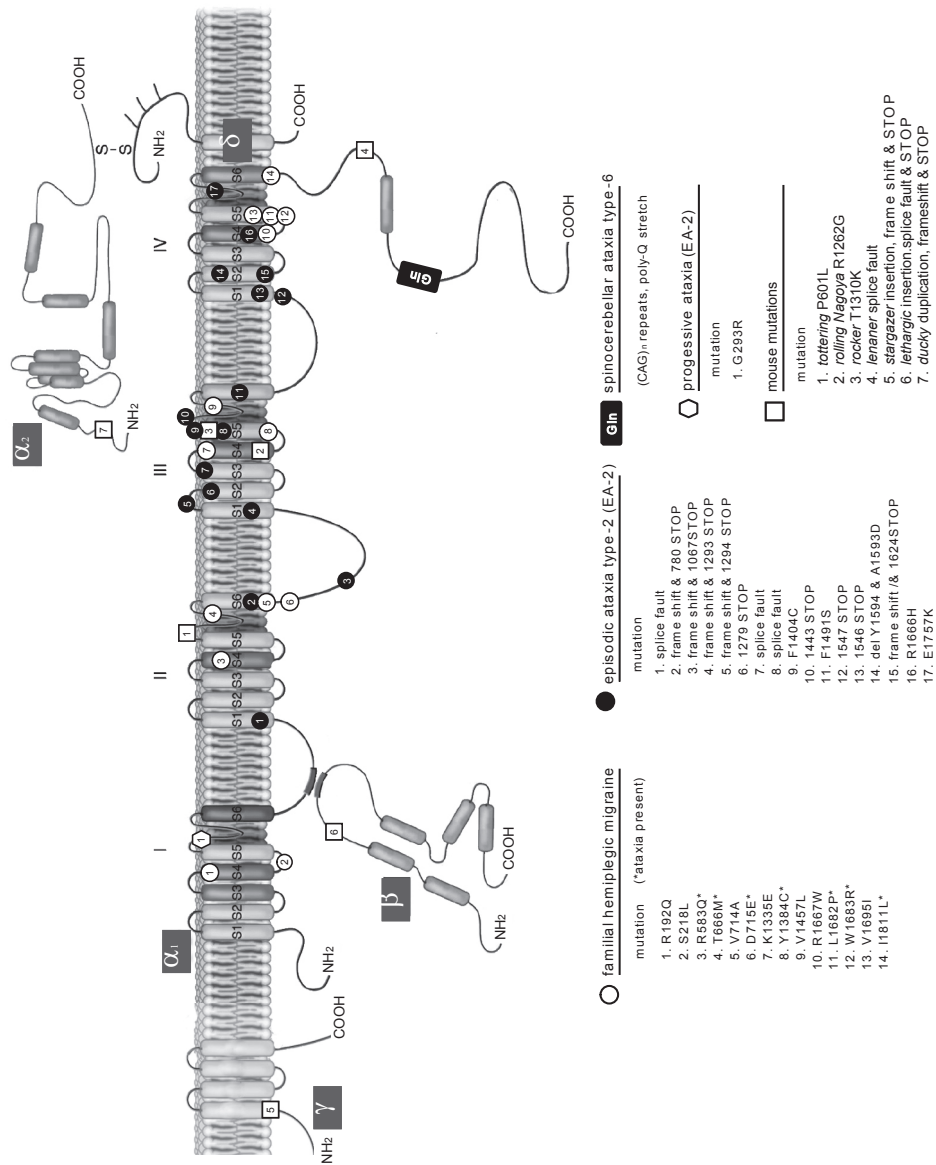
Finally, there are acquired immune-mediated motor coordination problems in patients with LEMS<sup>[71]</sup> and GBS<sup>[72]</sup> that are caused by auto-antibodies that target Ca<sub>v</sub>2.1 channels. LEMS is characterized by muscle weakness, impaired tendon reflexes, and autonomic dysfunction and is associated with small-cell lung cancer of endocrine origin in approximately 60% of patients<sup>[73]</sup>. GBS is an acquired peripheral neuropathy that is characterized by an acute motor axonal neuropathy, and acute inflammatory demyelinating polyneuropathy<sup>[74]</sup>. Both LEMS and GBS are, at least partly, caused by Ca<sub>v</sub>2.1 auto-antibodies blocking and/or causing the removal of Ca<sub>v</sub>2.1 channels from the plasma membrane, resulting in the severe impairment of neurotransmission and the clinical symptoms<sup>[75]</sup>.

## MOUSE MODELS OF CA<sub>V</sub>2.1 CHANNELS

The discovery of Ca<sub>v</sub>2.1 gene mutations (*Fig. 5*) that result in motor coordination dysfunction, has stimulated the search and development of suitable experimental animal models. Over the last decade, several mouse strains were shown to have spontaneously occurred mutations in the Ca<sub>v</sub>2.1 channel genes (see *Naturally occurring Ca<sub>v</sub>2.1 mutant mice*). In the same time attempts were also made to generate transgenic mice in which the genome has been manipulated (see *Cacna1a transgenic mice*). Both types of mouse models have been a very useful in the study of disease mechanisms, not only of motor coordination dysfunction, but also of the other Ca<sub>v</sub>2.1-associated diseases such as epilepsy, dystonia, and in particular, migraine. A brief overview is given below of the phenotypes and main findings in these mouse models that were already available for investigation before the start of the research discussed in this thesis. For more detailed reviews on naturally occurring and transgenic Ca<sub>v</sub>2.1 channel mutant mice see *Fletcher & Frankel, 1999*, and *van de Ven et al., 2007*.

### Naturally occurring Ca<sub>v</sub>2.1 mutant mice

*Tottering* mice exhibit highly stereotyped episodes of motor dysfunction and, between episodes, a mild ataxic gait, beginning after four weeks of age<sup>[79, 80]</sup>. In addition, the mice exhibit absence seizures<sup>[81]</sup>. *Tottering* is caused by a recessive P601L missense mutation in the *Cacna1a* gene<sup>[82]</sup>. At their NMJs, *tottering* mice show an increased spontaneous acetylcholine release<sup>[83]</sup> and a decreased Ca<sub>v</sub>2.1-dependent neurotransmission<sup>[84]</sup>. Cellular studies of transfected and dissociated PCs have revealed a reduction in Ca<sub>v</sub>2.1 current density that is not associated with changes in single channel conductance<sup>[85]</sup>. Relevant to this thesis, PF-PC synapses of *tottering* mice show a decreased synaptic efficacy<sup>[86]</sup>, although there is controversy on the subject as compensation from other Ca<sup>2+</sup> channel types can occur<sup>[87]</sup>. The *tottering* mutation causes increased irregularity of PC firing, offering a likely explanation for the motor discoordination in these mice<sup>[25, 88]</sup>. With respect to (ultra)structural abnormalities in *tottering* mice, mild PC loss<sup>[89]</sup>



