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ABSTRACTS

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### P01 Effects of cerebellar transcranial Direct Current Stimulation (tDCS) on acquisition, extinction and savings of classically conditioned eyeblink responses

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Earlier work of our group showed that transcranial direct current stimulation (tDCS) of the cerebellum modulated acquisition of classically conditioned eyeblink responses (Zuchowski et al. Brain Stim 2014). Anodal tDCS led to enhanced acquisition whereas cathodal tDCS reduced it. The first aim of the present study was to replicate these earlier findings. The second aim was to study possible effects of cerebellar tDCS on extinction and savings of conditioned eyeblink responses.
Three experiments were performed using a delay eyeblink conditioning paradigm. Thirty young and healthy subjects participated in each experiment (total of 90 subjects). In the first two experiments, tDCS ($7 \times 5$ cm$^2$; 2 mA) was applied over the cerebellar hemisphere during acquisition (20 min). An extracephalic reference (deltoid muscle) was used in the first experiment, a cephalic reference (buccinator muscle) in the second. In the third experiment cerebellar tDCS was applied during extinction (12 min) using the cephalic reference, and saving effects were tested on the next day. Subjects received anodal, cathodal or sham stimulation in a double-blinded fashion (10 subjects per subgroup).
In the first experiment, using the extracephalic reference, cerebellar tDCS had no significant effect on acquisition of conditioned eyeblinks. In the second experiment, using a cephalic reference, anodal (and cathodal) cerebellar tDCS led to faster acquisition but only on a trend level. In the third experiment, cerebellar tDCS during extinction had no significant effect on extinction and savings effects.
Overall, we were unable to show robust effects of cerebellar tDCS on the acquisition of conditioned eyeblink responses. Future experiments are needed to optimize the parameters of cerebellar tDCS. Lack of cerebellar tDCS on extinction (and subsequent saving effects) suggests that the underlying neural mechanisms of acquisition and extinction are different.
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### P02 Gene transfer of brain-derived neurotrophic factor prevents cerebellar degeneration triggered by frataxin knockdown

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Friedreich's ataxia (FRDA) is the most common hereditary ataxia and there is currently no effective cure or treatment. FRDA is caused by autosomal recessive mutations that produce a deficiency in frataxin, a protein which is thought to play an important role in the regulation of mitochondrial function. FRDA is characterized by a severe neurological deterioration resulting from neurodegeneration which predominantly affects the dorsal root ganglia, spinal cord, brainstem and cerebellum. Neurotrophic factors have been shown to inhibit neurodegeneration in a variety of experimental models of neurodegenerative diseases. However, the possible impact of neurotrophic factors into the progression and therapy of FRDA has not been studied so far.
Here we have used a herpesviral vector carrying the cDNA encoding for brain-derived neurotrophic factor (BDNF) to drive its overexpression in neuronal cells and test for its effect on frataxin-deficient neurons in the mouse cerebellum in vivo.
The stereotaxic injection into the mouse cerebellar cortex of a lentiviral vector carrying a minigene encoding for a frataxin-specific shRNA triggers a frataxin deficit which is accompanied by apoptosis of granule cells and other neurons as well as a marked atrophy of Purkinje cells. These pathological changes are accompanied by a moderate but significant loss of motor coordination of treated mice as assayed by the rotarod test. Co-injection of a herpesviral vector carrying the cDNA encoding for BDNF efficiently prevents both neuronal apoptosis and Purkinje cell atrophy in addition to rescue the ataxic phenotype. In view of these data, we suggest that BDNF gene transfer might be of therapeutic use in FRDA since BDNF may protect frataxin-deficient neurons from degeneration.

### P03 Modular organization of cerebello-cerebral connections in the rat

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The zonal, or modular, organization of Purkinje cells (PCs) is well established. Recently, using transneuronal retrograde transport of rabies virus (RV), we have shown in the rat that individual PC zones receive input from different functional areas of the cerebral cortex (Suzuki et al., 2012 J. Neurosci. 32: 10854–10869). We now study to what extent these features of the cerebro-cerebellar organization are reciprocated by cerebello-cerebral connection patterns.
Injections with a 4:1 mixture of RV and choleratoxin b-subunit (CTb) were made at various places of the sensorimotor cortex (M1, M2 or S1) of 21 male Wistar rats. After a survival time of 66–70 h, the brains were processed for RV, CTb and combined RV/zebrin immunohistochemistry. CTb-labeling enabled evaluation of the injection sites, which were indicated on flattened diagrams of the cerebral cortex.
The survival time used was sufficient for third-order transneuronal RV-labeling. This ensured that after 1st-order labeling of thalamic cells, 2nd-order labeling of cerebellar nuclear cells, 3rd-order labeling of PCs was observed in all studied cases. No evidence of 4th-order labeling was noted. Labeled PCs were organized in zonal patterns that were most prominent in the contralateral cerebellum. Aided by the zebrin pattern, the zonal identity of the labeled PCs could be established. The results indicate that: 1) all studied cortical injections (including injections restricted to S1) resulted in labeling within multiple PC zones; 2) the pattern

of zonal labeling co-varied with the location of the injection; 3) a single PC zone distributes its influence to various cortical regions.
We conclude that within a single PC zone not only input from various cortical regions is processed (Suzuki et al., 2012), but also that its output is distributed to different regions of the cerebral cortex. Additionally, output of multiple PC zones converges onto a single functional region of the cerebral cortex.

### P04 Disinhibition of dentate nuclear disinhibition of dentate nuclear cells generates output from the cerebrocerebellum

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The cerebrocerebellum receives input from the cerebral cortex and generates output to the cerebral cortex through the dentate nucleus (DN). Many previous studies showed that DN neurons are activated during a movement and their activity is essential for precise limb movements. Yet, it is not understood how DN cells are released from the dominant inhibitory drive provided by Purkinje cells (PCs), and how they are activated to modulate movement. The two excitatory inputs to DN, collaterals of mossy fibers and climbing fibers, do not appear to have sufficient strength for generation of burst activity in DN neurons: mossy fiber collaterals are minor in DN and climbing fiber input is too infrequent (~1 Hz). Two alternative mechanisms to activate DN cells are: post-inhibitory rebound excitation and disinhibition by PCs. If rebound excitation is the primary mechanism, a phasic excitation of PCs and concomitant inhibition of DN cells should precede excitation of DN cells. Alternatively, if disinhibition plays a primary role, phasic suppression of PCs should be observed without preceding excitation, with a nearly simultaneous activation of DN cells. We examined these two hypotheses by comparing the activity patterns of PCs and DN cells in the cerebrocerebellum during step-tracking wrist movements in three Japanese monkeys. We found that the majority of wrist-movement-related PCs showed suppression prior to movement onset, and the majority of wrist-movement-related DN cells showed concurrent burst activity without evidence of a prior suppression. These activity patterns led us to conclude that the initial activation in DN cells is generated by suppression of the inhibitory PCs, i.e., by disinhibition. We posit that molecular layer interneurons provide the early suppression of PCs. Our observations provide a new perspective on the functional operation of the cerebrocerebellum for limb motor control and learning.

### P05 Cerebellar substrates of writing

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Current theories state that the functional neuroanatomy of the cerebellum is subdivided in a motor and a cognitive part (Mariën et al., 2014). The anterior lobe is considered the sensorimotor cerebellum and the posterior lobe (lobules VI/VII) the neurocognitive cerebellum with the affective functions primarily situated in the posterior vermis. In addition, it is believed that the left cerebellum typically mediates right, nondominant hemisphere functions, while the right cerebellum is involved in left, dominant hemisphere processes, via cerebello-cerebral connections (Mariën et al., 2014).

In writing, right cerebellar activation is consistently found, with a similar subdivision in an anterior motor component (lobule V) and a posterior cognitive component (lobule VI) (Planton et al., 2013). Although Planton et al. (2013) remain skeptical about a nonmotor role of the cerebellum in writing, an increasing number of studies on central (Mariën et al., 2009) and peripheral agraphia (Silveri et al., 1997, 1999; Mariën et al., 2007; De Smet et al., 2011; Mariën et al., 2013) following right cerebellar damage strongly suggests a role for the cerebellum in written language processing. Several theories have been proposed to explain the involvement of the cerebellum in cognitive processing, including writing, both at the pathophysiological and at the cognitive level. Mariën et al. (2007) suggested that crossed cerebello-cerebral diaschisis might serve as the underlying pathomechanism of cerebellar-induced cognitive deficits including apraxic agraphia. Silveri et al. (1997, 1999) hypothesised that disrupted timing and coordination of proprioceptive and/or visual feedback may be the cognitive substrate of cerebellar-induced dysgraphia. Both theories rely on the crossed cerebello-cerebral connections by which the cerebellum can exert an influence on the supratentorial areas involved in writing. Most subsequent theories (dysmetria of thought (Schmahmann, 1998), sequencing hypothesis (Molinari, 2008), etc.) rely on the same basic principles: control of the cerebellum over incoming and outgoing motor and cognitive processes.

### P06 Induction of LTD at parallel fiber-Purkinje cell synapses in mice having mutated GluA2, C-terminus

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Long-term depression (LTD) of synaptic transmission from parallel fiber (PF) to Purkinje cell (PC) in the cerebellum has been considered to provide a mechanism for motor learning. Recently, however, discrepancy between LTD and motor learning was reported in mutant mice that target the expression of PF-PC LTD by blocking internalization of AMPA receptors (Schonewille et al., 2011). They reported that motor learning was normal in these mutant mice, while PF-PC LTD was not induced. For LTD induction, however, they used only one type of stimulation protocol (PF and climbing fiber (CF) stimulation, 1 Hz, 5 min). Here, we reassessed whether other stimulation protocols could induce LTD in these mutant mice.
We examined LTD in slice of 3 to 6 month-old GluR2K882A and GluR2D7 knockin mutants, which were gift from Prof. Richard L. Huganir. First, PF and CF stimulation at 1 Hz for 5 min induced LTD in wild type (WT) mice (75.4±2.4 %, $n$=6). In K882A, small LTD (86.6±7.2 %, $n$=6) was induced, but in D7, LTD was not induced (104.3±9.3 %, $n$=6). Second, two PF-stimuli (ISI: 50 ms) and CF stimulus was applied. Difference in mean EPSC amplitudes between K882A (79.2±3.1 %, $n$=6) and D7 (96.2± 4.8 %, $n$=6) was statistically significant ($p$<0.05). Finally, two PF stimuli and somatic depolarization (50 ms, 100–150 mV) were applied under voltage clamp condition. This conjunction decreased the mean EPSC amplitude to 67.0±3.9 % ($n$=6) in WT, 71.7±3.7 % ($n$=7) in K882A, and 80.6±7.3 % ($n$=6) in D7. There was no significant differences between them ($p$>0.2, ANOVA). The present results demonstrate that LTD is inducible under certain stimulating conditions even in K882A and D7 mutants, indicating no contradiction against the LTD hypothesis.

### P07 Cerebellar connectivity during a motor timing task in cervical dystonia

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Dystonia research has been recently showing significant interest in cerebellum which is hypothesized to play an important role in its pathophysiology. Despite the absence of traditional neurology signs of cerebellar dysfunction, functional cerebellar abnormities have been found using various methods. In our study, we evaluated cerebellum connectivity differences between normal population and cervical dystonia patients.
Twenty five patients and twenty five healthy controls underwent fMRI examination during an interception of a moving target – a task previously shown to be associated with cerebellum, specifically complex predictive motor timing. Left cerebellar lobule VI, based on the area of decreased activity in dystonia patients in simple activation maps, was used as the seed for the psychophysiologic interaction model. In addition to lower performance in the task itself (lower hit ratio) in dystonia patients, we found decreased connectivity of the above specified seed to basal ganglia complex (specifically left putamen, pallidum and caudate head, and right putamen) in dystonia patients.
This difference is well in accord with the newly forming definition of dystonia as a network disorder consisting of basal ganglia, cerebellum and specific cortical areas.
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**P08 Moderation of enhanced metabotropic glutamate receptor type 1 mediated synaptic signalling restores motor learning in a mouse model of human spino-cerebellar ataxia, type 1, SCA1**

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Cerebellar ataxias are a rare and incurable group of neurodegenerative disorders. Several ataxias, including SCA1, are inherited polyQ disorders caused by expansion of unstable CAG repeats. Using a transgenic Purkinje neuron (PN)-specific mouse model of human ataxia, SCA1 (82Q expansion in the gene for ataxin-1) that is also doxycycline repressible (82Q OFF-ON) we aim to identify specific early driver(s) of ataxia progression.
Motor performance and gait analysis revealed mild ataxia in 6 and 12 week old 82Q ON mice ($P<0.01$, one and two-way ANOVAs) whereas 12 week old mice where 82Q expression is repressed during weeks 0–6 (82Q OFF-ON) behaved normally.
In contrast, PNs from pre-symptomatic 82Q OFF-ON mice and all ataxic 82Q ON mice exhibited abnormally long-lasting mGluR1-mediated synaptic currents ($P<0.0001$, two-way ANOVAs), suggesting that enhanced mGluR1 signalling occurs before the onset of ataxia. To determine the functional significance of enhanced mGluR1 function we administered a very low dose of a potent, negative allosteric modulator of mGluR1, JNJ 16259685 (0.03 mg/kg, sub cutaneous) to 6 and 12 week 82Q ON mice prior to an acute motor learning test. JNJ (but not vehicle) treatment significantly improved performance in 82Q ON mice ($P<0.0001$, two way ANOVAs) whilst the performance of JNJ and vehicle-treated wild type mice was unaffected.
We conclude that mGluR1 is an early cellular mechanism that may mark the beginning of SCA1 neurodegeneration and may be a useful therapeutic target for treatment at the early stages of ataxia.

