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The Ataxic *Cacna1a*-Mutant Mouse *Rolling Nagoya*: An Overview of Neuromorphological and Electrophysiological Findings

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Abstract Homozygous *rolling Nagoya* natural mutant mice display a severe ataxic gait and frequently roll over to their side or back. The causative mutation resides in the *Cacna1a* gene, encoding the pore-forming α_1 subunit of $Ca_v2.1$ type voltage-gated Ca^{2+} channels. These channels are crucially involved in neuronal Ca^{2+} signaling and in neurotransmitter release at many central synapses and, in the periphery, at the neuromuscular junction. We here review the behavioral, histological, biochemical, and neurophysiological studies on this mouse mutant and discuss its usefulness as a model of human neurological diseases associated with $Ca_v2.1$ dysfunction.

Keywords Ataxia · Rolling Nagoya mouse · Cacna1a mutation · $Ca_v2.1$ Ca^{2+} channel · Synaptic transmission

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Introduction

The ataxic mouse *rolling Nagoya* (RN) is a natural mutant of which the neurological phenotype and cerebellar characteristics have been studied quite extensively in the first years following its initial report back in 1973 by Oda [1]. Research on this mouse mutant revived in 2000 when the causative mutation was identified in the *Cacna1a* gene, encoding the pore-forming α_1 subunit of $Ca_v2.1$ (P/Q-type) voltage-gated Ca^{2+} channels [2]. This type of channel is involved in neuronal Ca^{2+} signaling and also in neurotransmitter release at many central synapses as well as the neuromuscular junction (NMJ) in the periphery [3, 4]. The discovery of the RN mutation in *Cacna1a* was of particular interest because mutations in the orthologous human gene had in the meantime been identified in patients suffering from inherited forms of migraine and ataxia [5]. Besides, the same $Ca_v2.1$ channels were shown to be the autoimmune targets at the NMJ in the paralytic disorder Lambert–Eaton myasthenic syndrome (LEMS) [6]. These developments, therefore, designated the RN mouse (together with other $Ca_v2.1$ mouse mutants) as a potential model for $Ca_v2.1$-channelopathies. In this review, we will provide an overview on the neurochemical, -physiological, and -morphological findings in the RN mouse and will discuss its usefulness in studying ataxia, migraine, and neuromuscular synapse dysfunction.

Rolling Nagoya Phenotype

The RN mutant mouse was first described 35 years ago by Oda [1]. It was identified as a natural mutant among descendants of a cross between the SIII and C57Bl/6JNA strains but maintained on a C3Hf/Nga background [1]. Later studies showed that the RN mutation was allelic to the *tottering* mutation, which had been mapped on chromosome 8 [7]. A prominent phenotype in homozygous RN mice is a broad-based, severe ataxic gait with motor deficits that are characterized by frequent lurching of the mice and abnormal cyclic movements of the hind limbs when walking (Fig. 1). These symptoms of motor disturbances of the hind limbs and balancing difficulties become noticeable between postnatal days 10 and 14. RN mice do not show trunk tremor during movement or at rest. In addition, they have a 25–30% reduction in body weight [8, 9]. The motor symptoms in RN males make coitus difficult, causing a reduced breeding capacity. Females are fertile but produce less surviving offspring due to poor nursing abilities [7]. Still, once successfully gone through the weaning period, RN mice have a normal life span [7]. Heterozygous RN mice display no overt neurological symptoms.

A more detailed characterization of ataxia in RN revealed abnormalities in several motor tasks [10]. For instance, compared to wild-type littermates, RN mice underperformed by frequent falling from a 2-mm-thin horizontal wire or by falling or showing persistent exhibition of head-upward descent from a thick vertical rope, whereas wild-type mice from postnatal day 16 predominantly used a head-downward descent. In addition, footprint analysis revealed that RN mice used "double stepping of the same hind limb" in an attempt to compensate for their locomotor disability and being able to transverse forward as well as possible. In addition to the motor coordination defects and body weight reduction, RN mice exhibit muscle weakness [9]. This was shown in grip strength measurements that revealed a 62% reduction in pulling force compared to wild type. In addition, fatigability of limb muscles of RN mice was demonstrated in the inverted grid hanging test: hanging times of RN mice ranged from only 7 to 16 s, whereas almost all wild-type mice completed the maximum recording period of 300 s.