**Figure 5. Schematic representation of Ca<sub>v</sub>2.1 channels and their subunits with the position of disease-causing mutations in mice and humans that are relevant to this thesis.** The α<sub>1</sub> subunit of Ca<sub>v</sub>2.1 channels has four repeats (I-IV), each consisting of six (S1-S6) transmembrane domains. N - amino terminus; C - carboxyl terminus. Naturally occurring mouse mutations and human mutations introduced in knockin mouse models are depicted. *Ducky*, *lethargic*, and *stargazer* mice are knockouts of the subunits α<sub>2</sub>δ-2, β<sub>4</sub>, and γ<sub>2</sub>, respectively. Adapted from *van de Ven et al. (2007)* and *Barrett et al. (2008)*.

and an increase in the multisynaptic index of the PF contacts, somatic inclusions, and axonal swellings<sup>[90]</sup> have been reported.

*Leaner* mice are severely affected: they remain much smaller than their wild-type littermates and develop progressive ataxia, absence seizures, and dystonia starting at P10<sup>[79]</sup>. If left unaided, *leaner* mice will die between P20 and P28. The phenotype is caused by exon-skipping and the inclusion of intronic sequences in the aberrantly spliced C-terminus, resulting in shorter and longer *Cacna1a* transcripts<sup>[82]</sup>. Electrophysiological analysis showed a nearly 50% reduction in the neurotransmitter release at *leaner* NMJs<sup>[91]</sup>. Ca<sub>v</sub>2.1 channels of *leaner* PCs revealed a right shift in voltage dependence of activation and inactivation as well as a reduction in channel open probability<sup>[92, 93]</sup>. Reduced Ca<sub>v</sub>2.1 channel function was associated with a decrease in excitatory neurotransmission at PF-PC synapses<sup>[94]</sup> and, like in *tottering*, an increased irregularity of intrinsic PC firing<sup>[26]</sup>. Cerebellar granule cell loss in *leaner* mice, starts at P10, but Purkinje and Golgi cell loss is not detected until P40<sup>[95]</sup>. Other PC abnormalities include multiple dendritic spines contacting single parallel fiber varicosities<sup>[90]</sup>.

*Rolling Nagoya* mice exhibit severe dyscoordination of the hind limbs and an ataxic gait<sup>[96, 97]</sup>. This phenotype is the result of a recessive R1262G missense mutation<sup>[98]</sup>. Muscle weakness and fatigue have been reported for this strain of mutant mice. Evoked neurotransmission release at NMJs is 50-75% reduced in *rolling Nagoya*<sup>[99]</sup>. Dissociated *rolling Nagoya* PCs revealed a reduced voltage-sensitivity and a Ca<sub>v</sub>2.1 Ca<sup>2+</sup> current density<sup>[98]</sup>. Dysfunctions in the parallel and climbing fiber systems, but also basal ganglia, have been suggested to underlie the ataxic phenotype in *rolling Nagoya* mice<sup>[86, 100]</sup>. At the (ultra)structural level, *rolling Nagoya* mice exhibit a reduced cerebellar weight, a decreased number of granule cells and PCs, and PC abnormalities, such as multiple dendritic spines synapsing on single parallel fiber varicosities and axonal swelling<sup>[101, 102]</sup>. Notably, an increased expression of Ca<sub>v</sub>2.1 channels was reported for deep cerebellar nuclei of *rolling Nagoya* mice<sup>[103]</sup>.

Finally, *Rocker* mice display absence seizures as well as cerebellar ataxia. This phenotype is caused by a recessive T1310K missense mutation in the *Cacna1a* gene<sup>[104]</sup>. Abnormal PC morphology without their loss has been reported, likely underlying the motor and cerebellar dysfunction in this strain of natural mutants<sup>[104]</sup>.

There are also natural mutants relevant to this thesis that have mutations in a Ca<sub>v</sub>2.1 channel subunit other than the  $\alpha_1$  subunit. These include *ducky*, *lethargic*, and *stargazer* that lack functionally auxiliary subunits  $\alpha_2\delta$ -2,  $\beta_4$ , and  $\gamma_2$ , respectively. Their main characteristics are described below.

*Ducky* mice exhibit a wide-open gait, severe ataxia, spike-wave discharges, and paroxysmal dyskinesia<sup>[105]</sup>. The *ducky* phenotype results from a homozygous partial genomic duplication giving rise to two possible transcripts of the *Cacna2d2* gene, only one of which is actually translated into a protein, but lacks most of the  $\alpha_2$  domain. As a consequence, the *ducky* mutation is a functional  $\alpha_2\delta$ -2 knockout<sup>[106]</sup>. Dissociated



*ducky* PCs show approximately 35% reduction in  $\text{Ca}_v2.1 \text{ Ca}^{2+}$  current density<sup>[105]</sup>, that is associated with increased irregularity of intrinsic PC firing<sup>[26]</sup>. At the (ultra)structural level, mutant PCs present with severely reduced dendritic arborization, thickening of the dendrites, and a so-called “weeping willow”-like structure<sup>[106]</sup>.

*Lethargic* mice exhibit a phenotype of severe ataxia and slow (lethargic) movement<sup>[107]</sup>. The onset of the phenotype is around P10, and mice pass through a critical period between P15 and P60, during which they show increased mortality rate, weight loss and severe lymphocytopenia<sup>[108]</sup>. The *lethargic* mutation is a homozygous four nucleotide insertion of a splice site of the *Cchb4* gene, which results in the translation of a truncated, non-functional  $\beta_4$  protein<sup>[39]</sup>.  $\text{Ca}_v2.1 \text{ Ca}^{2+}$  current density in *lethargic* PCs is not affected<sup>[39]</sup>. At the (ultra)structural level, no abnormalities in the cerebellum were reported.

Finally, *stargazer* mice display ataxia, paroxysmal dyskinesia, spike-wave discharges (indicative of absence epilepsy), and typical head-tossing movements<sup>[109]</sup>. The *stargazer* mutation is a homozygous insertion in the *Cacng2* gene, leading to a non-functional  $\gamma_2$  protein product<sup>[110, 111]</sup>. The cerebellar morphology of *stargazer* mice appears normal however, loss and/or reduction of the receptors in cerebellar granule cells has been noted<sup>[112]</sup>.

