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Controlling cerebellar output to treat refractory epilepsy

Trends in Neurosciences 2015:
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Generalized epilepsy is characterized by recurrent seizures caused by oscillatory neuronal firing throughout thalamo-cortical networks. Current therapeutic approaches often intervene at the level of the thalamus or cerebral cortex to ameliorate seizures. Here, we review the therapeutic potential of cerebellar stimulation. The cerebellum forms a prominent ascending input to the thalamus and, whereas stimulation of the foliated cerebellar cortex exerts inconsistent results, stimulation of the centrally located cerebellar nuclei reliably stops generalized seizures in experimental models. Stimulation of this area indicates that the period of stimulation with respect to the phase of the oscillations in thalamo-cortical networks can optimize its effect, opening up the possibility of developing on-demand DBS treatments.

2.1 Neurostimulation for drug-resistant epilepsy

Epilepsy, defined as the occurrence of recurrent, unprovoked seizures, is one of the most common neurological disorders, affecting approximately 65 million people worldwide [40, 41]. The disorder can have devastating effects on one's life, not only directly due to the clinical effects, which may include injury and hospitalization, but also due to socio-economic effects such as social isolation, stigmatization, educational difficulties and unemployment. These various consequences of epilepsy result in a high comorbidity with psychiatric disorders and an increased suicide rate [42]. Anti-epileptic drugs (AEDs), which induce considerable side-effects, provide a decrease in seizure occurrence of more than 50% in ~70% of epilepsy patients [1, 41-43]. In the remaining 30% of patients the next line of treatment is often invasive. If the focus (or foci) of the seizures can be localized, and if the tissue involved is accessible and non-eloquent (**Glossary**), patients can be treated by neurosurgical resection [44]. If patients cannot be operated upon or show refractory epilepsy following resection, they are potential candidates for neurostimulation, which comprises ~30% of the medication-resistant cases.

Selecting the optimal stimulation target to treat these severely affected patients is a challenging task. However, the current surge of data from various clinical trials on the impact of vagal nerve stimulation (VNS) and deep brain stimulation (DBS) in the thalamus or epileptic focus reveals that for various types of drug-resistant epilepsies specific neurostimulation paradigms have therapeutic value (**Box 1**). Moreover, recent experimental evidence indicates that neurostimulation of the cerebellum can have potential therapeutic benefits [45, 46]. In contrast to the cerebellar cortex, which has been probed for treatment of epilepsy since the dawn of deep brain stimulation (DBS) [47], the cerebellar nuclei (CN) have rarely been targeted for seizure control in epilepsy patients [48, 49]. However, the CN are in a key position to affect a wide range of thalamic nuclei (**Box 2**) and can therefore, in our opinion, be of potential therapeutic interest for the treatment of particular types of epilepsy. Here, we will address why the CN should be targeted and how the impact of the CN on thalamo-cortical networks should be studied in experimental epilepsy models. We aim to provoke a re-evaluation of the potential use of cerebellar neurostimulation to stop epileptic seizures. Given the outcome of this evaluation we propose that single pulse stimulation of CN should be considered for novel closed-loop approaches that refine on-demand seizure control.

Box 1. Common neurostimulation treatment in the clinic

-Vagal nerve stimulation

Regardless of the type of seizures, the first line of neurostimulation treatment for refractory epilepsy [50] is vagal nerve stimulation (VNS). A meta-analysis on the results of VNS in thousands of epilepsy patients revealed that on average 50% of patients showed a 51% reduction in seizure frequency, with the important side notes that generalized seizures are more effectively treated than focal seizures and that very few patients will become seizure-free following VNS treatment [50]. The mechanisms underlying the therapeutic effect of VNS have only recently been described to rely at least partially on the prevention of hyper-synchronized neuronal activity (**Box 2**) [51-53].

-DBS for partial seizures

Apart from VNS various other neurostimulation trials have been conducted to treat refractory epilepsy of various types [39]. The SANTE study aimed to treat frontal and temporal lobe partial seizures in patients with drug-resistant epilepsy by stimulating the anterior nucleus of the thalamus (ANT) [54]. High-frequency stimulation effectively lowered seizure frequency by ≥50% in 43% of patients during the first year and by 69% in 68% of patients in the fifth year of stimulation [15]. These results indicate that continuous, i.e. non-responsive, ANT stimulation, is to some extent effective in treating patients with an epileptic focus in the frontal and temporal lobes.

In addition to this open-loop approach, responsive neurostimulation has also been tested in patients with refractory partial seizures. Patients enrolled in the 'Neuropace' study received a patient-tailored electrical stimulation in the epileptic focus upon the onset of epileptogenic activity patterns in frontal or temporal lobe [55]. The recently published findings revealed a stable level of seizure reduction up to 66% [56]. Together these data indicate that partial seizures originating from the frontal or temporal lobe may be adequately treated using responsive focal stimulation and continuous stimulation of the interconnected anterior thalamus nucleus.