**P09 Cerebellar Ataxia with Neuropathy and Vestibular Areflexia Syndrome (CANVAS), a novel vestibulo-cerebellar ataxia: clinical phenotype, pathology, imaging abnormalities, differential diagnoses and a quantitative bedside test**

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**Background:** As neuro-otological investigative modalities evolve it has become increasingly apparent that a greater number of patients with imbalance have a multifactorial cause for their dizziness. Whilst a number of these patients may have accrued multiple independent causes of their imbalance, our improved diagnostic methods have highlighted the possibility of further single diseases with multiple underlying foci of pathology.
Objective: To elucidate the underlying pathology and clinical characteristics in patients who present with a combination of a bilateral vestibulopathy, cerebellar impairment and peripheral sensory loss.
**Methods:** Prospective examination and investigation of 80 patients identified with idiopathic cerebellar ataxia and bilateral vestibulopathy, who were also found to have a somatosensory loss. Investigation included quantitative neuro-otologic oculomotor evaluation, MRI brain and spine imaging and peripheral somatic neurophysiology.
**Results:** We describe a novel balance disorder, Cerebellar Ataxia with Neuropathy and Vestibular Areflexia Syndrome (CANVAS), which is characterized by the triad of a bilateral peripheral vestibulopathy, cerebellar ataxia and a somatosensory deficit. The bilateral peripheral vestibulopathy has been quantified using rapid video-oculography and has been pathologically demonstrated to be a vestibular neuronopathy (ganglionopathy). The characteristic pattern of cerebellar atrophy has been elucidated (on MRI and validated by three post-mortem samples), whilst the sensory deficit has been shown to be a neuronopathy, with marked dorsal root ganglia neuronal loss.
**Conclusion:** CANVAS is a newly described balance disorder with clear clinico-pathological correlations, diagnostic criteria and given the existence of 13 kindred amongst the 80 patients described, is most likely a late-onset recessive disorder. Clinically, CANVAS may be a differential diagnosis for various spinocerebellar ataxias, particularly SCA 3 and 6, and Friedreich's ataxia.

**P10 MRI abnormalities of the cerebellar cortex and nuclei in SCA3, SCA6, and Friedreich's ataxia**

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In the present study, susceptibility weighted imaging (SWI) was used to show atrophy of cerebellar nuclei in spinocerebellar ataxia type 6 (SCA6), Friedreich ataxia (FRDA), and spinocerebellar ataxia type 3 (SCA3). We tested the hypothesis that cerebellar nuclei are smaller in FRDA and SCA3 compared to healthy controls, but relatively preserved in SCA6. In addition, 7 T fMRI was performed with optimized normalization methods to assess function of the cerebellar cortex and nuclei during simple hand movements. Twelve SCA6 patients (age range 41–76 years, five female), twelve FRDA patients (age range 21–55 years, seven female), and ten SCA3 patients (age range 34–67 years, three female), and age- and gender-matched control groups (total $n=23$, age range 22–75 years, ten female) were included. As expected, volume of the cerebellum was most markedly reduced in SCA6, with no significant reduction in FRDA and a slight decrease in SCA3. Atrophy of the cerebellar nuclei was not only present in FRDA and SCA3, but also in SCA6. In fact, volume reduction of the nuclei was most marked in SCA6. On a functional level, fMRI signal was altered both within the cerebellar cortex and the nuclei in the three disorders. In the nuclei, the reduction was most prominent in FRDA. Different from initial expectations, the decrease of fMRI signal in the cerebellar cortex appeared more prominent in SCA3 and FRDA than in SCA6. These findings will be discussed in further detail in the light of the known pathohistology of the diseases.
The study was supported by the EU Marie Curie Initial Training Network (ITN) grant C7 ("Cerebellar-Cortical Control: Cells, Circuits, Computation, and Clinic").

### P11 A quantitative bedside test of balance function: the Video Visually Enhanced Vestibulo-Ocular Reflex (VVOR)

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**Background**: Initially utilized to investigate the visual-vestibular interaction, the visually enhanced vestibulo-ocular reflex (VVOR) has only recently found clinical utility in the form of a qualitative bedside test. We describe the next increment in the evolution of the clinical application of the visual-vestibular interaction, by describing the quantitative bedside VVOR, which employs rapid video-oculographic (rVOG) diagnosis of vestibulo-cerebellar disease. Portable rVOG is a new field of diagnostic eye movement quantification, whose utility has been facilitated by the recent development of a lightweight, minimum-slip high-speed video eye tracking system. Underlying the efficacy of the VVOR as a robust and sensitive clinical sign is the knowledge that its perturbation represents a compromise in all three key compensatory oculomotor reflexes; smooth pursuit (SP), optokinetic nystagmus (OKN) and vestibulo-ocular reflex (VOR). The clinical utility of the VVOR sign is it's unique ability to simultaneously test for the co-existence of vestibular and cerebellar pathology. Conditions where this compound deficit may been found include spinocerebellar ataxia 3 and 6, Friedreich's ataxia, Cerebellar Ataxia with Neuropathy and Vestibular Areflexia Syndrome (CANVAS), multiple system atrophy of the cerebellar subtype (MSAc) and idiopathic cerebellar ataxia with bilateral vestibulopathy (iCABV).
**Objective:** To identify a robust and easily performed quantitative bedside clinical test of vestibular and cerebellar function.
Methods: A prospective observational study.
Results: We present data on 156 patients with combined vestibular and cerebellar pathology; 81 with Cerebellar Ataxia with Neuropathy and Vestibular Areflexia Syndrome (CANVAS), 23 with Friedreich's ataxia, 16 with SCA6, 7 with SCA3, 15 with MSAc, 9 with iCABV and 5 patients with rare presentations.
**Conclusion:** The video VVOR readily allows identification and quantification of combined vestibular and cerebellar pathology at the time of consultation. This process previously involved referral for specialized neuro-otology testing and so, improves clinical pathway efficiency and directs the diagnostic algorithm.

### P12 Purkinje cell learning of multiple responses to a single stimulus

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Classical conditioning of motor responses, such as the eyeblink, is an experimental model of associative learning and of adaptive response timing, since a conditioned blink response amplitude will be maximal near the expected onset of the unconditioned blink-eliciting stimulus. A critical neural circuitry for eyeblink conditioning is located in the cerebellar cortex, in a blink controlling microzone in the C3 zone of lobule VI. Purkinje cells in this microzone receive convergent signals from the conditioned stimulus via mossy/parallel fibers and signals from the unconditioned stimulus via climbing fibers, respectively.
Extracellular recordings of Purkinje cell activity in vivo in the decerebrate ferret have shown that Purkinje cells, over several hours of training with stimuli delivered either subcutaneously or directly to the cerebellar afferents, acquire a conditioned pause response that matches conditioned blink responses. In this project we investigate the conditioned Purkinje cell responses acquired under a mixed interstimulus interval protocol, i.e., when the conditioned stimulus is followed by the unconditioned stimulus after an interval of either 150, 300 or 450 milliseconds in different (i.e. separate) trials. In rabbit eyeblink conditioning, this kind of protocol is known to produce acquisition of multimodal responses, where the temporal profiles of the response components match the different interstimulus intervals used. Our data show that Purkinje cells too acquire such complex multimodal conditioned responses, with latencies that correspond to the different intervals.
There is thus a plasticity mechanism in the cerebellar cortex that can enable a single conditioned stimulus to elicit a complex sequential response pattern, consisting of both spiking and pausing over several hundreds of milliseconds, and is shaped by the temporal

### P13 Resting state functional connectivity of cerebello-cerebral network in patients with Autism Spectrum Disorders

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Autism Spectrum Disorders (ASDs) are neurodevelopmental conditions known to be characterized by impaired social communication and core

deficits in social reciprocity mainly including "Theory of Mind" (ToM) processes (American Psychiatric Association, 2013). Cerebellum has emerged as one of the brain regions affected by ASDs. As the cerebellum is known to influence cerebral activity via cerebello-thalamo-cortical (CTC) circuits (Middleton and Strick, 2000), it has been proposed that cerebello-cortical 'disconnection' could in part underlie autistic symptoms (Rogers et al., 2013). Resting state (RS) functional magnetic resonance imaging (fMRI) is an emerging technique that allows to probe functional connectivity (FC). As the superior cerebellar peduncle (SCP) is the main cerebellar outflow tract, in the present study we investigated the RS functional connectivity between the cerebellar Dentate Nucleus (DN), where SPC fibers originate from, and the cerebro-cortical targets. Nine adults with Asperger syndrome [mean(SD) age=24.4 (6.1); M/F=6/3] and 35 typically developing subjects [mean(SD) age=26.83 (3.57); M/F=19/17] were recruited for the study. All participants underwent an MRI acquisition protocol at 3.0 T, including T1 and BOLD RS-fMRI scans. The mean time course was extracted for every participants from the left and right DN separately in order to explore differences in connectivity between groups. When comparing ASDs patients to controls, we found decreased connectivity between the left and right DN and cerebral areas consistent with the Default Mode network, a RS network related to social deficits described in ASD (e.g. ToM) (Assaf et al., 2010). The presented RS-fMRI data provide the first evidence that DN FC is altered in ASDs patients. This suggests the cerebellum might play a crucial role in determining social behavior features of ASD via interaction with key cortical social brain regions, such as Default Mode structures.

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### P14 Cerebellar contributions to language: a tDCS-fMRI pilot study

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The right posterolateral cerebellum is structurally and functionally connected to language regions of the cerebral cortex. Clinical, neuroimaging, and neuromodulation studies suggest that the cerebellum is involved in many aspects of language, but the specific mechanisms underlying the cerebellar contributions to language remain unknown. It has been proposed that the cerebellum is important in the acquisition and training of internal models that enable prediction. Both damage to and neuromodulation of the right posterolateral cerebellum impair performance on language prediction tasks. Our aim was to examine the effects of cerebellar transcranial direct current stimulation (tDCS) on neural activation patterns and predictive language processing. We hypothesized that right cerebellar tDCS would modulate activation throughout the language network, specifically affecting performance and activation patterns during predictive trials.

We combined 20 min of 1.5 mA anodal tDCS over the right posterolateral cerebellum with functional MRI in healthy adults ($n$=7; $\mu$=26.46 years). Functional images were acquired pre- and post-tDCS while participants viewed a series of four words and decided which word best completed the sentence. In some sentences the final word was highly predictable based on the preceding context, while others sentences were non-predictive. Post-tDCS, participants exhibited a practice effect on non-predictive trials but did not improve on predictive trials. TDCS modulated activation throughout the reading/language network. Decreased activation in right Crus I/II was observed post-tDCS specifically during the predictive condition. Functional connectivity between right Crus I and the left inferior frontal gyrus was decreased post-tDCS.

These preliminary results are consistent with the proposed role of the right posterolateral cerebellum in optimizing performance during predictive language processing, and suggest that tDCS modulates supratentorial language networks.