The severity of the phenotype in RN mice is intermediate to that of other natural $Ca_v2.1$ mouse mutants (Table 1). The ataxia is more severe than in *tottering* but less severe than in *leaner* mice. Notably, RN mice do not show the absence or motor seizures present in the latter mutants [11], nor do they exhibit paroxysmal dyskinesis as seen in *tottering* mice [12]. Interestingly, compound heterozygous mice with RN and *tottering* alleles show abnormal locomotor activities and a wobbly gait of the RN mice, but not the typical epileptiform seizures seen in the *tottering* mice.

Locus of the *Rolling Nagoya* Mutation

The RN mouse mutation was only relatively recently mapped to the *Cacna1a* gene, located on mouse chromosome 8, encoding the pore-forming α_1-subunit of neuronal $Ca_v2.1$ (P/Q-type) Ca^{2+} channels [2]. The mutation is a C-to-G change at nucleotide position 3784 of the gene that results in a charge-neutralizing amino acid change from a highly conserved arginine to glycine at position 1262 in the $Ca_v2.1$-α_1 protein (Fig. 2). The R1262G mutation disturbs the characteristic pattern of positively charged amino acids of one of the channel's voltage sensors, localized in the fourth transmembrane segment of the third repeating domain, which reduces the voltage sensitivity of the channel (see below).

$Ca_v2.1$ channels belong to the group of high voltage-activated Ca^{2+} channels that also includes Ca_v1 (L-type), $Ca_v2.2$ (N-type), and $Ca_v2.3$ (R-type) channels. Localized in the membranes of both cell bodies and presynaptic terminals [3, 4], $Ca_v2.1$ channels are involved in neuronal Ca^{2+} signaling pathways, including those involved in gene expression [13], and are key mediators of neurotransmitter release in both the central and the peripheral nervous

Fig. 1. Homozygous *rolling Nagoya* mice while rolling on their back (*left panel*) or side (*right panel*). Wild-type littermate at *top of left panel*

Table 1 Genetic, behavioral, neuropathological, and electrophysiological characteristics of the *rolling Nagoya* mouse in comparison to other natural and induced *Cacna1a* mouse mutants

Mutant	*Cacna1a* mutation	Neurological phenotype		Cerebellar atrophy	Main electrophysiological defects $Ca_v2.1$	References
		Symptoms	Dominant or recessive			
rolling Nagoya	Missense R1262G	Ataxia	r	?	Positive shift activation voltage Reduced current density	[2]
tottering	Missense P601L	Ataxia, epilepsy, dyskinesia, dystonia	r	−	Reduced current density (?)	[2, 6, 59-62]
leaner	Splice site mutation: truncated and aberrant cytoplasmatic C-terminus	Severe ataxia, epilepsy, premature death	r	+	Reduced current density Positive shift activation voltage (?)	[61-63]
rocker	Missense T1310K	Ataxia, epilepsy, intention tremor	r	−	Reduced current density	[64, 65]
wobbly	Missense R1255L	Ataxia	d	+	Not studied yet	[66]
Cacna1a$^{tg\text{-}4J}$	Missense V581A	Ataxia, epilepsy	r	−	Positive shift activation voltage	[67]
Cacna1a$^{Tg\text{-}5J}$	Missense R1252Q	Ataxia, premature death (of homozygote)	d	−	Negative shift activation voltage	[67]
R192Q knockin	Missense R192Q	None	na	−	Negative shift activation voltage	[54]
knockout	*null*	Ataxia, epilepsy	r	+	Absent current	[68, 69]

d dominant, *r* recessive, *na* not applicable, + present, − absent, *?* controversial

system. Immunohistochemical and in situ hybridization studies have shown that $Ca_v2.1$ protein and mRNA are abundantly and broadly distributed over almost all brain areas, with a particularly high expression in the cerebellum [3, 14-16]. In the periphery, $Ca_v2.1$ channels are present at presynaptic motor nerve terminals at the NMJ [4, 17]. In vivo, the *Cacna1a*-encoded $Ca_v2.1$-α_1 subunit is associated with auxiliary subunits of the $\alpha_2\delta$, β, and γ families, which modulate the properties of the channel. In recent years, a large number of mutations in the $Ca_v2.1$ channel has been identified and shown to underlie several human neurological disorders, including inherited forms of migraine, episodic ataxia, and epilepsy (Table 2) [18].

