Cerebello-Cortical Coherence of Local Field Potentials following Patterned Stimulation of the Cerebellar Vermis

Stéfanie Tremblay

A thesis
in
the Department
of
Health, Kinesiology, and Applied Physiology

Presented in Partial Fulfillment of the Requirements
for the Degree of Master of Science (Exercise Science) at
Concordia University
Montreal, Quebec, Canada

December, 2018

CONCORDIA UNIVERSITY
School of Graduate Studies

This is to certify that the thesis prepared

By: Stéfanie Tremblay

Entitled: Cerebello-Cortical Coherence of Local Field Potentials following Patterned Stimulation of the Cerebellar Vermis

and submitted in partial fulfillment of the requirements for the degree of

MSc Exercise Science

complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the final examining committee:

Chair

Dr. Nancy St-Onge

Examiner

Dr. Andrew Chapman

Examiner

Dr. Thien Thanh Dang Vu

Thesis Supervisor(s)

Dr. Richard Courtemanche

Approved by

Chair of Department or Graduate Program Director

Dean,

Date December 10th, 2018

Cerebello-Cortical Coherence of Local Field Potentials following Patterned Stimulation of the Cerebellar Vermis

Stéfanie Tremblay, MSc candidate

ABSTRACT

The cerebellum is involved in sensorimotor, cognitive, and emotional functions through cerebello-cerebral connectivity. Non-invasive cerebellar neurostimulation has been used to treat neurological disorders and has positive effects on cognition and mood, which have been related to modulation in frontal oscillations. To explore the mechanisms, we studied the effects of cerebellar stimulation at various frequencies on oscillations and coherence across a cerebello-cortical network, in the anesthetized rat. Local field potentials were recorded continuously with monopolar and bipolar electrodes in the lateral cerebellum (crus I/II), and in the prefrontal cortex (FrA), in six adult male Sprague-Dawley rats anesthetized with urethane. Stimulation patterns were delivered to the cerebellar vermis (lobule VII) in a randomized order: single pulses (0.2 Hz for 60 s), and repeated pulses at 1 Hz (30 s), 5 Hz (10 s), 25 Hz (2 s), and 50 Hz (1 s). Low frequency stimulation (1 Hz and 5 Hz) enhanced coherence in the cerebello-cortical network in theta and alpha, while high frequency stimulation (50 Hz) had an enhancing effect on beta and low gamma coherence. Stimulation effects were influenced by the initial oscillatory state, perhaps due to cyclic stages under urethane anesthesia. Low frequency stimulation was more efficient when delivered in a state dominated by slow waves, while high frequency stimulation showed the opposite relationship. We have found here that cerebellar stimulation can drive synchronization of cerebello-cortical and cortico-cortical networks. The present results could provide basic mechanisms underlying the therapeutic effects of cerebellar stimulation by promoting large-scale synchronization of neural networks.

Acknowledgments

I would like to thank my supervisor, Dr. Richard Courtemanche, for his guidance and support over the past two and a half years. Despite my frequent travels for international judo tournaments, he has been very understanding and has made sure to transmit not only his knowledge, but also his views on several important topics related to research and careers in science. In that sense, he has acted as a great mentor for me. Thank you also to Dr. Andrew Chapman for sharing his surgical expertise with me and for providing insightful advice at important times throughout my Master's project. The continuous support from my parents has allowed me to take risks and pursue my goals in life; I am truly grateful for that. Lastly, I would like to thank Marie who has been so patient in teaching me some programming skills and who has even made me kind of like Matlab. But thank you especially for your constant love and support.

Table of Contents

LIST OF FIGURES AND TABLES

Tables

Figures

Abbreviations

DA: Dopamine

DBS: Deep brain stimulation

DCN: Deep cerebellar nuclei

DMN: Default-mode network

EEG: Electroencephalography

FrA: Frontal association area (L FrA: left side; R FrA: right side)

iTBS: Intermittent theta burst stimulation

LFP: Local field potential

LTP: Long term potentiation

MEG: Magnetoencephalography

MEP: Motor evoked potentials

MRI: Magnetic resonance imaging

NE: Norepinephrine

NREM: Non-rapid eye movement

PET: Positron emission tomography

PFC: Prefrontal cortex

REM : Rapid eye movement

R Cb: Right cerebellum

rTMS: Repetitive transcranial magnetic stimulation

tDCS: Transcranial direct current stimulation

TMS : Transcranial magnetic stimulation

Chapter 1

GENERAL INTRODUCTION

There has been growing evidence supporting cerebellar involvement in cognitive and affective functions (Hoppenbrouwers et al., 2008; Strick et al., 2009). More than just a motor control structure, the cerebellum is now seen as a large-scale network synchronizer that can exert its influence through numerous cortical and subcortical projections (Strick et al., 2009; Courtemanche et al., 2013; Farzan et al., 2016). The different types of cerebellar neurons are organized into microcircuits, which are repeated across the whole cerebellar cortex (Kandel et al., 2013). A uniform organization suggests a machine-like functioning, with similar neural computation taking place in all regions of the cerebellum (Ito, 2006). However, recent evidence suggests that regional anatomical variations in the cerebellar cortex lead to functional differences (Cerminara et al., 2015) and that cerebellar functions expand well beyond motor control (Hoppenbrouwers et al., 2008; Strick et al., 2009).

The discovery of an increasing number of cerebellar projections to nonmotor areas, such as the prefrontal and posterior parietal cortices and limbic structures (Snider and Maiti, 1976; Strick et al., 2009), led to the hypothesis of a universal cerebellar transform. This theory suggests that the cerebellum, due to its repeating microcircuits, performs the same computation on a wide variety of inputs, modulating several different behaviors or states, depending on the specific loop a cerebellar subregion is part of (Schmahmann, 2004). Thus, connections to cortical motor areas such as the primary motor cortex permit the modulation of sensorimotor functions, while projections from the vermis to limbic structures and prefrontal cortex areas could play a role in emotional processing (Schmahmann, 2004; Wessel and Hummel, 2017). On the other hand, projections from the lateral hemispheres of the posterior cerebellum, and dentate nuclei, to the associative areas (prefrontal and posterior parietal cortex) could allow the modulation of cognitive functions (Sasaki et al., 1975; Wiesendanger et al., 1979; Schmahmann and Pandyat, 1997). Indeed, the dentate nuclei displayed strong bilateral activation on MRI during problem-solving, highlighting the contribution of the lateral cerebellum to cognitive functions (Kim et al., 1994). This role in cognition was shown to be independent of motor control functions. Allen and colleagues (1997) reported the activation of cerebellar subregions, distinct from those activated during motor control tasks, during an attention task that does not involve any motor planning aspect (Allen et al., 1997). Moreover, it was suggested that the enlargement of the prefrontal cortex during human evolution drove the expansion of the whole prefrontal loop, which includes the lateral

cerebellum and ventral dentate nuclei (Leiner et al., 1993; Matano, 2001; Schoenemann et al., 2005; Ramnani, 2006). This would explain why the lateral cerebellum is much larger in humans than in other primates and how our language and cognitive capacities have expanded throughout evolution. Since the cerebellum contributes to a wide array of functions, lesions lead to impairments in motor control (ataxias), cognition, or affect (Cerebellar Cognitive Affective Syndrome), depending on the specific cerebellar subregion that is damaged (Schmahmann, 2004).

In the last 40 years, increasing evidence has suggested cerebellar involvement in emotional functions (Sacchetti et al., 2005). Affective changes, such as lethargy, depression, anxiety and aggression, were observed in patients with both acquired and congenital cerebellar lesions (Schmahmann et al., 2007; Tavano et al., 2007). Stimulation of the vermis in rodents induced evoked potentials in the amygdala, the hippocampus and the septum (Snider and Maiti, 1976). In humans, electrical stimulation of the cerebellum during neurosurgery provoked emotions of anxiety and fear in patients, further reinforcing the idea of the implication of the cerebellum in a network regulating emotional processes (Nashold Jr, Wilson, & Slaughter, 1969) .

Cerebellar contribution in the pathophysiology of neuropsychiatric disorders has long been overlooked. In fact, since the cerebellum was traditionally looked upon as solely a motor structure, it was often used as a reference point in neuroimaging studies investigating psychiatric disorders (Schutter and Van Honk, 2005a; b). In the last decades however, growing evidence supporting cerebellar involvement in emotion led to the idea of a contribution to mood disorders, such as major depression (Hoppenbrouwers et al., 2008). Several cerebellar abnormalities were found in patients suffering from major depressive disorder: patients performed poorly on tasks associated with cerebellar function (Sweeney et al., 1998; Greer et al., 2005) and have decreased brainstem and vermis volumes (Shah et al., 1992). The cerebellum has also been implicated in the pathogenesis of schizophrenia and autism (Andreasen et al., 1996; Andreasen and Pierson, 2008; Fatemi et al., 2012).

Because of its contribution in several neurological disorders and its wide connectivity with extra-cerebellar structures, the cerebellum has been proposed as a therapeutic target for non-invasive stimulation techniques such as transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS) (Hoppenbrouwers et al., 2008; van Dun et al., 2017; van Dun et

al., 2018). Notably, stimulation of the vermis, the most medial part of the cerebellum, has resulted in positive effects on cognition and mood, perhaps through modulation of frontal oscillations (Schutter et al., 2003; Schutter and van Honk, 2006; 2009; Demirtas-Tatlidede et al., 2010). A better understanding of how the cerebellum modulates activity in cortical networks and how cerebellar dysfunctions contribute to neuropsychiatric disorders could perhaps provide useful leads in improving treatments.

Oscillations

Synchrony as a mean of communication

Communication between neurons, as well as between different brain structures, is essential to perform complex processing tasks and to simply ensure the proper functioning of an organism. While anatomical connectivity is an obvious prerequisite, synchronization of neural oscillations is a key mechanism for communication (Fries, 2015). Oscillations occur when neural electrical activity fluctuates at the same rhythm in a population of nearby cells. Synchronization of the oscillatory activity between neurons within a neural circuit allows local communication, while synchrony between neuronal ensembles in distinct structures allows long-range communication (Buzsaki and Draguhn, 2004). Networks with similar frequencies have a higher chance to synchronize (Strogatz and Stewart, 1993). Traditionally, the frequency of oscillations was thought to determine the range of its influence, with slow frequency oscillations (<30 Hz) allowing communication between distant networks, and faster frequencies (>30 Hz) synchronizing more local networks (Buzsaki and Draguhn, 2004). However, recent evidence has shown that neuronal groups in distant brains areas can synchronize at frequencies as high as gamma (30-80 Hz) (Gregoriou et al., 2009; Fries, 2015).

Other functions of synchrony

Synchrony can also be interpreted as a binding mechanism. Two events or stimulus are perceived as related if they share common features; the brain links those events by binding neuronal activity in remote areas (Singer, 1999). Synchrony thus allows for the integration of information by linking neuronal activity (Singer, 1999; Schnitzler and Gross, 2005). Perceptual binding is not the only function associated with synchrony of oscillations. Synchronization

between distinct structures of the brain, in the gamma frequency range (30-80 Hz), was also linked to various cognitive processes (Gray et al., 1989; Singer, 1999; Engel et al., 2001; Ward, 2003; Buzsaki and Draguhn, 2004). During a cognitive task where participants had to identify successive targets, the parietal cortex and frontal cortex synchronized in the beta range (Gross et al., 2004). This study also underlined the importance of desynchronization as it allows for efficient transition between processing states. Oscillations play a role in motor control through synchronization of a cerebello-thalamo-cortical loop (Gross et al., 2002), and in movement preparation through synchronization of a thalamo-cortical loop in the beta range (Paradiso et al., 2004). Lastly, oscillatory phenomena can represent idling states (Leung and Yim, 1993), as well as various stages during sleep (Destexhe and Sejnowski, 2003).

Neural recording methods

Neural oscillations can be assessed invasively, through extracellular recording of local field potentials (LFPs), or non-invasively, with electroencephalography (EEG) or magnetoencephalography (MEG). LFPs represent voltage fluctuations among a population of cells within a given field and are measured with electrodes inserted in the extracellular space of brain tissues (Buzsáki et al., 2012). EEG and MEG record neural activity with surface electrodes placed on the head and provide a measure that represents a spatial summation of superficial LFPs in that area. These non-invasive techniques allow recording of brain activity in humans with good temporal resolution but are less spatially specific than LFPs (Buzsáki et al., 2012).

In the first few decades after the invention of EEG, research on oscillations focused mainly on the cerebral cortex (Courtemanche et al., 2013). Besides the observation of rhythmic olivo-cerebellar activity, rendered hyperactive by harmaline administration in rodents (De Montigny and Lamarre, 1973; Llinas and Volkind, 1973), very few studies focused on oscillatory phenomena in the cerebellum. In fact, for several years, the rhythmic brain waves were considered a mere by-product of the more essential spikes of the brain and the study of oscillations was pushed aside (Buzsaki and Draguhn, 2004). Because oscillations are most evident during sleep, anesthesia, and seizures, the rhythmic electrical activity was initially associated only to idling states and pathology (Steriade, 2001; Buzsaki and Draguhn, 2004). Later on, research breakthroughs linking oscillations to cognitive and motor function rekindled an interest in the electrical waves of the brain (Buzsaki

and Draguhn, 2004). The observation of oscillations in the granule cell layer of the cerebellum then opened the door to a more in-depth investigation of cerebellar oscillatory phenomena (Pellerin and Lamarre, 1997; Hartmann and Bower, 1998).

Pathological synchronization

Abnormal synchronization patterns were reported in several neuropsychiatric disorders such as Parkinson's disease, epilepsy, schizophrenia, Alzheimer's disease, and other forms of dementia (Schnitzler and Gross, 2005). The movement disturbances seen in Parkinson's patients are associated with increased oscillatory activity in basal ganglia structures that are vital in supporting normal movement in healthy individuals (Levy et al., 2000; Raz et al., 2000). Moreover, tremors, a main symptom of Parkinson's disease, are often synchronized with low-frequency oscillations (Timmermann et al., 2003). Alzheimer's patients typically show diminished synchronization in the alpha, beta and gamma frequency bands, whereas synchronization in delta is increased (Stam et al., 2002; König et al., 2005). Schizophrenia is characterized by an inability to oscillate at high frequencies, specifically in gamma (Spencer et al., 2004).

Disturbances in oscillatory activity have also been reported in mood disorders. Cortical activity and excitability are reduced in the left prefrontal cortex (PFC) of patients suffering from major depressive disorder (Baxter et al., 1989; Maeda et al., 2000a). Other pathological changes can be seen in the EEGs of depressive patients during sleep. This is not surprising considering the high incidence of sleep disturbances in depression (Reynolds and Kupfer, 1987). Indeed, sleep ultradian rhythms of unmedicated depressed patients, recorded in parietal and central sites, have lower temporal coherence than normal in interhemispheric beta and theta bands, as well as in intrahemispheric beta and delta bands (Armitage et al., 1999). Coherence is a measure frequently used to quantify the similarity, in terms of frequency content, between EEG signals in remote brain areas (Schnitzler and Gross, 2005; Fries, 2015). Another EEG study investigating sleep rhythms during major depressive episodes concluded that synchronization is decreased in the delta (1-3 Hz), theta (3-8 Hz), and sigma (11.5-15.5 Hz) bands, as assessed by *synchronization likelihood* (Leistedt et al., 2009). Synchronization likelihood is a statistical measure of the temporal relationship between two time series, which present the same pattern recurrence at a given frequency. According to the computational model used in this study, neural networks of depressed

patients have reduced global and local connectivity, as shown by high characteristic path lengths and low clustering coefficients. Optimal networks, which obey the *small-world* mathematical model, are characterized by high clustering, reflecting local connectedness, and short path length, a measure of global efficiency. Leistedt and colleagues (2009) suggest that depressed patients lack *small-world* network properties and that this abnormal neural organization could underlie the pathology of major depressive disorders.

Abnormalities involving subcortical structures have also been described. A functional MRI study revealed a reduction in functional coupling between the amygdala and anterior cingulate cortex in unmedicated depressive patients, and this decrease was proportional to the severity of symptoms (Matthews et al., 2008). Lastly, evidence in monkeys suggests oscillatory abnormalities in the cerebellum and hippocampus as a result of early stress, a common risk factor for depression. Indeed, epileptiform spike and sharp-wave activity were observed in the fastigial nucleus and hippocampus of monkeys raised in isolation (Heath, 1972). Our current knowledge of oscillatory abnormalities in neuropathology highlights the need for more research on the mechanisms underlying pathological oscillations.

Connectivity between the Prefrontal cortex and Cerebellum

The PFC is one of the regions that has received the most attention for its implication in the pathophysiology of depression (Nestler and Carlezon Jr, 2006). The interest for frontal regions in mood disorders arose from neuroimaging studies showing decreased blood flow and metabolism in the PFC of unipolar and bipolar depressive patients (Drevets et al., 1997). Asymmetries in interhemispheric cortical activity were also reported in depression. Positron emission tomography (PET) showed decreased metabolism in the left PFC, particularly in the dorsolateral region, while transcranial magnetic stimulation (TMS) highlighted decreased excitability in this region (Baxter et al., 1989; Maeda et al., 2000a).