### P15 A case of "Cerebellar cognitive affective syndrome" (Schmahmann's syndrome) after isolated brainstem stroke

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Although research on the role of the cerebellum in cognition and affect is rapidly expanding, little is known about a possible involvement of the brainstem. A 60-year-old right-handed woman is presented who was admitted to hospital after acute onset of dizziness, dysarthria and right hemiparesis. MRI of the brain showed an acute ischemic lesion in the pons, more pronounced on the left side. Formal neuropsychological investigations revealed a complex of executive, linguistic (confrontational word naming), attentional and verbal memory deficits, in addition to emotional lability (laughing-crying spells) and behavioural disinhibition. Visuospatial skills were normal. A Tc-99 m ECD SPECT scan disclosed a decreased tracer uptake bilaterally distributed in the medial frontal and left parietal areas as well as in the right anterior basal ganglia. The neurobehavioural and neuroimaging findings in this case are compared with a recent review of the literature (1) in which it was shown that cognitive deficits and behavioural changes represent common findings after isolated brainstem stroke if looked for. In addition, the available SPECT data of patients with vascular damage of the brainstem indicate functional suppression of frontal, parietal and temporal areas. It is hypothesized that the brainstem is an inherent functional part of the cerebellocerebral network subserving cognition and affect, and that brainstem lesions may interrupt the modulatory role of the cerebellum in cognitive and affective processing as a consequence of damage to the corticopontocerebellar tracts. This may result in diaschisis phenomena that reflect a constellation of symptoms closely resembling the cerebellar cognitive affective syndrome (Schmahmann's syndrome (2)) observed in patients with cerebellar pathology. Further research is needed to delineate the exact role of the cerebellum in the development of cognitive and affective disturbances in patients with brainstem lesions.

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### P16 Effects of anodal transcranial direct current stimulation of the cerebellum on EEG activities during self-paced finger movements

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Transcranial direct current stimulation (tDCS) of the cerebellum offers novel perspectives to modulate non invasively the activity of cerebellar networks, acting in particular on the excitability of the cerebellar cortex. Given the oscillatory properties of the cerebellar circuits, the role of the cerebellum in motor control and the participation of the cerebellum in cerebello-cerebral networks, we wondered whether cerebellar tDCS modulates the EEG rhythms during the execution of self-paced movements. Twenty control subjects (mean age +/− SD : 24,0 years +/− 8.3; 8 F/12 M) participated in the study. EEG activities were recorded with a wireless EEG, at baseline, after sham tDCS and after anodal tDCS (1.5 mAmp; active electrode located over the cerebellar hemisphere on the side of the dominant hand; duration of stimulation: 20 min). Subjects performed self-paced movements of the fingers.
The following sub-bands of frequencies were studied (spectral power was computed): delta (1–4 Hz), theta (4-7 Hz), alpha (8-12 Hz), beta (13-30 Hz) and the respective ratios were computed. We found a tDCS effect (ANOVA followed by post-hoc tests) for the following parameters: increase in ratios theta/ delta (mean AF3/F7/F3: $p$=0.039 and T8: $p$= 0.001), increase in ratios alpha/delta (F3: $p$=0.017; F4: $p$=0.005; mean AF4/F8/F4: $p$=0.013; P8: $p$=0.049), increase in ratios beta/delta (T8: $p$= 0.004), decrease in ratios beta/alpha (P7: $p$<0.001).
Our results highlight that anodal tDCS of the cerebellum modulates brain rhythms. This is the first demonstration that anodal tDCS of the cerebellum impacts on fundamental rhythms during the execution of self-paced finger movements. The cerebello-thalamo-cortical projections might be the anatomical substrate subserving these effects.

### P17 Acute focal brain damage alters mitochondrial dynamics and autophagy in axotomized neurons

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Mitochondria are key organelles for the maintenance of life and death of cell, and their morphology is controlled by continual and balanced fission and fusion dynamics. A balance between these events is mandatory for normal mitochondrial and cellular function, and emerging evidence indicates that mitochondria undergo extensive fission at an early stage during programmed cell death in several neurodegenerative diseases. A pathway for selective degradation of damaged mitochondria by autophagy, known as mitophagy, has been described, and is of particular importance to neurons viability. There is still much to be learned about mithophagy in neurodegenerative diseases, but it appears that the regulation of mitophagy shares key steps with the macroautophagy pathway, while exhibiting distinct regulatory steps specific for mitochondrial autophagic turnover.
In the present study we analysed the effect of autophagy stimulation on mitochondrial function and dynamics in a model of remote degeneration after focal cerebellar lesion (hemicerebellectomy). The lesion causes mitochondria depolarization in axotomized precerebellar neurons associated with PTEN-induced putative kinase 1 accumulation and Parkin translocation to mitochondria, block of mitochondrial fusion by Mfn1 degradation, increase of calcineurin activity and dynamin-related protein 1 translocation to mitochondria, and consequent mitochondrial fission. The neuroprotective effect of rapamycin is the result of a dual role: (1) stimulation of autophagy leading to damaged mitochondria removal and (2) enhancement of mitochondria fission to allow their elimination by mitophagy.
The involvement of mitochondrial dynamics and mitophagy in brain injury, especially in the context of protection from remote degeneration after acute focal brain damage, has not been investigated, and these findings may offer new target for therapeutic intervention to improve functional outcomes following acute brain injury.

### P18 Characterization of multiple contacts synapses in physiological and pathological models of the cerebellum

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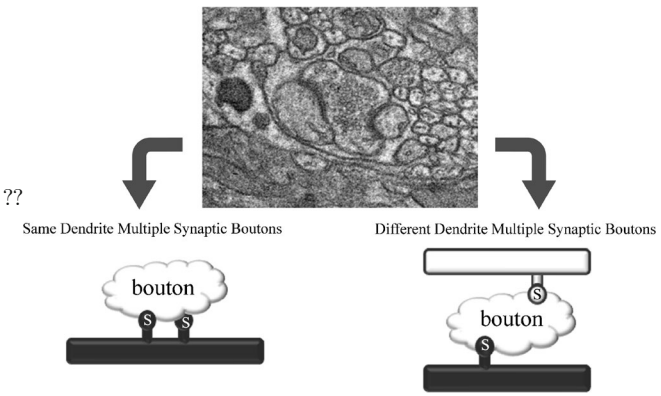
The synapses between parallel fiber varicosity (PFV) originating from granule cell and dendritic spines of Purkinje cells (PCs) have two kinds of synaptic composition in the cerebellum. More than 95 % of the synapse is composed of one PFV and one PCs and less than 5 % is composed of one PVF and two or more PCs (multiple synaptic boutons: MSBs). An increased number of MSBs is observed in two different conditions: one is observed in the cerebellum in a physiological model such as environmental enrichment housing condition (EE), the other is observed in the cerebellum of the ataxic animal model tottering mutant mice (tg/tg). We investigated synaptic connectivity of the apparently same MSB underling different conditions.
The MBSs from each animal model were observed using serial sectioning transmission electron microscopy (ssTEM) and we analyzed dendritic spine origin. A three dimensional reconstruction image for the dendritic spine organization was performed. The MSBs observed in EE showed that PCs originate from the same dendrites (sdMSBs). However MSBs observed in the tottering mouse showed an increased proportion of PCs originating from different dendrites (ddMSBs). Both groups showed an increased total proportion of MSBs (significantly higher than the normal condition), but the characteristics of synapses were different. These results imply that synaptic connectivity is closely related to the functional status. These differences in dendritic spine origination may offer insight into the morphological event underlying neuronal plasticity of the cerebellum.



### P19 Preventive cell replacement strategies in a mouse model of Purkinje cell degeneration

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Most of the degenerative disorders of the cerebellum are characterized by motor incoordination and impairment due to the selective loss of cerebellar projection neurons, the Purkinje cells (PCs), whose progenitors derive from cerebellar ventricular neuroepithelium. Here we aim to set up preventive cell replacement strategies to treat the PC degeneration that occurs in tambaleante (tbl) cerebella. This mouse line is characterized by an autosomal recessive mutation in the gene encoding the E3 ubiquitin ligase HERC1 that causes ataxia with progressive neurodegeneration of PCs, starting by two months of age. We transplanted healthy PC progenitors in immature wild-type (wt) and tbl mice, and we tested grafted and non grafted animals for motor performances and coordination by means of accelerated rotarod, beam walking test and footprint analysis. Our data showed that grafted PCs are able to acquire a fully mature phenotype and integrate into tbl mutant environment replacing lost endogenous PCs. However, grafted PCs did not restore appropriate cortico-nuclear connections and, consequently, a significant behavioral rescue was not detected in grafted animals. In order to improve graft's effectiveness, we set up a protocol of motor training including running wheels, climbing exercise and balance tasks and administered that to juvenile wt and tbl mice We observed significant behavioral and histological changes in both wt and tbl cerebella after a 6-weeks period of motor training protocol. Our preliminary results suggest that preventive motor training on mutant tbl mice could delay the progression of motor impairment and PCs death.
Support: Fondo per l'Incentivazione della Ricerca di Base research grant RBFR10A01S (to KL)

### P20 The role of novel subset of mesencephalic derived neurons in cerebellar nuclei development

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**Introduction:** The cerebellum functions in motor coordination and also implicated in non-motor behaviors including emotion and cognition. Purkinje cells (Pcs) are the sole output of the cerebellar cortex and they project to the cerebellar nuclei (CN). The CN provide the main output of the cerebellum. During cerebellar development, the CN neurons and Pcs are the earliest born among the different neuronal subtypes. However, they are generated from two distinct germinal zones: the ventrally located ventricular zone, which produces Pcs and the dorsally located rhombic lip, which produces large CN neurons. We have found a new subset of the neurons derived from mesencephalon that appears to play an important role in cerebellar development.
**Methods:** This study utilized whole mount/section immunohistochemistry, western blotting and primary dissociated cerebellar and embryonic cultures to examine the origin and the role of a new subset of CN neurons.
**Results:** Our results showed that a subset of CN neurons, which are immunopositive for α-Synuclein (SNCA) and Otx2 (a marker of mesencephalic-derived cells), originate from the mesencephalon and migrate to the rostral end of nuclear transitory zone. Double immunostainings using SNCA and p75 neurotrophin receptor antibodies suggest that these cells are derived from a combination of neurons and nerve fibers of neural crest, that terminate to the subpial surface of putative lobules VI/VII. Interestingly, the SNCA+/Otx2+/p75+ cells which divide the cerebellar primordium into rostrodorsal and caudoventral compartments undergo apoptosis programmed cell death via activation of caspases.
**Conclusion:** The temporary present of mesencephalic-derived early CN neurons in the nuclear transitory zone suggests a regulatory role as a "transient signaling center" that may play role as an intrinsic organizer during early cerebellar development.

### P21 Knockdown of HCN1 channels in the inferior olive results in motor behaviour deficits

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Molecular mechanisms that configure neuronal responses to synaptic input are critical for coordinated behaviour. Evidence from mice with global deletion of the HCN1 gene, which encodes hyperpolarization-activated cyclic nucleotide-gated (HCN) channels with rapid kinetics, suggests that this channel is important for synaptic integration underlying learned motor behaviours. However, while HCN1 is strongly expressed in cerebellar Purkinje cells, deletion of the channel solely from these neurons is not sufficient to account for behavioural deficits in mice with global deletion of HCN1. An alternative possibility is that HCN1 channels strongly expressed by neurons in the inferior olive mediate behavioural deficits in HCN1 knockout mice. To address this we investigated effects of knockdown of HCN1 in the inferior olive (IO) using AAV co-expressing EGFP to label transduced neurons and interfering RNAs targeted against HCN1. EGFP expression was found in the IO and transduction of neurons expressing HCN1-miRNA resulted in a hyperpolarized resting membrane potential, an increase in input resistance, and a reduction in the voltage sag generated by a hyperpolarizing current compared with neurons expressing miRNA targeted to a control Luciferase sequence. To test the effect of the HCN1 knockdown in the IO on motor output we used an accelerating rotarod test. Four weeks after AAV injections, mice were trained on the accelerating rotarod (4–40 rpm in 300 s) for four sessions per day, during four days. Mice injected with HCN1-miRNA-AAV ($n=19$) performed worse than mice injected with Luciferase-miRNA-AAV ($n=14$, $p=0.024$, ANOVA). These data suggest that HCN1 channels in the IO are important for motor learning. To address the mechanism for this role of HCN1 we are now investigating how HCN1 channels affect neuronal activity in the cerebellar cortex of awake behaving mice.

### P22 Early life stress induced the retardation of cerebellar function in adulthood

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It is well known that the early-life stress, such as physical/sexual abuse and neglect from the parent, induces several mental disorders in adulthood. Although such abuse become serious social problem in most countries, and appropriate care of abused child needs to be done, the neural

mechanisms inducing adulthood mental disorders has not yet fully understood. Early-life stressed mice, such as maternal deprivation (MD; separated from the dam for several time period) during lactation, is good model for studying the effect of neglect of human. Early-life stress induces structural/functional change of neuron in hippocampus, prefrontal cortex, and amygdala, and causes the mental disorder in adulthood. Recently, we reported that the homeostasis of glutamate release is disrupted in somatosensory cortex by early-life stress inducing the structural/functional changes (Takatsuru et al., 2009, Toya et al., 2014). We also found the functional retardation of the cerebellum in early-life stressed mice. MD mice were received the early-life stress during postnatal day (P) 2 to P14, 3 h per day (Takatsuru et al., 2009). Rotarod test was performed in 10 ~ 16 weeks old male MD or control mice. The duration on the rotarod was significantly decreased in MD mice. In this presentation, we will also show the retardation of the motor learning function in early-life stressed mice.