-Thalamic DBS for primarily generalized seizures

Another form of refractory epilepsy is primarily generalized epilepsy. Neurostimulation treatment for these types of seizures requires a structure that projects to wide areas of the cerebral cortex. The centromedian (CM) thalamus region projects diffusely to agranular layers of cerebral cortices as well as to subcortical structures [57-59]. A recent single-blinded study on the effects of high frequency CM stimulation reported that all six patients of generalized epilepsies showed a reduced occurrence of seizures [60], which was in line with earlier findings of the Velasco group on dozens of patients [61-65].

Box 2. Anatomy of the cerebello-thalamo-cortical tract

The robust anatomical connectivity between cerebellum and thalamocortical networks has given substantial impetus to the general interest in cerebellar stimulation in epilepsy. Due to the anatomical accessibility of the cerebellar cortex, and the purely inhibitory nature of its sole output neuron, the Purkinje cell, this structure was initially stimulated in various experimental setting [39]. Nonetheless, owing to the complex foliation of the cerebellar cortex and its division in functional zones (i.e., 'zebrin bands') [79], the impact of stimulation on the activity of CN neurons is presumably highly variable (**Online Table S1**). One particularly relevant aspect of this pathway is that Purkinje cells' action potential firing seems determined by their anatomical location: higher firing frequencies have been recorded in zebrin-negative bands and lower frequencies in zebrin-positive bands [80]. Although the impact of these differences on the activity of CN neurons, which form the final output of the cerebellum, remains to be elucidated, cerebellar cortical stimulation has profound effects on the firing patterns of CN neurons mediated by perisomatic inhibitory axon terminals [81-83].

Axons of CN neurons form the superior cerebellar peduncle (brachium conjunctivum) and project to a variety of brain regions, including several pre-motor nuclei in the mesodiencephalic junction, inferior olive, thalamus, superior colliculus and zona Incerta [84-89]. The cerebellar axons that innervate the primary 'relay', secondary 'associative' and intralaminar nuclei of the thalamus form mainly large diameter, excitatory terminals on proximal dendrites that are believed to provide a potent excitatory input [86, 89-92]. In the figure panel we provide an overview of the cerebello-thalamo-cortical connectivity for the rodent thalamus. It should be noted that for the human and primate brain the cerebello-thalamic connectivity has been mostly studied for the laterally-located dentate nucleus, with a particular focus on axons innervating the nuclei analogous to the rodent ventrolateral thalamus [79, 87, 93]. However, because the CN in other mammalian species have been shown to connect to many thalamic nuclei other than the ventrolateral complex, we assume that the CN also provide dense projections throughout the thalamic complex in the primate brain. Thalamic connectivity with cortical areas as well as interspecies differences are reviewed in detail elsewhere [86, 94].

2.2 Cerebellar stimulation – the cortex

From the first half of the 20th century, electrophysiological recordings have revealed that in addition to the thalamus and cerebral cortex the cerebellum also shows oscillatory neuronal activity during generalized epileptic seizures (**Box 3 and 4**). Following the work of Moruzzi in the 1940s on the regulatory effect of cerebellar stimulation on clonic motor behavior [66], several studies were undertaken to investigate the potential use of stimulation of the cerebellum to stop epileptic seizures of various kinds in rats, cats and monkeys, yielding mainly positive results (as reviewed by [67]). Subsequent studies on electrical stimulation

of the cerebellum in epileptic patients indicated that stimulation of the cerebellar cortex could effectively stop psychomotor, generalized tonic-clonic, myoclonic, partial or focal seizures (**Online Table 1 in supplement**) [47, 68-75]. Yet, two out of three subsequent and independently conducted double-blind studies on the effects of cerebellar cortical stimulation in epilepsy patients reported a much more limited and inconsistent effect, shifting the general opinion away from cerebellar cortex stimulation [76-78]. In these studies, the efficacy of stimulation treatment appeared to depend on many factors, such as: the location and size of cerebellar cortical stimulation sites and the type and severity of seizures involved.

There are several reasons that could underlie the variable and inconsistent effects of cerebellar cortex stimulation on epileptic seizures. First, the overall density and complexity of the deeply penetrating foliation of the cerebellar cortex and the pronounced convergence of the inhibitory Purkinje cell projections to CN neurons complicates the entrainment of CN firing by cortical stimulation (**Figure 1**) [83, 95]. These limits prevent effective reduction of oscillatory firing in the cerebello-thalamo-cortical networks (**Box 3**).