The cerebellum could contribute to frontal cortex abnormalities seen in depression through cerebello-cortical pathways. Indeed, several cerebellar subregions project to PFC areas via the ventromedial thalamus (Steriade, 1995; Middleton and Strick, 2001). This connectivity was shown in humans (O'reilly et al., 2009; Buckner et al., 2011; Sang et al., 2012; Farzan et al., 2016), non-human primates (Middleton and Strick, 1994; Middleton and Strick, 2001; Kelly and Strick, 2003), and rodents (Watson et al., 2009; Suzuki et al., 2012; Watson et al., 2014). Tracing studies showed indirect projections between the dorsolateral PFC (area 46) and Crus II, vermal lobule VII and vermal lobule IX in the cerebellum of monkeys (Kelly and Strick, 2003). Moreover, stimulation of the prelimbic cortex in rats resulted in evoked field potentials in the lobule VII of the contralateral vermis and stimulation of the fastigial nucleus evoked field potential responses (onset latency ~13ms) in the prelimbic cortex, demonstrating reciprocal connections between the two regions (Watson et al., 2009; Watson et al., 2014). Simultaneous recording of on-going LFP activity from the fastigial nucleus and the prelimbic cortex while rats were at rest showed significant coherence in the theta frequency band (5–10 Hz) (Watson et al., 2014). Furthermore, distinct portions of the dentate nucleus project to either motor areas (ventral dentate projects to primary motor cortex and premotor areas) or to non-motor areas (PFC and posterior parietal cortex) of the cerebrum (Dum et al., 2002). These connections between the PFC and the cerebellum are thought to be reciprocal, and thus to form closed loops (Middleton and Strick, 1997; Kelly and Strick, 2003; Ramnani, 2006).

Cerebellar Stimulation

Electrical stimulation of the cerebellum has been used to probe cerebellar function and connectivity in animals and humans (Harper and Heath, 1973; Snider and Maiti, 1976; Dempesy and Richardson, 1987; Schutter et al., 2003; Demirtas-Tatlidede et al., 2010; Farzan et al., 2016). Several studies have also investigated the effects of cerebellar stimulation on symptoms of various neurological disorders, as well as on cognition, speech, mood, and emotional processing (Heath, 1977; Heath et al., 1980; Schutter and van Honk, 2006; Schutter et al., 2009; Schutter and van Honk, 2009; Demirtas-Tatlidede et al., 2010; Farzan et al., 2016). In fact, cerebellar stimulation has been used since the 1970s to treat epilepsy, schizophrenia, and depression (Heath et al., 1980). Recent technological advances now allow stimulation of the brain non-invasively, making it a safe and easy to apply intervention tool in the treatment of disease (Kadosh et al., 2012). The use of neurostimulation to enhance psychological function in the absence of pathology, and the wide accessibility of some neurostimulation methods, especially transcranial direct current stimulation (tDCS) devices which are particularly inexpensive and portable, has however raised ethical concerns (Kadosh et al., 2012). Even though there are several complex cerebello-cerebral connections to explore, we will focus here on the effects of stimulation of the cerebellar vermis.

High-frequency repetitive TMS (rTMS) at 25 Hz on the cerebellar vermis induced a shift in PFC activity, from left to right dominance, in the gamma spectrum, and led to elevations in mood (Schutter et al., 2003). Activity in the gamma band has been linked to emotional processing, for instance when viewing pictures that feature affective contents (Müller et al., 1999). Knowing depressive patients often display PFC asymmetry, the shift induced by rTMS could provide an interesting treatment avenue, with the cerebellum as a target (Baxter et al., 1989; Maeda et al., 2000a; Schutter et al., 2003). TMS is already used to modulate the hypofrontality characteristic of depression but usually targets the PFC directly (Dubin, 2017). On the other hand, single-pulse TMS on the vermis resulted in increased frontal theta EEG activity in healthy subjects (Schutter and van Honk, 2006). Frontal theta activity has been linked with emotional and memory processes, and is associated with reduced anxiety and increased approach behaviour (Inanaga, 1998; Schutter and van Honk, 2006). The septo-hippocampal complex and the amygdala are also related to theta activity in cognition, emotion and fear memory (Denham and Borisyuk, 2000; Pape et al., 2005). Theta activity, in a limbic-thalamo-cortical network, may thus represent an important neural

correlate for emotion and memory processes and, interestingly, the cerebellum can facilitate neural activity in this frequency band (Schutter and van Honk, 2006). Since anxiety and avoidance behaviour are common symptoms of major depression, this line of evidence suggests abnormal frontal theta activity may be another component in the pathophysiology of major depressive disorders (Leventhal, 2008).

Intermittent theta burst stimulation (iTBS) is a novel form of rTMS that is used to increase cortical excitability and leads to long-lasting effects (Huang et al., 2005). Farzan and colleagues (2016) used iTBS to investigate the effects of stimulating different cerebellar nodes on the neural circuit each node is connected with. They found network-specific effects on oscillatory power and complexity. Complexity measures reflect the structural organization of biological time series, such as neural signals, and the adaptive capacity of higher order species (Costa et al., 2005). Higher complexity of neural signal is linked with greater cognitive and behavioral capacity (Costa et al., 2005; Liang et al., 2014), while decreased complexity has been reported in neurological disorders (Mizuno et al., 2010; Mišic et al., 2014). Stimulation of the vermis (lobule VII) increased complexity in fronto-parietal regions (bilateral dorsolateral PFC, dorsal anterior cingulate cortex, frontal eye field, and parietal cortex), while stimulation of the lateral cerebellum increased complexity in fronto-temporal leads (bilateral inferior and middle temporal gyrus and triangular and opercular gyri) (Farzan et al., 2016). Moreover, iTBS on the vermis resulted in increased power in 20-35 Hz oscillations (high beta/low gamma) in fronto-parietal areas. iTBS on the lateral cerebellum resulted in decreased power in theta globally, and increased power in gamma in fronto-temporal areas (Farzan et al., 2016). Reduction in frontal theta activity is associated with activation of the default-mode network (DMN), which is often found to be hyperactive in depressed individuals and is associated with the degree of negative ruminative state (Zhu et al., 2012; Northoff, 2016). Along with the fact that increased theta activity promotes approach behavior and decreases anxiety (Inanaga, 1998), this suggests that single-pulse stimulation of the vermis may have beneficial effects on symptoms of depression (Schutter and van Honk, 2006), whereas stimulation of the lateral cerebellum with iTBS may aggravate depressive symptoms or result in negative effects on mood (Farzan et al., 2016). TMS targeting the PFC has also been used to normalize the connectivity of the DMN, which is hyperactive in depressed individuals (Liston et al., 2014; Salomons et al., 2014). The relationship between frontal theta activity and connectivity

of the DMN, as well as the modulating effects of various types of stimulation on those components, is illustrated in **Figure 1.1**.

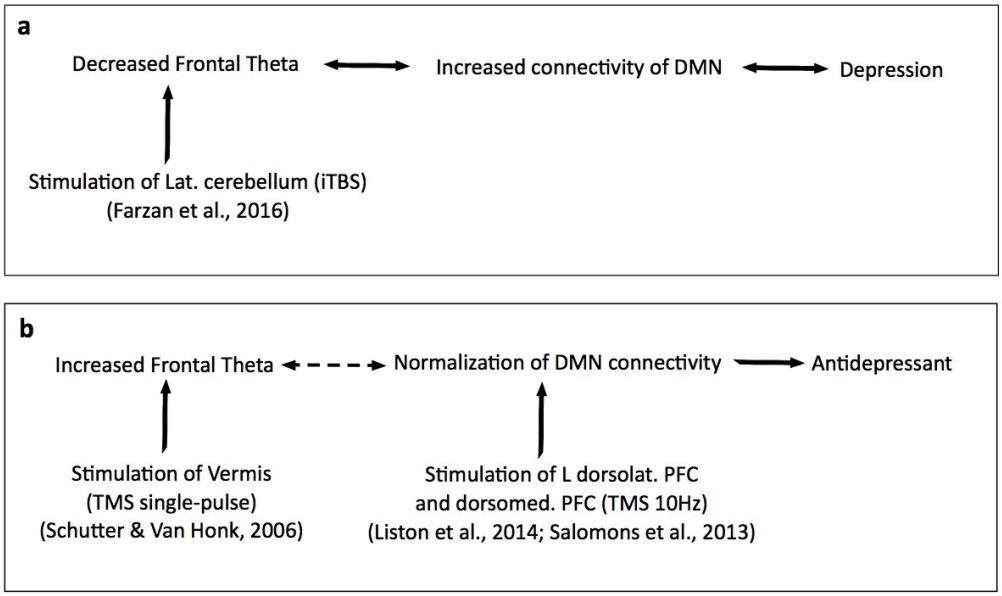

FIGURE 1.1. Relationship between theta activity and DMN connectivity.

Cerebellar stimulation in animals has also shown some interesting results. For instance, subdural electrical pulses on the vermis (lobule V) of rats led to increases in dopamine and noradrenaline levels in the nucleus accumbens (Dempesy and Richardson, 1987). Moreover, stimulating the vermis in cats and monkeys resulted in evoked responses in several limbic structures, including the amygdala, hippocampus, and septum (Harper and Heath, 1973; Snider and Maiti, 1976). Stimulating the fastigial nucleus in a rat model of post-stroke depression improved neuroprotection by suppressing cerebellar Purkinje cells death (apoptosis) and alleviated depressive-like behaviors in these animals (Zhang et al., 2017).

Oscillations in the prefrontal cortex

Prior to studying oscillatory phenomena following intervention such as stimulation, knowledge of normal oscillations in the areas of interest is essential. In the frontal cortex, oscillations in the gamma and beta ranges synchronize the PFC with the visual cortex and

contribute in mediating attention processes (Benchenane et al., 2011). The PFC also displays theta activity that is coherent with hippocampal theta and plays a crucial role in working memory and learning (Siapas et al., 2005; Benchenane et al., 2011). Lastly, slow oscillations (1 Hz), considered the signature of slow-wave sleep and anesthesia, are thought to mediate memory consolidation during sleep in a cortico-hippocampal circuit (Sirota et al., 2003; Greenberg et al., 2016). Those slow oscillations can group sharp-waves and ripples of the hippocampus, providing a mechanism for consolidation of memory through replay of awake experiences during sleep (Sirota et al., 2003).

Oscillations in the cerebellar cortex

Several types of oscillations have been reported in the cerebellum, the majority of which have been recorded in the paramedian lobules and in crus I-II. The main oscillatory patterns reported are 4-25 Hz oscillations in the granule cell layer and fast oscillations (150-300 Hz) in the Purkinje cell layer (Courtemanche et al., 2013). Slow oscillations (1 Hz) were also recorded in mice, under ketamine anesthesia (Ros et al., 2009). An in vitro study showed that the cerebellum could generate gamma rhythms and very fast oscillations (80-160 Hz). The researchers hypothesized that these rhythms, along with corresponding rhythms in the neocortex, may be a key component in somatosensory processing (Middleton et al., 2008). Oscillations in the theta range (5-10 Hz) were reported in the fastigial nucleus of the rat and were synchronized with the medial PFC (Watson et al., 2014).

Table 1.1. Summary of stimulation studies.

Authors	Stimulation site	Parameters	Results
(Dempesy & Richardson, 1987)	Vermis, 5th lobe (rat)	Over vermis (subdural), biphasic square pulse, 100 Hz, 5 min On-5 min Off	↑ DA and NE in NAc and lasts until 1h after
(Snider & Maiti, 1976)	Vermis & Fastigial nucleus (cats)	Biphasic square, 1-10 Hz, 0.1-1msec for 5-15 sec	Evoked response in amygdala, hippocampus, and septum
(Heath, 1977)	Vermis (humans)	Subdural, chronic, monophasic pulse, 0.25msec, 100 Hz, 3-6 V	Normalize behavior in psychiatric patients
(D. J. Schutter et al., 2003)	Vermis (healthy humans)	rTMS, 80% motor threshold, 25 Hz (beta), 10 sec On-5 sec Off, 20 min	Shift in PFC asymmetry (left to right) in 30-50 Hz
(D. J. Schutter & van Honk, 2006)	Vermis (healthy humans)	Single-pulse TMS (0.2 Hz), 60 pulses delivered	↑ Frontal Theta activity (↓ anxiety, ↑ approach behavior)
(Schutter et al., 2009)	Vermis (humans)	rTMS 20 Hz (beta), 15 min	↑ emotional responses to happy facial expressions
(Schutter and van Honk, 2009)	Cerebellum (humans)	rTMS 1 Hz (delta), 20 min (hypothesized to inhibit the cerebellum)	↑ negative mood
(Ferrucci et al., 2012)	Cerebellum & Left PFC (humans)	tDCS 2mA	tDCS over cerebellum enhanced emotional processing of negative facial expressions. No change with tDCS over PFC.
(Salomons et al., 2014)	Dorsomedial PFC (humans)	rTMS 10 Hz (alpha)	Normalize functional connectivity in DMN and neurotransmitter levels
(Liston et al., 2014)	Left dorsolat. PFC (humans)	rTMS 10 Hz (alpha)	

(Wu and Baeken, 2017)	Left dorsolat. PFC (humans)	rTMS 10 Hz and 20 Hz	Longer depressive episode linked to lower baseline cerebellar metabolism and ↓ efficacy of rTMS on PFC in those patients. Suggest stimulating the cerebellum instead.
Farzan et al., 2016	Right lateral cerebellar Crus I/II and vermis lobules VIIA/VIIB (humans)	iTBS (three biphasic waveform pulses at 50 Hz applied at 5 Hz, every 10 s) for 600 pulses	- Vermis: ↑ Power in high beta/low gamma (20-35 Hz) in fronto-parietal leads (bilateral dorsolat. PFC, dorsal anterior cingulate cortex, frontal eye field, and parietal regions) ↑ complexity in same regions. - Lat. Cb: ↓ Power in theta globally (activation of DMN) and ↑ Power of gamma in fronto-temporal leads. ↑ complexity fronto-temporal
Zhang et al., 2017	Fastigial nucleus (post-stroke depression rat model)	70μA direct square-wave current. 50 Hz	Alleviates depressive-like behaviors (↓ weight loss and normalizes sucrose preference, locomotor and rearing activities) Restores resting cerebral blood flow (CBF) and improve neuroprotection of purkinje cells (via suppression of apoptosis). ↓ mRNA level of inflammatory cytokines in hippocampus
Parker et al., 2016	Lat cerebellar projections in thalamus (rat model of schizophrenia)	Optogenetic stim 2 Hz	Increase power in delta in medial frontal cortex (in which delta activity is attenuated)
Park et al., 2015	Dentate nucleus (rat model of stroke and controls)	DBS at 20, 30, 50, and 100 Hz	Stroke group: ↑ contralateral cortical excitability (↑ MEP) maximally with 50 Hz DBS Naïve group: DBS of 30, 50 and 100 Hz were effective in ↑ MEP

RATIONALES AND OBJECTIVES

The cerebellar vermis and the dorsolateral PFC are involved in several neurological disorders including major depressive disorder, autism, and schizophrenia (Baxter et al., 1989; Andreasen et al., 1996; Maeda et al., 2000a; Andreasen and Pierson, 2008; Koenigs and Grafman, 2009; Fatemi et al., 2012). The cerebellum plays a role in various types of behaviors, from sensorimotor to higher-order functions, through numerous cerebello-cortical loops and connections with subcortical regions (Schmahmann, 2004; O'reilly et al., 2009; Strick et al., 2009). For this reason, non-invasive brain stimulation targeting the cerebellum has been used to modulate large-scale network activity and has become an important field of research (van Dun et al., 2017; van Dun et al., 2018). A better understanding of how cerebello-cortical networks operate and how they respond to different stimulation patterns is thus essential in the development of new treatments for neurological disorders in which the cerebellum is involved.

In this study, we investigated the effects of cerebellar vermis (lobule VII) stimulation, at various frequencies (single-pulse, 1 Hz, 5 Hz, 25 Hz, and 50 Hz), on LFP oscillations in the lateral cerebellum (Crus I/II) and dorsolateral PFC (frontal association area) and on coherence across a cerebello-cortical network, in the urethane-anesthetized rat. We recorded in the dorsolateral PFC because this region shows abnormalities in patients suffering from major depressive disorder, autism, and schizophrenia, in which the cerebellar vermis is also implicated (Baxter et al., 1989; Andreasen et al., 1996; Maeda et al., 2000a; Andreasen and Pierson, 2008; Koenigs and Grafman, 2009; Fatemi et al., 2012). More specifically, we recorded in the frontal association area (FrA), which is part of the dorsolateral PFC in the rat (Uylings et al., 2003). Lobule VII of the vermis was targeted because anatomical connections and functional connectivity have been shown between the vermis lobule VII and the dorsolateral PFC, in monkeys and in humans respectively (Kelly and Strick, 2003; Buckner et al., 2011). Since the stimulation was delivered in the most superficial layer of the cerebellar cortex, the electrical impulses were likely to travel laterally to the cerebellar hemispheres through parallel fibers, in addition to being transmitted from the Purkinje cells (PCs) to the fastigial nucleus and then to cortical areas via the thalamus (Akgören et al., 1996; Kandel et al., 2013). We thus decided to record LFP activity in the lateral cerebellum. Rats were anesthetized with urethane, which is an anesthetic agent that allows brain oscillations and induces a state similar

to sleep, with cyclic alternations between slow-wave (nREM) and active (REM) stages (Clement et al., 2008; Ros et al., 2009; Robinson et al., 2017). The anesthesia provided a controlled resting-state-like condition that allowed us to test various stimulation patterns. Indeed, patterns of resting-state functional connectivity, first described in idling states and during early stages of sleep in fMRI studies, have also been identified in the anesthetized state in humans, monkeys, and rodents (Lu et al., 2007; Raichle, 2015).

The goals were:
(1) To describe physiological interactions between the cerebellar vermis and FrA by assessing the cortical response (change in oscillatory power in the FrA) to vermal stimulation.
(2) To characterize LFP activity in the lateral cerebellum and FrA, as well as coherence in this network;
(3) To determine how different cerebellar stimulation patterns modulate activity in this network.