### *Cacna1a* transgenic mice

Various transgenic knockout (KO) models (see Section 6.4; *Generation of transgenic mice*) have been generated that target the *Cacna1a* gene<sup>[91, 113, 114]</sup>. By disrupting the *Cacna1a* gene sequence, the KO mice lack functional  $\text{Ca}_v2.1$  channels. Homozygous KO mice exhibit a severe phenotype of ataxia and dystonia that starts around P10 and is very similar to what is seen in *leaner* mice. KO mice die around 3 weeks after birth. Heterozygous KO mice have been considered a possible model for EA2, but they do not exhibit the (relevant) phenotype. Notably, only one strain of KO mice shows decrease in  $\text{Ca}_v2.1 \text{ Ca}^{2+}$  current density in cerebellar granule neurons of heterozygous mice<sup>[114]</sup>, whereas in the other strain  $\text{Ca}_v2.1 \text{ Ca}^{2+}$  current density is normal<sup>[113]</sup>, possibly due to the fact that, in the latter strain, truncated  $\alpha_1$  proteins may have been produced that prevent transcriptional compensation of the wild-type allele. A mouse model has been generated also for SCA6. Transgenic knockin mice expressing a “hyperexpanded” (i.e., far beyond the expansion seen in SCA6 patients) glutamine repeat in the  $\alpha_1$  protein developed progressive motor impairment and aggregation of mutant  $\text{Ca}_v2.1$  channels, but no changes in intrinsic electrophysiological properties of the mutant channels were observed<sup>[115]</sup>.

Finally, knockin mice were generated that harbor the human pathogenic R192Q mutation in the *Cacna1a* gene<sup>[116]</sup>. In humans, mutation R192Q causes FHM *without* cerebellar ataxia<sup>[51]</sup>. R192Q mutant mice did not exhibit an overt phenotype. At the molecular level, several *gain-of-function* effects were found in homozygous mutants

that include increased  $\text{Ca}_v2.1$  current density in cerebellar granule cell neurons, enhanced spontaneous and evoked neurotransmission at NMJs, and, in the intact animal, an increased susceptibility for CSD<sup>[116]</sup>. A detailed analysis of NMJs revealed a gene dosage-dependent increase in neurotransmitter release that was not accompanied by overt structural adaptations<sup>[118]</sup>.

### Limitations of the existing mouse models

Attempts to unravel the neuronal basis of motor coordination dysfunction using natural mutants and transgenic knockout and knockin  $\text{Ca}_v2.1$  mice have been hampered by the widespread expression of  $\text{Ca}_v2.1$  channels throughout the brain. That is, the relative contribution of a cell type cannot be assessed in the existing mice. This is especially relevant since a lack of  $\text{Ca}_v2.1$  channel expression during development has been shown to cause cell-specific compensatory upregulations of other  $\text{Ca}^{2+}$  channel types (for review, see *Urbano et al., 2002*). Expression or lack of expression of mutant and/or normal  $\text{Ca}_v2.1$  channels in *specific cell types* is needed to address the problem. Notwithstanding, it would also be very interesting to compare knockin mouse models harboring FHM1 mutations that are (i.e., S218L) or are not (i.e., R192Q) associated with cerebellar ataxia.

### Generation of transgenic mice

There are two main strategies to generate transgenic mice: *conventional transgenesis* and *gene targeting*.

In the conventional transgenesis strategy, one or more copies of a transgene (often a cDNA with a specific promoter or a piece of genomic DNA) gets randomly integrated into the mouse genome after the microinjection of DNA into the male pronucleus of a fertilized oocyte. Relevant to this thesis, a modified promoter of the *L7* gene can be used to obtain expression of the cDNA of a gene of interest exclusively in cerebellar Purkinje cells<sup>[118]</sup>.

In the gene targeting strategy, a specific part of the mouse genome of embryonic stem cells is replaced by sequences from the targeting construct by a process called homologous recombination. There are three frequently used types of gene targeting: *knockout*, *conditional knockout*, and *knockin*. The use of knockouts to obtain mice that lack, for instance,  $\text{Ca}_v2.1$  channels was discussed above. This can be achieved by either introducing the resistance cassette (mostly neomycine that is used to select for targeted embryonic stem cells) into an exonic sequence or by using a deletion construct in such a way that the expression of the target gene is abnormal and can no longer lead to translation of a functional protein. In a conditional knockout mice, the resistance cassette is placed in an intron. The cassette is flanked by two so-called loxP sites. A third loxP site is placed in another intron in such a way that the floxed resistance cassette and the third loxP site flank one or more exons. Sequences between loxP sites are deleted at the genomic level in cells where the enzyme Cre recombinase is expressed. Crossing of

gene-targeted mice containing the three loxP sites with conventional transgenic mice expressing Cre recombinase driven by the *EIIA* promoter (i.e., Cre-deleter mice)<sup>[119]</sup>, can result in mice that contain two loxP sites and no longer the resistance cassette. These mice are called *conditional knockout* mice. In the subsequent crossing of the conditional knockout mice with Cre-transgenic mice that express the recombinase in a specific spatial and temporal manner, novel mutant mice can be generated in which the target gene product is deleted *only* in these cells in which Cre is expressed. For example, transgenic L7-driven Cre mice can be used to specifically ablate a gene product in PCs<sup>[120]</sup>. The third type of gene targeting yields knockin mice. In knockin mice, a floxed resistance cassette is placed in an intron close to the exon in which, for instance, a mutation is introduced. Using the Cre-deleter mice, the cassette is removed, yielding knockin mice that only contain the mutation and essentially one remaining intronic loxP site. Heterozygous, homozygous, and wild-type control mice are usually compared in experimental designs.

## OUTLINE OF THE THESIS

The aim of the studies described in this thesis was to investigate how abnormal Ca<sub>v</sub>2.1 channel function can cause disease, in particular motor coordination dysfunction. The chapters illustrate how various neuronal cell types in the periphery (peripheral nervous system: PNS) and the central nervous system (CNS) are affected by mutations in subunits of Ca<sub>v</sub>2.1 channels. Using existing and newly generated mouse models, the consequences of such mutations were investigated at the molecular, cellular, and systems level so as to unravel pathways involved in motor coordination.