Second, responses in the CN to partially synchronized Purkinje cell input is also variable, in that not all types of CN neurons show a post-inhibitory rebound in membrane potential and/or action potential firing [105, 107-111]. Third, the distribution of synaptic afferents is likely to differ for individual types of neurons [112]. Fourth, because the patients included in these early cerebellar cortex stimulation trials suffered from various types of seizures involving dedicated parts of their brains, the gross positioning may have been suboptimal for seizure intervention (**Online Table 1 in supplement**). Together, these factors may have induced variability in the effects of chronic stimulation of the cerebellar cortical stimulation on the seizure frequency, which had been reported previously in a range of experimental animal models [113]. However, it was recently shown that optogenetic stimulation of parvalbumin-expressing Purkinje cells and interneurons in the cerebellar cortex were effective in shortening the pharmacologically induced temporal lobe seizures [45]. Moreover, stimulating vermal, but not the lateral, cerebellum was effective in decreasing seizure frequency, which may be related to the pronounced connectivity of the vermal cerebellum with the temporal lobe [45, 114]. Although this study did not report the estimated number of optogenetically-modulated Purkinje cell or CN neurons, the applied light intensities [115] modulated a sufficiently large portion of cerebellar neurons to shorten temporal lobe seizures.

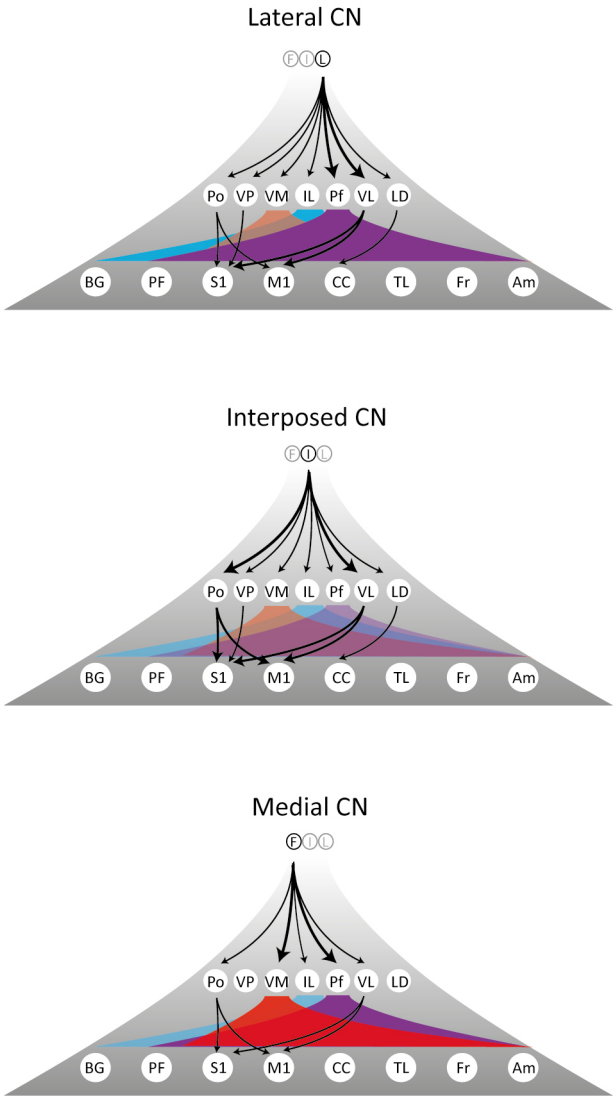

Figure 1. Rodent cerebellar projections to dorsal thalamus and connected cortical regions

Note that thickness of the arrows indicate the putative strength of the indicated cerebellar connections to thalamic nuclei. Thickness of the arrows and transparency of the shaded areas indicate the putative strength of cerebellar impact on thalamocortical connections. VM, IL and PF project particularly diffuse to cortical areas and are therefore represented differently (shaded areas) than the more focused projections from Po, VP, VL and LD (arrows).

LD = Lateral dorsal thalamic nucleus, VP = Ventral posterior thalamic nucleus, VL = Ventral lateral thalamic nucleus, Po = Posterior thalamic nuclear complex, Pf = Parafascicular thalamic nucleus, VM = Ventral medial thalamic nucleus, IL, intralaminar thalamic nuclei (i.e., centromedian, centrolateral and mediodorsal), Am = Amygdala, PF = Prefrontal cortex, S1 = Primary somatosensory cortex, M1 = Primary motor cortex, CC = Cingulate cortex, Fr = Frontal cortex.