HYPOTHESES

Based on previous anatomical and connectivity studies (Akgören et al., 1996; Kelly and Strick, 2003; Buckner et al., 2011; Farzan et al., 2016), we hypothesized that LFP activity in the FrA and in the lateral cerebellum would be modulated by stimulation of the cerebellar vermis.

We predicted that stimulation at slow frequencies (single-pulse, 1 Hz, and 5 Hz) would increase cerebello-cortical coherence in theta, and that stimulating at faster frequencies would mainly affect activity in the beta and gamma bands.

Chapter 2

MANUSCRIPT

INTRODUCTION

There has been growing evidence supporting cerebellar involvement in cognitive and affective functions (Hoppenbrouwers et al., 2008; Strick et al., 2009). More than just a motor control structure, the cerebellum is now seen as a large-scale network synchronizer that can exert its influence through numerous cortical and subcortical projections (Courtemanche et al., 2013; Farzan et al., 2016). The notion of a universal cerebellar transform supports this view, stating that the uniform circuitry of the cerebellum allows for the same computation to take place in all cerebellar subregions, but each modulating different behaviors or states depending on the specific circuit a subregion is connected with, whether it be motor control, cognition, or affect (Schmahmann, 2004). Therefore, depending on the subregions affected, disorders of the cerebellum can manifest as ataxia, cognitive deficits, emotional disturbances, or a combination of these (Cerebellar Cognitive Affective Syndrome) (Schmahmann, 2004). Because of its contribution in several neurological disorders and its wide connectivity with extra-cerebellar structures, the cerebellum has been proposed as a therapeutic target for non-invasive stimulation techniques such as transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS) (Hoppenbrouwers et al., 2008; van Dun et al., 2017; van Dun et al., 2018). Notably, stimulation of the vermis, the most medial part of the cerebellum, has resulted in positive effects on cognition and mood, perhaps through modulation of frontal oscillations (Schutter et al., 2003; Schutter and van Honk, 2006; 2009; Demirtas-Tatlidede et al., 2010).

Evidence from studies in rats also suggest potential benefits of targeting the medial cerebellum with stimulation. For instance, stimulating the fastigial nucleus in a rat model of post-stroke depression (middle cerebral artery occlusion combined with chronic mild stress) improved neuroprotection by suppressing cell death of cerebellar Purkinje cells and alleviated depressive-like behaviors in these animals (Zhang et al., 2017). The effects of cerebellar stimulation have also been investigated on neocortical local field potentials (LFPs), in anesthetized cats. High frequency (300 Hz) stimulation of the fastigial nucleus attenuated slow rhythms and enhanced 20-40 Hz oscillations in the frontal cortex (Steriade, 1995).

Functional imaging studies have shown connectivity between the cerebellum and PFC in humans (O'reilly et al., 2009; Buckner et al., 2011; Sang et al., 2012; Farzan et al., 2016). Connections between the cerebellum and PFC were also demonstrated in tracing studies in non-human primates (Middleton and Strick, 1994; Middleton and Strick, 2001; Kelly and Strick, 2003; Strick et al., 2009) and with neuroanatomical and electrophysiological approaches in rodents (Watson et al., 2009; Suzuki et al., 2012; Watson et al., 2014). Reciprocal connections between the medial PFC (prelimbic cortex) and contralateral vermis (lobule VII) were demonstrated in *in vivo* electrophysiological studies in the urethane-anesthetized rat (Watson et al., 2009; Watson et al., 2014). Watson and colleagues (2014) also reported synchronous LFP activity between the fastigial nucleus and prelimbic cortex in the theta range (5-10 Hz) during active locomotion and rest. Synchronization, often expressed with measures of coherence, provides a means of communication between distant neuronal ensembles and allows them to collaborate in a task (Engel et al., 2001; Fries, 2015). There is growing interest in understanding how cerebello-cortical network interactions synchronize to modulate higher-order and affective functions. The dorsolateral PFC and the vermis are thought to be involved in the pathogenesis of several neurological disorders such as autism, schizophrenia, and depression (Baxter et al., 1989; Andreasen et al., 1996; Maeda et al., 2000a; Andreasen and Pierson, 2008; Koenigs and Grafman, 2009; Fatemi et al., 2012). Anatomical evidence in monkeys (Kelly and Strick, 2003) and functional connectivity studies in humans (Buckner et al., 2011; Farzan et al., 2016) have shown connections between the vermal lobule VII and dorsolateral PFC, but to our knowledge this has not been demonstrated in the rat.

In this study, we investigated the effects of cerebellar vermis stimulation, at various rhythms, on LFP oscillations in the lateral cerebellum (Crus I/II) and dorsolateral PFC (frontal association area) and on coherence across a cerebello-cortical network, in the urethane-anesthetized rat. Urethane is an anesthetic agent that allows brain oscillations and induces states similar to sleep, with cyclic alternations between slow-wave (nREM) and active (REM) stages (Clement et al., 2008; Ros et al., 2009; Robinson et al., 2017). The goals were to (1) describe physiological interactions between the cerebellar vermis and dorsolateral PFC (FrA in the rat (Uylings et al., 2003)) by assessing the cortical response to vermal stimulation, (2) characterize LFP activity in the lateral cerebellum and FrA, as well as coherence in this network, and (3) determine how different cerebellar stimulation patterns modulate activity in this network. Based on previous anatomical and connectivity studies

(Akgören et al., 1996; Kelly and Strick, 2003; Buckner et al., 2011; Farzan et al., 2016), we hypothesized that LFP activity in the FrA and in the lateral cerebellum would be modulated by stimulation of the cerebellar vermis. Since the stimulation was delivered in the most superficial layer of the cerebellar cortex, the electrical impulses were likely to travel laterally to the cerebellar hemispheres through parallel fibers, in addition to being transmitted from the Purkinje cells (PCs) to the fastigial nucleus and then to cortical areas via the thalamus (Akgören et al., 1996; Kandel et al., 2013). Lastly, we expected low frequency stimulation to impact slow oscillatory activity and coherence in the network and higher frequencies to drive cortical beta and gamma oscillations.

MATERIALS AND METHODS

Animals and anesthesia

Six adult male Sprague-Dawley rats were used in this study. The anesthesia procedures were the same as used by Frederick and colleagues (2015). Briefly, rats were anesthetized with a 5% isoflurane and 95% oxygen mixture to allow catheter placement in the jugular vein. We then injected urethane (0.8g/ml) intravenously through the catheter to continue anesthesia. We ensured sufficient anesthesia level by verifying that the foot-pinch reflex was absent throughout the experiment. Rats were placed in a stereotaxic apparatus and heating blankets were used to maintain body temperature around 37°C. All procedures were in accordance with the guidelines of the Canadian Council on Animal Care and with the Concordia University Animal Research Ethics Committee.

Surgery

The skin was cut to expose the skull and a 2-2.5 mm craniotomy was performed in the occipital bone, over the right cerebellum (R Cb). Holes were drilled in the skull bilaterally over the Frontal Association area (FrA; AP 4.7; ML +/-1.8; Depth 2.2), right FrA (AP 4.7; ML 1.8; Depth 2.2), and over the cerebellar vermis lobule VII (AP -13; ML 0; Depth 3.3). Bipolar electrodes constructed from Teflon-coated stainless steel twisted wire (125 μm tip diameter), with each tip at 1 mm apart in depth, were attached to flagpole holders and anchored to the stereotaxic apparatus. Electrodes were inserted stereotaxically in the vermis, for stimulation, and in each recording site (L FrA, R FrA and R Cb Crus II; **Figure 2.1**). This electrode arrangement resulted in one bipolar and two monopolar recordings for each site (3 channels per site). Cerebellar electrodes were inserted in the Crus I/II through the opening in the occipital bone, at a 45 degrees angle (3.2 mm from the midline, at a depth of about 1 mm from the surface of the cerebellum). A bare stainless steel reference electrode of about 5 mm long was placed between the skull and the surface of the temporal lobe. The stereotaxic apparatus was grounded.

Procedure

A the beginning of each experiment, LFPs in the R Cb and FrA (see **Table 2.1** for recording sites for each animal) were recorded for a baseline period of 2 minutes. Biphasic square-wave pulses (0.1 ms duration) were delivered through the bipolar electrode in the vermis at set frequencies using a stimulus generator (A-M Systems, Model 2100; Sequim, WA, USA). Single-pulses delivered every 5 s for 60 s, and repeated pulses at 1 Hz (30 s duration), 5 Hz (10 s), 25 Hz (2 s), and 50 Hz (1 s) were applied to the vermis. These stimulation frequencies were used previously to assess the effects of non-invasive neurostimulation targeting the cerebellum on cerebello-cortical connectivity via EEG rhythms (Schutter et al., 2003; Schutter and van Honk, 2006; 2009). Each stimulation pattern was first delivered at 500, 750, and 1000 μA, resulting in at least three trials for each stimulation frequency (trials were doubled in one animal). In three rats, stimulation was delivered from the lowest to the highest frequency (single-pulse to 50 Hz) and LFPs were recorded in only one side of the FrA: ipsilateral to the Cb in two animals, and contralateral in the other (6 channels experiments). In the next three rats, the order of stimulation was randomized to control for potential additive effect and LFPs were recorded bilaterally in the FrA (9 channels) (**Table 2.1**). Each recording period, with a given stimulation frequency, lasted 2 minutes, including a 30-seconds baseline, the stimulation period, and a post-stimulation phase (**Figure 2.2**). Once all stimulation patterns were delivered, the animal was euthanized with an intravenous overdose of urethane.

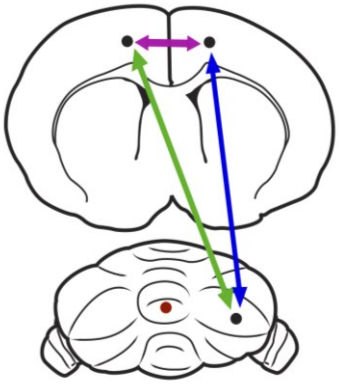

FIGURE 2.1. Anatomical representation of recording and stimulation sites. Recordings sites (R Cb, L FrA and R FrA) are represented by black dots, and stimulation site (Cb vermis) by a red dot. Arrows represent coherence between sites. Modified from (Frederick et al., 2015).

Table 2.1. Recording sites, number of trials per stimulation pattern and order of stimulation pattern delivery for each animal.

Rat	Recording sites	# trials/stim pattern	Order of stim delivery
1	R Cb, R FrA	3	Low to high frequency
2	R Cb, R FrA	3	Low to high freq
3	R Cb, L FrA	3	Low to high freq
4	R Cb, R FrA, L FrA	3	Randomized
5	R Cb, R FrA, L FrA	6	Randomized
6	R Cb, R FrA, L FrA	3	Randomized

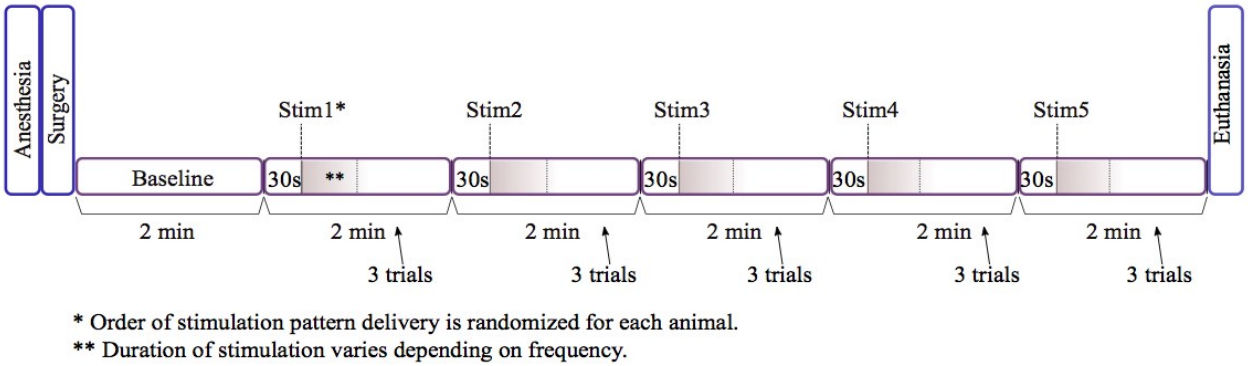

FIGURE 2.2. Experimental timeline. The animal was anesthetized, electrodes were inserted stereotaxically during surgery, and then a baseline period of 2 min was recorded. The first stimulation pattern was delivered after another short baseline of 30 s. The duration of the stimulation period varied depending on the stimulation frequency, leaving a post-stimulation period of varying length (total of 2 min recording). Three trials were conducted for each stimulation pattern. Once all stimulation patterns were delivered, the animal was euthanatized.

Signal processing and analysis

LFP signals were band-pass filtered between 0.01-500 Hz, amplified (x1000; A-M Systems Model 1700), and digitized onto the computer's hard drive at a sampling rate of 1024 Hz with a Datawave SciWorks system (Datawave Technologies, Loveland, CO, USA). Recordings were then imported into MATLAB (Mathworks, Natick, MA, USA) for analysis. The signal was filtered using the function *filtfilt*, with a FIR equiripple low-pass at 250 Hz. Power spectral density analyses (short-time Fourier transform) were conducted using the *spectrogram* function with windows of 512 samples (0.5 s) and a 50% overlap for all channels. This window width allowed to capture slow components of the signal with good temporal resolution. Spectrograms were constructed in MATLAB to represent the frequency content of the signal as a function of time.

For coherence analysis, the filtered signal was divided into epochs of 2 seconds. The magnitude-squared coherence, which indicates how closely related two signals (x and y) are in frequency was computed between each electrode pair using the *mscohere* function.

$$C_{xy}(f) = \frac{|P_{xy}(f)|^2}{P_{xx}(f)P_{yy}(f)},$$

where $P_{xx}(f)$ and $P_{yy}(f)$ are the power spectral densities of the signals x and y, and $P_{xy}(f)$ is the cross spectral density of the two signals.

This resulted in 15 comparisons for the experiments with 6 recorded channels and 36 comparisons for those with 9 channels. Since several of the comparisons were redundant and some were of lesser interest (e.g., coherence between the two cerebellar monopolar channels, which were too close to compare), comparisons of interest were selected and grouped together, resulting in three main comparison types: contralateral Cb-FrA, ipsilateral Cb-FrA, and left FrA-right FrA. Redundant channels were grouped as well (e.g., the two Cb monopolar channels), unless an artefact was present in a channel, in which case it was excluded from further analyses, resulting in three channel types: R Cb, L FrA, R FrA. Each type of comparison and channel comprised both monopolar and bipolar.

Power and coherence values were integrated within each frequency band (delta: 0.01-3 Hz, theta: 3-8 Hz, alpha: 8-14 Hz, beta: 14-30 Hz, low gamma: 30-55 Hz, high gamma: 65-80 Hz, and fast: 80-200 Hz). Frequencies between 55-65 Hz were left out to remove the 60 Hz noise. The integrated values were then normalized per frequency bin (e.g. integrated power values in the theta band were divided by 5), which allowed comparisons across frequency bands. After careful observation of spectrograms and coherograms to determine the duration of changes following stimulation, we decided to analyze periods of 6 s. Normalized integrated values of power and coherence were thus averaged across periods of 6 s, resulting in five pre-stimulation periods (30 s period) and a varying number of post-stimulation periods depending on the condition (5 to 15 periods).

Statistical analysis

Statistical analyses were conducted using Tibco Statistica (Dell Software, Round Rock, Texas, USA). The dependent variables were oscillatory power and coherence, while the independent variable was the type of stimulation. Repeated-measures ANOVAs, with 7 levels of frequency bands and levels of time (5 Pre and 5 to 8 Post windows, depending on condition) as repeated measures, and stimulation frequency (Stim-SP, Stim-1 Hz, Stim-5 Hz, Stim-25 Hz, and Stim-50 Hz) and channel (R Cb, L FrA, R FrA; monopolar and bipolar) as categorical factors, were performed for oscillatory power. A similar analysis was done for coherence but included the comparison type as a categorical factor. Results were considered significant if $p < 0.05$. The different stimulation intensities were compared at first, but this variable was found to have a negligible effect (1 rat, 2 trials per intensity with 4 stimulation patterns: $F(2, 288)=1.92$, $p=0.15$) and was excluded from further analysis. The three trials (6 in one animal) were thus grouped together independently of stimulation intensity. As mentioned in the General Discussion section below, future work should investigate the effect of intensity more specifically.

In a second analysis, we averaged pre-stimulation values (baseline) and calculated the relative changes (%) in power from baseline, for each site and in each frequency band, for all animals. The percent changes were then averaged across experiments. The same was done for coherence for each comparison type. Specific repeated-measures ANOVAs, each focusing on the effects of one stimulation frequency on the power, or coherence, in a given band, were conducted on the relative

changes from baseline using levels of time (1 Baseline and 5 to 8 Post windows, depending on the condition) as repeated measures. These analyses were performed for each stimulation frequency and frequency band, for power and coherence. Fisher's post-hoc analysis ($p<0.05$) were conducted to identify which time window (Post) was statistically different from baseline. Given the amount of variables and factors, separating the condition and frequency bands allowed to better visualize and characterize the effects of each stimulation frequency on the power, or coherence, in a given band. Expressing the changes as relative values served as a normalization per animal, which all have different baselines, allowing us to evaluate the effects of stimulation in all animals. With a view of simplification, we reported only the results of this analysis here.