Using the peripheral neuromuscular junction (NMJ) synapse as a model, *Chapters 2* and *3* describe the role of various subunits of Ca<sub>v</sub>2.1 channels on neurotransmission. Naturally occurring mouse models, i.e., *ducky*, *lethargic*, and *stargazer*, with mutations in Ca<sub>v</sub> subunits  $\alpha_2\delta$ -2,  $\beta_4$ , and  $\gamma_2$ , respectively, were the subject of detailed investigations (*Chapter 2*). As NMJ functioning did not seem to be affected in these mutants, either these subunits are not expressed at the mouse NMJ or adequate compensation by other subunit proteins occurs. The generation of a conditional mouse model that carries a so-called “floxed” *Cacna1a* allele is described in *Chapter 3*. This model allows for the spatial and temporal ablation of the *Cacna1a*-encoded Ca<sub>v</sub>2.1- $\alpha_1$  protein, and thereby the specific ablation of Ca<sub>v</sub>2.1 channels. Crossing the floxed mice with *EIIA* promoter-driven Cre-deleter mice resulted in KO mice that had a severe, early postnatal lethal phenotype that is identical to that seen in conventional Ca<sub>v</sub>2.1 KO mice.

The consequences of Ca<sub>v</sub>2.1- $\alpha_1$  mutations in various neuronal cell types of the CNS are described in *Chapters 4* and *5*, focusing on cell types in the cerebellar cortex where Ca<sub>v</sub>2.1 channels are highly expressed. We used cell-specific Cre recombinase-expressing transgenic mice to study the effect of specific ablation of Ca<sub>v</sub>2.1 channels in either Purkinje (i.e., Purkinje Ca<sub>v</sub>2.1 KO or L7<sup>Cre</sup>*Cacna1a* KO) (*Chapter 4*) or granule

(i.e., granule  $\text{Ca}_v2.1$  KO or  $\alpha6^{\text{Cre}}\text{Cacna1a}$  KO) (*Chapter 5*) cell neurons to dissect their  $\text{Ca}_v2.1$ -mediated role on cerebellar motor coordination. It was shown that the lack of  $\text{Ca}_v2.1$  channels in PCs, but not in granule cells is sufficient to cause cerebellar ataxia. Moreover, it could be demonstrated that  $\text{Ca}_v2.1$  channels in granule cells exert an as yet undiscovered role in the consolidation of newly acquired motor learning. *Chapter 6* describes transgenic mice expressing mutant human  $\text{Ca}_v2.1-\alpha_1$  from a transgene, driven by a Purkinje cell-specific promoter, that contained a *CACNA1A* cDNA construct with the T666M mutation. In humans, this T666M mutation causes familial hemiplegic migraine type 1 (FHM1) and in most patients with this mutation also cerebellar ataxia. The transgenic mice did not, however, exhibit a noticeable phenotype, which may be due to the relatively low expression level of the transgene.

*Chapter 7* elucidates several phenotypic and neurobiological features in transgenic mouse models that are associated with mutant  $\text{Ca}_v2.1$  channels when expressed in the PNS and CNS. Two knockin mouse models were compared that carry human pathogenic  $\text{Ca}_v2.1-\alpha_1$  mutations that are at both ends of the clinical spectrum of FHM1. While the FHM1 R192Q mutation in patients causes pure FHM without additional neurological symptoms, a much more severe phenotype of FHM with cerebellar ataxia, seizures, and severe, sometimes lethal, head trauma-triggered edema is observed in patients with the FHM1 S218L mutation. The molecular consequences of the S218L mutation can explain why S218L KI mice, and not R192Q KI mice, exhibit permanent ataxia and an increased susceptibility to epilepsy. In *Chapter 8*, the consequences of the S218L mutation in the cerebellum of S218L KI are explored in much greater detail and reveal that abnormal synapse organization leads to a relevant disturbance of the amount of  $\text{Ca}^{2+}$  influx in neurons, which in turn causes irregular firing of Purkinje cells and thereby to cerebellar ataxia.

A general discussion on the experimental findings presented in this thesis is presented in *Chapter 9*, and suggestions for future research are included.

## REFERENCES

1. WHO, Neurological disorders: public health challenges, 2006.
2. Calton JL, Taube JS. Where am I and how will I get there from here? A role for posterior parietal cortex in the integration of spatial information and route planning. *Neurobiol Learn Mem.* 2009; 91:186-96.
3. Ito M, Controller-regulator model of the central nervous system. *J Integr Neurosci.* 2002; 1:129-43.
4. Plomp JJ, van den Maagdenberg AM, Ferrari MD, Frants RR, Molenaar PC. Transmitter release deficits at the neuromuscular synapse of mice with mutations in the Cav2.1 (alpha1A) subunit of the P/Q-type  $\text{Ca}^{2+}$  channel. *Ann N Y Acad Sci.* 2003;998:29-32.
5. Sanes JR, Lichtman JW. Development of the vertebrate neuromuscular junction. *Annu Rev Neurosci.* 1999;22:389-442.
6. Wood SJ, Slater CR. Safety factor at the neuromuscular junction. *Prog Neurobiol.* 2001; 64:393-429.
7. Urbano FJ, Rosato-Siri MD, Uchitel OD. Calcium channels involved in neurotransmitter release at adult, neonatal and P/Q-type deficient neuromuscular junctions. *Mol Membr Biol.* 2002; 19:293-300.
8. Voogd J, Glickstein M. The anatomy of the cer-