Box 3. Pathophysiology in cerebello-thalamo-cortical tract during generalized epilepsy seizures

Although it is widely recognized that various brain structures are involved in generalized epilepsy, the role of thalamocortical networks in its pathogenesis has been studied in particular detail [96, 97]. During generalized seizures thalamic and cortical neurons show repetitive, synchronous action potential firing (and pausing), which can be recorded as generalized spike-and-wave discharges (GSWDs – see **Glossary**) in the electro-encephalogram (EEG) and electrocorticogram (ECoG) [96, 98, 99]. This particular firing pattern is caused by the local interplay between excitatory and inhibitory neurons; upon inhibition I_h-current and $Ca_V3.1$-channels are activated, which depolarize the membrane and subsequently evoke bursts of action potentials [29, 99]. Moreover, both thalamocortical relay neurons and corticothalamic neurons potently drive action potential firing in the inhibitory reticular thalamic nucleus neurons, which in turn are particularly tuned to transmit bursts of action potential firing [100], thereby facilitating the oscillatory network activity that underlies GSWDs [97]. Also, the interconnected cerebellar neurons show epilepsy-related activity changes, which are most likely caused by enhanced excitatory collateral input from pontine and olivary nuclei that relay oscillatory firing patterns from the cerebral cortex (see also **Box 2**) [90, 101]. In fact, experimental studies show that epileptic seizures are generally accompanied by oscillatory action potential firing in the sole output neurons of the cerebellar cortex (i.e. Purkinje cells) as well as CN neurons [45, 46, 102-104]. These principle neurons are susceptible to oscillatory activity, as they, like thalamic and cerebral cortical neurons, encompass I_H- and/or $Ca_V3.1$- and $Ca_V3.3$-channels that activate upon inhibitory input, supporting burst firing [81]. In principle, these ion conductances could also start epileptic oscillatory activity, which might occur in the sparse cases of cerebellar epileptogenesis (**Box 4**). At the very least these conductances allow CN projection neurons to transmit the oscillatory spiking patterns to downstream targets, i.e., thalamic relay neurons [82, 91, 92, 105, 106]. Stimulating CN neurons, which have an exclusively glutamatergic impact on thalamic neurons, should in principle be effective in preventing hyperpolarization of the thalamic membrane potentials and thereby prevent the aforementioned burst-firing [29, 100]. A similar mechanism to prevent too high levels of thalamic hyperpolarization have been the aim of various pharmacological interventions (e.g. [37]).

2.3 Cerebellar stimulation – The CN

Electrical stimulation of the fastigial, interposed and/or dentate nucleus as well as that of the *brachium conjunctivum* (superior cerebellar peduncle) (**Box 2**) has been reported to shorten and reduce the occurrence of seizures in various epilepsy seizures. For instance, both cobalt- and electrically-evoked generalized and hippocampal seizures in cats were effectively stopped using low (20-40 Hz) and high (200-400 Hz) stimulation of the interposed and dentate nuclei [116, 117]. However, other studies on the impact of CN stimulation

in comparable chemically- and electrically induced epilepsy models showed limited or no positive effects [113, 118]. A recent study showed that CN stimulation is effective in stopping generalized spike and wave discharges (GSWDs; see **Glossary**) in genetic mouse models of absence epilepsy (**Figure 2)**. Increasing the neuronal activity in the interposed nuclei in particular, was highly effective in stopping GSWDs, stopping up to 100% of positive responses even following unilateral stimulation [46]. One of the potential causes for the difference in efficacy between this recent study and previous ones is the use of optogenetic stimulation. Yet, this seems a rather unlikely source of the sharp difference between the findings, since optogenetic stimulation in the cerebellum and various other regions has been a validated approach to modulate neuronal spiking during epileptic seizures, just as electrical stimulation [45, 115, 126]. Unlike in the situation of cerebellar cortex stimulation, it is unlikely that inadequate entrainment of CN action potential firing evoked the variable effects in stopping seizures (**Figure 1**), since the volume of the stimulated CN is limited: even in the adult human brain the cerebellar dentate nucleus has a rather limited volume of ~400 mm^3, while the emboliformis, globose and fastigial nucleus each cover at most ~50 mm^3 [127, 128]. Moreover, electrically stimulating the dentate nucleus in epilepsy patients was reported highly effective [48, 49, 129]. Therefore we argue that the variability in efficacy of stopping epileptic seizures is most likely due to inadequate modulation of CN neuronal spiking or a mismatch between the stimulated region and the type of seizures.

Based upon the anatomical connections between the individual CN and the thalamic subnuclei and interconnected cortical networks, electrical stimulation of CN should in principle be effective to reach vast areas of the cerebrum (**Box 2**). Indeed, stimulating medial CN that project more densely to the limbic system, appears more effective in stopping temporal lobe seizures [45], whereas manipulation of the more laterally located CN was most effective in manipulating the occurrence of GSWDs in genetic mouse models of absence epilepsy [46]. These findings corroborate earlier studies on the differential impact of electrical stimulation of the lateral and medial cerebellum on various types of epilepsy seizure occurrence (as reviewed by [67]). Thereby these results advocate the application of CN stimulation in a subnucleus specific manner to investigate the differential impact on thalamo-cortical networks.