Lastly, in an attempt to control for the potential influence of the stage of anesthesia on our results, we identified periods of slow-wave state in each animal. To achieve this, we looked at the averaged power values in delta during the periods preceding each stimulation (Pre periods) in a given condition. Neocortical activity at ~1 Hz (delta) has been associated with the slow-wave state in sleep and under urethane anesthesia, while low-amplitude faster cortical oscillations are present during the activated state (Clement et al., 2008). To our knowledge this has not been reported in the cerebellum hence why we focused on cortical channels to identify states. We classified periods with higher power in delta as periods of slow-wave and periods with lowest delta power as activated state. This allowed us to determine whether the animal was in a slow-wave state (peaks in delta power) or in an activated state prior to stimulating at a given frequency. For each condition of stimulation, two subgroups were thus formed (slow-wave state group and activated state group) and compared to infer whether the anesthesia stage had an impact on the efficacy of stimulation and whether this was frequency dependent.

RESULTS

Oscillatory activity and coherence at baseline

Prior to assessing the effects of cerebellar stimulation, we evaluated oscillatory activity and coherence in the network at baseline. Oscillatory power was highest in the delta band in all animals, which is consistent with the predominant slow wave activity reported in anesthetized rodents in other studies (Clement et al., 2008; Frederick et al., 2015). Baseline power spectra showed peaks in delta, at about 2 Hz, in all animals (**Figure 2.3**). Refer to **Table 2.2** for the mean power values (+/- SEM) in each frequency band at baseline, for each site and each animal. Consistent with previous physiological evidence in the cerebellum and neocortex (O'connor et al., 2002), coherent cerebello-cortical (contralateral) activity was found in the alpha band (10 Hz) (**Figure 2.4 A-C**). In cortical sites, fast activity (20-40 Hz) was observed on top of a slow oscillation (2 Hz). The left and right FrA also showed coherent activity in both these frequency bands (**Figure 2.4 D-F**). Oscillations in the gamma and beta ranges in the PFC are thought to synchronize with the visual cortex to mediate attentional processes (Benchenane et al., 2011).

Table 2.2. Mean power at baseline in each frequency band and each site, for all animals. (dB/Hz, +/- SEM)

Rat	Channel	Delta	SEM	Theta	SEM	Alpha	SEM	Beta	SEM	Low Gamma	SEM	High Gamma	SEM	Fast	SEM
1	R Cb	36053.6	4072.1	12284.3	825.6	3495.7	344.7	1504.6	100.4	749.2	32.1	347.1	8.0	159.9	5.5
	R FrA	12529.5	1109.8	3876.5	308.9	1356.7	88.1	524.8	17.5	218.9	10.0	124.6	3.9	67.8	1.2
2	R Cb	49494.0	1692.4	19211.0	897.6	5294.7	238.7	1793.5	44.3	868.4	17.4	521.9	7.8	220.7	3.2
	R FrA	444402.7	123979.7	171467.0	19111.2	61161.3	1464.1	17598.0	1560.2	7801.1	509.8	3860.1	278.9	1305.2	75.1
3	R Cb	24342.3	655.4	15155.0	281.4	4847.6	143.5	1635.8	56.0	705.5	22.3	303.9	3.3	128.4	1.3
	L FrA	421211.2	45707.8	238887.3	21846.8	68891.4	4079.4	13766.4	351.5	6724.9	250.9	3522.5	97.9	1189.7	23.9
4	R Cb	14514.6	606.1	7024.3	257.4	2551.8	60.2	1214.8	20.2	791.8	5.9	335.2	2.9	152.0	1.2
	R FrA	367131.7	38539.1	138852.1	5007.2	29068.7	957.6	9828.1	137.4	5063.7	92.5	2396.4	33.2	895.3	5.3
	L FrA	481383.7	28061.7	199882.0	3456.8	44259.3	841.0	14432.8	176.6	7857.9	166.8	4180.0	64.7	1794.2	28.2
5	R Cb	20234.3	249.9	8161.0	72.3	3142.0	35.0	1108.0	13.3	493.0	13.8	202.2	7.4	93.3	1.8
	R FrA	40816.5	4268.7	8892.9	1498.4	3142.9	619.9	1207.7	196.5	623.2	85.8	314.1	25.1	218.4	5.4
	L FrA	11443.4	511.5	2863.3	314.5	1132.4	76.2	511.6	33.3	338.3	21.9	247.3	5.5	182.3	1.4
6	R Cb	42000.4	2552.9	12122.4	594.7	2908.3	85.1	1226.0	23.1	820.2	13.7	377.3	7.4	197.5	5.2
	R FrA	92836.5	12317.7	32273.1	1075.8	11185.1	445.2	4298.8	218.4	1695.4	42.6	924.5	27.4	381.7	8.0
	L FrA	52537.3	1339.2	15494.9	175.0	4716.9	187.5	1665.2	77.4	653.8	8.5	385.3	6.0	214.4	1.4

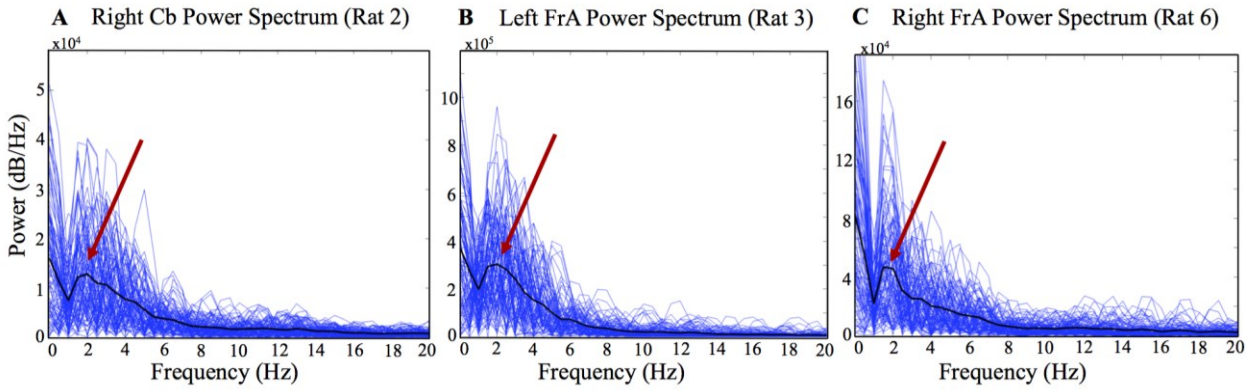

FIGURE 2.3. Power spectra at baseline showing peaks in delta (2 Hz) in different animals. **(A)** Power spectrum of the right cerebellum in Rat 2. **(B)** Power spectrum of the left FrA in Rat 3. **(C)** Power spectrum of the right FrA in rat 6. Each blue line represents the power spectra of one window. There are 100 windows in each graph, which corresponds to 25 s of the recorded baseline signal (width of the windows=512 samples; overlap=50%; one FFT is calculated per 0.25 s of signal). Thick black lines represent the mean of the power spectra for the 25 s period. Red arrows show the peaks at 2 Hz.

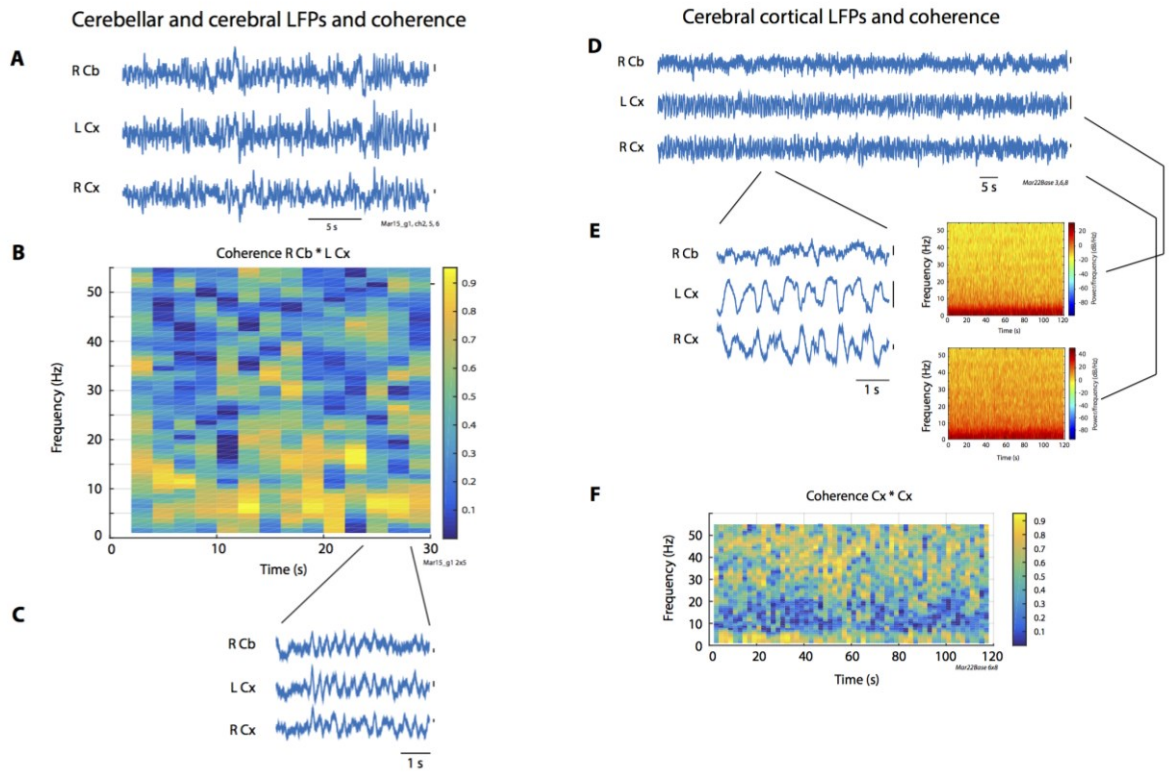

FIGURE 2.4. Oscillatory activity and coherence at baseline. Cerebello-cortical coherence at 10 Hz. (A) LFP traces recorded in the R Cb, L FrA and R FrA, from top to bottom. **(B)** Coherogram (R Cb-L FrA coherence across time) shows coherence at 10 Hz (theta/alpha band). **(C)** Closer view of 1 s of LFP signal shows 10 Hz activity. **Cortico-cortical coherence at slow and fast frequencies. (D)** LFP traces in R Cb, L FrA and R FrA, from top to bottom. **(E)** Left panel: Closer view of 1 s of LFP signal shows activity between 20-40 Hz at cortical sites on top of slow activity (2 Hz). Right panel: Slow activity can also be seen on spectrograms. **(F)** Coherogram (FrA-FrA coherence across time) shows coherent activity at about 2 Hz and in low gamma (20-40 Hz). Vertical bar is 100 µV.

Main effects of stimulation and interactions

Changes in Power. A main effect of stimulation was present for changes in power ($F(4, 112)=2.84$; $p<0.05$), with Stim-50 Hz resulting in the greatest increase and Stim-25 Hz resulting in the largest decrease. We observed a main effect of time windows ($F(5, 560)=2.77$; $p<0.05$); there was a global increase at Post1, with all stimulation patterns, and in all sites and frequency bands. A significant interaction between stimulation and time windows was also present ($F(20, 560)=4.39$; $p<0.05$): with Stim-50 Hz there was an increase at Post1, with Stim-SP there were increases following stimulation (Post1 and Post3) and with Stim-25 Hz all Post windows were below baseline.

Changes in Coherence. For coherence, there was no main effect of stimulation ($F(4, 81)=1.84$; $p=0.13$). There was a significant interaction between frequency band and time windows for coherence ($F(30, 2430)=2.18$; $p<0.05$). Those worth noting are a reduction in coherence in the delta band and an increase in alpha, both at Post1.

Because these main effects and interactions combine several factors (comparisons or channels, frequency bands), more specific ANOVAs were conducted in which those variables were carefully separated, allowing for the evaluation of each stimulation patterns on specific channels/comparisons and frequency bands. These results are presented in the next sections.

Effects of Single-Pulse Stimulation

Mean power increased in the delta band in the L FrA (13.3%; $p<0.05$ post-hoc Fisher) following single-pulse stimulation. In the theta band, power decreased in the R Cb and L FrA (8% in both; $p<0.05$ post-hoc Fisher), while there was an increase in the R FrA (9.5%; $p<0.05$ post-hoc Fisher). There were no significant changes in coherence. A summary of the effects of stimulation can be found in **Table 2.3** and in **Figure 2.6**.

Effects of Stimulation at 1 Hz

Stimulating at 1 Hz led to a decrease in delta mean power in the R FrA (20.4%; $p<0.05$ post-hoc Fisher) and to an increase in the L FrA (13%; $p<0.05$ post-hoc Fisher). Mean power decreased in theta in the R FrA post-stimulation (10.4%; $p<0.05$ post-hoc Fisher) while it increased in the L FrA in alpha (8%; $p<0.05$ post-hoc Fisher).

Ipsilateral cerebello-cortical coherence decreased (25.6%; $p=0.054$ post-hoc Fisher) in delta. Cortico-cortical coherence in theta was higher (23.6%; $p<0.05$ post-hoc Fisher) post-stimulation. Increased cerebello-cortical coherence in alpha was also observed ipsilaterally and contralaterally (24.7%; 19.3%; $p<0.05$ post-hoc Fisher).

Effects of Stimulation at 5 Hz

Mean delta power decreased in the R Cb, L FrA, and R FrA (9.9%; 12.1%; 13.5%; $p<0.05$ post-hoc Fisher). In the alpha band, power increased in the L and R FrA (9%; $p=0.057$; 13.9%; $p<0.05$ post-hoc Fisher; **Figure 2.5**). Power increased in the low gamma band in the L FrA (5.6%; $p<0.05$ post-hoc Fisher).

Cortico-cortical coherence decreased in delta (33.2%; $p<0.05$ post-hoc Fisher) after stimulation. In alpha, cortico-cortical (20.1 Post1, 22.2% Post2; $p<0.05$ post-hoc Fisher) and contralateral cerebello-cortical (21.8%; $p<0.05$ post-hoc Fisher) coherence increased following stimulation at 5 Hz (**Figure 2.5**). Coherence between the Cb and FrA (9.3%; $p<0.05$ post-hoc Fisher) increased ipsilaterally in the low gamma band.

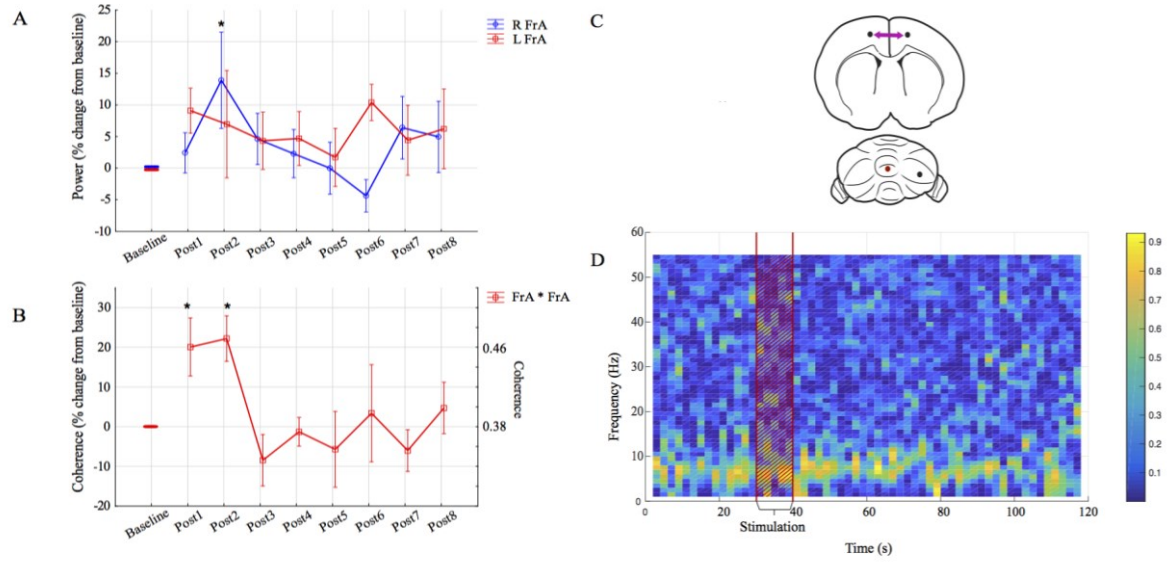

FIGURE 2.5. Effects of stimulation at 5 Hz on cortical power and cortico-cortical coherence in the alpha band. (A) Relative changes (%) in power from baseline showing increases in the L FrA (red line; 9% at Post1) and R FrA (blue line; 13.9% at Post2) in the alpha band. **(B)** Increase in FrA-FrA coherence (20.1% Post1 and 22.2% Post2; $p<0.05$ post-hoc Fisher) in the alpha band. Absolute coherence values are indicated on the right y axis and show that coherence increases from a mean value of 0.38 to 0.46. Each Post period lasts 6 s. **(C)** Anatomical representation of the sites of interest. Purple arrow represents coherence between the L and R FrA. **(D)** The coherogram shows cortico-cortical coherence across time in one animal. Cross-hatched area represents the stimulation period (10 s). Slightly more coherence, indicated by bright yellow, at about 10 Hz (alpha) is visible following the stimulation period and lasts ~10 s. Power Current effect: $F(8, 56)=1.17$, $p=.33$. Coherence Current effect: $F(10,60)=2.16$, $p=.03$. * Represent significant changes from baseline (post-hoc Fisher $p<0.05$).