Morphological Studies of *Rolling Nagoya* Cerebellum and Other Brain Areas

Many studies investigated RN brain anatomy and morphology as well as the expression and distribution of neurotransmitter receptors in the RN brain. Since the early studies on cerebellar anatomy, there has been a controversy on the presence or absence of cerebellar atrophy and apoptosis. While some of the older studies showed a small cerebellar volume, reduced weight and a reduction in the total number of granule, basket, and superficial stellate cells, others found a normal anatomy (for summary overview, see "Introduction" of [19]). More recent studies

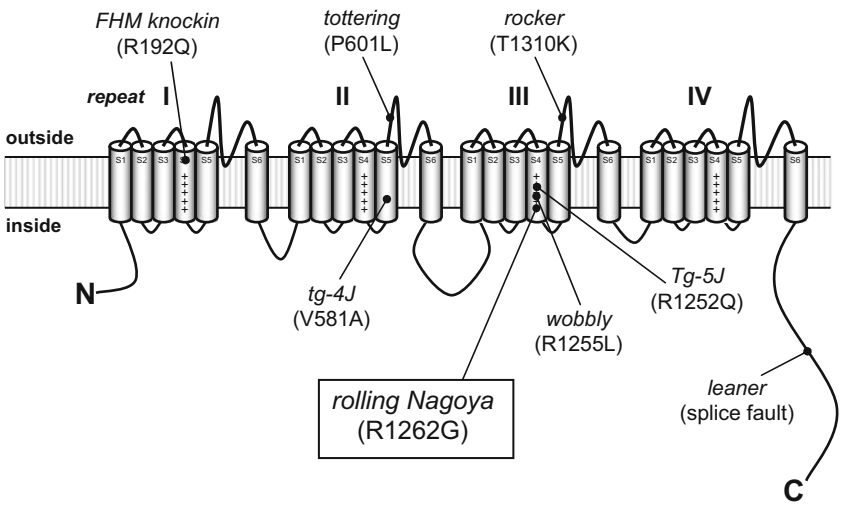

Fig. 2. Transmembrane topology of the $Ca_v2.1$-α_1 protein, with the location of the *rolling Nagoya* arginine-to-glycine mutation at position 1262 (R1262G) in the voltage-sensing S4 segment of the third repeating domain. Also indicated are the localizations of the mutations of other *Cacna1a* mouse mutants

Table 2 Human $Ca_v2.1$ channelopathies

Disorder	Main symptoms	Etiology	References
Familial hemiplegic migraine type-1	Migraine attacks with aura Hemiparesis during migraine aura Sometimes (progressive) ataxia	Genetic: missense mutations in *CACNA1A*	[5, 18]
Episodic ataxia type-2	Ataxic episodes with mildly progressive baseline ataxia Interictal nystagmus Sometimes muscle weakness	Genetic: truncation, deletion and missense mutations in *CACNA1A*	[5, 18, 49]
Spinocerebellar ataxia type-6	Late onset, slowly progressive ataxia Dysarthria Nystagmus	Genetic: CAG trinucleotide expansion in *CACNA1A*	[48, 70]
Rare forms of epilepsy	Generalized tonic-clonic epileptic seizures Absence epilepsy Episodic ataxia	Genetic: missense and truncation mutations in *CACNA1A*	[71, 72]
Lambert–Eaton myasthenic syndrome	(Proximal) muscle weakness Often small-cell lung cancer Sometimes autonomic dysfunction (dry mouth, impotence) Sometimes ataxia	Autoimmune: anti-$Ca_v2.1$ auto-antibodies	[73]

have re-addressed but not solved this question. In 3- to 4-week-old RN mice, no abnormalities in cerebellar anatomy nor apoptosis was observed [2], and deep cerebellar nuclei of 4- to 8-month-old RN mice had a normal cell density [20]. In contrast, others have reported (cerebellar granule cell) apoptosis in 4-month-old [21] and, especially in the anterior lobe, in 3-week-old RN mice [22]. The reasons for these discrepancies remain unclear.

In deep cerebellar nuclei, increased numbers of $Ca_v2.1$-α_1 positive neurons have been shown with immunohistochemistry, possibly as a compensatory response to reduced $Ca_v2.1$ activity due to the RN mutation (see below) [20].