Box 4: Cerebellar epileptogenesis

Apart from thalamic and cerebral cortical foci, epilepsy can also arise from other neuronal structures [119]. Among these is the cerebellum, which has been linked to focal and generalized seizures as early as the 17th century (reviewed by [120]). The general scientific interest in cerebellar involvement in epilepsy was initiated by John Hughling Jackson's description of a 5 year old boy, who experienced 'tetanus-like' seizures and was found to have a tumor in the cerebellar vermis [121]. Ever since, many case reports have shown that cerebellar tumors or lesions can indeed result in various types of epileptic seizures, including generalized seizures, which disappear after complete resection of the affected region (as reviewed by [122]). Still, it remains to be elucidated whether pathophysiological cerebellar activity by itself can be sufficient to cause epileptic seizures.

Few experimental reports exist on cerebellar epileptogenesis. It has been shown that using the well-described *L7/Pcp2-Cre* mouse line, which is generally used for Purkinje cell specific gene manipulations but also reveals extra-cerebellar *Cre*-expression, the conditional deletion of the *Cacna1a* gene, which codes for the pore-forming subunit of $Ca_V2.1$ calcium channels, resulted in the occurrence of GSWDs [123]. More recently, it was also reported that the *Cacna1a* ablation from cerebellar granule cells using the *Gabra6-Cre* mouse line evoked spontaneously occurring GSWDs [124]. These data implicate abnormal cerebellar output induces secondary changes in the activity of thalamic relay neurons and their cortical targets; thus provoking oscillations in thalamo-cortical networks. Given that the deletion of *Cacna1a* from the majority, but not all, cerebellar granule cells did not evoke increased levels of irregular cerebellar action potential firing, nor ataxic motor behavior nor other gross pathologies like epilepsy [125], it could be that particular aspects of the cerebellar activity patterns provide protective effects against epilepsy. Future studies should further denote under which conditions aberrant cerebellar output can protect from or induce hyper-synchronous thalamo-cortical activity.

2.4 Correlations between epilepsy and cerebellar atrophy: implications for neurostimulation

Even before the advent of volumetric brain imaging, patients with generalized seizures due to mesial temporal lobe epilepsy were found to have cerebellar pathology upon postmortem examination (reviewed by [130]). In general, patients with chronic epilepsy retain the gross morphology of their cerebellar cortex and nuclei, but the total volume of their cerebellum as well as the density of their main cortical output neuron, i.e. the Purkinje cell, eventually decreases [131]. Moreover, the degree of cerebellar atrophy can often be correlated to the number and intensity of seizures [130]. Together, these associations raise questions regarding the extent to which co-occurrence of epilepsy and cerebellar atrophy reflect a common cause and/or cerebellar atrophy facilitates epileptic seizures.

In principle, both causal conditions may occur. On the on hand, there are many possible genetic mutations that can cause both epilepsy and cerebellar atrophy independent from one another. For example, mutations in the *CACNA1A*-gene and *NPC (Niemann-Pick disease type C)* gene, which are known to cause Familial Hemiplegic Migraine type 1 and Niemann-Pick disease, respectively, directly affect expression profiles in various types of neurons in both the cerebral cortex and cerebellum and these in turn are probably directly capable of inducing epileptic seizures and cerebellar atrophy, respectively [132, 133]. In addition the genetic aberrations that cause progressive myoclonic epilepsies, such as Unverricht-Lundborg (*CSTB*-gene) and LaFora disease (*EPM2A*-gene) are also known to directly cause cerebellar atrophy [134, 135]. Yet it is also known that cerebellar atrophy in turn may also facilitate epileptogenesis, in that structural impairment of the cerebellum facilitates a relapse of generalized seizures following neurosurgical resection of the temporal lobules [136] (see also **Box 4**). Moreover, pharmacological silencing of CN activity in rat and mouse models of epilepsy results in a pronounced increase in seizure occurrence [46, 137], just as the surgical removal of the interposed and lateral CN reduces the threshold for secondarily generalized seizures induced by amygdaloid kindling (**Glossary**) [138].

When studying the cause and consequence of epilepsy and cerebellar atrophy in patients, one should note that anti-epileptic drugs such as phenytoin [139] and benzodiazepine derivatives [140] can compromise cerebellar anatomy in the long-run; thus even though such drugs may reduce the seizures in the initial stage of the treatment, when permanently applied they may damage the cerebellum and thereby worsen the level of epilepsy. Indeed, the iconic epilepsy patient Henry Molaison ('patient H.M.'), who was treated for many years with phenytoin before he was subjected to a bilateral hippocampal resection, also suffered from a severe cerebellar atrophy [141, 142] and probably even from white matter lesions that affected the cerebellar output to thalamo-cortical networks [143-145].

Given the complex interactions between epilepsy and cerebellar pathology over the course of the disease, it is critical to assess the integrity of the cerebellar output to the thalamo-cortical networks before implementing DBS. Clearly, cerebellar stimulation will be relatively ineffective in patients with lesions in their superior cerebellar peduncle. However, despite the considerable size of the cerebello-thalamo-cortical tract (**Box 2**), until recently it was not possible to adequately assess its complex pathology in a clinical setting [93]. Only since the development of dedicated magnetic resonance imaging (MRI) sequences that allow accurate diffusion tensor imaging (DTI; **Glossary**) is it possible to non-invasively and quantitatively evaluate the anatomy of the cerebello-thalamo-cortical tract in epileptic patients [87]. In principle DTI can also be used to position stimulus electrodes in grey and/or white matter structures [146, 147].