Effects of Stimulation at 25 Hz

Stimulating at 25 Hz led to decreases in power in the R FrA in six frequency bands: delta, theta, alpha, beta, low gamma, and high gamma (15.3%; 7.3%; 7%; 5.3%; 5.2%; 5.3%; $p<0.05$ post-hoc Fisher). Alpha power in the R Cb also decreased (6.6%; $p<0.05$ post-hoc Fisher). In the beta band, there was an increase in power of the L FrA (6.2%; $p<0.05$ post-hoc Fisher). Cerebello-cortical coherence (ipsilateral) decreased in the delta, theta, and alpha bands (25.4%; 19.3%; 16.7%; $p<0.05$ post-hoc Fisher).

Effects of Stimulation at 50 Hz

High frequency stimulation resulted in increases in delta power of the R FrA (18.9%; $p<0.05$ post-hoc Fisher) and in theta power of the R Cb (16.1%; $p<0.05$ post-hoc Fisher). Contralateral cerebello-cortical coherence decreased in the delta and alpha bands (33.5%; 25.9%; $p<0.05$ post-hoc Fisher), while ipsilateral cerebello-cortical coherence decreased in the theta band (36.1%; $p<0.05$ post-hoc Fisher). In the beta band, there were increases in cortico-cortical (15.7%; $p<0.05$ post-hoc Fisher) and contralateral cerebello-cortical coherence (16.8%; $p<0.05$ post-hoc Fisher) post-stimulation. Contralateral cerebello-cortical coherence in low gamma increased (10.8%; $p<0.05$ post-hoc Fisher), while there was a decrease between cortical sites (8.9%; $p<0.05$ post-hoc Fisher) post-stimulation. Cortico-cortical coherence decreased (14.9%; $p<0.05$ post-hoc Fisher) in high gamma.

Table 2.3. Summary of the effects of stimulation on power and coherence.

	Freq. band	Power	Coherence
Stim-SP	Delta	↑ L FrA	No sign. changes
	Theta	↓ R Cb ↑ R FrA ↓ L FrA	No sign. changes
Stim-1 Hz	Delta	↓ R FrA ↑ L FrA	↓ Cb-FrA ipsi (p=0.054)
	Theta	↓ R FrA	↑ FrA-FrA
	Alpha	↑ L FrA	↑ Cb-FrA contra ↑ Cb-FrA ipsi
Stim-5 Hz	Delta	↓ R Cb ↑ R FrA ↓ L FrA	↓ FrA-FrA
	Alpha	↑ R FrA ↑ L FrA (p=0.057)	↑ Cb-FrA contra ↑ FrA-FrA
	Low Gamma	↑ L FrA	↑ Cb-FrA ipsi
Stim-25 Hz	Delta	↓ R FrA	↓ Cb-FrA ipsi
	Theta	↓ R FrA	↓ Cb-FrA ipsi
	Alpha	↓ R Cb ↓ R FrA	↓ Cb-FrA ipsi
	Beta	↓ R FrA ↑ L FrA	
	Low Gamma	↓ R FrA	
	High Gamma	↓ R FrA	
Stim-50 Hz	Delta	↑ R FrA	↓ Cb-FrA contra
	Theta	↑ R Cb	↓ Cb-FrA ipsi
	Alpha		↓ Cb-FrA contra
	Beta		↑ FrA-FrA ↑ Cb-FrA contra
	Low Gamma		↑ Cb-FrA contra ↓ FrA-FrA
	High Gamma		↓ FrA-FrA

*All changes mentioned in this table are significant post-hoc Fisher ($p<0.05$) unless another p value is indicated.

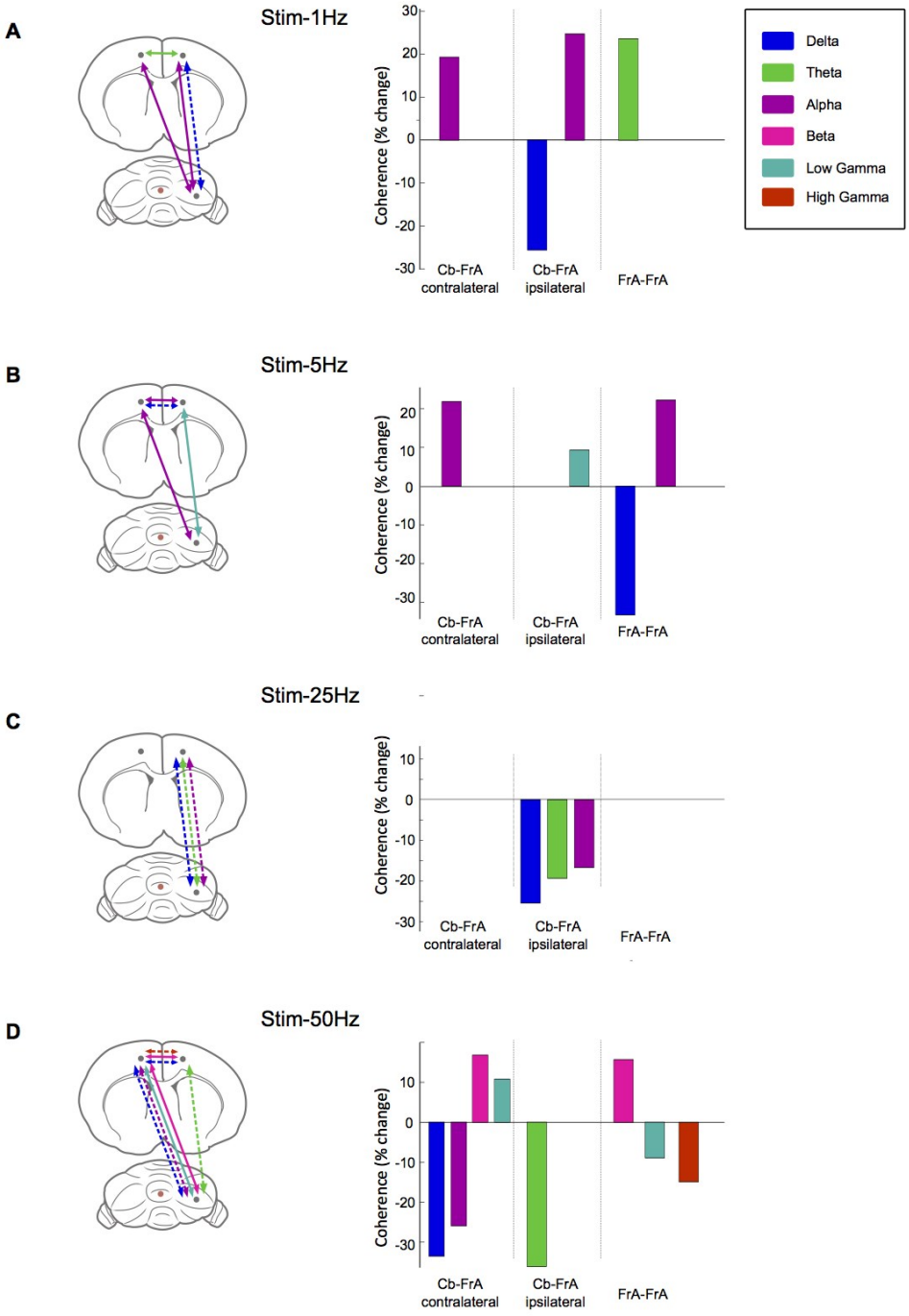
A
Stim-1Hz
B
Stim-5Hz
C
Stim-25Hz
D
Stim-50Hz
Coherence (% change)
Cb-FrA contralateral
Cb-FrA ipsilateral
FrA-FrA
Delta
Theta
Alpha
Beta
Low Gamma
High Gamma

FIGURE 2.6. Summary of the effects of each pattern of stimulation on relative changes (%) in coherence, in each frequency band. Solid arrows in left panels represent increases in coherence while dotted arrows represent decreases. Bar graphs show the magnitude of changes (% change from baseline). Different frequency bands are represented by different colors (refer to legend). **(A)** Stim-1 Hz increased Cb-FrA (contra and ipsi) in alpha and cortico-cortical coherence in theta. Ipsilateral Cb-FrA coherence in delta decreased following stimulation at 1 Hz. **(B)** Stim-5 Hz increased FrA-FrA and Cb-FrA (contralateral) coherence in alpha. FrA-FrA coherence decreased in delta and there was a small increase in low gamma Cb-FrA (ipsilateral) coherence. **(C)** Stim-25 Hz decreased ipsilateral Cb-FrA coherence in delta, theta, and alpha. **(D)** Stim-50 Hz increased Cb-FrA (contra) and FrA-FrA coherence in beta. Low gamma coherence also increased between the Cb and contralateral FrA, but it decreased between the left and right FrA. There were also decreases in delta and alpha (Cb-FrA contra), in theta (Cb-FrA ipsi) and in high gamma (FrA-FrA) following stimulation at 50 Hz.

Impact of baseline oscillatory state and anesthesia stage on the effects of stimulation

We investigated whether the oscillatory state of the animal before stimulation had an impact on the effects of stimulation. We thus attempted to identify periods of slow-wave state in each animal, akin to the sleep-like brain states under urethane suggested by Clement et al. (2008). Refer to **Table 2.4** for the temporal localization of the slow-wave state in each animal and **Figure 2.7** (**A-B**) for examples of slow-wave state localization from peaks in cortical delta power curves. To evaluate the impact of anesthesia stage on the effects of stimulation, we compared situations in which we stimulated, at a given frequency, while in the slow-wave state to those when we stimulated in the activated state. Two subgroups were thus created for each stimulation pattern (slow-wave state group and activated state group). When stimulating at low frequency (Stim-SP and Stim-1 Hz), the effects of stimulation on coherence were greater if the animal was initially in an activated state than if they were in slow-wave (**Figure 2.7 C-D**). Following single-pulse stimulation, FrA-FrA coherence in delta stayed near baseline in the slow-wave group, while a decrease of about 50% was seen in the activated state group ($p<0.05$ post-hoc Fisher at Post2). The difference between the two states was also significant post-hoc ($p<0.05$) in Cb-FrA (contralateral) coherence in the theta band, where coherence increased in the activated state only.

At higher frequencies (Stim-25 Hz and Stim-50 Hz), the opposite relationship was seen with a greater effect on coherence when the animal was in slow-wave prior to stimulating (**Figure 2.7 E-F**). Differences between slow-wave and activated states were significant when stimulating at 50 Hz ($F(8, 8)=25.49$; $p<0.01$). FrA-FrA coherence in alpha increased by 25-29% in the slow-wave group, whereas it remained near baseline in the slow-wave group. Differences between groups were non-significant when stimulating at 25 Hz but a trend can be seen despite a low sample size (2 rats in slow-wave group, 4 in activated state group). There were no instances when Stim-5 Hz was delivered in slow-wave making state comparisons impossible. We could deduce that Stim-5 Hz was always delivered in the activated state and since we observed large effects when stimulating at 5 Hz, we could speculate that, akin to other low frequency stimulations, this pattern was more effective when delivered in the activated state. However this would have to be verified. This state-dependent effect of stimulation was only present for coherence, with power changes showing an inconsistent relationship with state.

Table 2.4. Temporal localization of slow-wave state in each animal.

Rat	Slow-wave state
1	Stim-1 Hz, Stim 25 Hz*
2	Stim-SP, Stim-1 Hz, Stim-50 Hz
3	Stim-SP, Stim-1 Hz
4	Stim-SP, Stim-50 Hz
5	Stim-SP, Stim-1 Hz
6	Stim-25 Hz, Stim-1 Hz

* For example, rat 1 was in the slow-wave state before delivery of Stim-1 Hz and Stim-25 Hz.

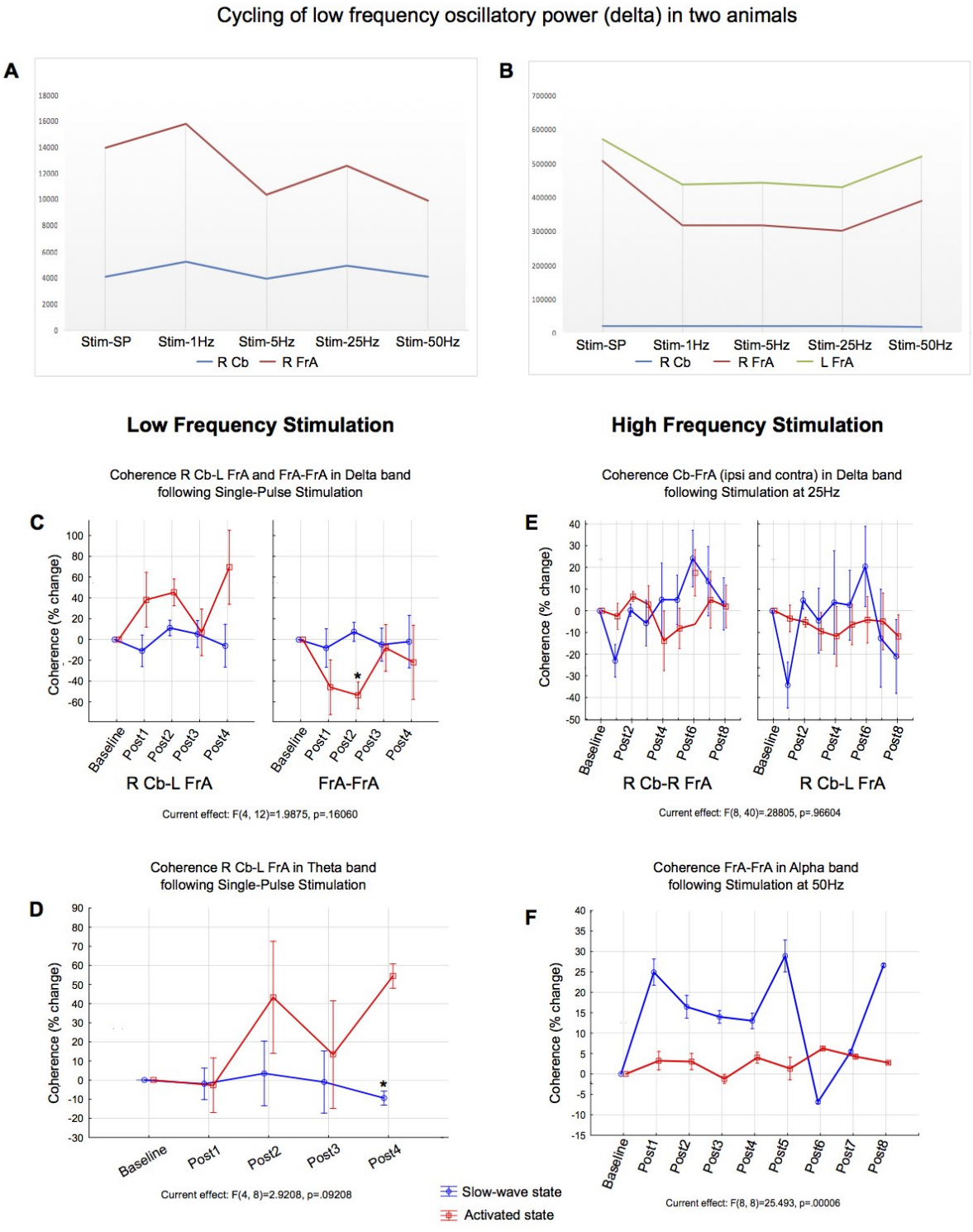
Cycling of low frequency oscillatory power (delta) in two animals
A
B
Stim-SP
Stim-1Hz
Stim-5Hz
Stim-25Hz
Stim-50Hz
R Cb
R FrA
L FrA
Low Frequency Stimulation
High Frequency Stimulation
Coherence R Cb-L FrA and FrA-FrA in Delta band
following Single-Pulse Stimulation
C
Coherence (% change)
Baseline
Post1
Post2
Post3
Post4
R Cb-L FrA
FrA-FrA
Current effect: F(4, 12)=1.9875, p=.16060
Coherence Cb-FrA (ipsi and contra) in Delta band
following Stimulation at 25Hz
E
Post6
Post8
R Cb-R FrA
R Cb-L FrA
Current effect: F(8, 40)=.28805, p=.96604
Coherence R Cb-L FrA in Theta band
following Single-Pulse Stimulation
D
Current effect: F(4, 8)=2.9208, p=.09208
Coherence FrA-FrA in Alpha band
following Stimulation at 50Hz
F
Post5
Post7
Current effect: F(8, 8)=25.493, p=.00006
Slow-wave state
Activated state

FIGURE 2.7. Impact of anesthesia state on the effects of stimulation at low and high frequencies. (A) Example of slow-wave state localization from the delta power curve of the R FrA in one animal (6 channel experiment). Peaks in delta power indicate that the animal was in the slow-wave state prior to Stim-1 Hz and Stim-25 Hz. **(B)** Example of localization from the L and R FrA in a 9 channel experiment. The trough is more visible than the peaks here, suggesting the animal was in the activated state prior to Stim-1 Hz, Stim-5 Hz and Stim-25 Hz, and in the slow-wave state prior to Stim-SP and Stim-50 Hz. **Low frequency stimulation has a greater effect on coherence when the animal is in the activated state. (C)** Relative changes (%) in coherence in delta following Stim-SP between the R Cb and L FrA (left graph), where the increase in coherence is mostly in the activated state group (red line), and between the L FrA and R FrA (right graph), where the decreased coherence occurs almost exclusively in the activated state. **(D)** Relative changes (%) in R Cb-L FrA coherence in theta following Stim-SP, where the increase in coherence is again mostly in the activated state. **High frequency stimulation has a greater effect on coherence when the animal is in slow-wave. (E)** Decreases in delta coherence in ipsilateral Cb-FrA (left graph) and in contralateral Cb-FrA (right graph) following Stim-25 Hz are greater in the slow-wave state group (blue lines). **(F)** Increased FrA-FrA coherence in the alpha band following Stim-50 Hz is largely due to the increase in the slow-wave group ($p<0.01$). * represent differences significant with post-hoc Fisher.