### P23 Learning induced pause responses in Purkinje cells reduce the probability that a subsequent periorbital stimulus elicits a complex spike

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During eyeblink conditioning, in which a CS is repeatedly paired with a blink eliciting US until the CS evokes a conditioned blink response (CR), cerebellar Purkinje cells develop a conditioned pause response that is thought to trigger the overt CR. Moreover, Purkinje cells control the activity of cells in the inferior olive that send climbing fibers back to the Purkinje cells. This negative feedback suggests that Purkinje cell pause responses can inhibit the US signal, which enters the cerebellum via the inferior olive. To test this we trained 12 Purkinje cells in a classical conditioning paradigm, using two different CSs (whisker air puff and forelimb), until both CSs induced a suppression of Purkinje cell activity. At this point we presented, in succession, each of the CSs as well as both CSs combined, followed by a single periorbital stimulus pulse, and then recorded whether this pulse elicited a complex spike in the Purkinje cell. We found that the probability of a complex spike following the periorbital stimulus was significantly lower if a CS preceded the stimulus, compared to when the periorbital stimulus was presented alone. Moreover, when we analyzed the effect of each CS individually, we found a strong correlation between the amount of suppression induced by a given CS and the probability that the periorbital stimulus elicited a complex spike. This tight correspondence between the strength of the pause response and the probability that a periorbital stimulus elicits a complex spike offers a plausible explanation for several behavioral phenomena, such as blocking and overexpectation, which were derived from the Rescorla & Wagner model of associative learning. The central tenet of their model is that the reinforcement value of a paired CS-US presentation depends on the existing associative strength between these two variables. Our data suggest that the reduced reinforcement value of a given a trial is due to the increased inhibition of the inferior olive.

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### P24 Dendritic integration in an advanced multicompartmental Purkinje cell model

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The Purkinje cell (PC) is among the most complex neurons in the brain and plays a key role for cerebellar functioning. In vivo, PCs operate as fast pacemakers modulated by synaptic inputs but can switch from simple spikes (SS) to complex spikes (CS) firing and, in some conditions, show bistability. The mechanisms sustaining these properties were reconstructed in an advanced multicompartmental model (Masoli et al., 2015) requiring an explicit representation of soma, dendrites, initial segment (AIS) and Ranvier nodes (RNs). This model was extended here to implement PC synapses precisely located on the different sections of the neuron. Excitatory activity at synapses formed by parallel fibers (PFs) on proximal dendrites and by ascending axons (AAs) on terminal dendrites of the PC generated a short SS burst. Inhibitory activity at synapses formed by stellate cells (SC) on PC proximal dendrites generated a pause in SS firing reproducing the "burst/pause" behavior observed in vivo. Excitatory activity transmitted by bursts in the climbing fibers (CFs) reached the dendritic trunk and generated CS. The model highlighted several fundamental properties of the PC. (1) While AIS currents do not propagate well into the dendrites, synaptic currents propagate well to the soma and AIS modulating SS generation. (2) The SS initiated in the AIS and protracted discharges were stabilized in the soma through Na-dependent mechanisms. (3) The CS was mostly generated by dendritic Ca channels. (4) The pause in SS firing following PF activity depends on synaptic inhibition and therefore reflects a network property. The model, challenged with different geometrical combinations of the pf, aa, and SC inputs, is revealing how the PC responds to afferent granular layer patterns.

### P25 Distribution patterns of histamine and 5-HT6 receptors in the cerebellar cortex and nuclei

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There is developing interest in monoaminergic influences on neural excitability and plasticity underpinning learning and memory. In the cerebellum, histamine has a modulatory influence on the consolidation of some types of memory and yet the distribution of histamine receptors is poorly understood. Serotonergic afferents project heavily to the cerebellar cortex where several 5-HT receptor subtypes have been reported. The activity of Lugaro cells is significantly modulated by 5-HT but their receptor subtype is unknown. Here we have used fluorescence immunohistochemistry with confocal microscopy to analyse the cellular and regional distribution of H1 and H2 receptors and a variety of 5-HT receptor subtypes to identify the Lugaro cell 5-HT receptor subtype, in the rat cerebellum.

H1 receptors were seen to be expressed in Purkinje cells, as has been reported. But the distribution of this expression is heterogeneous. More Purkinje cells located towards the apex of all lobules express H1 receptor protein than those towards the base of the lobules. Within the apical regions, this expression is also

patterned and related to Zebrin II expression. H2 receptors are also expressed in Purkinje cells, contrary to previous reports, in other molecular layer neurons and in the granule cell layer. The Purkinje cell expression is heterogeneous but with no clear relationship to zonal or lobular regions. There is strong expression of H2 and weak expression of H1 receptors in the cerebellar nuclei.
Lugaro cells were identified by calretinin immunoreactivity and they clearly express the 5-HT6 receptor subtype. But 5-HT6 expression is not confined to the Lugaro cell. We also found 5-HT6 expression in unipolar brush cells and a wide variety of calretinin-negative cortical interneurons, including basket, stellate, and Golgi cells.
The identification of H1, H2 and 5-HT6 receptor expression distribution offers opportunities for their manipulation in physiological and behavioural studies.

### P26 Defective dolichol metabolism disrupts protein N-Glycosylation and cerebellar granule cell migration in mouse

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During cerebellar development, neural stem cells rely heavily on N-glycosylated proteins for differentiation, adhesion, migration and synaptogenesis. The disruption of protein N-glycosylation causes Congenital Disorders of Glycosylation (CDG), a group of genetic diseases frequently associated with intellectual disability (ID) and cerebellar atrophy/hypoplasia. So far, no CDG genetic model has been generated to understand how this metabolic defect impacts cerebellum development. Mutations in SRD5A3 gene are responsible for a CDG caused by disruption in the synthesis of the lipid dolichol. This lipid is used to build the lipid-linked oligosaccharide, a precursor necessary to initiate the protein-N-glycosylation process. SRD5A3 patients show serum protein hypo-glycosylation, ID and cerebellar defects, but the exact mechanisms underlying the neurological symptoms are unknown.
To assess the importance of the N-glycosylation process during cerebellar development and to overcome the embryonic lethality of the Srd5a3−/− mouse, we created a conditional knockout mouse for the Srd5a3 gene. We used an En1-Cre line to achieve cerebellum-specific disruption. We could detect more than 40 % reduction in the levels of the glycoprotein LAMP1, suggesting that N-glycosylation is significantly impaired. The histological evaluation showed the presence of ectopic cells that accumulate in the cerebellum molecular layer during development and are present until adulthood. These ectopic cells were identified as granule cells that are unable to migrate to the internal granule cell layer. By achieving a granule cell specific disruption of Srd5a3 using an Atoh1-Cre line, we could observe the same defect, suggesting a cell autonomous mechanism.
These results, in combination with the up-coming studies should help to better understand the pathophysiological mechanisms underlying this CDG, and by doing so, to gain insights into the implication of the N-glycosylation pathway during brain development.

### P27 Detailed modeling of Golgi cell connectivity reveals the inner structure of granular layer activity

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Located at the input stage of the cerebellar cortex, the granular layer is thought to perform a complex spatiotemporal reconfiguration of mossy fibers inputs. However, the way this operation occurs is still unclear. Beyond the specific combinatorial arrangement of mossy fiber - granule cell connections (mf-GrC), Golgi cells (GoCs) are revealing a number of unpredicted properties, whose impact on microcircuit computation remains speculative. GoCs, which control granular layer activity through their inhibitory action on GrCs, were recently shown to receive excitatory synapses from both mfs and GrC ascending axons (aa) and parallel fibers (pf). Moreover, GoCs have been proposed to form an inhibitory interneuron network through gap junctions and reciprocal inhibition. In order to account for the multiplicity of structural and dynamical aspects involved, we have developed a realistic large-scale model of the granular layer incorporating a detailed representation of GoC connectivity respecting experimentally derived ratios and geometries. In the model, GoCs were excited by mfs and GrCs through aa and pf synapses, and inhibited GrCs. Furthermore, GoCs were coupled through gap junctions and reciprocal inhibitory synapses. We analyzed the network response to a short mf burst (5 spikes, 100 Hz) delivered over a 6-Hz background with GoCs either fully integrated into the network or disconnected from the GrCs. Activation of a mossy fibre bundle caused dense clusters activation with a center-surround profile reflecting the presence of lateral inhibition. The aa and mf synapses contributed to concentrate GoC activation inside the cluster and generate the center-surround effect with properties compatible with experimental measurements in vitro and in vivo. The relationship between activity in the cluster and in the inhibitory interneuron network is currently under investigation.

### P28 Cerebellar tDCS does not improve learning of a complex whole body dynamic balance task

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Transcranial direct current stimulation (tDCS) of the primary motor cortex has been shown to improve motor learning in healthy subjects and patients with cerebral stroke.
Because of the known role of the cerebellum in motor learning, cerebellar tDCS may lead to similar effects. In a previous experiment we studied whether tDCS of cerebellar midline structures was able to improve learning of a complex whole body motor skill in healthy subjects. This was not the case. Because learning of motor strategies may be central in learning the whole body motor skill, the present study investigated whether stimulation of the lateral cerebellar hemisphere may be more beneficial.
Thirty young healthy subjects participated. They performed balance training on a Lafayette Instrument 16030 stability platform® on two consecutive days. On the first day subjects received anodal, cathodal or sham tDCS in a double blinded fashion while performing the dynamic balance task (10 in each group).
Electrode size was 5×7 cm2. The center of the vertically orientated electrode was positioned over the inion and current intensity was 2.8 mA. Mean platform angle and balance time were registered. While all subjects showed significant effects of learning, no significant effects of tDCS could be observed on day 1. On day 2 there

was a trend towards worse performance in the anodal and cathodal groups compared to sham.
Neither cerebellar tDCS primarily located over the vermis nor cerebellar tDCS primarly located over both hemispheres appear to improve learning of a complex whole body dynamic balance task. Cerebellar tDCS effects may be task dependent, e. g. cerebellar tDCS effects may be more pronounced in motor adaptation than during skill acquisition. On the other hand, as yet, optimized parameters of cerebellar tDCS still need to be established.

### P29 Atypical cerebral and cerebellar language organisation: a case report

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**Introduction:** Language is subserved by an extensive network of specialised areas connected by subcortical pathways. In most right-handed subjects, the left cerebral hemisphere (perirolandic and peri-insular region) and contralateral right posterior cerebellum are involved in language processing. Lesions in the left anterior insular region frequently lead to motor speech disorders while lesions in the right cerebellar hemisphere induce a large variety of linguistic deficits due to disruption of crossed cerebello-cerebral connections. We report some unexpected anatomoclinical findings in a right-handed patient.
**Methodology:** A right-handed patient was formally examined by means of a standardised battery of neurolinguistic and neurocognitive tests and functional and structural neuroimaging including fMRI, DTI, and SPECT.
**Results:** Despite an old ischemic lesion in the left insular and frontoopercular region and a recent stroke in the vascular territory of the right posterior inferior cerebellar artery (PICA), this patient never presented with any speech or language deficits. By contrast, as reflected by crossed cerebello-cerebral diaschisis on SPECT, executive and behavioural disturbances were found after the cerebellar infarct. Evidence from fMRI and DTI strongly suggested a congenital bilateral language representation at the cerebral level (Laterality Index=+0,11). At the cerebellar level, significantly more voxels were activated in the left than in the right, lesioned cerebellum.
**Discussion:** Based on these results, we developed the hypothesis that the exceptional condition of congenital bilateral language representation forms a more powerful neurobiological basis to compensate acute damage to a critical language area than a more typical strongly lateralised representation of language functions. As reflected by the fMRI findings, the loss of right cerebellar activation in the language task is possibly compensated by a stronger activation of the left cerebellum. However, more research is needed to confirm this hypothesis.