RN mice show ectopic tyrosine hydroxylase (TH) expression in the cerebellum [2, 20, 23], as also found in the other $Ca_v2.1$ mouse mutants *tottering* [24] and *leaner* [25]. TH is normally expressed only during development and ectopic TH expression in $Ca_v2.1$ mutants may thus be a sign of delayed neuronal maturation. Interestingly, no enzymatically active form of TH, i.e., phosphorylated at serine residue 40, was identified in the RN cerebellum [26], suggesting that there is no aberrant catecholamine synthesis. Because the Ca^{2+} concentration in Purkinje cells is an important determinant of TH expression [27, 28], the ectopic TH expression in RN cerebella is likely the direct result of Ca^{2+} dysregulation following from $Ca_v2.1$ dysfunction.

Increased levels of corticotropin-releasing factor (CRF) were found in some climbing fibers as well as in mossy fibers and inferior olive neurons of the RN cerebellum [20, 29, 30]. Interestingly, increased CRF immunoreactivity in climbing fibers correlated with TH-positive Purkinje cells [20]. CRF is a neuropeptide that is widely expressed throughout the central nervous system (CNS) where it acts as a neuromodulator. In Purkinje cells, CRF increases glutamate and reduces γ-aminobutyric acid (GABA) sensitivity [31]. Furthermore, it can potentiate Ca_v1 (L-type) currents [32]. It is perceivable how a similar mechanism in the RN cerebellum could result in Ca^{2+} dysregulation and cause ectopic TH expression. Of note, $Ca_v1.2$ channels are selectively upregulated in the cerebellum, but not forebrain, of *tottering* mice [33], which also show prominent ectopic TH expression.

Expression of ryanodine receptors type 1 and 3 is altered in the RN cerebellum [34], indicating a possible disturbance of intracellular Ca^{2+} mobilization from the endoplasmatic reticulum. Autoradiography studies have shown reduced levels of $GABA_A$ and adenosine A_1 receptors in the cerebellum and of A_1 receptors in the cerebral cortex and caudate-putamen. Furthermore, benzodiazepine binding sites were found reduced in the cerebral cortex and increased in the CA1 subfield of the hippocampus [35].

Although the motor disturbances in RN mice are generally typified as *cerebellar* ataxia, based on behavioral, histological, and physiological analyses (see below), some features also suggest *extrapyramidal* disturbances. Increased local cerebral glucose utilization (indicating enhanced neuronal activity) in the basal ganglia (including the globus pallidus, entopeduncular nucleus, substantia nigra reticulate, and subthalamic nucleus) as well as electrophysiological abnormalities recorded in the globus pallidus have led to the hypothesis that motor disturbances of RN mice may perhaps in the end be not so much due to cerebellar dysfunction but rather due to striatial dysfunction [19, 36]. In addition, radiochemical studies have shown increased preproenkephalin and preprotachykinin mRNA in the striatum [37]. More research is clearly needed to shed light

on how (combined) striatal and cerebellar dysfunction causes motor dysfunction in RN mice.

Taken together, there is much histological and biochemical evidence of altered expression levels of a multitude of intracellular and membrane proteins in many structures of the RN brain. These changes may, in principle, all contribute to motor dysfunction but must be secondary (developmental or compensatory) phenomena resulting from the primary defect in RN, namely a disturbed Ca^{2+} signaling due to the dysfunction of $Ca_v2.1$ channels resulting from the RN missense mutation.

Functional Consequences of the *Rolling Nagoya* Mutation in $Ca_v2.1$ Channels

The consequences of the RN mutation on the biophysical properties of $Ca_v2.1$ channels have been investigated both in primary Purkinje cell cultures obtained from RN mice and in a heterologous expression system [2]. When expressing RN-mutated $Ca_v2.1$-α_1 in baby hamster kidney cells that also stably express the auxiliary subunits $\alpha_2\delta$ and β_{1a}, whole cell peak current density (with Ba^{2+} as charge carrier) was reduced by nearly 75%, compared with the wild-type control. Furthermore, the mutation affected the voltage dependence of activation of $Ca_v2.1$ channels, shifting the midpoint of activation by ~10 mV in the positive direction and increasing the slope factor by ~2 mV, demonstrating a shallower voltage dependence. In contrast, the voltage of inactivation of RN $Ca_v2.1$ channels was unaffected in these experiments. $Ca_v2.1$ type Ca^{2+} currents measured in native cerebellar Purkinje cell bodies were similarly affected by the RN mutation, showing reduced density (~25%), a positive shift (~8 mV) of the midpoint of the voltage of activation, and a ~1 mV increase of the slope factor. In contrast to heterologously expressed channels, the inactivation voltage midpoint was shifted by ~9 mV in the positive direction in native RN Purkinje cells. The finding of a reduced voltage sensitivity nicely demonstrates that the R1262G mutation indeed affects the function of the voltage sensor of $Ca_v2.1$ channels, resulting in diminished $Ca_v2.1$ activity in Purkinje and other cells expressing this channel, which likely is the initial factor in the cascade that ultimately results in the ataxic RN phenotype.