DISCUSSION

As we expected, based on previous research investigating the effects of vermal stimulation on frontal oscillations in humans, cats, and rodents (Steriade, 1995; Schutter et al., 2003; Schutter and van Honk, 2006; Parker et al., 2017), low frequency stimulation had an effect mainly on slow activity while stimulating at higher frequencies increased activity in faster bands. Another key finding in our study is that the effects of stimulation were influenced by the stage of anesthesia, as described by Clement and colleagues (2008). This highlights the pertinence of detecting the initial oscillatory state when stimulation is applied so that appropriate states can be identified and targeted. Studies in humans have started to explore the impact of EEG fluctuations on the response to non-invasive brain stimulation techniques such as TMS (Ferreri et al., 2014). This state-dependent effect of stimulation could be of clinical relevance given the fact that the cerebellum is being increasingly used as a target for non-invasive neurostimulation in the treatment of neurological disorders (Schutter and Van Honk, 2005b; van Dun et al., 2017; van Dun et al., 2018).

Effects of Stimulation on network coherence

The changes in power and coherence observed following cerebellar vermis stimulation are consistent with findings of other studies investigating the effects of cerebellar stimulation, in various species. See **Table 2.3** and **Figure 2.6** for a summary of the results. Single-pulses had no effect on coherence but resulted in power changes in the delta and theta bands. Increases in the delta range have also been reported in a study by Parker and colleagues (2017) using optogenetic stimulation (2 Hz) to re-establish normal levels of delta activity in a rat model of schizophrenia, which displays abnormal oscillations in the medial frontal cortex. Increased frontal theta activity was also observed with single-pulse TMS applied to the vermis in humans (Schutter and van Honk, 2006). In the current study however, theta power increased only in the right prefrontal cortex and decreased in the left. Stimulation at 5 Hz enhanced alpha power in cortical sites as well as cerebello- and cortico-cortical coherence in the alpha band.

High frequency stimulation (50 Hz) resulted in increases in cortico-cortical and cerebello-cortical coherence in the beta band. Steriade (1995) demonstrated an attenuation of slow rhythms and

enhancement of 20-40 Hz (beta/low gamma) oscillatory activity in the frontal cortex in response to stimulation of the fastigial nucleus, but using a much higher stimulation frequency of 300 Hz, in the cat. Deep brain stimulation of the dentate nucleus at 50 Hz increased contralateral cortical excitability, measured as motor evoked potentials, in both a rat model of stroke and in controls (Park et al., 2015). These findings are in line with the hypothesis that high frequency stimulation modulates fast activity in the frontal cortex. However, to our knowledge, the effects of 50 Hz stimulation on coherence in this network has not been described specifically. Stimulating at 25 Hz, on the other hand, led to decreases in ipsilateral cerebello-cortical coherence in slow frequency bands. Interestingly, we obtained asymmetrical changes in power in the beta band, with a decrease in the right FrA and an increase in the left. This is akin to the prefrontal asymmetrical shift reported by Schutter and colleagues (2003) after applying rTMS at 25 Hz to the vermis in humans, but in this study the power changes observed were in gamma. This difference in frequency could be due to different axon conduction speed and synaptic delays characteristic of different species (Buzsáki et al., 2013). Although targeting the cerebellum with stimulation has become a popular field of research (Schutter and Van Honk, 2005b; van Dun et al., 2017; van Dun et al., 2018), the effects of stimulation on coherence in these cerebello-prefrontal loops remain largely unexplored in the rat.

Potential Mechanisms

Stimulation of cerebellar Purkinje cells (PCs) leads to changes in the output of the cerebellar cortex, which in turn modulates the output of deep cerebellar nuclei (DCN) (Oulad Ben Taib and Manto, 2013; 2016; Das et al., 2017). Because the inputs from the PCs on the DCN are inhibitory, increased activation of PCs with high frequency stimulation (Maeda et al., 2000b; Hallett, 2007) inhibits the tonic activity of the DCN. Subsequent activation of extra-cerebellar areas via the thalamus is thus likely to occur through rebound excitation within the DCN (Buzsaki, 2006; Hoebeek et al., 2010). This phenomenon has been reported mainly in thalamic, cortical, and DCN neurons (Grenier et al., 1998; Buzsaki, 2006; Hoebeek et al., 2010; Boehme et al., 2011). After the initial inhibition induced by stimulation, the T-channel is activated causing Ca^{2+} influx which leads to a slow rebound spike. Thus, the initial hyperpolarization of the fastigial nuclei, induced by electrical stimulation of the vermis cerebellar cortex, would lead to a burst of rebound spikes in

the DCN up to 100 ms after the hyperpolarization ceases. If these spikes occur in synchrony and are coupled with the necessary opposing currents (mixed cation current, I_h), oscillations could be generated and would then propagate to thalamocortical pathways (McCormick and Pape, 1990; Buzsaki, 2006). The emergence of different oscillatory patterns thus depends not only on the strength and frequency of the applied stimuli, but also on pre-existing intracellular properties, such as intracellular concentration of Ca^{2+}. Indeed, in this mode, when the effects of stimulation on oscillations rely on rebound excitation mechanisms (high frequency stimulation), the initial state of the neuron strongly impacts the effects of inputs (Buzsaki, 2006). This is in line with the state-dependent effects of stimulation that we have found here, with stimulation at high frequencies (25 and 50 Hz) leading to greater changes when initially in a slow-wave state. Since the neuron is in a refractory period while it discharges and during hyperpolarization, and since the spike lasts longer in this mode, it may be easier to catch the right timing (when the neuron can respond to afferent inputs) if the neurons oscillate at a lower frequency when stimulating. This is assuming however that the cerebellar vermis and DCN also exhibit periods of slow-wave and that they are in a similar state as the prefrontal cortex at a given time.

Low frequency stimulation on the other hand may cause a decrease in activity of PCs (Chen et al., 1997), which would reduce inhibitory input to the fastigial and transmit a greater excitatory drive to the thalamus. In a study investigating rebound responses to different types of stimulation, they found that single-pulse stimulation of the cerebellar cortex (paravermal lobules VI/VII) increased the chance of spiking for a short period post-stimulation (after a latency of ~8 ms), but did not alter the firing frequency of DCN neurons (Hoebeek et al., 2010). Although the mechanisms are not fully understood, this effect on spike timing occurred in the absence of rebound excitation.

In this study, the changes we observed following stimulation were short-lasting, indicating our protocol had an entrainment effect on the cerebello-cortical network. However, given the duration of the changes observed, stimulation was unlikely to have induced long-term potentiation (LTP). For instance, high frequency stimulation (100 Hz in bursts of 15 pulses, for a total of 1500 pulses) applied to the parallel fibers (in the most superficial layer of the cerebellar cortex) has been shown to induce LTP at parallel fibers-PCs synapses (Jörntell and Ekerot, 2002). Therefore, the number of pulses delivered in our study may have been too low to lead to plastic changes.

Modulation of alpha and beta activity

Activity and coherence in the alpha band were enhanced following stimulation at 1 Hz and at 5 Hz. Activity in this frequency range may be easily entrained in the cerebellum and neocortex where oscillating at 5-20 Hz could be a natural frequency of communication between those areas (O'connor et al., 2002). Coherence in alpha may be useful in the context of functional inhibition, which allows the optimization of performance by suppressing unimportant inputs (Jensen and Mazaheri, 2010). Given the role of the cerebellum in adaptation and learning, cerebello-coherence in alpha may provide a mechanism by which the cerebellum contributes to modulating various types of behavior (Simpson and Alley, 1974; Ito, 1982). For example, the cerebellum is known to play an important role in associative learning tasks such as eyeblink conditioning, which has been demonstrated in several different species (Logan and Grafton, 1995; Kim and Thompson, 1997; Medina et al., 2002). Multiple brain regions, including the amygdala, hippocampus, medial prefrontal cortex, and cerebellum must coordinate to acquire a learned response, such as the conditioned eyelid response in the eyeblink conditioning paradigm (Lee and Kim, 2004). Indeed, synchronization of LFPs between the medial prefrontal cortex and the cerebellum at 5-12 Hz has been linked with adaptive performance in eyeblink conditioning during the early stages of learning (Chen et al., 2016). Coherent activity between the cerebellum and prefrontal cortex in the theta/alpha bands may thus contribute in acquiring appropriate behaviors through associative learning. However, this would have to be verified with the proper experimental protocol.

Prefrontal cortex networks have been shown to generate and respond preferentially to beta and gamma rhythms (Sherfey et al., 2018). Furthermore, the functional role of the cerebellum in gamma-band coherence between areas of the cerebral cortex has been demonstrated in rats (Popa et al., 2013). In this study, inactivating the cerebellum with a muscimol injection disrupted cortical coherence in gamma between the sensory and motor cortices, interrupting transmission of sensorimotor information between these areas. Consistent with these lines of evidence, we have found here that high frequency stimulation (50 Hz) of the cerebellum can enhance coherence between the left and right frontal association cortices in the beta and low gamma bands. Intermittent theta burst stimulation (iTBS) of the vermis was also found to modulate 20-35 Hz

(beta/gamma) activity in the dorsolateral prefrontal cortex (bilaterally) (Farzan et al., 2016). They reported increased oscillatory power at 20-35 Hz and enhanced EEG complexity, in fronto-parietal regions. Since the complexity of brain signals is an indication of cognitive and information processing capacity, which requires optimal communication amongst brain areas involved in a given cognitive process, greater complexity may reflect stronger coherence between the brain regions involved (Tononi et al., 1998; Fries, 2015; Farzan et al., 2016). Given the role of the dorsolateral PFC in executive functions and its implication in disorders such as depression, stimulating the cerebellar vermis could contribute in improving cognitive function and may provide an interesting treatment avenue for disorders in which the dorsolateral PFC is dysfunctional (Baxter et al., 1989; Maeda et al., 2000a; Koenigs and Grafman, 2009).

State-dependent effect of stimulation

We were interested in knowing if the initial oscillatory state, prior to stimulation, had an impact on the effects of cerebellar stimulation. Indeed, it has been shown that LFP activity fluctuates in urethane-anesthetized rats and that these cyclic alternations are similar to sleep stages (Clement et al., 2008). We also sought to determine if this relationship was the same for all patterns of stimulation. Our results show that the effects of stimulation are state-dependent. Furthermore, the influence of the anesthesia state depended on the frequency of stimulation and was divided in two categories with inverse relationships to state. High frequency stimulation had a greater effect on coherence, whether it was an increase or a decrease, when the animal was in a slow-wave state (i.e. a state dominated by activity in the delta range). As mentioned above, this could be due to the requirements in terms of the initial state of the neurons for rebound excitation to occur at the DCN level (Buzsaki, 2006). Because high frequency stimulation initially inhibits the fastigial nucleus, oscillatory effects in extra-cerebellar regions as a result of stimulation likely depend on this phenomenon. On the other hand, low frequency stimulation had a greater effect when the animal was in the activated state. (See **Figure 2.7**). The idea of an influence of brain state on the effects of stimulation, whether it be behavioral changes or oscillatory modulation, is of interest and has been investigated in humans, in studies using TMS or direct cortical stimulation, as well as in rats (Jackson et al., 2008; Alagapan et al., 2016; Connolly et al., 2016; Silvanto et al., 2017). However,

to our knowledge, this is the first study directly investigating the influence of the cyclic stage of urethane anesthesia on the effects of stimulation.

CONCLUSION

Although reciprocal anatomical connections have been shown between the cerebellum and PFC (cerebello-thalamo-cortical and cortico-ponto-cerebellar pathways) (Kelly and Strick, 2003; Strick et al., 2009; Watson et al., 2009; Buckner et al., 2011; Watson et al., 2014; Farzan et al., 2016), much is unknown regarding the degree to which cerebellar output modulates cortical activity and the exact spatial distribution of these connections. Ros and colleagues (2009) have shown that the cerebellum can generate slow oscillations that are synchronized with those of the neocortex and that neocortical oscillations drive cerebellar rhythms (Ros et al., 2009). We have found here that stimulation of the cerebellar vermis can modulate oscillatory activity and coherence in a large cerebello-cerebral network involving the dorsolateral PFC, under urethane anesthesia. This provides basic network interaction mechanisms in a resting-state condition. Indeed, patterns of resting-state intrinsic activity, first described in idling states and during early stages of sleep in fMRI studies, have also been identified in the anesthetized state in humans, monkeys, and rodents (Lu et al., 2007; Raichle, 2015). Similar mechanisms as those found here may thus be involved in states of quiet wakefulness and during sleep, in rodents, and warrant investigation.

These findings show that even under anesthesia, cerebello-cortical network interactions can be modulated through cerebellar stimulation. Future studies could investigate the effects of different patterns of cerebellar stimulation on awake animals, during behavior or rest, and on clinical animal models. More attention should be paid to the initial oscillatory state, as this was shown to impact the effects of stimulation. The cerebellum contributes to higher-order functions, in addition to its role in sensorimotor functions, and is thought to play a role in cognition and emotional behaviors through cerebello-cerebral loops. Understanding how this network operates and how it responds to stimulation is essential in developing new methods to address the numerous disorders in which the cerebellum is involved.

Chapter 3

GENERAL DISCUSSION

Limitations

In this study, we tested five different stimulation frequencies on oscillatory power in three sites, and coherence between these sites, in urethane-anesthetized rodents. This resulted in a lengthy experimental protocol. For this reason, we did not conduct a high number of trials per condition. This is one of the main limitation of the current study. This protocol allowed us to compare the effects of different stimulation frequencies, but diminished the power of the current study. Furthermore, since we focused on the frequency of stimulation, we did not consider the effects of different stimulation intensities (500-1000 μA).

Our study aimed at characterizing the response of the network to various stimulation patterns, by measuring the changes from baseline. Electrodes were thus inserted in the areas of interest, adjusting slightly their depth to obtain a good bipolar recording, but we did not isolate a specific oscillatory phenomena. The advantage of this method is that we could characterize baseline activity, and changes in activity, in a wide range of frequencies (power and coherence were evaluated in 7 frequency bands). However, the electrodes were variably located relative to an oscillatory generator and this may have resulted in a weaker recorded oscillatory signal and in a lower signal-to-noise ratio. Because extracellular field potentials are composed of multiple sources of current, the further the electrode is from the current source, the more noise, some of biological origin, will be captured (Buzsáki et al., 2012). Thus, if the oscillatory signal recorded is weak, biological noise, or 1/f spectral trend, will be relatively higher. This 1/f trend, also called pink noise, is a natural behavior in physiological systems and involves an inverse relationship between power and frequency (i.e. power decreases as frequency increases) (Demanuele et al., 2007). The power law of LFP, which describes the diminished amplitude of LFPs with increasing frequency, contributes to the 1/f power scaling of extracellular signals (Buzsáki et al., 2012). Furthermore, the low pass filtering property of dendrites causes an attenuation of high frequency inputs at the level of the soma (Lindén et al., 2010). Although physiological, this trend may render the detection of power spectra peaks difficult since low frequency peaks are drowned in the superimposed 1/f and because high frequency LFP component, which already have a lower amplitude (due to phase-amplitude coupling and to the power law), are attenuated by dendritic low pass filtering (Buzsáki et al., 2012). Several methods have been used to normalize signals by removing the 1/f trend

(Mäkinen et al., 2004), but some methods are based solely on the frequency domain in which case, phase information is lost. However, methods that combine both a frequency domain and a phase domain approach can be effective in removing the 1/f trend without sacrificing phase information and allow to capture event-related peaks in power spectra (Demanuele et al., 2007).

Another limitation of this study concerns the analysis of the impact of the anesthesia state. To analyze the influence of the anesthesia state on our results, the sample (n=6) was divided into two subgroups, depending on the state the animal was in prior to the delivery of a given stimulation pattern. The procedure was repeated for each condition. This led to small sample sizes (e.g. n_1=2 and n_2=4; see **Table 2.4**) and thus, to high variability in the measures. Despite the low sample sizes, a few differences were significant post-hoc (Fisher $p<0.05$) between groups.

Future directions

As mentioned previously, the intensity of stimulation was not taken into consideration in this study. The effects of intensity should thus be investigated more specifically in future work. Moreover, applying a spectral normalization method to remove the 1/f trend may allow the identification of peaks which were initially obscured by the 1/f trend in power spectra (Demanuele et al., 2007).

Lastly, to obtain a more in-depth picture of the impact of the anesthesia stage, a re-analysis of our data, in which each individual trial would be classified in either one of the two states (i.e. slow-wave or activated state), could result in more significant differences between states. Since we averaged the trials of each condition for the main analysis, we classified the average in either one of the two states, with the assumption that the state would remain the same during the 6-min period that each condition lasts (3 trials of 2 min). However, it is possible that the anesthesia stage (duration of ~11 min per state) shifted during a condition, so re-classifying trials would result in a more accurate evaluation of the impact of state on the effects of stimulation. If judged necessary after re-analysis, more experiments could be conducted which would lead to larger sample sizes of the subgroups.

REFERENCES

Akgören, N., Dalgaard, P., and Lauritzen, M. (1996). Cerebral blood flow increases evoked by electrical stimulation of rat cerebellar cortex: relation to excitatory synaptic activity and nitric oxide synthesis. *Brain research* 710(1-2)**,** 204-214.

Alagapan, S., Schmidt, S.L., Lefebvre, J., Hadar, E., Shin, H.W., and Fröhlich, F. (2016). Modulation of Cortical Oscillations by Low-Frequency Direct Cortical Stimulation Is State-Dependent. *PLOS Biology* 14(3)**,** e1002424. doi: 10.1371/journal.pbio.1002424.