- ebellum. *Trends Neurosci.* 1998; 21:370-5.
9. Marr D. A theory of cerebellar cortex. *J Physiol.* 1969; 202:437-70.
  10. Ambrosi G, Flace P, Lorusso L, Girolamo F, Rizzi A, Bosco L, Errede M, Virgintino D, Roncali L, Benagiano V. Non-traditional large neurons in the granular layer of the cerebellar cortex. *Eur J Histochem.* 2007; 51:59-64.
  11. Simat M, Parpan F, Fritschy JM. Heterogeneity of glycinergic and gabaergic interneurons in the granule cell layer of mouse cerebellum. *J Comp Neurol.* 2007; 500:71-83.
  12. Gundappa-Sulur G, De Schutter E, Bower JM. Ascending granule cell axon: an important component of cerebellar cortical circuitry. *J Comp Neurol.* 1999; 408:580-96.
  13. Simat M, Ambrosetti L, Lardi-Studler B, Fritschy JM. GABAergic synaptogenesis marks the onset of differentiation of basket and stellate cells in mouse cerebellum. *Eur J Neurosci.* 2007; 26:2239-56.
  14. Groenewegen HJ, Voogd J, Freedman SL. The parasagittal zonation within the olivocerebellar projection. II. Climbing fiber distribution in the intermediate and hemispheric parts of cat cerebellum. *J Comp Neurol.* 1979; 183:551-601.
  15. Ito M. The modifiable neuronal network of the cerebellum. *Jpn J Physiol.* 1984; 34:781-92.
  16. Glickstein M, Doron K. Cerebellum: connections and functions. *Cerebellum.* 2008; 7:589-94.
  17. Houk JC. On the role of the cerebellum and basal ganglia in cognitive signal processing. *Prog Brain Res.* 1997; 114:543-52.
  18. D'Angelo E, De Zeeuw CI. Timing and plasticity in the cerebellum: focus on the granular layer. *Trends Neurosci.* 2009; 32:30-40.
  19. Wang VY, Zoghbi HY. Genetic regulation of cerebellar development. *Nat Rev Neurosci.* 2001; 2:484-91.
  20. Glickstein M. Mossy-fibre sensory input to the cerebellum. *Prog Brain Res.* 1997; 114:251-9.
  21. D'Angelo E. The critical role of Golgi cells in regulating spatio-temporal integration and plasticity at the cerebellum input stage. *Front Neurosci.* 2008; 2:35-46.
  22. Harvey RJ, Napper RM. Quantitative studies on the mammalian cerebellum. *Prog Neurobiol.* 1991; 36:437-63.
  23. Bower JM, Woolston DC. Congruence of spatial organization of tactile projections to granule cell and Purkinje cell layers of cerebellar hemispheres of the albino rat: vertical organization of cerebellar cortex. *J Neurophysiol.* 1983; 49:745-66.
  24. Isope P, Franconville R, Barbour B, Ascher P. Repetitive firing of rat cerebellar parallel fibres after a single stimulation. *J Physiol.* 2004; 554:829-39.
  25. Hoebeek FE, Stahl JS, van Alphen AM, Schonewille M, Luo C, Rutteman M, van den Maagdenberg AM, Molenaar PC, Goossens HH, Frens MA, De Zeeuw CI. Increased noise level of purkinje cell activities minimizes impact of their modulation during sensorimotor control. *Neuron.* 2005; 45:953-65.
  26. Walter JT, Alviña K, Womack MD, Chevez C, Khodakhah K. Decreases in the precision of Purkinje cell pacemaking cause cerebellar dysfunction and ataxia. *Nat Neurosci.* 2006; 9:389-97.
  27. Yamamoto K, Kawato M, Kotosaka S, Kitazawa S. Encoding of movement dynamics by Purkinje cell simple spike activity during fast arm movements under resistive and assistive force fields. *J Neurophysiol.* 2007; 97:1588-99.
  28. Gibson AR, Horn KM, Pong M. Activation of climbing fibers. *Cerebellum.* 2004; 3:212-21.
  29. Sugihara I. Organization and remodeling of the olivocerebellar climbing fiber projection. *Cerebellum.* 2006; 5:15-22.
  30. Dittman JS, Regehr WG. Calcium dependence and recovery kinetics of presynaptic depression at the climbing fiber to Purkinje cell synapse. *J Neurosci.* 1998; 18:6147-62.
  31. van Alphen AM, De Zeeuw CI. Cerebellar LTD facilitates but is not essential for long-term adaptation of the vestibulo-ocular reflex. *Eur J Neurosci.* 2002; 16:486-90.
  32. Koekkoek SK, van Alphen AM, van der Burg J, Grosveld F, Galjart N, De Zeeuw CI. Gain adaptation and phase dynamics of compensatory eye movements in mice. *Genes Funct.* 1997; 1:175-90.
  33. Mintz IM, Sabatini BL, Regehr WG. Calcium control of transmitter release at a cerebellar synapse. *Neuron.* 1995; 15:675-88.
  34. Fatt P, Katz B. Membrane potentials at the motor end-plate. *J Physiol.* 1950; 111:46-7.
  35. Curtis BM, Catterall WA. Purification of the calcium antagonist receptor of the voltage-sensitive calcium channel from skeletal muscle transverse tubules. *Biochemistry.* 1984; 23:2113-8.
  36. Doering CJ, Zamponi GW. Molecular pharmacology of high voltage-activated calcium channels. *J Bioenerg Biomembr.* 2003; 35:491-505.