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### P30 Bilateral language representation in a patient with a large porencephalic cyst

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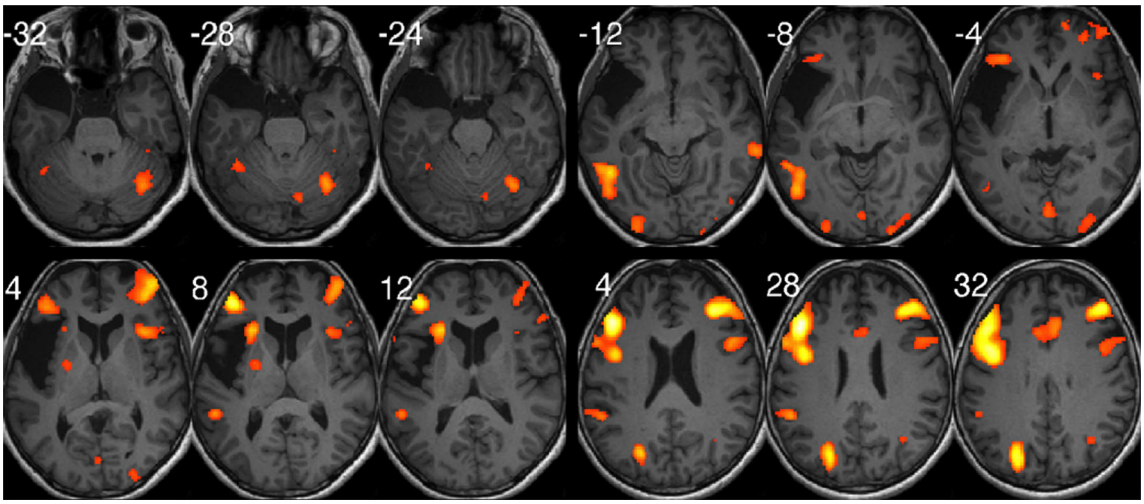
To test the hypothesis of crossed cerebro-cerebellar language dominance in atypical populations, the pattern of cerebral and cerebellar language organisation in a neurologically healthy right-handed woman with a large porencephalic cyst in the left temporal lobe was studied by means of an fMRI-language paradigm.
Extensive neuropsychological examinations were performed to formally rule out cognitive dysfunctions and revealed an asymmetrical distribution of the IQ-levels (VIQ=108, PIQ=125). The fMRI task, consisting of a covert controlled oral word generation task, disclosed a pattern of bilateral activity in the frontal language areas, slightly more pronounced in the left hemisphere, and unilateral activation of the left inferior and superior temporal and supramarginal gyrus. This pattern of supratentorial activations was reflected at the infratentorial level by bilateral activations in the posterior lobe of the cerebellum with slightly more activity located in the right cerebellar hemisphere.

This pattern of bilateral cerebral and cerebellar activation seems to confirm that the distribution of supratentorial language dominance is intrinsically reflected at the level of the cerebellum. Bilateral frontal language representation might be the consequence of neurofunctional compensation for the structural anomaly affecting eloquent brain regions resulting in an operational inefficiency of the neural network subserving language in the left hemisphere.




Figuur 1: Significant fMRI activations
Values represent MNI Z-coordinates;
−32 until 4: extent of the porencephalic cyste in the left temporal lobe;
−32, −28 & -24: bilateral cerebellar activation;
−16, −12 & -8: left visual association cortex activation,
−4, 0 & 4: 24, 28, 32: bilateral frontal activation
Orientation of the images according to the neurological convention

development to specific morphological, microvascular or biochemical features of the diseased host tissue in various cerebellar degenerations may help to identify factors determining the fate of grafted cells and potential of their functional integration.

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**P31 Transplantation and stem cell therapy for cerebellar degenerations**

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Stem cell-based and regenerative therapy may become a hopeful treatment for neurodegenerative diseases including hereditary cerebellar degenerations. Nevertheless, by now, there are still serious limitations. In principle, intracerebellar transplantation of various stem cells or embryonic neural tissue may lead to functional restoration by several mechanisms: specific replacement of lost cells, enhancement of neural plasticity and/or rescue of degenerating cells. There is a wide spectrum of human hereditary cerebellar degenerations as well as numerous cerebellar mutant mouse models. Disease-specific pathological features of the cerebellum could significantly influence the fate of the grafts and their functional effects. For instance, Lurcher mutant mouse cerebellum appears to be not permissive for some types of grafts. Grafted cells tended to avoid the cerebellum and did not establish massive connections with the host cerebellum. This corresponds with a moderate functional benefit of the treatment which could be attributable rather to trophic mechanisms. On the other hand, in Purkinje cell degeneration mice (pcd), grafted tissue became more integrated in the host cerebellum. However, the cerebellar degeneration is in these two mutants very similar regarding the spectrum of affected cell types. Investigation of the relation of graft

**P32 Predictive functions of the cerebellum: a transcranial magnetic stimulation study**

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Neuropsychological evidence from patients with cerebellar lesions and neuroimaging findings in healthy participants suggest that the cerebellum is involved in motor and sensory sequences processing, acting as a sort of "forward controller". Furthermore, the cerebellum seems to play a role also in processing repetitions of events (i.e., sequences) that are not sensory- or motor-based but pertain to the cognitive level (i.e., numerical or alphabetic sequences, ordinal sequences). The main aim of the present study was to investigate the cerebellar contribution in processing of sequences based on different relational rules by means of transcranial magnetic stimulation (TMS). In a series of experiments, participants were asked to detect possible violations in sequences responding to different rules (e.g., sequences composed by geometrical shapes increasing or decreasing in size or organized in specific ordered patterns, but also letters in alphabetic order and numbers in numerical order). Results showed that cerebellar TMS was able to impair participants' performance in detecting sequences' violations. These results are consistent with the

hypothesis of a cerebellar predictive ability (i.e., forward controller) in the cognitive domain.

### P33 Selective modulation of histaminergic inputs on projection neurons of cerebellum rapidly promotes motor coordination via HCN channels

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Circuits between the cerebellum and the hypothalamus have been strongly implicated in generation of somatic-nonsomatic integration. Thus, insights into function of central histaminergic system, a general modulator originating from the hypothalamus for whole brain activity, in cerebellar motor control are critical for understanding the mechanism underlying somatic-nonsomatic integration. Here, we show a novel selective role of histamine in the cerebellar nuclei, the final integrative center and output of the cerebellum. Histamine depolarizes projection neurons but not interneurons in the cerebellar nuclei via the hyperpolarization-activated cyclic nucleotide-gated (HCN) channels coupled to histamine H2 receptors, which are exclusively expressed on glutamatergic and glycinergic projection neurons. Furthermore, blockage of HCN channels to block endogenous histaminergic afferent inputs in the cerebellar nuclei significantly attenuates motor balance and coordination. Therefore, through directly and quickly modulation on projection neurons but not interneurons in the cerebellar nuclei, central histaminergic system may act as a critical biasing force to not only promptly regulate ongoing movement but also realize a rapid integration of somatic and nonsomatic response (Supported by grants 31171050, 31330033, 91332124, 31471112 and NSFC/RGC Joint Research Scheme 31461163001 from NSFC; SRFDP/RGC ERG grant 20130091140003 and NCET Program from the State Educational Ministry of China; grants BK2011014 and BK20140599 from the NSF of Jiangsu Province, China).

### P34 Cerebellar compartmentation of neurodegeneration and inflammation in prion disease

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The conversion of the cellular prion protein into a pathogenic conformer PrPD is a cardinal event in prion diseases which are featured by deposits of amyloid PrPD, spongiosis, astrogliosis and neuronal loss in the brain. In addition to major parameters related to prion "strain" and invasion route to the brain, host cell-specific parameters modulate prion-induced degeneration. To gain insight into such local influences, we investigated the pathology induced by 22 L scrapie and 6 PB1 bovine spongiform encephalopathy prions in the mouse cerebellum. Irrespective of the neuroinvasion route, PrP22L but not PrP6PB1 accumulated in a reproducible banding pattern reminiscent of the parasagittal zebrin pattern in the cerebellar cortex. This suggests that local factors related to the cerebellar compartmentation as well as the prion "strain" properties modulate the production of PrP22L. Investigating the effects of 22 L prions on Purkinje cells (PCs) the biochemistry and connectivity of which delineate the cerebellar compartments showed that most of PCs were lost in the bands where PrP22L accumulated. This differential sensitivity to 22 L matched zebrins expression patterns, with zebrin-expressing PCs being the most resistant, indicating a link between the pathogenesis of the 22 L prions and the cerebellar compartmentation. Interestingly, reactive astrogliosis occurred in the PrP22L-rich bands of lost PCs suggesting that it follows the topographic pattern of PC death. However, the tumor necrosis factor receptor 1, a key initiator of inflammation, was increased in the PC-rich bands at the membrane of those astrocytes enveloping PC excitatory synapses in the infected cerebellar cortex. The response of synaptic complexes to prions is thus likely to involve a glial component. Our results suggest that local differences related to the compartmentation of the cerebellum modulate cell sensitivity to neurodegenerative and inflammatory effects of 22 L prions in the mouse cerebellar cortex.

### P35 Social cognition and the cerebellum: a meta-analytic & multi-study connectivity analysis

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What is the functional connectivity of the cerebellum with the cerebrum in social cognitive processes? In a recent meta-analysis, Van Overwalle and colleagues (2014) documented that the cerebellum is critically implicated in social processes of "body" (i.e., mirroring) and "mind" reading (i.e., mentalizing), and most strongly so in more abstract and complex forms of social mentalizing, often involving the reconstruction of past, future and hypothetical events. The overlap of these findings with the cerebellar topography of a recent functional connectivity study (Buckner et al., 2011) suggests that the involvement of the cerebellum in social reasoning critically depends on its functional connectivity with the cerebrum. To test this hypothesis, we first explored the meta-analytic functional connectivity between the cerebellum and the cerebrum during social cognition ($n$=38 studies). The meta-analytic results confirm substantial and distinct connectivity with respect to the functions of (a) social mentalizing ("mind" reading) and (b) action understanding ("body" reading). A follow-up multi-study connectivity analysis ($n$=92 participants) confirms a domain-specific mentalizing functionality that is strongly connected with the corresponding mentalizing network in the cerebrum. Specifically, there was reliable connectivity from the right temporo-parietal junction (TPJ) into the right posterior cerebellum, and back to the left TPJ. There is also connectivity from the right TPJ to the left TPJ, which is the common endpoint of both connectivity loops. The discussion centers on the role of this meeting point in matching predictions/inferences of the cerebrum based on the external context with internal predictions generated by the cerebellum. Together, the consistent and strong connectivity findings of these analyses suggest that cerebellar activity during social judgments reflects a domain-specific mentalizing and

mirroring functionality, and that these functions are strongly connected with the corresponding functional networks in the cerebrum.

### P37 Blocking mGluR7 prevents expression of conditioned Purkinje cell responses

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In Pavlovian eyeblink conditioning, an animal learns to emit a motor response to a neutral conditional stimulus at a particular time. This response is driven by a learned, adaptively timed pause in the spiking of cerebellar Purkinje cells. The timing of this cell response is usually attributed to a temporal code in the conditional stimulus signal in the parallel fibres to the Purkinje cell in combination with long-term depression of the parallel fiber to Purkinje cell synapses. We have recently shown that such timing can be learned even if the conditional stimulus is direct, repetitive stimulation of the pre-synaptic parallel fibers, so that no temporal code in the input signal is possible.
These responses must therefore depend upon a novel intrinsic cellular timing mechanism. Contrary to current views of cerebellar function, the learned pause responses are resistant to blockade of GABAergic input from interneurons, a finding that raises the puzzling question of how the responses are elicited. Here we combine in vivo electrophysiology with nanoliter micro pressure ejections of selective glutamate receptor antagonists near the dendrites of the recorded Purkinje cells. We show that the timed pause can be abolished by antagonists of the metabotropic glutamate receptor 7, but that it is not significantly affected by antagonists of ionotropic glutamate receptors or of metabotropic glutamate receptor 1.
These results support the existence of an intrinsic cellular temporal memory mechanism, different from the change in synaptic weight previously thought to underlie learning and memory. They also demonstrate an unexpected mechanism, in vivo post-synaptic inhibition mediated by glutamate acting on the rarely studied mGluR7, for generating adaptively timed pause responses in cerebellar Purkinje cells.