2.5 Optimizing paradigms for cerebellar stimulation

In addition to tailoring the placement of DBS electrodes to the patho-anatomical characteristics of cerebello-thalamo-cortical tracts, the potential impact of DBS on epilepsy can also be optimized by applying dedicated stimulation paradigms that are adjusted in the temporal domain. The parameters that should be taken into consideration in this respect include the frequency, regularity, synchronicity, duration and phase of stimulation (**Figure 2**).

2.5.1 Low vs high frequency

DBS can be used to activate neuronal structures, but also to inactivate structures [148]. Injecting negative current in the neuropil depolarizes membranes and thereby evokes action potentials in neurons or passing axons, inducing release of neurotransmitters at axonal terminals. High-frequency stimulation (typically ≥130 Hz) may deplete neurotransmitters from terminals and thereby temporarily 'silence' the stimulated neurons, whereas low-frequency stimulation continuously evokes enhanced activity according to the stimulated pattern [148]. Although this differentiation appears rather clear cut, several issues prevent a straightforward setting of stimulus frequency.

First, different types of neurons are endogenously active at widely ranging frequencies. Therefore, it is of utmost importance to consider the experimental evidence available for the stimulation target. For example, the CN contain a heterogeneous, partially interconnected population of neurons, which fire at a wide range of firing frequencies in awake behaving animals [46, 81, 105, 112, 149]. Given this variation and connectivity, DBS at a particular frequency may continuously activate one particular type of neuron, while temporarily silencing another one, and thereby exert its downstream effects quite differently from those at other frequencies.

Second, neurotransmitter release from specific cell types is tuned to particular frequencies of the stimulated cells and to properties of the downstream targets. For example, the cerebellar input to thalamic nuclei has been described to be of a 'driver' type, which typically indicates that neurotransmitter release evoked by high frequency stimulation is subject to paired-pulse depression [150]. Thus, if CN cells are stimulated at a frequency that far exceeds their endogenous firing frequency, their transmission onto thalamic neurons may be dampened. In line with this notion, clinical studies report that stimulation below 100 Hz can be highly effective in treating epilepsy [48, 49] or tremor [148, 151].

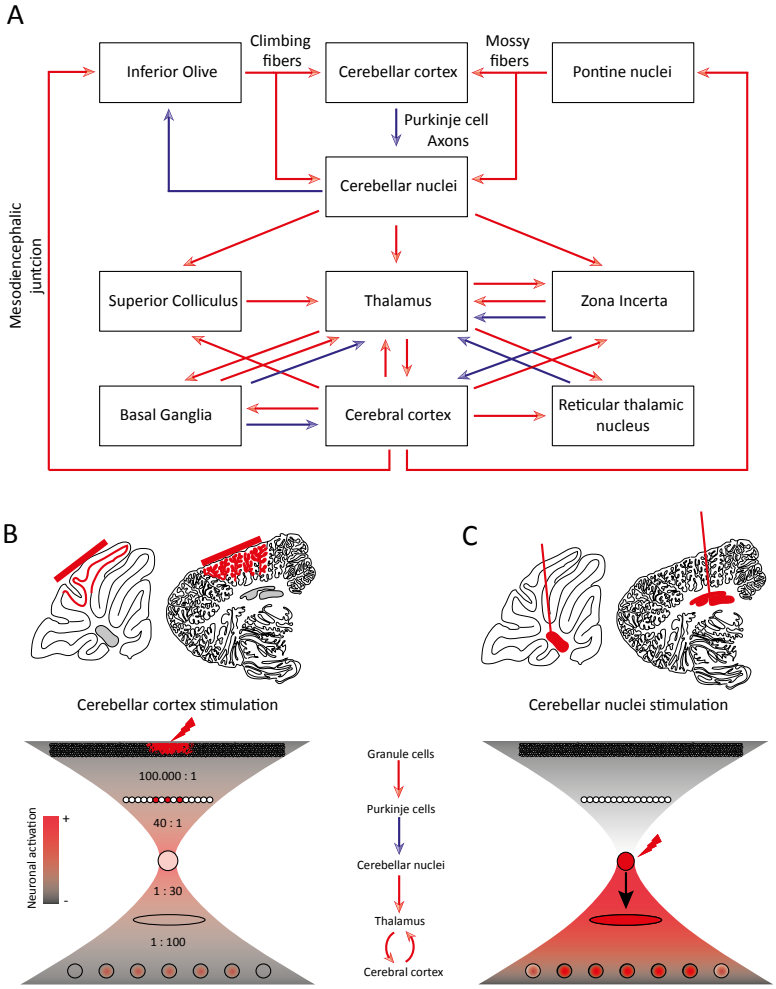

Figure 2. Cerebellar stimulation and the impact on thalamo-cortical networks.