37. Ertel EA, Campbell KP, Harpold MM, Hofmann F, Mori Y, Perez-Reyes E, Schwartz A, Snutch TP, Tanabe T, Birnbaumer L, Tsien RW, Catterall WA. Nomenclature of voltage-gated calcium channels. *Neuron*. 2000; 25:533-5.
38. Dolphin AC. Calcium channel diversity: multiple roles of calcium channel subunits. *Curr Opin Neurobiol*. 2009; 19:237-44.
39. Burgess DL, Biddlecome GH, McDonough SI, Diaz ME, Zilinski CA, Bean BP, Campbell KP, Noebels JL. Beta subunit reshuffling modifies N- and P/Q-type Ca<sup>2+</sup> channel subunit compositions in lethargic mouse brain. *Mol Cell Neurosci*. 1999; 13:293-311.
40. Ludwig A, Flockerzi V, Hofmann F. Regional expression and cellular localization of the alpha1 and beta subunit of high voltage-activated calcium channels in rat brain. *J Neurosci*. 1997; 17:1339-49.
41. Bourinet E, Soong TW, Sutton K, Slaymaker S, Mathews E, Monteil A, Zamponi GW, Nargeot J, Snutch TP. Splicing of alpha 1A subunit gene generates phenotypic variants of P- and Q-type calcium channels. *Nat Neurosci*. 1999; 2:407-15.
42. Westenbroek RE, Sakurai T, Elliott EM, Hell JW, Starr TV, Snutch TP, Catterall WA. Immunohistochemical identification and subcellular distribution of the alpha 1A subunits of brain calcium channels. *J Neurosci*. 1995; 15:6403-18.
43. Craig PJ, McAinsh AD, McCormack AL, Smith W, Beattie RE, Priestley JV, Yip JL, Averill S, Longbottom ER, Volsen SG. Distribution of the voltage-dependent calcium channel alpha(1A) subunit throughout the mature rat brain and its relationship to neurotransmitter pathways. *J Comp Neurol*. 1998; 397:251-67.
44. Mintz IM, Adams ME, Bean BP. P-type calcium channels in rat central and peripheral neurons. *Neuron*. 1992; 9:85-95.
45. Mintz IM, Sabatini BL, Regehr WG. Calcium control of transmitter release at a cerebellar synapse. *Neuron*. 1995; 15:675-88.
46. Uchitel OD, Protti DA, Sanchez V, Cherksey BD, Sugimori M, Llinás R. P-type voltage-dependent calcium channel mediates presynaptic calcium influx and transmitter release in mammalian synapses. *Proc Natl Acad Sci U S A*. 1992; 89:3330-3.
47. Garwicz M. Spinal reflexes provide motor error signals to cerebellar modules--relevance for motor coordination. *Brain Res Brain Res Rev*. 2002; 40:152-65.
48. Manto M, Marmolino D. Cerebellar ataxias. *Curr Opin Neurol*. 2009; 22:419-29.
49. Jen J, Kim GW, Baloh RW. Clinical spectrum of episodic ataxia type 2. *Neurology*. 2004; 62:17-22.
50. Strupp M, Zwergal A, Brandt T. Episodic ataxia type 2. *Neurotherapeutics*. 2007; 4:267-73.
51. Ophoff RA, Terwindt GM, Vergouwe MN, van Eijk R, Oefner PJ, Hoffman SM, Lamerdin JE, Mohrenweiser HW, Bulman DE, Ferrari M, Haan J, Lindhout D, van Ommen GJ, Hofker MH, Ferrari MD, Frants RR. Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca<sup>2+</sup> channel gene CACNL1A4. *Cell*. 1996; 87:543-52.
52. Denier C, Ducros A, Vahedi K, Joutel A, Thierry P, Ritz A, Castelnovo G, Deonna T, Gérard P, Devoize JL, Gayou A, Perrouy B, Soisson T, Autret A, Warter JM, Vighetto A, Van Bogaert P, Alamowitch S, Roulet E, Tournier-Lasserre E. High prevalence of CACNA1A truncations and broader clinical spectrum in episodic ataxia type 2. *Neurology*. 1999; 52:1816-21.
53. van den Maagdenberg AM, Kors EE, Brunt ER, van Paesschen W, Pascual J, Ravine D, Keeling S, Vanmolkot KR, Vermeulen FL, Terwindt GM, Haan J, Frants RR, Ferrari MD. Episodic ataxia type 2. Three novel truncating mutations and one novel missense mutation in the CACNA1A gene. *J Neurol*. 2002; 249:1515-9.
54. Jeng CJ, Chen YT, Chen YW, Tang CY. Dominant-negative effects of human P/Q-type Ca<sup>2+</sup> channel mutations associated with episodic ataxia type 2. *Am J Physiol Cell Physiol*. 2006; 290:1209-20.
55. Page KM, Hebllich F, Davies A, Butcher AJ, Leroy J, Bertaso F, Pratt WS, Dolphin AC. Dominant-negative calcium channel suppression by truncated constructs involves a kinase implicated in the unfolded protein response. *J Neurosci*. 2004; 24:5400-9.
56. Frontali M. Spinocerebellar ataxia type 6: channelopathy or glutamine repeat disorder? *Brain Res Bull*. 2001; 56:227-31.
57. Zhuchenko O, Bailey J, Bonnen P, Ashizawa T, Stockton DW, Amos C, Dobyns WB, Subramony SH, Zoghbi HY, Lee CC. Autosomal dominant cerebellar ataxia (SCA6) associated with small polyglutamine expansions in the alpha 1A-voltage-dependent calcium channel. *Nat Genet*. 1997; 15:62-9.