### P38 Characterization of the anatomical pathways between the cerebellum and the hippocampus

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The role of the cerebellum has been long confined to the coordination of motor activity. Recently, a consensus emerged in favor of its participation in numerous cognitive and affective functions (Buckner et al., 2013; Koziol et al., 2014). Convergent evidences point towards an anatomo-functional link between the hippocampus and the cerebellum. Functionally, we showed that cerebellar processing influences hippocampal place cells activity in mice during navigation (Rochefort et al., 2011). In a rabbit model, works based on electrophysiological analysis also suggested a functional cooperation between the cerebellum and the hippocampus (Hoffmann et al., 2009; Wikgrenet al., 2010). Recently, optogenetic intervention on the cerebellum of a mouse model for epilepsy was able to reduce spontaneous hippocampal seizures (Krook-Magnuson et al., 2014). In humans, hippocampo-cerebellar interaction has been found for behaviours requiring spatio-temporal organization (Onuki et al., 2013; Igloi et al., 2014). Previous and more recent results suggest that those functional interactions could be subtended by a short pathway between the two structures in both rodents (Heath and Harper, 1971) and humans (Arrigo et al., 2014).
However, the anatomical basis of such interaction remains unknown. To address this issue in mice, we used the rabies virus (RV) as a retrograde transneuronal tracer. RV was inoculated in the hippocampus of C57BL/6 mice, together with analexafluor 488-associated cholera toxin β subunit (CTb). The CTb labeling within the hippocampus allowed us to identify the injection site. Using different survival times after RV infection we have identified several anatomical pathways from different zones of the cerebellar cortex to the hippocampus.

### P39 Temporal integration in an interneuron circuit model

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Inhibitory interneurons are integral parts of all brain circuits, and are commonly assumed to provide brisk control of spike timing, synchronization, circuit oscillation, etc. Theoretical studies have shown, however, that inhibitory circuits may also act as temporal integrators through a slow process of progressive disinhibition (Cannon, Robinson and Shamma, Biol. Cybern., 1983).
We simulated a circuit of molecular layer interneurons, comprising a volume of about 100 (parallel-fibre axis) x 700 (sagittal) x 300 (depth) micrometers of cerebellar cortex. Each interneuron was implemented as a 22-compartment unit (an active soma and three passive dendrites, following Abrahamsson et al., Neuron, 2012).
A population of 400 to 800 interneurons were interconnected through chemical (GABAA receptor) and electrical synapses, and activated by a pool of more than 10,000 parallel fibers firing Poisson spike trains. A narrow beam of parallel fibres conveyed the time-modulated, rectangular or sinewave stimulus.
In the complete absence of inhibition, all interneurons spiked very fast with little variation across the network (mean±s.d. 102±10.4 spikes per second, CV 0.1), and they modulated their spike rates in-phase with the parallel-fibre stimulus. When the inhibition was strengthened, however, the overall spike rate decreased and could vary markedly across the network (13.5±17.8, CV 1.3). Moreover, the most responsive interneurons spiked with a considerable phase lag of about 45° at 0.2-0.5 Hz stimulation, corresponding to an integration time-constant of about 4 s. Three factors were able to enhance this integration time-constant: the strength of inhibition, the strength of electrical coupling, and the interneuron density.
In conclusion, the present simulation results indicate that networks of interneurons may act as low-pass filters, and they suggest that

interneurons may be a component of the neural integrator thought to be distributed across the cerebellar – brainstem circuit.

### P40 Cerebellar immunohistochemical staining in idiopathic sporadic ataxia: towards a new marker for Primary Autoimmune Cerebellar Ataxia (PACA)

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Immune mediated ataxias include paraneoplastic cerebellar degeneration, gluten ataxia, ataxia with anti-GAD antibodies and Primary Autoimmune Cerebellar Ataxia (PACA). Idiopathic sporadic ataxia (ISA) is defined as a late onset progressive ataxia of undetermined aetiology and accounts for 20 % of all ataxias. A proportion of ISA patients have PACA. The identification of such patients relies on indirect evidence eg the presence of additional autoimmune diseases in the patients or their first-degree relatives, the preferential involvement of the vermis on MR imaging and spectroscopy of cerebellum. We used indirect immunohistochemistry to determine if cerebellar staining may help in the diagnosis of PACA.
Forty-eight patients with ISA were recruited from the Sheffield Ataxia Centre, UK. Reactivity of patient sera on adult Sprague–Dawley rat cerebellar tissue was assessed by indirect immunohistochemistry. Mouse anti-Calbindin-D-28 K monoclonal antibody and sera from patients with other immune mediated ataxias were used as positive controls. Negative controls included sections incubated without patient sera. Secondary antibodies consisted of a horseradish peroxidase-conjugated IgG antibody. Sections were developed with a 3,3'- diaminobenzidine (DAB) substrate kit. Patient and control samples were run simultaneously. Two blinded observers performed semi-quantitative evaluation of the staining independently. The 1:600 dilution was identified as the optimum sera dilution in determining reactivity. There was 79 % concordance between the 2-blinded observers.
Positive Purkinje cell staining was demonstrated in 17/48 (35 %) patients with ISA including 13 with weak and 4 with strong staining. Five per cent of patients with genetic ataxias had weak Purkinje cell staining and none of healthy controls had any staining. Strong Granular layer staining was seen in 31/48 (65 %) patients. Strong Purkinje cell and Granular layer staining was seen in 12/48 (25 %) patients.
This technique may help identify patients with PACA potentially amenable to treatment with ummunomodulation

### P41 A novel kinematic parameter to assess prism adaptation during the e-CAM test

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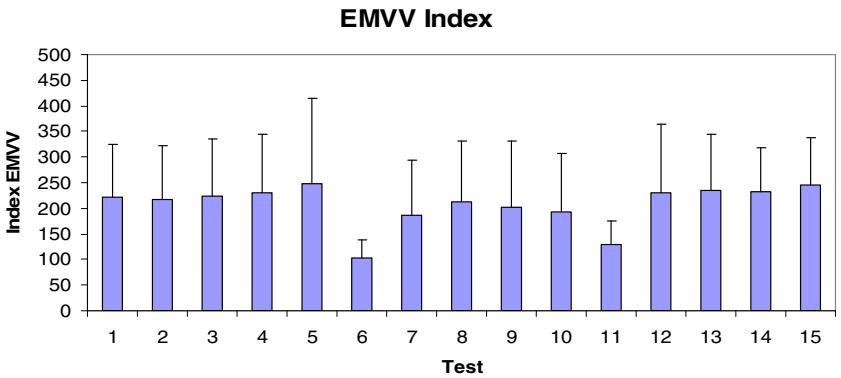
The e-CAM test is a novel transportable platform designed to assess fast alternate upper limb pointing movements in the vertical plane under visual guidance. We wondered how healthy subjects adapt themselves when they perform this task during a prism adaptation procedure.
Fifteen control subjects (mean age: 25.5 +/− 10.5 years; 10 F/5 M) participated in the study. Subjects performed 15 series of alternate movements: baseline: series 1 to 5, prisms on (adaptation): series 6 to 10, prisms off (after-effect): series 11 to 15. Duration of every series was 15 s, with a 20 s break time between each acquisition. Subjects viewed the targets binocularly through 30 diopter Fresnel prisms (3 M) mounted on safety glasses. Kinematic parameters were collected using six-axis (gyro + accelerometer) microelectromechanical systems (MEMS) fixed on the dominant upper limb. We also collected the surface electromyographic (EMG) activities of the flexor carpi radialis (FCR), extensor carpi radialis (ECR), biceps brachii (Bi) and triceps brachii (Tri) to extract patterns of EMG activities.
We extracted a novel index (EMVV) taking into account the number of movements (E: efficiency), the mean velocity of the hand (MV) and the coefficient of variation of the delta times between successive movements (V: variability). The EMVV index dropped markedly at series 6 and 11 ($p<0.001$). The Student-Newman-Keuls test showed a statistically significant difference between trial 6 and trial 11. The ratios of delta times between successive peak velocities were significantly higher for trial 5/trial 1 as compared to trial 10/trial 6 and trial 15/trial 11 ($p=0.002$).



We also identified abnormal patterns of agonist/antagonist EMG activities during adaptation characterized by deletions (pauses) and insertions (intrusions of burst of EMG activities). The EMVV parameter combines several key-aspects of fast alternate movements. These results provide a basis to assess patients with neurological disorders, especially cerebellar patients.

### P42 Neurophysiologic features as indicative for cerebellar action in emotional processing

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Neuroscientific research successively outlines specific cerebellar implementations to emotional processing. In opposite to neuroimaging approaches demarking topographic peculiarities, neurophysiologic measurements may give a precise assessment to the temporal features of cerebellar activities in cerebral processing of emotional contents. One prinicpal hypothesis requests the entailing of early and late cerebellar response to pure emotional stimuli, whereas another focus might cover the temporal mode of cerebellar activitiy to more complex demands such as the emotional information within social conditions. Own studies of Event-related potentials indicated lesions of the lobule VII as predominately responsible for impaired modulation of the early and late processing steps. As substantially to own observations, the cerebellum influences early perception and forwarding of pure emotional cues, and presumably only late processing stages of evaluating complex emotional informations. Moreover, connections to the prefrontal cortex seem to be a major route of the cerebellum at both domains of emotional perception. Nonetheless, studying Event-related potentials in paradigms scheduled for displaying neurophysiological patterns of emotional responses in cerebellum warranted a certain dependency to cognitive attentional capacities. As a summary, own findings rather close up to contemporary understanding of large-scaled networks of involved cortical and subcortical brain structures for building functional domains in emotional processing. In face of this concept of extensive multi-leveled networks guiding distinct brain actions in perceiving and forwarding emotional cues, confined protocols of neurophysiologic measurements such as recording of Event-related potentials in respect to their functional significance suggest a valuable approach of evaluating circumscribed cerebellar areas for understanding the relationship to questioned contributions in emotional processing.

### P43 How does cerebellar disorders and frontal lobe pathology affect the detection of emotional facial expressions and metacognition?

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For many decades the cerebellum was considered only to play a role in sensorimotor function, excluding it from higher level cognitive-affective processing, such as emotional and affective facial processing and metacognition. In this exploratory study, we investigated the possible role of the cerebellum in emotional change detection and subsequent subjective, metacognitive ratings via an experimental task addressing mostly frontal lobe function. Four patients with cerebellar disorders were compared to four patients with frontal lobe pathology and a group of eight normal control participants. In the task, video morphs of gradual changes of facial expressions (from a neutral to a happy or an angry face) were presented to track the moment where subjects become aware of a new facial display. Participants were instructed to press a button at the moment they detected a change towards the new display. In addition, the subjects were invited to identify the type of emotional change they had detected (accuracy) and to rate their level of confidence over their emotional identifications (subjective experience). The findings of this exploratory study reveal that the only significant difference between the three groups was related to the metacognitive ratings. Only the cerebellar group showed significantly lower confidence ratings when compared to the frontal lobe and the control group. The difference was greater with regard to the rating of certainty over the identification of happy facial expressions. This difference was also observed at the individual level. Furthermore, correlational analyses showed that this finding was not related to mood as assessed by a battery of emotion regulation and emotional perception questionnaires.

### P44 A role of the cerebellum in semantic integration?

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The cerebellum has historically been associated with motor function (Bastian et al., 1999; Courchesne & Allen, 1997; Ito, 1984; Marr, 1969; Thach et al., 1992) but it may also be involved in language (Desmond & Fiez, 1998; Fulbright et al., 1999). Recent research suggests that the cerebellum may even play a role in language-based prediction (Lesage et al., 2012). Here we used continuous theta-burst stimulation (cTBS) over the right lateral cerebellum to disrupt the hypothetical predictive function. We monitored changes indexed by the N400 wave of event-related potentials. This ERP component relies upon language prediction and appears to semantic violations. Stimulation was administered to one cerebellar hemisphere in a first session and, 1 week later, to the other hemisphere. Before and after stimulation in each session, participants read 40 congruent and 40 incongruent sentences, in which the final word is semantically expected (very high cloze probability) or unexpected in the sentence context. Final incongruent words were different between sessions and counterbalanced between participants, but the sentence contexts were repeated.
Considering that the cerebellar hemisphere contralateral to the language network may facilitate semantic integration, we expect right cerebellar cTBS to affect semantic priming. Preliminary results from six participants support this prediction: an anticipated priming effect (overall N400 modulation) is found between pre- and post-stimulation sessions when the stimulation is applied over the left cerebellar hemisphere (Figure 1); whereas no such priming effect is found for right cerebellar stimulation (Figure 2). We interpret this lack of reduction in the N400

amplitude as a sign that the right cerebellum is involved in semantic processing and particularly language-based prediction, since exogenous disruption of its function abolishes semantic priming.