(a) Schematic representation of epilepsy-relevant afferent and efferent cerebellar projections. Red arrows represent excitatory projections and blue arrows represent inhibitory connections. (b) (Top panel) Sagittal view of the mouse (left) and human (right) vermal cerebellum. The thin outlines indicate the cerebellar surface and the thicker lines indicate the Purkinje cell layer. The grey volumes embedded within the cortex represent the medial cerebellar nuclei ('fastigial nuclei'). Red rectangular shapes above the cerebellum represent electrical stimulation electrodes (e.g. [78]) and the red lines within the cerebellum indicate the area activated by this stimulation. (Bottom panel) Schematic representation of the convergence and divergence of the cerebello-thalamo-cortical tract: Purkinje cells receive a strongly convergent input from granule cells [129] and subsequently provide a converging input to the cerebellar nuclei [83]. The cerebellar nuclei project divergently to the thalamic nuclei (see also **Box 2**) [84], each of which can innervate numerous cortical neurons [86]. The red lightning bolt represents the site and extent of cerebellar cortical stimulation and the red color indicates the stimulus-evoked increase in neuronal action potential firing. (c) As in B but for cerebellar nuclei stimulation. Note that due to the degree of convergence and divergence of their respective afferent and efferent projections, direct stimulation of the cerebellar nuclei can alter the neuronal action potential firing in larger thalamocortical areas as opposed to cerebellar cortical stimulation.

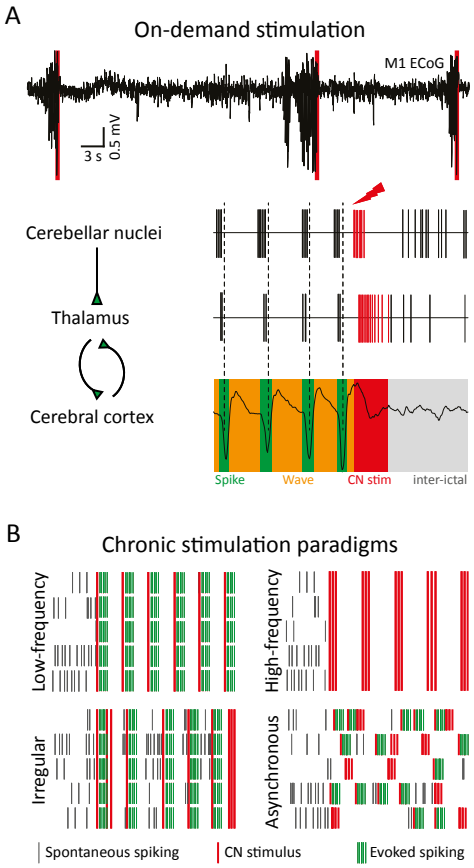

Figure 3. Cerebellar stimulation to stop epilepsy.

(a) (Top panel) On-demand stimulation effectively stops spontaneously occurring GSWDs as shown by primary motor cortex (M1) ECoG recordings from awake *C3H/HeOuJ* mice [8]. Vertical red lines indicate the optogenetic stimulations. (Bottom panels) Schematic representation of the CN, thalamic and cortical activity patterns during an absence seizure that is stopped by increasing the neuronal action potential firing in CN during the 'wave' phase of the GSWD, i.e., the phase during which most CN, thalamic and cortical neurons remain silent [46, 99]. (b) Schematic representation of chronic stimulation paradigms and their putative effect on CN action potential firing. When delivered by a conventional lead that contains a single contact point, the stimulation pulses ('CN stimulus' - red bold lines) delivered at low frequency (top left) are likely to entrain the local neuronal spiking ('evoked spiking' - green lines) synchronously; a change from spontaneous spiking ('spontaneous spiking' – dark lines). In contrast, high frequency stimulation is likely to stop action potential firing [148]. Also irregular stimulus patterns are likely to evoke arbitrary levels of synchronicity when applied through the conventional stimulus leads. Yet, by using novel stimulation leads equipped with tens of contact points [151, 156] it should be feasible to randomize the neuronal firing patterns and thereby mimic interictal neuronal firing.