58. Ishikawa K, Owada K, Ishida K, Fujigasaki H, Shun Li M, Tsunemi T, Ohkoshi N, Toru S, Mizutani T, Hayashi M, Arai N, Hasegawa K, Kawanami T, Kato T, Makifuchi T, Shoji S, Tanabe T, Mizusawa H. Cytoplasmic and nuclear polyglutamine aggregates in SCA6 Purkinje cells.

- Neurology. 2001; 56:1753-6.
59. Piedras-Renteria ES, Watase K, Harata N, Zhuchenko O, Zoghbi HY, Lee CC, Tsien RW. Increased expression of alpha 1A Ca<sup>2+</sup> channel currents arising from expanded trinucleotide repeats in spinocerebellar ataxia type 6. *J Neurosci*. 2001; 21:9185-93.
  60. Toru S, Murakoshi T, Ishikawa K, Saegusa H, Fujigasaki H, Uchihara T, Nagayama S, Osanai M, Mizusawa H, Tanabe T. Spinocerebellar ataxia type 6 mutation alters P-type calcium channel function. *J Biol Chem*. 2000; 275:10893-8.
  61. Lauritzen M. Pathophysiology of the migraine aura. The spreading depression theory. *Brain*. 1994; 117:199-210.
  62. Goadsby PJ. Migraine pathophysiology. *Headache*. 2005; 45:S14-24.
  63. Bolay H, Reuter U, Dunn AK, Huang Z, Boas DA, Moskowitz MA. Intrinsic brain activity triggers trigeminal meningeal afferents in a migraine model. *Nat Med*. 2002; 8:136-42.
  64. van den Maagdenberg AM, Haan J, Terwindt GM, Ferrari MD. Migraine: gene mutations and functional consequences. *Curr Opin Neurol*. 2007; 20:299-305.
  65. Kors EE, Melberg A, Vanmolkot KR, Kumlien E, Haan J, Raininko R, Flink R, Ginjaar HB, Frants RR, Ferrari MD, van den Maagdenberg AM. Childhood epilepsy, familial hemiplegic migraine, cerebellar ataxia, and a new CACNA1A mutation. *Neurology*. 2004; 63:1136-7.
  66. Hans M, Luvisetto S, Williams ME, Spagnolo M, Urrutia A, Tottene A, Brust PF, Johnson EC, Harpold MM, Stauderman KA, Pietrobon D. Functional consequences of mutations in the human alpha1A calcium channel subunit linked to familial hemiplegic migraine. *J Neurosci*. 1999; 19:1610-9.
  67. Tottene A, Fellin T, Pagnutti S, Luvisetto S, Striessnig J, Fletcher C, Pietrobon D. Familial hemiplegic migraine mutations increase Ca<sup>2+</sup> influx through single human CaV2.1 channels and decrease maximal CaV2.1 current density in neurons. *Proc Natl Acad Sci U S A*. 2002; 99:13284-9.
  68. Tottene A, Pivotto F, Fellin T, Cesetti T, van den Maagdenberg AM, Pietrobon D. 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*. 2005; 280:17678-86.
  69. Cao YQ, Piedras-Rentería ES, Smith GB, Chen G, Harata NC, Tsien RW. Presynaptic Ca<sup>2+</sup> channels compete for channel type-preferring slots in altered neurotransmission arising from Ca<sup>2+</sup> channelopathy. *Neuron*. 2004; 43:387-400.
  70. Moskowitz MA, Bolay H, Dalkara T. Deciphering migraine mechanisms: clues from familial hemiplegic migraine genotypes. *Ann Neurol*. 2004; 55:276-80.
  71. Kim YI, Neher E. IgG from patients with Lambert-Eaton syndrome blocks voltage-dependent calcium channels. *Science*. 1988; 239:405-8.
  72. Nakatani Y, Hotta S, Utsunomiya I, Tanaka K, Hoshi K, Ariga T, Yu RK, Miyatake T, Taguchi K. Cav2.1 voltage-dependent Ca<sup>2+</sup> channel current is inhibited by serum from select patients with Guillain-Barré syndrome. *Neurochem Res*. 2009; 34:149-57.
  73. O'Neill JH, Murray NM, Newsom-Davis J. The Lambert-Eaton myasthenic syndrome. A review of 50 cases. *Brain*. 1988; 111:577-96.
  74. Griffin JW, Li CY, Ho TW, Tian M, Gao CY, Xue P, Mishu B, Cornblath DR, Macko C, McKhann GM, Asbury AK. Pathology of the motor-sensory axonal Guillain-Barré syndrome. *Ann Neurol*. 1996; 39:17-28.
  75. Liao YJ, Safa P, Chen YR, Sobel RA, Boyden ES, Tsien RW. Anti-Ca<sup>2+</sup> channel antibody attenuates Ca<sup>2+</sup> currents and mimics cerebellar ataxia in vivo. *Proc Natl Acad Sci U S A*. 2008; 105:2705-10.
  76. van de Ven RC, Kaja S, Plomp JJ, Frants RR, van den Maagdenberg AM, Ferrari MD. Genetic models of migraine. *Arch Neurol*. 2007; 64:643-6.
  77. Barrett CF, van den Maagdenberg AM, Frants RR, Ferrari MD. Familial hemiplegic migraine. *Adv Genet*. 2008; 63:57-83.
  78. Fletcher CF, Frankel WN. Ataxic mouse mutants and molecular mechanisms of absence epilepsy. *Hum Mol Genet*. 1999; 8:1907-12.
  79. Meier H, MacPike AD. Three syndromes produced by two mutant genes in the mouse. Clinical, pathological, and ultrastructural bases of tottering, leaner, and heterozygous mice. *J Hered*. 1971; 62:297-302.
  80. Fureman BE, Jinnah HA, Hess EJ. Triggers of paroxysmal dyskinesia in the calcium channel mouse mutant tottering. *Pharmacol Biochem Behav*. 2002; 73:631-7.
  81. Kaplan BJ, Seyfried TN, Glaser GH. Spontaneous polyspike discharges in an epileptic mutant mouse (tottering). *Exp Neurol*. 1979; 66:577-86.