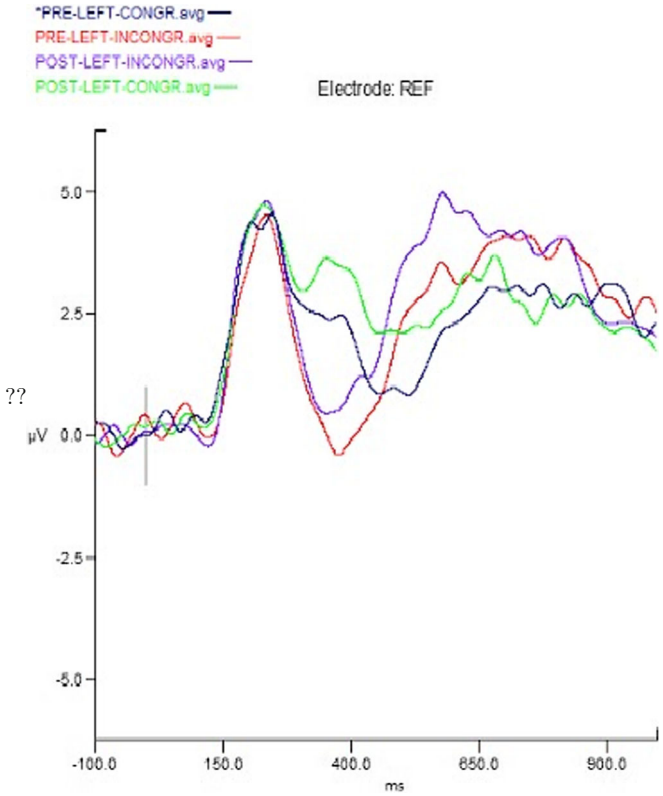


Figure 1. The between session semantic priming takes the form of a ~1 microvolt N400 modulation between pre- and post-stimulation sessions.

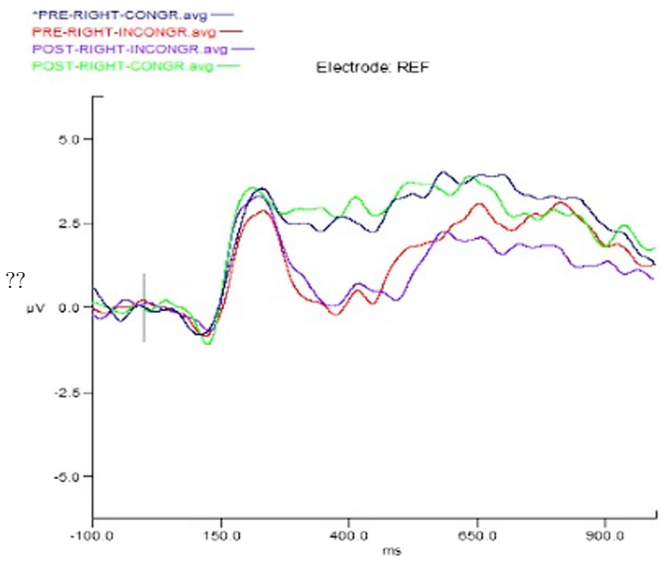


Figure 2. The N400 modulations between sessions is absent.

**P45 Role of the reelin signalling pathway in cerebellar cortex corticogenesis in lysosomal acid phosphatase (Acp2)- mutant mice**

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**Introduction:** The mouse mutant, nax (naked-ataxia), results from a spontaneous mutation in the lysosomal acid phosphatase (Acp2) gene and shows severe defects and neuronal degeneration in the cerebellum. In the Acp2 mutant mouse, the three-layer cortex (granule cells (gcs), Purkinje cells (Pcs) and the molecular layer) is hypotrophic and Pcs are multilayered and disorganized, with ectopic positioning in the molecular layer. Reelin is an extracellular signaling protein that is expressed by gc precursors and it is required for Pc redistribution from the "clusteric stage" to monolayer organization. We hypothesize that the establishment of the Pc monolayer is independent of the Reelin signalling pathway, but that this pathway has a migratory role in corticogenesis.
**Materials and methods:** Acp2 mutant mice were used for this study and molecular expression and distribution were assessed using immunohistochemistry and Western blotting.
**Results:** Pcs in the Acp2 mutant mouse cerebellar cortex are arranged in a random, dispersed manner that spans the entire molecular layer compared with a monolayer arrangement in wild type mice. The pattern of Reelin expression shows down-regulation in both wild type and nax mice postnatally, while less protein is detected in the nax mutant at P4 (at approximately the time of Pc monolayer formation) compared to wild type mice.
**Conclusion:** Pc differentiation is severely delayed in the Acp2 mutant cerebellar cortex while the presence of Reelin is similar to that of wild type mice during early postnatal development. This shows the effect of Reelin during the "clusteric stage", but it is not involved in Pc monolayer formation. Thus, multilayer Pcs may result from failure of appropriate cross-talk between Acp2 and the Reelin signalling pathway during early postnatal cerebellar development.

**P46 Roles of two types of internal models of the cerebellum in prism adaptation of hand-reaching movement**

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It is generally assumed that, to make automatic and precise motor control, the cerebellum acquires and update internal models by learning from errors. In the present study, we investigated how internal models of voluntary movements are updated under the prism adaptation of hand-reaching movement in humans. The subjects were requested to touch the small target presented on the touchscreen with their right index finger while wearing the prism-goggle which shifted the visual field 25° rightward. The electrical shutter mounted on the goggle prevented the subjects to visually confirm the finger-touch positions in non-confirming tasks, but not in confirming tasks. The adaptation was evaluated by the error in the hand reaching, i.e., the distance between the target and finger-touch position. Training with non-confirming tasks did not induce any adaptation in any of the subjects examined. Meanwhile, training with confirming tasks induced adaptation in all of the healthy subjects ($n$= 5), whereas it induced little adaptation in patients of cerebellar diseases ($n$=10). After the completion of adaptation, the healthy subjects correctly touched the target when tested with the non-confirming task whereas

cerebellar patients did not. When another five healthy subjects were tested 14 times repetitively with combinations of 10 tests with the confirming and 5 tests with the non-confirming tasks, they adapted rapidly in the confirming tasks (fast adaptation), but slowly in non-confirming tasks (slow adaptation). Thus, two types of adaptation with different time course should occur in parallel during prism adaptation. We consider that the fast and slow adaptations reflect the adjustment of forward and inverse models, respectively. Our computer simulation study implementing both the forward and inverse models reproduced these results, suggesting that cerebellar diseases impair learning in the inverse or forward models.

### P47 The effect on verbal working memory load on cerebellar activity

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The cerebellum is thought to play an important role in verbal working memory. However, a relationship between working memory load and cerebellar activity has yet to be definitively demonstrated.
Twenty right handed healthy participants underwent a high-resolution functional MRI study at 3 T (in-plane resolution 1.8 mm). The Sternberg verbal working memory task was used in an event related fMRI design, with memory load of two, four, six or eight letters. Subjects were instructed to memorise the letter sequence, which was then assessed with a probe letter, which was either present (match) or not present (mismatch) in the previously shown letter sequence – and responses obtained via a button box.
Analysis was performed with FSL software, and included correction for motion, local field inhomogeneity and physiological signals of no interest (cardiac and respiratory). Group activity and its correlation with working memory load were assessed using mixed effects models, with cluster forming threshold $Z>3.09$ and corrected significance level of $P<0.05$.
During letter string encoding activity was observed in right lobule VI, Crus II and VIIb and vermis VI (the latter presumably related to eye movement). As string length increased, activity in right lobule VI extended into Crus I. At the highest memory load, activity was additionally found in left lobule VI, in right lobules VIIb-VIIIa and left Crus II-VIIb. Cerebral activation included bilateral occipital cortex, supramarginal, paracingulate and precentral gyri bilaterally.
Our results are consistent with previous work showing involvement of lobule VI in verbal working memory. However, a linear relationship between working memory load and BOLD activity was also evident across numerous cerebellar regions, in addition to lobule VI.

### P48 A variant of Nesprin1 giant underlies the molecular etiology of autosomal recessive cerebellar ataxia type I

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Nonsense mutations scattered across the whole coding sequence of Syne1/ Nesprin1 have been linked to Autosomal Recessive Cerebellar Ataxia Type I (ARCA1), a late onset pathology characterized by diffuse cerebellar atrophy, limb and gait ataxia and dysarthria. Canonical Nesprins (encoded by four distinct genes) redundantly mediate nuclear migration and anchorage through their interaction with the nuclear envelope via an evolutionary-conserved C-terminal KASH (Klarsicht/Anc1/Syne1 Homology) domain. Nothing is currently known about the disease-causing mechanism of Nesprin1 mutations. Furthermore, the exclusive involvement of Nesprin1 is difficult to reconcile with the functional redundancy of Nesprins at the nuclear envelope. Here, we report that the 1MDa giant isoform of Nesprin1 is specifically expressed in CNS tissues and especially abundant in the cerebellum. Interestingly, we identified a CNS-specific splicing event that leads to the abundant expression of a new variant of Nesprin1giant devoid of KASH domain (KLNes1g) in the cerebellum. Accordingly, despite very high expression levels of Nesprin1 transcripts in the granule cell layer, Nesprin1 immunoreactivity did not localize to the nuclear envelope of granule cell neurons (GCNs) but rather formed immunoreactive "patches" corresponding to interfaces between cerebellar mossy fibers and CGNs. Nesprin2, by contrast, exclusively localized at the nuclear envelope of all cerebellar neurons. In immunogold electron microscopy, KLNes1g colocalized both with synaptic vesicles of mossy fibers and with dendritic membranes of CGNs. This localization was further consistent with the identification of vesicle- and membrane-associated proteins that co-immunoprecipitate with KLNes1g. Interestingly, mutations of these binding partners are also associated to human cerebellar ataxia phenotypes. Together, our results strongly support the hypothesis that Nesprin1 nonsense mutations underlying ARCA1 act through the functional inactivation of KLNes1g and affect trafficking and/or structural integrity of mossy fibers/CGNs synapses. Mouse models that may further our understanding of ARCA1 progression are currently being developed.

### P49 Three neurorobotic testing paradigms for a cerebellar spiking neural network with distributed plasticity

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Neurorobotics is a useful tool to challenge neural models in closed-loop tasks in a noisy world. We present three neurorobotic paradigms to test a cerebellar spiking neural network model. It was composed of 2160 Integrate&Fire specific neurons that replicated the cerebellar structure, with firing rates of each cells population within neurophysiological ranges. The learning mechanisms involved three different synaptic connections: Parallel Fibers-Purkinje Cells (cortical plasticity), Mossy Fibers-Deep Cerebellar Nuclei and Purkinje Cells-Deep Cerebellar Nuclei (nuclear plasticities). Neurophysiologists suggest that different time-scales of cerebellar learning depend on different mechanisms. In particular, the cortical plasticity is supposed to correspond to a fast learning, whereas the other two nuclear plasticities correspond to a slower adaptation and modulation of the output activity. We embedded the cerebellar model within a robot controller and we tested it in three sensorimotor tasks: a Pavlovian timing association between two stimuli, a combined learning of timing and gain in the vestibulo-ocular reflex and in a voluntary arm reaching perturbed by a viscous force-field. The model parameters were the same, whereas the input and output signals were protocol-dependent. Each task consisted of two sessions of 80 trials of acquisition, where the model had to learn the appropriate response, and 20 trials of extinction, where the model had to extinguish the previously acquired behaviour. We compared the performances of two models: one equipped with the distributed plasticity and the other with only the cortical plasticity. Both models were able to learn and extinguish the proper response in all the three tasks, proving the generalizability of the proposed neural network. The model with distributed plasticity demonstrated better performances than the other one; it was able to modulate the output activity, with a synergic action of the three plasticities, and to transfer information from the cortical to the nuclear plasticities, exhibiting memory consolidation.

### P50 Responses of Golgi cells to activation of pontocerebellar mossy fibres

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In classical accounts Golgi cells are activated by dendritic parallel fibre inputs, and in turn inhibit granule cells. This picture has become considerably more complex in recent years. One unusual feature of Golgi cells is that many respond to stimulation of peripheral nerve afferents with a long lasting depression of firing via a brainstem pathway involving the lateral reticular nucleus (LRN). This widespread response depends at least partly upon mGluR2 receptors.
A question which arises is whether this response is specific to LRN inputs, or do other sources of mossy fibres generate similar responses? To examine this issue we recorded from Golgi cells in vivo and studied their responses to stimulation of pontocerebellar mossy fibres.
Extracellular recordings were made from Golgi cells in the cerebellar hemispheres, identified by their spontaneous firing statistics. Many, but not all, showed long lasting depressions of firing following peripheral afferent stimulation.
The majority of Golgi cells showed short latency activation followed by a depression of firing in response to pontocerebellar mossy fibre stimulation, although this depression was of shorter duration than the depression generated by peripheral stimulation. By separating out trials in which short latency activation failed, it is possible to show that this short depression occurs independently of short latency activation. In some Golgi cells no short latency activation was observed, yet similar depressions occurred.
The common incidence of firing depressions indicate that they are not uniquely evoked by LRN inputs and that they are an important part of the in vivo response of Golgi cells to incoming mossy fibre activity. Nonetheless direct stimulation of pontocerebellar mossy fibres did not generate reductions in firing with long durations like those generated by peripheral afferent stimulation.