Neurophysiological Effects of the *Rolling Nagoya* Mutation

Aberrant Firing Pattern in Purkinje Neurons

Current-clamp analyses at Purkinje cell somata in RN brain slices revealed a disturbed firing pattern of action potentials upon stimulation with large depolarizing currents [2]. The repetitive firing of Na^+ action potentials was aborted due to interspike depolarization, reminiscent of the effect of blocking Ca^{2+}-activated K^+ channels by Cd^{2+} in wild-type neurons. These channels are important for post-spike repolarization and are presumably activated by the Ca^{2+} influx through $Ca_v2.1$ channels on the soma and dendritic tree of the Purkinje cell. Apparently, reduced $Ca_v2.1$ function in RN Purkinje cell dendritic tree and/or soma leads to impaired repolarization, causing impairment of high-frequency spiking. Reduced current through RN-mutated $Ca_v2.1$ channels was further indicated by the observation that Ca^{2+} spikes were hard to evoke in RN cells. Together, these experimental findings suggest that the RN mutation impairs the neuronal firing behavior of Purkinje cells (in response to synaptic integration) and thus affects cerebellar neuronal network function, contributing to the ataxia. Similar observations have been made in other $Ca_v2.1$-mutant mice [38]. These findings do not exclude involvement of brain areas other than the cerebellum in causing the movement abnormalities of RN mice. For instance, spontaneous firing rate of globus pallidus neurons in the basal ganglia is increased, likely resulting from a diminished inhibitory input [19].

Synaptic Dysfunction

Cerebellum

Cerebellar synaptic dysfunction in RN mice is highly likely in view of the demonstrated causative mutation in $Ca_v2.1$-α_1 and the abundance of this channel at cerebellar nerve terminals [3]. Cerebellar cortical Purkinje cell dendrites receive extensive (excitatory) synaptic input from nerve terminals of climbing and parallel fibers, and there are many other synaptic connections within the cerebellum that contribute to network function [39]. There has been only very limited study of the details of cerebellar synaptic transmission in the RN mouse. Neurochemistry studies in cerebellar homogenates showed increased concentration of neurotransmitters glutamate, serotonin, noradrenaline, and dopamine and decreased glycine [8, 40]. Morphological studies showed abnormally shaped Purkinje cell dendritic spines and single parallel fiber varicosities making multiple synaptic contacts, not observed in the wild-type [21]. While these biochemical and histological studies roughly indicated neurotransmission deficits in the RN cerebellum, they did not provide detailed insight in the synaptic dysfunction. To our knowledge, there is only one study in which cerebellar synaptic function in RN mice was characterized with direct and detailed electrophysiological measurements. Matsushita and colleagues [41] measured with voltage-clamp methods in brain slices the glutamatergic synaptic currents originating

from neurotransmission in parallel fiber as well as climbing fiber synapses on Purkinje cells. They found reduced excitatory postsynaptic currents at parallel fiber synapses, with increased paired-pulse facilitation. With Ca^{2+} channel type-selective toxins, it was shown that presynaptic Ca^{2+} influx at wild-type parallel fiber synapses is jointly mediated by $Ca_v2.1$, -2.2, and presumably -2.3 type channels, where $Ca_v2.1$ is the predominant subtype. In RN, the $Ca_v2.1$ contribution is somewhat reduced, while those of $Ca_v2.2$ and -2.3 are somewhat increased. Interestingly, the situation in climbing fiber synapses is completely different. Excitatory postsynaptic currents in these synapses are *enhanced* and display a slower decay phase, compared to wild type. There is normal triggering of Purkinje cell spikes by climbing fiber synaptic transmission. Specific $Ca_v2.1$ contribution to neurotransmitter release was found clearly reduced, while that of $Ca_v2.2$ was increased. Pharmacological analyses indicated that the increased and broadened excitatory postsynaptic currents are rather due to increased postsynaptic sensitivity to glutamate than to increased presynaptic release.