82. Fletcher CF, Lutz CM, O'Sullivan TN, Shaughnessy JD Jr, Hawkes R, Frankel WN, Copeland NG, Jenkins NA. Absence epilepsy in tottering mutant mice is associated with calcium channel defects. *Cell*. 1996; 87:607-17.
83. Plomp JJ, Vergouwe MN, Van den Maagdenberg AM, Ferrari MD, Frants RR, Molenaar PC. Abnormal transmitter release at neuromuscular junctions of mice carrying the tottering alpha(1A) Ca(2+) channel mutation. *Brain*. 2000; 123:463-71.
84. Kaja S, Van de Ven RC, Ferrari MD, Frants RR, Van den Maagdenberg AM, Plomp JJ. Compensatory contribution of Cav2.3 channels to acetylcholine release at the neuromuscular junction of tottering mice. *J Neurophysiol*. 2006; 95:2698-704.
85. Wakamori M, Yamazaki K, Matsunodaira H, Teramoto T, Tanaka I, Niidome T, Sawada K, Nishizawa Y, Sekiguchi N, Mori E, Mori Y, Imoto K. Single tottering mutations responsible for the neuropathic phenotype of the P-type calcium channel. *J Biol Chem*. 1998; 273:34857-67.
86. Matsushita K, Wakamori M, Rhyu IJ, Arai T, Oda S, Mori Y, Imoto K. Bidirectional alterations in cerebellar synaptic transmission of tottering and rolling Ca2+ channel mutant mice. *J Neurosci*. 2002; 22:4388-98.
87. Zhou YD, Turner TJ, Dunlap K. Enhanced G protein-dependent modulation of excitatory synaptic transmission in the cerebellum of the Ca2+ channel-mutant mouse, tottering. *J Physiol*. 2003; 547:497-507.
88. Hoebeek FE, Khosrovani S, Witter L, De Zeeuw CI. Purkinje cell input to cerebellar nuclei in tottering: ultrastructure and physiology. *Cerebellum*. 2008; 7:547-58.
89. Sawada K, Kalam Azad A, Sakata-Haga H, Lee NS, Jeong YG, Fukui Y. Striking pattern of Purkinje cell loss in cerebellum of an ataxic mutant mouse, tottering. *Acta Neurobiol Exp (Wars)*. 2009; 69:138-45.
90. Rhyu IJ, Abbott LC, Walker DB, Sotelo C. An ultrastructural study of granule cell/Purkinje cell synapses in tottering (tg/tg), leaner (tg(la)/tg(la)) and compound heterozygous tottering/leaner (tg/tg(la)) mice. *Neuroscience*. 1999; 90:717-28.
91. Kaja S, van de Ven RC, Broos LA, Frants RR, Ferrari MD, van den Maagdenberg AM, Plomp JJ. Characterization of acetylcholine release and the compensatory contribution of non-Ca(v)2.1 channels at motor nerve terminals of leaner Ca(v)2.1-mutant mice. *Neuroscience*. 2007; 144:1278-87.
92. Dove LS, Abbott LC, Griffith WH. Whole-cell and single-channel analysis of P-type calcium currents in cerebellar Purkinje cells of leaner mutant mice. *J Neurosci*. 1998; 18:7687-99.
93. Lorenzon NM, Lutz CM, Frankel WN, Beam KG. Altered calcium channel currents in Purkinje cells of the neurological mutant mouse leaner. *J Neurosci*. 1998; 18:4482-9.
94. Liu S, Friel DD. Impact of the leaner P/Q-type Ca2+ channel mutation on excitatory synaptic transmission in cerebellar Purkinje cells. *J Physiol*. 2008; 586:4501-15.
95. Herrup K, Wilczynski SL. Cerebellar cell degeneration in the leaner mutant mouse. *Neuroscience*. 1982; 7:2185-96.
96. Oda S. The observation of rolling mouse Nagoya (rol), a new neurological mutant, and its maintenance. *Jikken Dobutsu*. 1973; 22:281-8.
97. Plomp JJ, van den Maagdenberg AM, Kaja S. The ataxic Cacna1a-mutant mouse rolling nagoya: an overview of neuromorphological and electrophysiological findings. *Cerebellum*. 2009; 8:222-30.
98. Mori Y, Wakamori M, Oda S, Fletcher CF, Sekiguchi N, Mori E, Copeland NG, Jenkins NA, Matsushita K, Matsuyama Z, Imoto K. Reduced voltage sensitivity of activation of P/Q-type Ca2+ channels is associated with the ataxic mouse mutation rolling Nagoya (tg(rol)). *J Neurosci*. 2000; 20:5654-62.
99. Kaja S, van de Ven RC, van Dijk JG, Verschuuren JJ, Arahata K, Frants RR, Ferrari MD, van den Maagdenberg AM, Plomp JJ. Severely impaired neuromuscular synaptic transmission causes muscle weakness in the Cacna1a-mutant mouse rolling Nagoya. *Eur J Neurosci*. 2007; 25:2009-20.
100. 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*. 1992; 598:38-44.
101. Rhyu IJ, Oda S, Uhm CS, Kim H, Suh YS, Abbott LC. Morphologic investigation of rolling mouse Nagoya (tg(rol)/tg(rol)) cerebellar Purkinje cells: an ataxic mutant, revisited. *Neurosci Lett*. 1999; 266:49-52.
102. Suh YS, Oda S, Kang YH, Kim H, Rhyu IJ. Apoptotic cell death of cerebellar granule cells in rolling mouse Nagoya. *Neurosci Lett*. 2002; 325:1-4.
103. 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*. 2001; 316:87-90.
104. Zwingman TA, Neumann PE, Noebels JL, Herrup K. Rcker is a new variant of the voltage-dependent calcium channel gene *Cacna1a*. *J Neurosci*. 2001; 21:1169-78.
  105. Barclay J, Balaguero N, Mione M, Ackerman SL, Letts VA, Brodbeck J, Canti C, Meir A, Page KM, Kusumi K, Perez-Reyes E, Lander ES, Frankel WN, Gardiner RM, Dolphin AC, Rees M. Ducky mouse phenotype of epilepsy and ataxia is associated with mutations in the *Cacna2d2* gene and decreased calcium channel current in cerebellar Purkinje cells. *J Neurosci*. 2001; 21:6095-104.
  106. Brodbeck J, Davies A, Courtney JM, Meir A, Balaguero N, Canti C, Moss FJ, Page KM, Pratt WS, Hunt SP, Barclay J, Rees M, Dolphin AC. The ducky mutation in *Cacna2d2* results in altered Purkinje cell morphology and is associated with the expression of a truncated  $\alpha 2$  delta-2 protein with abnormal function. *J Biol Chem*. 2002; 277:7684-93.
  107. Dickie MM. New *splotch* alleles in the mouse. *J Hered*. 1964; 55:97-101.
  108. Dung HC, Swigart RH. Histo-pathologic observations of the nervous and lymphoid tissues of "lethargic" mutant mice. *Tex Rep Biol Med*. 1972; 30:23-39.
  109. Noebels JL, Qiao X, Bronson RT, Spencer C, Davisson MT. Stargazer: a new neurological mutant on chromosome 15 in the mouse with prolonged cortical seizures. *Epilepsy Res*. 1990; 7:129-35.
  110. Letts VA, Valenzuela A, Kirley JP, Sweet HO, Davisson MT, Frankel WN. Genetic and physical maps of the stargazer locus on mouse chromosome 15. *Genomics*. 1997; 43:62-8.
  111. Sharp AH, Black JL 3rd, Dubel SJ, Sundarraj S, Shen JP, Yunker AM, Copeland TD, McEnery MW. Biochemical and anatomical evidence for specialized voltage-dependent calcium channel  $\gamma$  isoform expression in the epileptic and ataxic mouse, stargazer. *Neuroscience*. 2001; 105:599-617.
  112. Qiao X, Meng H. Nonchannel functions of the calcium channel  $\gamma$  subunit: insight from research on the stargazer mutant. *J Bioenerg Biomembr*. 2003; 35:661-70.
  113. Jun K, Piedras-Rentería ES, Smith SM, Wheeler DB, Lee SB, Lee TG, Chin H, Adams ME, Scheller RH, Tsien RW, Shin HS. Ablation of P/Q-type  $\text{Ca}(2+)$  channel currents, altered synaptic transmission, and progressive ataxia in mice lacking the  $\alpha(1A)$ -subunit. *Proc Natl Acad Sci U S A*. 1999; 96:15245-50.
  114. Fletcher CF, Tottene A, Lennon VA, Wilson SM, Dubel SJ, Paylor R, Hosford DA, Tessarollo L, McEnery MW, Pietrobon D, Copeland NG, Jenkins NA. Dystonia and cerebellar atrophy in *Cacna1a* null mice lacking P/Q calcium channel activity. *FASEB J*. 2001; 15:1288-90.
  115. Watase K, Barrett CF, Miyazaki T, Ishiguro T, Ishikawa K, Hu Y, Unno T, Sun Y, Kasai S, Watanabe M, Gomez CM, Mizusawa H, Tsien RW, Zoghbi HY. Spinocerebellar ataxia type 6 knockin mice develop a progressive neuronal dysfunction with age-dependent accumulation of mutant  $\text{CaV}2.1$  channels. *Proc Natl Acad Sci U S A*. 2008; 105:11987-92.
  116. van den Maagdenberg AM, Pietrobon D, Pizzorusso T, Kaja S, Broos LA, Cesetti T, van de Ven RC, Tottene A, van der Kaa J, Plomp JJ, Frants RR, Ferrari MD. A *Cacna1a* knockin migraine mouse model with increased susceptibility to cortical spreading depression. *Neuron*. 2004; 41:701-10.
  117. Kaja S, van de Ven RC, Broos LA, Veldman H, van Dijk JG, Verschuuren JJ, Frants RR, Ferrari MD, van den Maagdenberg AM, Plomp JJ. Gene dosage-dependent transmitter release changes at neuromuscular synapses of *CACNA1A R192Q* knockin mice are non-progressive and do not lead to morphological changes or muscle weakness. *Neuroscience*. 2005; 135:81-95.
  118. Oberdick J, Smeyne RJ, Mann JR, Zackson S, Morgan JL. A promoter that drives transgene expression in cerebellar Purkinje and retinal bipolar neurons. *Science*. 1990; 248:223-6.
  119. Lakso M, Pichel JG, Gorman JR, Sauer B, Okamoto Y, Lee E, Alt FW, Westphal H. Efficient in vivo manipulation of mouse genomic sequences at the zygote stage. *Proc Natl Acad Sci U S A*. 1996; 93:5860-5.
  120. Barski JJ, Dethleffsen K, Meyer M. Cre recombinase expression in cerebellar Purkinje cells. *Genesis*. 2000; 28:93-8.