### P51 Cerebro-cerebellar pathways with prominent involvement of associative areas in humans in vivo

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The cerebellar involvement in both cognitive and motor functions is increasingly recognised. This is thought to occur through the cerebro-cerebellar loop composed of two main pathways: the cerebello-thalamo-cortical (CTC) and, on the way back, the cortico-ponto-cerebellar (CPC) pathway. The anatomical basis of these connections has been established using virus retrograde transport techniques in animals ex vivo, while evidence in humans in vivo is limited due to technical challenges of assessing the long polysynaptic connections of the cerebro-cerebellar loop. Moreover, there is no direct evidence that these pathways operate in closed-loop in the context of cognitive processing. Using advanced diffusion MRI tractography we aimed to characterise the cerebro-cerebellar circuit in terms of functional and anatomical areas encompassing the tracts.15 healthy subjects underwent diffusion MRI on a 3 T scanner. Data were preprocessed using FSL and whole brain tractography was performed with MRtrix by combining the Constrained Spherical Deconvolution technique with probabilistic tractography. CTC and CPC pathways were reconstructed separately using two regions of interest. Cerebral and cerebellar cortices were parcellated according to anatomical and functional atlases. This method successfully reconstructed contralateral cerebro-cerebellar pathways allowing the investigation of their properties in terms of anatomical and functional areas encompassing the tracts. The main finding is that both CTC and CPC pathways connected cerebral associative areas with the cerebellar cognitive counterpart, providing a plausible way through which the cerebellum can influence cognition. Whether these connections could generate cerebro-cerebellar closed loops directly or passing intracortical connection between associative cortices remains to be clarified. Further tractographic and functional investigations are needed to determine the effective involvement of these loops in cognition.

### P52 Cerebellar hyper-plasticity in the IB2 KO Mouse Model of ASD

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Autism spectrum disorders (ASD) are pervasive neurodevelopmental disorders including syndromes with familial conditions. Among these, the Phelan-McDermid syndrome is associated with the co-deletion of SHANK3 and IB2 genes at the chromosome 22q terminus. Although much attention has been devoted to characterize SHANK3 mutations, very little is known about the role of IB2 in ASD. The IB2 protein is expressed at synapses and takes part to the NMDA receptor interactome in the postsynaptic densities. Here we further investigate the synaptic and circuit alterations in the IB2 KO mice, that already proved to be a reliable ASD model (Giza et al., 2010). Starting from the previously described enhancement of NMDA receptor-mediated synaptic currents at the cerebellar mossy fiber - granule cell synapse of IB2 KO mice, we investigated the impact of IB2 gene deletion on excitatory/inhibitory balance and synaptic plasticity in cerebellar cortex microcircuits. In particular, electrophysiological experiments showed increased granule cell excitability in IB2 KO compared to WT mice, together with a 3-fold enhanced NMDA receptor-mediated current. Using voltage sensitive dye imaging (VSDi), the spatial distribution of excitation (E) and inhibition (I) in the granular layer was assessed, showing an unbalanced E/I ratio in IB2 KO, mirrored in the distribution of the enhanced NMDA component of excitation in IB2 KO mice. Moreover, long-term potentiation (LTP) proved to be enhanced, while long-term depression (LTD) was reduced, in the IB2 KO compared to WT. Interestingly, the spatial distribution in center (LTP) - surround (LTD) structures showed alterations in IB2 KO mice (with larger center and less deep surround) suggesting a shift from a classic "mexican hat" to a "stove-pipe" shape. These data show impressive cerebellar microcircuit alterations in the IB2 KO model, according to the growing number of evidence accounting for a major role of the cerebellum in ASD.

### P53 Afferent PSA-NCAM and PAX3 mediate BDNF-induced olivocerebellar pathway reinnervation

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In the olivocerebellar pathway (OCP), climbing fibers (CFs), which are the terminal processes of inferior olivary nucleus (ION) axons, innervate Purkinje cells (PCs) in the cerebellum with a precisely-organised topography. Following unilateral transection of the OCP, addition of the neurotrophic factor BDNF into the denervated hemi-cerebellum induces CF reinnervation to PCs. How a BDNF-activated mechanism opens this plastic window in the mature OCP is unclear. Using an ex vivo model of the mouse OCP, we show that addition of exogenous BDNF into the denervated hemi-cerebellum modulates expression of the neuroplasticity biomarker Polysialic acid-neural cell adhesion molecule (PSA-NCAM) in both cerebellar and ION region, and that PSA-NCAM mediates the amount of BDNF-induced reinnervation. Moreover olivary overexpression of the PSA-NCAM synthetic enzyme, ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase (Sia2), allows CF reinnervation of denervated PCs. Although little is known about the gene regulation of Sia2, it is induced by the transcription factor PAX 3, which in turn can be upregulated by BDNF. We have found that addition of BDNF into the deafferented hemi-cerebellum increases PAX3 expression in the reinnervating ION. Also PAX3 overexpression in the afferent ION induces significant PC re-innervation and potentiates exogenous BDNF-induced reinnervation. Our results show a novel afferent role for PAX3 in olivocerebellar reinnervation and suggest it may be a mechanism underlying BDNF-induced plasticity. We propose that BDNF induces PAX3 expression in olivary neurons and causes an increase of PSA-NCAM on their axons, which may increase their motility and therefore promote reinnervation of target PCs.

### P54 Cooperative coincidence detectors control mixed pre- and postsynaptic expression of spike-timing dependent plasticity at the cerebellar input stage

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Excitatory central synapses show a special form of persistent change, spike-timing dependent plasticity (STDP), in which long-term potentiation and depression (LTP and LTD) are related to the relative phase of occurrence of EPSPs and action potentials. At the cerebellar mossy fiber - granule cell synapse, LTP and LTD have been previously related to the duration and frequency of input busts but their EPSP-spike phase sensitivity was unknown. Here we show that EPSP-spike pairing on the 6 Hz band can reliably induce STDP in this synapse. LTP was confined to the + 5/+20 ms time-window, while LTD occurred at longer positive phases and at negative phases revealing a high temporal precision for LTP induction. STDP as a whole required NMDA receptor activation and calcium release from intracellular stores, but LTP also required mGluR activation and higher calcium levels. Importantly, STDP was 2–3 times larger than any forms of long-term synaptic plasticity previously reported at this same synapse (LTP:+61.4 %±20.2 %, $n$=5, $t$<0.05; LTD:-50.6 % ±12.6 %, $n$=5, $t$<0.05). While LTP and LTD induced by modulated burst duration and frequency were uniquely expressed by a release probability change, STDP showed a mixed pre- and postsynaptic expression attested by consistent changes in EPSC amplitude and coefficient of variation, EPSC paired-pulse ratio (PPR; LTP:-32.3 %±4.9 %, $n$=5, $t$<0.001; LTD:+21.0 %±14.9 %, $n$=5, $t$<0.05) and minis amplitude (LTP:+ 23.4 %±9.9 %, $n$=5, $t$<0.05; LTD:-16.1 %±5.2 %, $n$=5, $t$<0.05) and frequency (LTP:+18.1 %±8.7 %, $n$=5, $t$<0.05; LTD:-30.7 %±8.6 %, $n$= 5, $t$<0.05). Therefore STDP appears a powerful form of plasticity that binds LTP to the mossy fiber burst phase on the millisecond time-scale and could control granular layer functions binding it tightly to ongoing brain temporal dynamics.

### P55 Role of stress in spatial task acquisition in mouse model of olivocerebellar degeneration

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Cerebellum is involved not only in motor control, but also in cognitive and emotional processing. Mouse model of olivocerebellar degeneration, Lurcher mutant mouse, is characterized by impaired spatial performance in the Morris water maze (MWM). We hypothesized that the spatial task acquisition deficit could be potentiated by stress-related behavioral disinhibition. The aim of this study was to test the reaction of the hypothalamo-pituitary-adrenal (HPA) axis and basolateral amygdala (BLA) to repeated exposure to the MWM task. Adult Lurcher and wild type males were tested for spatial learning using the modified MWM protocol with the first day-session without the escape platform and following 17 day-sessions with hidden platform. The level of urinary corticosterone was measured before the MWM task for basal level assessment and on the first, second and last day of the of the MWM task always after the test. After the last day-session, the mice were sacrificed to examine adrenal gland volume and cFos expression in the paraventricular nuclei (PN) and BLA. Although, Lurcher mice did not show higher basal level of corticosterone, its increase after exposure to the water environment was significantly higher compared to the control mice. Nevertheless, the Lurcher mutants were able to significantly reduce corticosterone level during the MWM task acquisition. Lurcher mice also showed significantly increased volume of the right adrenal glands that were significantly smaller compared to the left glands in wild type mice. However, no significant differences in PN and BLA volume and cFos positive cell density between Lurcher and wild type mice were found. The results indicated the relationship between the stress and the MWM spatial task in Lurcher mice, however it was not confirmed along the whole HPA axis. The affection of emotional processing should be considered for the evaluation of cognitive functions in mouse models of cerebellar dysfunction.

### P56 The anterior and posterior cerebellum engagement during a non-invasive visuomotor task: Beyond what it is thought it should be

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**Aim:** To investigate how the cerebellum behaves during a dynamic power grip functional magnetic resonance imaging (fMRI) task performed using

dominant (DH) and non-dominant (NDH) hand with different force levels.

**Methods:** 14 right-handed healthy subjects performed unimanually, using a squeezeball, a *visually* guided event-related fMRI paradigm. The power grip design comprised 75 trials divided into 3 GF targets (20, 40, and 60 % of Maximum Voluntary Contraction). Signal changes were investigated for each GF in a factorial design.

**Results:** Three major findings: 1) Performing the task using the NDH revealed widespread cerebellar activations compared to DH, irrespective of GF, including the anterior and posterior lobes; 2) The NDH, unexpectedly, activated the contralateral cerebellum (lobule VI) at each GF; 3) When looking at the force related effects, both hands produced increased activations with increased force, localized mainly in lobule V of the ipsilateral cerebellum.

**Conclusion:** We showed, using a non-invasive technique, how the cerebellum was strongly engaged during different GF tasks using DH and NDH. The widespread activations seen using the NDH may suggest the much needed increased neuronal recruitment when using the non-preferred hand. In addition, the significant involvement of the posterior cerebellum may reflect the complexity of our task. Indeed our task requires whole hand movements, squeezing a ball that invokes somatosensory feedback (from the whole palm and fingers) to different GF levels, in addition to attending to external visual cues, i.e. engaging an additional ecologically-relevant cognitive component. Finally, the consistent bilateral involvement of the cerebellum suggests its role in error tracking or in synkinetic processes between DH and NDH.

### P57 Synapse Identity in Purkinje cells: Role of the C1QL1/BAI3 complex

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Proper innervation by specific afferents on a given neuron is required to obtain a functional neuronal network. During development, synaptogenesis shows an exquisite specificity in terms of partner selection and choice of innervating territory. How this process is regulated for each type of synapse remains to be understood.

The adhesion-G protein-coupled receptors, Brain Angiogenesis Inhibitors (BAI), are poorly studied receptors, which are highly expressed in the developing brain. Our in vivo functional analysis showed that BAI3 in cerebellar Purkinje cells (PCs) regulates dendrite differentiation and orientation, spinogenesis and the development of excitatory synapses. The secreted proteins C1QL, members of the complement C1Q-related family, are ligands for BAI3. C1QL1 is highly expressed by inferior olivary neurons, which give rise to climbing fibers, throughout postnatal development. Knockdown of C1QL1 in inferior olivary neurons of neonatal mice impairs the ability of climbing fibers to form functional synapses on PCs and reduces their synaptic territory, without affecting their translocation. Misexpression of C1QL1 in the molecular layer in the cerebellum also leads to a reduction in the synaptic territory of climbing fibers. Our results demonstrate that C1QL1 is a promoter of excitatory synaptogenesis in vivo, and that its specific expression by climbing fibers underlies their proper connectivity on the PC targets.

Parallel fiber connectivity on PCs involves another pathway implicating a C1Q-related protein, the CBLN1/GluRd2 complex. Thus, the specific expression of C1Q-related genes by each presynaptic input and their interaction with different signaling pathways postsynaptically might contribute to the"chemoaffinity code" proposed by Sperry to control the formation of specific neuronal circuits. Given the broad expression of BAI receptors and C1Q-related proteins in various neuronal populations during brain development, our study reveals a general mechanism, and explains why these molecular complexes are potentially associated with synaptopathies such as schizophrenia.