2.5.2 Irregular vs regular

So far, electrical stimulation paradigms designed to treat epilepsy are of a regular pattern. However, several *in vivo* studies in awake animals have indicated that dentate neurons do not show a regular firing pattern, but instead reveal an irregular firing pattern, reflecting the integration of intrinsic pacemaking activity with inhibitory and excitatory synaptic inputs [81-83]. Using a conventional stimulation electrode, regular stimulation patterns at frequencies known to drive activity are likely to induce a regular firing pattern in all neurons within reach of the current field generated by the electrode lead. Inducing regular firing patterns for extended periods of time may exert artificial long-term effects on synaptic transmission in the downstream nuclei (e.g., [81]). To address this issue, a different stimulus pattern may be considered resembling the endogenous, i.e., irregular, neuronal firing pattern. A recent experimental study in the field of neuro-rehabilitation on the impact of irregular CN stimulus patterns on stroke-affected cerebral cortex reported increased recovery rates compared to regular stimulation patterns [152]. Irregular stimulation patterns are currently also under consideration for patients with Parkinson's disease and essential tremor [153]. Using this approach, the stimulation paradigms may be utilized to mimic CN interictal firing patterns and thereby better control epileptic seizures.

2.5.3 Synchronous vs asynchronous

Both the anatomy of the cerebellar cortex and its axonal afferents promote synchronous action potential firing in cortical Purkinje cells [154], which potently evokes well timed-spiking in CN (**Box 2**) [81-83, 155]. These bouts of synchronous cerebellar activity can be evoked readily by both sensory and motor inputs [81]. During epileptic absence seizures and temporal lobe seizures cerebellar firing is phase-locked to oscillations in thalamocortical networks [45, 46, 104]. The putative hyper-synchronization of the cerebellar output during epileptic seizures is therefore likely to corroborate the synchronization of neuronal firing in thalamic nuclei (**Box 2** and **Figure 1**). Thus, supported by an optimal convergence - divergence ratio of its inputs and outputs, the CN appear to be suitable locus to provide an intervention stopping the hyper-synchronized neuronal activity in thalamocortical networks. The question is to what extent these intervening signals themselves should be synchronized or not. On one hand, asynchronous signals may gradually impose a desynchronizing effect, but on the other, synchronized bursts of activity out of phase with the hyper-synchronized neuronal activity in the thalamocortical networks may provide an initial boost to break the rhythm. Currently, several novel electrode designs are being tested that allow the generation of variable current density at multiple, individual contact

points [151, 156], which in principle should allow differential regulation of the level of asynchronous and synchronous stimulation in the CN.

2.5.4 On-demand vs chronic

Regardless of the spatiotemporal pattern, electrical stimulation can be applied for shorter or longer durations and with or without a particular phase relation to periodic biological or pathological events (like a seizure episode). Currently, most clinical stimulation paradigms are applied chronically for long periods of time or intermittently in a semi-chronic fashion. For diseases with a chronic effect, like Parkinsonism and essential tremor, continuous stimulation paradigms appear most suitable [151]. However, the episodic nature of epilepsy, with intense but relatively short periods of aberrant thalamocortical activity, may call for a more dynamic, on-demand, approach (**Glossary**) [157, 158]. Applying electrical stimulation only when the seizure occurs requires an optimal design of the stimulation paradigms to counteract the pathological activity patterns. Such a tailored stimulus approach has recently been successfully applied in studies of rodent models of generalized or spontaneous epilepsy [45, 46, 126, 159, 160] and of patients with temporal lobe epilepsy [56]; indeed, these studies showed that seizure-triggered single-pulse stimulation delivered shortly after onset is highly effective in stopping the spike and wave discharges. Moreover, with this on-demand approach, the moment of stimulation can be adjusted to the intrinsic phase of the seizures, allowing a highly precise level of temporal control. Recent analysis of varying the moment of stimulation with respect to the phase of the spike and wave discharges has indicated that increases in the excitatory input of CN neurons to thalamic neurons are most likely to stop seizures when they are initiated during the hyperpolarization phase (i.e. wave) of thalamo-cortical networks (**Figure 2**) [46]. Although further refinement of the exact phase between the onset of the single pulse stimulus and network oscillations is warranted [161, 162], these benefits of on-demand DBS further advocate the parallel avenue of closed-loop applications in which both the duration and phase of the stimulus are reproducibly controlled at a high temporal resolution based on the input of the EEG signals.

2.6 Concluding remarks and future directions

Following the initial studies on the therapeutic use of cerebellar stimulation to stop epilepsy, several decades of cerebellar research have substantially improved our understanding of cerebellar information processing. Future neurobiological experiments should aim to utilize the latest advancements in optogenetic tools or electrode design to apply stimulus

paradigms tailored to endogenous, local activity patterns [151, 153]. Due to the rapidly increasing understanding of the pathophysiological mechanisms underlying various types of epilepsies [97] it may soon become clear which particular DBS paradigms are optimal for treatment of the various types of drug-resistant epilepsies (see also **Box 1**). The anatomical and electrophysiological characteristics of the cerebello-thalamo-cortical tract provide sufficient possibilities to stop GSWD episodes in animal models of absence seizures [46]. Future research should elucidate whether the CN stimulation is also effective in stopping convulsive seizures characterized by the phasic occurrence of GSWDs, like generalized tonic-clonic seizures [163].