Allen, G., Buxton, R.B., Wong, E.C., and Courchesne, E. (1997). Attentional activation of the cerebellum independent of motor involvement. *Science* 275(5308)**,** 1940-1943.

Andreasen, N.C., O'Leary, D.S., Cizadlo, T., Arndt, S., Rezai, K., Ponto, L., et al. (1996). Schizophrenia and cognitive dysmetria: a positron-emission tomography study of dysfunctional prefrontal-thalamic-cerebellar circuitry. *Proceedings of the National Academy of Sciences* 93(18)**,** 9985-9990.

Andreasen, N.C., and Pierson, R. (2008). The role of the cerebellum in schizophrenia. *Biological psychiatry* 64(2)**,** 81-88.

Armitage, R., Hoffmann, R.F., and Rush, A.J. (1999). Biological rhythm disturbance in depression: temporal coherence of ultradian sleep EEG rhythms. *Psychological medicine* 29(06)**,** 1435.

Baxter, L.R., Schwartz, J.M., Phelps, M.E., Mazziotta, J.C., Guze, B.H., Selin, C.E., et al. (1989). Reduction of prefrontal cortex glucose metabolism common to three types of depression. *Archives of General Psychiatry* 46(3)**,** 243.

Benchenane, K., Tiesinga, P.H., and Battaglia, F.P. (2011). Oscillations in the prefrontal cortex: a gateway to memory and attention. *Current opinion in neurobiology* 21(3)**,** 475-485.

Boehme, R., Uebele, V.N., Renger, J.J., and Pedroarena, C. (2011). Rebound excitation triggered by synaptic inhibition in cerebellar nuclear neurons is suppressed by selective T-type calcium channel block. *Journal of Neurophysiology* 106(5)**,** 2653-2661. doi: 10.1152/jn.00612.2011.

Buckner, R.L., Krienen, F.M., Castellanos, A., Diaz, J.C., and Yeo, B.T. (2011). The organization of the human cerebellum estimated by intrinsic functional connectivity. *American Journal of Physiology-Heart and Circulatory Physiology*.

Buzsaki, G. (2006). The brain's default state: self-organized oscillations in rest and sleep. *Rhythms of the Brain***,** 175-205.

Buzsáki, G., Anastassiou, C.A., and Koch, C. (2012). The origin of extracellular fields and currents—EEG, ECoG, LFP and spikes. *Nature reviews neuroscience* 13(6)**,** 407.

Buzsaki, G., and Draguhn, A. (2004). Neuronal oscillations in cortical networks. *Science (New York, N.Y.)* 304(5679)**,** 1926. doi: 10.1126/science.1099745 [doi].

Buzsáki, G., Logothetis, N., and Singer, W. (2013). Scaling brain size, keeping timing: evolutionary preservation of brain rhythms. *Neuron* 80(3)**,** 751-764.

Cerminara, N.L., Lang, E.J., Sillitoe, R.V., and Apps, R. (2015). Redefining the cerebellar cortex as an assembly of non-uniform Purkinje cell microcircuits. *Nature Reviews Neuroscience* 16(2)**,** 79.

Chen, H., Wang, Y.-j., Yang, L., Sui, J.-f., Hu, Z.-a., and Hu, B. (2016). Theta synchronization between medial prefrontal cortex and cerebellum is associated with adaptive performance of associative learning behavior. *Scientific reports* 6**,** 20960.

Chen, R., Classen, J., Gerloff, C., Celnik, P., Wassermann, E., Hallett, M., et al. (1997). Depression of motor cortex excitability by low‐frequency transcranial magnetic stimulation. *Neurology* 48(5)**,** 1398-1403.

Clement, E.A., Richard, A., Thwaites, M., Ailon, J., Peters, S., and Dickson, C.T. (2008). Cyclic and sleep-like spontaneous alternations of brain state under urethane anaesthesia. *PloS one* 3(4)**,** e2004.

Connolly, M.J., Gross, R.E., and Mahmoudi, B. (2016). The influence of the pre-stimulation neural state on the post-stimulation neural dynamics via distributed microstimulation of the hippocampus. *Conf Proc IEEE Eng Med Biol Soc* 2016**,** 1810-1813. doi: 10.1109/embc.2016.7591070.

Costa, M., Goldberger, A.L., and Peng, C.-K. (2005). Multiscale entropy analysis of biological signals. *Physical review E* 71(2), 021906.
Courtemanche, R., Robinson, J.C., and Aponte, D.I. (2013). Linking oscillations in cerebellar circuits. *Frontiers in neural circuits* 7, 125.
Das, S., Spoor, M., Sibindi, T.M., Holland, P., Schonewille, M., De Zeeuw, C.I., et al. (2017). Impairment of Long-Term Plasticity of Cerebellar Purkinje Cells Eliminates the Effect of Anodal Direct Current Stimulation on Vestibulo-Ocular Reflex Habituation. *Frontiers in Neuroscience* 11(444). doi: 10.3389/fnins.2017.00444.
De Montigny, C., and Lamarre, Y. (1973). Rhythmic activity induced by harmaline in the olivo-cerebello-bulbar system of the cat. *Brain research* 53(1), 81.
Demanuele, C., James, C.J., and Sonuga-Barke, E.J. (2007). Distinguishing low frequency oscillations within the 1/f spectral behaviour of electromagnetic brain signals. *Behavioral and Brain Functions* 3(1), 62.
Demirtas-Tatlidede, A., Freitas, C., Cromer, J.R., Safar, L., Ongur, D., Stone, W.S., et al. (2010). Safety and proof of principle study of cerebellar vermal theta burst stimulation in refractory schizophrenia. *Schizophrenia research* 124(1-3), 91-100.
Dempesy, C.W., and Richardson, D.E. (1987). Paleocerebellar stimulation induces in vivo release of endogenously synthesized 3 H dopamine and 3 H norepinephrine from rat caudal dorsomedial nucleus accumbens. *Neuroscience* 21(2), 565.
Denham, M.J., and Borisyuk, R.M. (2000). A model of theta rhythm production in the septal - hippocampal system and its modulation by ascending brain stem pathways. *Hippocampus* 10(6), 698.
Destexhe, A., and Sejnowski, T.J. (2003). Interactions between membrane conductances underlying thalamocortical slow-wave oscillations. *Physiological Reviews* 83(4), 1401. doi: 10.1152/physrev.00012.2003 [doi].
Drevets, W.C., Price, J.L., Simpson Jr, J.R., Todd, R.D., Reich, T., Vannier, M., et al. (1997). Subgenual prefrontal cortex abnormalities in mood disorders. *Nature* 386(6627), 824.
Dubin, M. (2017). Imaging TMS: antidepressant mechanisms and treatment optimization. *International Review of Psychiatry*, 1.
Dum, R.P., Li, C., and Strick, P.L. (2002). Motor and nonmotor domains in the monkey dentate. *Annals of the New York Academy of Sciences* 978(1), 289-301.
Engel, A.K., Fries, P., and Singer, W. (2001). Dynamic predictions: oscillations and synchrony in top–down processing. *Nature Reviews Neuroscience* 2(10), 704.
Farzan, F., Pascual-Leone, A., Schmahmann, J.D., and Halko, M. (2016). Enhancing the temporal complexity of distributed brain networks with patterned cerebellar stimulation. *Scientific reports* 6, 23599.
Fatemi, S.H., Aldinger, K.A., Ashwood, P., Bauman, M.L., Blaha, C.D., Blatt, G.J., et al. (2012). Consensus paper: pathological role of the cerebellum in autism. *The Cerebellum* 11(3), 777-807.
Ferreri, F., Vecchio, F., Ponzo, D., Pasqualetti, P., and Rossini, P.M. (2014). Time-varying coupling of EEG oscillations predicts excitability fluctuations in the primary motor cortex as reflected by motor evoked potentials amplitude: An EEG-TMS study. *Human Brain Mapping* 35(5), 1969-1980. doi: doi:10.1002/hbm.22306.
Ferrucci, R., Giannicola, G., Rosa, M., Fumagalli, M., Boggio, P.S., Hallett, M., et al. (2012). Cerebellum and processing of negative facial emotions: cerebellar transcranial DC stimulation specifically enhances the emotional recognition of facial anger and sadness. *Cognition & emotion* 26(5), 786.
Frederick, A., Bourget-Murray, J., Chapman, C.A., Amir, S., and Courtemanche, R. (2015). Diurnal influences on electrophysiological oscillations and coupling in the dorsal striatum and cerebellar cortex of the anesthetized rat. *Distributed Networks-New Outlooks on Cerebellar Function*, 52.
Fries, P. (2015). Rhythms for cognition: communication through coherence. *Neuron* 88(1), 220-235.

Gray, C.M., Konig, P., Engel, A.K., and Singer, W. (1989). Oscillatory responses in cat visual cortex exhibit inter-columnar synchronization which reflects global stimulus properties. *Nature* 338(6213)**,** 334. doi: 10.1038/338334a0 [doi].
Greenberg, A., Whitten, T.A., and Dickson, C.T. (2016). Stimulating forebrain communications: Slow sinusoidal electric fields over frontal cortices dynamically modulate hippocampal activity and cortico-hippocampal interplay during slow-wave states. *NeuroImage* 133**,** 189.
Greer, T.L., Trivedi, M.H., and Thompson, L.T. (2005). Impaired delay and trace eyeblink conditioning performance in major depressive disorder. *Journal of affective disorders* 86(2)**,** 235.
Gregoriou, G.G., Gotts, S.J., Zhou, H., and Desimone, R. (2009). High-Frequency, Long-Range Coupling Between Prefrontal and Visual Cortex During Attention. *Science* 324(5931)**,** 1207-1210. doi: 10.1126/science.1171402.
Grenier, F., Timofeev, I., and Steriade, M. (1998). Leading role of thalamic over cortical neurons during postinhibitory rebound excitation. *Proceedings of the National Academy of Sciences* 95(23)**,** 13929-13934. doi: 10.1073/pnas.95.23.13929.
Gross, J., Schmitz, F., Schnitzler, I., Kessler, K., Shapiro, K., Hommel, B., et al. (2004). Modulation of long-range neural synchrony reflects temporal limitations of visual attention in humans. *Proceedings of the national Academy of Sciences* 101(35)**,** 13050-13055.
Gross, J., Timmermann, L., Kujala, J., Dirks, M., Schmitz, F., Salmelin, R., et al. (2002). The neural basis of intermittent motor control in humans. *Proceedings of the National Academy of Sciences of the United States of America* 99(4)**,** 2299. doi: 10.1073/pnas.032682099 [doi].
Hallett, M. (2007). Transcranial magnetic stimulation: a primer. *Neuron* 55(2)**,** 187-199.
Harper, J.W., and Heath, R.G. (1973). Anatomic connections of the fastigial nucleus to the rostral forebrain in the cat. *Experimental neurology* 39(2)**,** 285.
Hartmann, M.J., and Bower, J.M. (1998). Oscillatory activity in the cerebellar hemispheres of unrestrained rats. *Journal of neurophysiology* 80(3)**,** 1598.
Heath, R.G. (1972). Electroencephalographic studies in isolation-raised monkeys with behavioral impairment. *Diseases of the nervous system*.
Heath, R.G. (1977). Modulation of emotion with a brain pacemaker. *J Nerv Ment Dis* 165**,** 300.
Heath, R.G., Dempesy, C.W., Fontana, C.J., and Fitzjarrell, A.T. (1980). Feedback loop between cerebellum and septal-hippocampal sites: its role in emotion and epilepsy. *Biological psychiatry*.
Hoebeek, F.E., Witter, L., Ruigrok, T.J.H., and De Zeeuw, C.I. (2010). Differential olivo-cerebellar cortical control of rebound activity in the cerebellar nuclei. *Proceedings of the National Academy of Sciences*. doi: 10.1073/pnas.0907118107.
Hoppenbrouwers, S.S., Schutter, D.J.L.G., Fitzgerald, P.B., Chen, R., and Daskalakis, Z.J. (2008). The role of the cerebellum in the pathophysiology and treatment of neuropsychiatric disorders: a review. *Brain Research Reviews* 59(1)**,** 185.
Huang, Y.-Z., Edwards, M.J., Rounis, E., Bhatia, K.P., and Rothwell, J.C. (2005). Theta burst stimulation of the human motor cortex. *Neuron* 45(2)**,** 201-206.
Inanaga, K. (1998). Frontal midline theta rhythm and mental activity. *Psychiatry and clinical neurosciences* 52(6)**,** 555.
Ito, M. (1982). Cerebellar control of the vestibulo-ocular reflex--around the flocculus hypothesis. *Annual review of neuroscience* 5(1)**,** 275-297.
Ito, M. (2006). Cerebellar circuitry as a neuronal machine. *Progress in neurobiology* 78(3)**,** 272.
Jackson, J., Dickson, C.T., and Bland, B.H. (2008). Median raphe stimulation disrupts hippocampal theta via rapid inhibition and state-dependent phase reset of theta-related neural circuitry. *J Neurophysiol* 99(6)**,** 3009-3026. doi: 10.1152/jn.00065.2008.
Jensen, O., and Mazaheri, A. (2010). Shaping Functional Architecture by Oscillatory Alpha Activity: Gating by Inhibition. *Frontiers in Human Neuroscience* 4(186). doi: 10.3389/fnhum.2010.00186.
Jörntell, H., and Ekerot, C.-F. (2002). Reciprocal Bidirectional Plasticity of Parallel Fiber Receptive Fields in Cerebellar Purkinje Cells and Their Afferent Interneurons. *Neuron* 34(5)**,** 797-806. doi: https://doi.org/10.1016/S0896-6273(02)00713-4.

Kadosh, R.C., Levy, N., O'Shea, J., Shea, N., and Savulescu, J. (2012). The neuroethics of non-invasive brain stimulation. *Current Biology* 22(4), R108-R111.
Kandel, E., Schwartz, J., Jessell, T., Siegelbaum, S., and Hudspeth, A. (2013). "Chapter 42 The Cerebellum," in *Principles of Neural Science*. Fifth ed (United States of America: McGraw-Hill Companies).
Kelly, R.M., and Strick, P.L. (2003). Cerebellar loops with motor cortex and prefrontal cortex of a nonhuman primate. *Journal of neuroscience* 23(23), 8432-8444.
Kim, J.J., and Thompson, R.E. (1997). Cerebellar circuits and synaptic mechanisms involved in classical eyeblink conditioning. *Trends in neurosciences* 20(4), 177-181.
Kim, S., Ugurbil, K., and Strick, P. (1994). Activation of a cerebellar output nucleus during cognitive processing. *Science* 265(5174), 949-951.
Koenigs, M., and Grafman, J. (2009). The functional neuroanatomy of depression: distinct roles for ventromedial and dorsolateral prefrontal cortex. *Behavioural brain research* 201(2), 239-243.
König, T., Prichep, L., Dierks, T., Hubl, D., Wahlund, L.O., John, E.R., et al. (2005). Decreased EEG synchronization in Alzheimer's disease and mild cognitive impairment. *Neurobiology of aging* 26(2), 165.
Lee, T., and Kim, J.J. (2004). Differential effects of cerebellar, amygdalar, and hippocampal lesions on classical eyeblink conditioning in rats. *Journal of Neuroscience* 24(13), 3242-3250.
Leiner, H.C., Leiner, A.L., and Dow, R.S. (1993). Cognitive and language functions of the human cerebellum. *Trends in neurosciences* 16(11), 444-447.
Leistedt, S.J.J., Coumans, N., Dumont, M., Lanquart, J.P., Stam, C.J., and Linkowski, P. (2009). Altered sleep brain functional connectivity in acutely depressed patients. *Human brain mapping* 30(7), 2207.
Leung, L.S., and Yim, C.Y.C. (1993). Rhythmic delta-frequency activities in the nucleus accumbens of anesthetized and freely moving rats. *Canadian journal of physiology and pharmacology* 71(5-6), 311.
Leventhal, A.M. (2008). Sadness, depression, and avoidance behavior. *Behavior modification* 32(6), 759. doi: 10.1177/0145445508317167 [doi].
Levy, R., Hutchison, W.D., Lozano, A.M., and Dostrovsky, J.O. (2000). High-frequency synchronization of neuronal activity in the subthalamic nucleus of parkinsonian patients with limb tremor. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 20(20), 7766. doi: 20/20/7766 [pii].
Liang, W.-K., Lo, M.-T., Yang, A.C., Peng, C.-K., Cheng, S.-K., Tseng, P., et al. (2014). Revealing the brain's adaptability and the transcranial direct current stimulation facilitating effect in inhibitory control by multiscale entropy. *Neuroimage* 90, 218-234.
Lindén, H., Pettersen, K.H., and Einevoll, G.T. (2010). Intrinsic dendritic filtering gives low-pass power spectra of local field potentials. *Journal of computational neuroscience* 29(3), 423-444.
Liston, C., Chen, A.C., Zebley, B.D., Drysdale, A.T., Gordon, R., Leuchter, B., et al. (2014). Default mode network mechanisms of transcranial magnetic stimulation in depression. *Biological psychiatry* 76(7), 517.
Llinas, R., and Volkind, R.A. (1973). The olivo-cerebellar system: functional properties as revealed by harmaline-induced tremor. *Experimental Brain Research* 18(1), 69.
Logan, C.G., and Grafton, S.T. (1995). Functional anatomy of human eyeblink conditioning determined with regional cerebral glucose metabolism and positron-emission tomography. *Proceedings of the National Academy of Sciences* 92(16), 7500-7504.
Lu, H., Zuo, Y., Gu, H., Waltz, J.A., Zhan, W., Scholl, C.A., et al. (2007). Synchronized delta oscillations correlate with the resting-state functional MRI signal. *Proceedings of the National Academy of Sciences* 104(46), 18265-18269.
Maeda, F., Keenan, J.P., and Pascual-Leone, A. (2000a). Interhemispheric asymmetry of motor cortical excitability in major depression as measured by transcranial magnetic stimulation. *The British journal of psychiatry : the journal of mental science* 177, 169.