Neuromuscular Junction

Besides being a predominant presynaptic Ca^{2+} channel in the CNS, $Ca_v2.1$ channels are also present in the peripheral nervous system at intramuscular motor nerve terminals where they mediate the release of acetylcholine (ACh) at the NMJ [4, 17, 42]. Therefore, NMJ dysfunction is to be expected in *Cacna1a*-mutant mice. We have tested this hypothesis with detailed electrophysiological methods in several mutants [43-45], including RN [9]. We observed a large reduction (50–75%, depending on the muscle type) of nerve stimulation-evoked ACh release at RN NMJs (Fig. 3). Interestingly, this was accompanied by a ~3-fold increase of spontaneous ACh release, measured as miniature endplate potential frequency. RN is the only *Cacna1a* mouse mutant so far in which opposing effects on spontaneous and evoked ACh release were found by us. Most likely, they result from a complex effect of the mutation on different functional channel parameters, allowing for increased Ca^{2+} influx at resting potential while limiting Ca^{2+} influx upon depolarization by a nerve impulse. Compensatory non-$Ca_v2.1$ channels appear to be absent, as the selective $Ca_v2.1$ channel blocker ω-agatoxin-IVA reduced evoked ACh release by ~95% in both wild-type and RN NMJs. Reduced ACh release at NMJs most likely underlies the muscle weakness and fatigue we observed in grip strength and inverted grid hanging tests of RN mice; this was further substantiated by our finding of a reduced and decrementing compound muscle action potential with in vivo electromyography and a reduced safety factor of neuromuscular synaptic transmission in ex vivo muscle contraction experiments. Therefore, our NMJ studies indicate that the gait abnormality of RN mice is likely due to a combination of ataxia and muscle weakness and that the RN mouse models, besides ataxia, aspects of the NMJ dysfunction in LEMS (see below), where presynaptic $Ca_v2.1$ channels are targeted by auto-antibodies [6].

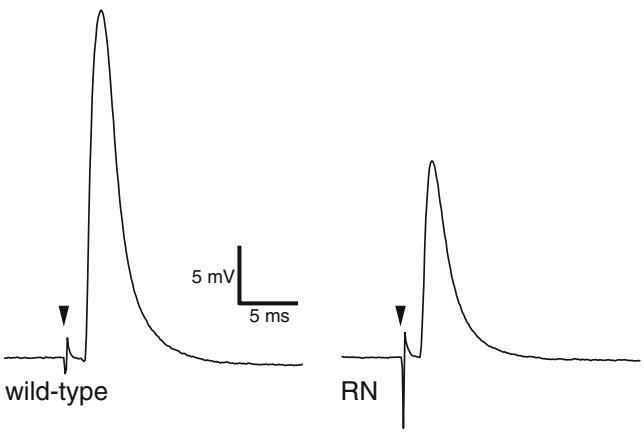

Fig. 3. Reduced amplitude of the endplate potential due to reduced acetylcholine release at the neuromuscular synapse of the *rolling Nagoya* mouse, recorded in an ex vivo diaphragm–phrenic nerve muscle nerve preparation with intracellular electrophysiological techniques. *Black triangle* indicates moment of nerve stimulation

Does the *Rolling Nagoya* Mouse Form a Good Model for (Aspects of) Human Neurological Disease?

Ataxia

The ataxic phenotype of RN mice has been described in much detail [10] and is not "contaminated" by epileptic seizures, as seen in other *Cacna1a*-mutant strains such as *tottering*, *leaner*, *rocker*, and *null*-mutants (Table 1) [46, 47]. Therefore, the RN mouse seems a valuable ataxia model, suitable for testing the anti-ataxic properties of (experimental) drugs, especially in the context of human *CACNA1A* mutation-related cerebellar ataxia [5, 48, 49]. Such studies are needed because current drug treatment of ataxia is not optimal [50]. Only a few compounds (such as acetazolamide in episodic ataxia type 2) have been reported to improve ataxia, but none of them has been studied in a controlled or comparative way [49]. Surprisingly, few anti-ataxic drug studies have been performed using the RN mouse mutant as ataxia model. Two studies have shown anti-ataxic effects of thyrotropin-releasing hormone and synthetic analogs (with only minor hormonal activity) in RN mice, possibly due to yet undefined neuroprotective or metabolic effects on RN brain areas [8, 51]. It would be of interest to test the effect of drugs acting on Ca^{2+}-activated K^+ channels, in view of the likely involvement of these channels in aberrant action potential firing of cerebellar Purkinje cells of RN [2] and other ataxic *Cacna1a*-mutant mice [38].

Migraine

One may wonder whether the RN mouse may also be a relevant animal model to study migraine, which is a common, neurovascular brain disorder of disabling attacks of headache and associated neurological symptoms [52]. After all, mutations in the *CACNA1A* gene in humans cause familial hemiplegic migraine type 1 (FHM1) [5], a rare subtype of migraine with aura with transient hemiparesis during the aura phase. FHM is considered a model for the common forms of migraine because of similar aura and headache characteristics. Moreover, the majority of FHM patients also have normal attacks of migraine without hemiparesis. Interestingly, some 20% of FHM1 patients also suffer from cerebellar ataxia that is also the prominent neurological phenotype in RN mice.

However, electrophysiological studies of FHM1-mutated $Ca_v2.1$-transfected cells and dissociated cerebellar neurons from a recently generated *Cacna1a* knockin mouse carrying the FHM1 mutation R192Q [18, 53, 54] indicate that the consequences of FHM1 mutations on $Ca_v2.1$ channel function are in some important respects opposite to those of the RN mutation [2]. Whereas FHM1 mutations cause gain-of-function effects on neuronal Ca^{2+} influx with a shift of channel activation voltage in the *negative* direction and increased $Ca_v2.1$ current density, the RN mutation causes *reduced* $Ca_v2.1$ current density and a shift of activation voltage in the *positive* direction. In view of these differences, the RN mouse seems not useful as a model for (familial hemiplegic) migraine.

Lambert–Eaton Myasthenic Syndrome

Our own neuromuscular electrophysiological analyses and muscle strength tests of RN mice [9] have revealed that this mutant shares certain aspects with the paralytic auto-immune disease LEMS, where auto-antibodies target presynaptic $Ca_v2.1$ channels at the NMJ. Electrophysiological analysis of synaptic signals at biopsied NMJs of LEMS patients showed severely reduced ACh release [55], as found at RN NMJs. A similar presynaptic defect was present at biopsy NMJs of three congenital myasthenic syndrome patients without anti-$Ca_v2.1$ antibodies or identified *CACNA1A* mutation but with symptoms of ataxia [56] and, furthermore, at biopsy NMJs of two episodic ataxia type 2 patients with *CACNA1A* truncation mutations [57]. Conversely, some LEMS patients have accompanying symptoms of ataxia [58]. In addition, electromyography performed in LEMS patients resembles that in RN mice, in that there is a low initial compound muscle action potential which decrements during low-frequency nerve stimulation (1–10 Hz). Thus, although different causes underlie the paralytic symptoms in RN mice and LEMS patients (i.e., genetic vs. auto-immune), these similarities indicate that RN mice can serve as a non-immunological model for (NMJ function) aspects of LEMS and could be useful for drug studies aiming to improve treatment of NMJ dysfunction.

Conclusion

The R1262G mutation in the $Ca_v2.1$-α_1 protein of the RN mouse causes a reduced voltage sensitivity of $Ca_v2.1$ Ca^{2+} channels. This presumably leads to reduced Ca^{2+} influx in cerebellar and other neurons that express the channel, causing disturbed Ca^{2+} signaling leading to aberrant expression of many neuronal proteins and possibly also to the apoptosis of some neurons. Also, $Ca_v2.1$-dependent central synaptic transmission is likely to be disturbed. Together, these complex phenomena culminate in the well-described motor coordination defects of RN mice. It is yet unclear to which extent noncerebellar regions such as the basal ganglia contribute to the motor symptoms and whether cerebellar atrophy is an important factor. Neuromuscular function analyses together with synaptic studies at the NMJ indicate that, in addition to the ataxia, the RN phenotype has a muscle weakness component. Although the RN mouse may not represent a very good model for common forms of migraine or not even for FHM1 (defined by *CACNA1A* mutations), it may be useful in the experimental study of new anti-ataxic drugs and drugs that restore disturbed NMJ function.

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