Maeda, F., Keenan, J.P., Tormos, J.M., Topka, H., and Pascual-Leone, A. (2000b). Interindividual variability of the modulatory effects of repetitive transcranial magnetic stimulation on cortical excitability. *Experimental Brain Research* 133(4)**,** 425.

Mäkinen, V., May, P., and Tiitinen, H. (2004). Spectral characterization of ongoing and auditory event-related brain processes. *Neurology & clinical neurophysiology: NCN* 2004**,** 104-104.

Matano, S. (2001). Brief communication: proportions of the ventral half of the cerebellar dentate nucleus in humans and great apes. *American Journal of Physical Anthropology: The Official Publication of the American Association of Physical Anthropologists* 114(2)**,** 163-165.

Matthews, S.C., Strigo, I.A., Simmons, A.N., Yang, T.T., and Paulus, M.P. (2008). Decreased functional coupling of the amygdala and supragenual cingulate is related to increased depression in unmedicated individuals with current major depressive disorder. *Journal of affective disorders* 111(1)**,** 13.

McCormick, D.A., and Pape, H.C. (1990). Properties of a hyperpolarization-activated cation current and its role in rhythmic oscillation in thalamic relay neurones. *The Journal of Physiology* 431(1)**,** 291-318. doi: doi:10.1113/jphysiol.1990.sp018331.

Medina, J.F., Christopher Repa, J., Mauk, M.D., and LeDoux, J.E. (2002). Parallels between cerebellum- and amygdala-dependent conditioning. *Nature Reviews Neuroscience* 3**,** 122. doi: 10.1038/nrn728.

Middleton, F.A., and Strick, P.L. (1994). Anatomical evidence for cerebellar and basal ganglia involvement in higher cognitive function. *Science* 266(5184)**,** 458-461.

Middleton, F.A., and Strick, P.L. (1997). "Cerebellar output channels," in *International review of neurobiology*. Elsevier), 61-82.

Middleton, F.A., and Strick, P.L. (2001). Cerebellar projections to the prefrontal cortex of the primate. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 21(2)**,** 700. doi: 21/2/700 [pii].

Middleton, S.J., Racca, C., Cunningham, M.O., Traub, R.D., Monyer, H., Knöpfel, T., et al. (2008). High-frequency network oscillations in cerebellar cortex. *Neuron* 58(5)**,** 763.

Mišic, B., Doesburg, S.M., Fatima, Z., Vidal, J., Vakorin, V.A., Taylor, M.J., et al. (2014). Coordinated information generation and mental flexibility: large-scale network disruption in children with autism. *Cerebral Cortex* 25(9)**,** 2815-2827.

Mizuno, T., Takahashi, T., Cho, R.Y., Kikuchi, M., Murata, T., Takahashi, K., et al. (2010). Assessment of EEG dynamical complexity in Alzheimer's disease using multiscale entropy. *Clinical Neurophysiology* 121(9)**,** 1438-1446.

Müller, M.M., Keil, A., Gruber, T., and Elbert, T. (1999). Processing of affective pictures modulates right-hemispheric gamma band EEG activity. *Clinical Neurophysiology* 110(11)**,** 1913-1920.

Nestler, E.J., and Carlezon Jr, W.A. (2006). The mesolimbic dopamine reward circuit in depression. *Biological psychiatry* 59(12)**,** 1151-1159.

Northoff, G. (2016). How do resting state changes in depression translate into psychopathological symptoms? From 'Spatiotemporal correspondence'to 'Spatiotemporal Psychopathology'. *Current opinion in psychiatry* 29(1)**,** 18-24.

O'connor, S.M., Berg, R.W., and Kleinfeld, D. (2002). Coherent electrical activity between vibrissa sensory areas of cerebellum and neocortex is enhanced during free whisking. *Journal of neurophysiology* 87(4)**,** 2137-2148.

O'reilly, J.X., Beckmann, C.F., Tomassini, V., Ramnani, N., and Johansen-Berg, H. (2009). Distinct and overlapping functional zones in the cerebellum defined by resting state functional connectivity. *Cerebral cortex* 20(4)**,** 953-965.

Oulad Ben Taib, N., and Manto, M. (2013). Trains of epidural DC stimulation of the cerebellum tune corticomotor excitability. *Neural plasticity* 2013.

Oulad Ben Taib, N., and Manto, M. (2016). The in vivo reduction of afferent facilitation induced by low frequency electrical stimulation of the motor cortex is antagonized by cathodal direct current stimulation of the cerebellum. *Cerebellum & Ataxias* 3(1)**,** 15. doi: 10.1186/s40673-016-0053-3.

Pape, H.C., Narayanan, R.T., Smid, J., Stork, O., and Seidenbecher, T. (2005). Theta activity in neurons and networks of the amygdala related to long‐term fear memory. *Hippocampus* 15(7)**,** 874.

Paradiso, G., Cunic, D., Saint-Cyr, J.A., Hoque, T., Lozano, A.M., Lang, A.E., et al. (2004). Involvement of human thalamus in the preparation of self-paced movement. *Brain : a journal of neurology* 127(Pt 12)**,** 2717. doi: 10.1093/brain/awh288 [doi].

Park, H.-J., Furmaga, H., Cooperrider, J., Gale, J.T., Baker, K.B., and Machado, A.G. (2015). Modulation of cortical motor evoked potential after stroke during electrical stimulation of the lateral cerebellar nucleus. *Brain stimulation* 8(6)**,** 1043-1048.

Parker, K., Kim, Y., Kelley, R., Nessler, A., Chen, K., Muller-Ewald, V., et al. (2017). Delta-frequency stimulation of cerebellar projections can compensate for schizophrenia-related medial frontal dysfunction. *Molecular psychiatry* 22(5)**,** 647.

Pellerin, J.P., and Lamarre, Y. (1997). Local field potential oscillations in primate cerebellar cortex during voluntary movement. *Journal of neurophysiology* 78(6)**,** 3502.

Popa, D., Spolidoro, M., Proville, R.D., Guyon, N., Belliveau, L., and Léna, C. (2013). Functional role of the cerebellum in gamma-band synchronization of the sensory and motor cortices. *Journal of Neuroscience* 33(15)**,** 6552-6556.

Raichle, M.E. (2015). The brain's default mode network. *Annual review of neuroscience* 38**,** 433-447.

Ramnani, N. (2006). The primate cortico-cerebellar system: anatomy and function. *Nature Reviews Neuroscience* 7(7)**,** 511.

Raz, A., Vaadia, E., and Bergman, H. (2000). Firing patterns and correlations of spontaneous discharge of pallidal neurons in the normal and the tremulous 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine vervet model of parkinsonism. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 20(22)**,** 8559. doi: 20/22/8559 [pii].

Reynolds, C.F., and Kupfer, D.J. (1987). Sleep research in affective illness: state of the art circa 1987. *Sleep: Journal of Sleep Research & Sleep Medicine*.

Robinson, J.C., Chapman, C.A., and Courtemanche, R. (2017). Gap junction modulation of low-frequency oscillations in the cerebellar granule cell layer. *The Cerebellum* 16(4)**,** 802-811.

Ros, H., Sachdev, R.N., Yu, Y., Sestan, N., and McCormick, D.A. (2009). Neocortical networks entrain neuronal circuits in cerebellar cortex. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 29(33)**,** 10309. doi: 10.1523/JNEUROSCI.2327-09.2009 [doi].

Sacchetti, B., Scelfo, B., and Strata, P. (2005). The cerebellum: synaptic changes and fear conditioning. *The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry* 11(3)**,** 217. doi: 11/3/217 [pii].

Salomons, T.V., Dunlop, K., Kennedy, S.H., Flint, A., Geraci, J., Giacobbe, P., et al. (2014). Resting-state cortico-thalamic-striatal connectivity predicts response to dorsomedial prefrontal rTMS in major depressive disorder. *Neuropsychopharmacology* 39(2)**,** 488.

Sang, L., Qin, W., Liu, Y., Han, W., Zhang, Y., Jiang, T., et al. (2012). Resting-state functional connectivity of the vermal and hemispheric subregions of the cerebellum with both the cerebral cortical networks and subcortical structures. *Neuroimage* 61(4)**,** 1213-1225.

Sasaki, K., Oka, H., Matsuda, Y., Shimono, T., and Mizuno, N. (1975). Electrophysiological studies of the projections from the parietal association area to the cerebellar cortex. *Experimental Brain Research* 23(1)**,** 91-102.

Schmahmann, J.D. (2004). Disorders of the cerebellum: ataxia, dysmetria of thought, and the cerebellar cognitive affective syndrome. *The Journal of neuropsychiatry and clinical neurosciences* 16(3)**,** 367.

Schmahmann, J.D., and Pandyat, D.N. (1997). "The cerebrocerebellar system," in *International review of neurobiology*. Elsevier), 31-60.

Schmahmann, J.D., Weilburg, J.B., and Sherman, J.C. (2007). The neuropsychiatry of the cerebellum—insights from the clinic. *The Cerebellum* 6(3)**,** 254.

Schnitzler, A., and Gross, J. (2005). Normal and pathological oscillatory communication in the brain. *Nature reviews neuroscience* 6(4)**,** 285.

Schoenemann, P.T., Sheehan, M.J., and Glotzer, L.D. (2005). Prefrontal white matter volume is disproportionately larger in humans than in other primates. *Nature neuroscience* 8(2)**,** 242.
Schutter, D.J.L.G., Enter, D., and Hoppenbrouwers, S.S. (2009). High frequency repetitive transcranial magnetic stimulation to the cerebellum and implicit processing of happy facial expressions.
Schutter, D.J.L.G., and Van Honk, J. (2005a). The cerebellum on the rise in human emotion. *The Cerebellum* 4(4)**,** 290.
Schutter, D.J.L.G., and Van Honk, J. (2005b). A framework for targeting alternative brain regions with repetitive transcranial magnetic stimulation in the treatment of depression. *Journal of psychiatry & neuroscience: JPN* 30(2)**,** 91.
Schutter, D.J.L.G., and van Honk, J. (2006). An electrophysiological link between the cerebellum, cognition and emotion: frontal theta EEG activity to single-pulse cerebellar TMS. *NeuroImage* 33(4)**,** 1227.
Schutter, D.J.L.G., and van Honk, J. (2009). The cerebellum in emotion regulation: a repetitive transcranial magnetic stimulation study. *The Cerebellum* 8(1)**,** 28.
Schutter, D.J.L.G., van Honk, J., d'Alfonso, A.A.L., Peper, J.S., and Panksepp, J. (2003). High frequency repetitive transcranial magnetic over the medial cerebellum induces a shift in the prefrontal electroencephalography gamma spectrum: a pilot study in humans. *Neuroscience letters* 336(2)**,** 73.
Shah, S.A., Doraiswamy, P.M., Husain, M.M., Escalona, P.R., Na, C., Figiel, G.S., et al. (1992). Posterior fossa abnormalities in major depression: a controlled magnetic resonance imaging study. *Acta Psychiatrica Scandinavica* 85(6)**,** 474.
Sherfey, J.S., Ardid, S., Hass, J., Hasselmo, M.E., and Kopell, N.J. (2018). Flexible resonance in prefrontal networks with strong feedback inhibition. *PLoS computational biology* 14(8)**,** e1006357.
Siapas, A.G., Lubenov, E.V., and Wilson, M.A. (2005). Prefrontal phase locking to hippocampal theta oscillations. *Neuron* 46(1)**,** 141.
Silvanto, J., Bona, S., and Cattaneo, Z. (2017). Initial activation state, stimulation intensity and timing of stimulation interact in producing behavioral effects of TMS. *Neuroscience* 363**,** 134-141. doi: 10.1016/j.neuroscience.2017.09.002.
Simpson, J.I., and Alley, K.E. (1974). Visual climbing fiber input to rabbit vestibulo-cerebellum: a source of direction-specific information. *Brain research* 82(2)**,** 302-308.
Singer, W. (1999). Neuronal synchrony: a versatile code for the definition of relations? *Neuron* 24(1)**,** 49.
Sirota, A., Csicsvari, J., Buhl, D., and Buzsaki, G. (2003). Communication between neocortex and hippocampus during sleep in rodents. *Proceedings of the National Academy of Sciences of the United States of America* 100(4)**,** 2065. doi: 10.1073/pnas.0437938100 [doi].
Snider, R.S., and Maiti, A. (1976). Cerebellar contributions to the Papez circuit. *Journal of neuroscience research* 2(2)**,** 133.
Spencer, K.M., Nestor, P.G., Perlmutter, R., Niznikiewicz, M.A., Klump, M.C., Frumin, M., et al. (2004). Neural synchrony indexes disordered perception and cognition in schizophrenia. *Proceedings of the National Academy of Sciences of the United States of America* 101(49)**,** 17288. doi: 0406074101 [pii].
Stam, C.J., van Walsum, A.M.v.C., Pijnenburg, Y.A.L., Berendse, H.W., de Munck, J.C., Scheltens, P., et al. (2002). Generalized synchronization of MEG recordings in Alzheimer's disease: evidence for involvement of the gamma band. *Journal of Clinical Neurophysiology* 19(6)**,** 562.
Steriade, M. (1995). Two channels in the cerebellothalamocortical system. *Journal of Comparative Neurology* 354(1)**,** 57-70. doi: doi:10.1002/cne.903540106.
Steriade, M. (2001). Impact of network activities on neuronal properties in corticothalamic systems. *Journal of neurophysiology* 86(1)**,** 1.
Strick, P.L., Dum, R.P., and Fiez, J.A. (2009). Cerebellum and nonmotor function. *Annual review of neuroscience* 32**,** 413-434.

Strogatz, S.H., and Stewart, I. (1993). Coupled oscillators and biological synchronization. *Scientific American* 269(6)**,** 102.
Suzuki, L., Coulon, P., Sabel-Goedknegt, E.H., and Ruigrok, T.J. (2012). Organization of cerebral projections to identified cerebellar zones in the posterior cerebellum of the rat. *Journal of Neuroscience* 32(32)**,** 10854-10869.
Sweeney, J.A., Strojwas, M.H., Mann, J.J., and Thase, M.E. (1998). Prefrontal and cerebellar abnormalities in major depression: evidence from oculomotor studies. *Biological psychiatry* 43(8)**,** 584.
Tavano, A., Grasso, R., Gagliardi, C., Triulzi, F., Bresolin, N., Fabbro, F., et al. (2007). Disorders of cognitive and affective development in cerebellar malformations. *Brain : a journal of neurology* 130(Pt 10)**,** 2646. doi: awm201 [pii].
Timmermann, L., Gross, J., Dirks, M., Volkmann, J., Freund, H.J., and Schnitzler, A. (2003). The cerebral oscillatory network of parkinsonian resting tremor. *Brain : a journal of neurology* 126(Pt 1)**,** 199.
Tononi, G., Edelman, G.M., and Sporns, O. (1998). Complexity and coherency: integrating information in the brain. *Trends in cognitive sciences* 2(12)**,** 474-484.
Uylings, H.B., Groenewegen, H.J., and Kolb, B. (2003). Do rats have a prefrontal cortex? *Behavioural brain research* 146(1-2)**,** 3-17.
van Dun, K., Bodranghien, F., Manto, M., and Marien, P. (2017). Targeting the cerebellum by noninvasive neurostimulation: a review. *The Cerebellum* 16(3)**,** 695-741.
van Dun, K., Mitoma, H., and Manto, M. (2018). Cerebellar cortex as a therapeutic target for neurostimulation. *The Cerebellum***,** 1-11.
Ward, L.M. (2003). Synchronous neural oscillations and cognitive processes. *Trends in cognitive sciences* 7(12)**,** 553.
Watson, T.C., Becker, N., Apps, R., and Jones, M.W. (2014). Back to front: cerebellar connections and interactions with the prefrontal cortex. *Frontiers in systems neuroscience* 8**,** 4.
Watson, T.C., Jones, M.W., and Apps, R. (2009). Electrophysiological mapping of novel prefrontal-cerebellar pathways. *Frontiers in integrative neuroscience* 3**,** 18.
Wessel, M.J., and Hummel, F.C. (2017). Non-invasive cerebellar stimulation: a promising approach for stroke recovery? *The Cerebellum***,** 1-13.
Wiesendanger, R., Wiesendanger, M., and Ru, D. (1979). An anatomical investigation of the corticopontine projection in the primate (Macaca fascicularis and Saimiri sciureus)—II. The projection from frontal and parietal association areas. *Neuroscience* 4(6)**,** 747-765.
Wu, G.-R., and Baeken, C. (2017). Longer depressive episode duration negatively influences HF-rTMS treatment response: a cerebellar metabolic deficiency? *Brain imaging and behavior* 11(1)**,** 8-16.
Zhang, L., Zhao, M., and Sui, R.-B. (2017). Cerebellar fastigial nucleus electrical stimulation alleviates depressive-like behaviors in post-stroke depression rat model and potential mechanisms. *Cellular Physiology and Biochemistry* 41(4)**,** 1403-1412.
Zhu, X., Wang, X., Xiao, J., Liao, J., Zhong, M., Wang, W., et al. (2012). Evidence of a dissociation pattern in resting-state default mode network connectivity in first-episode, treatment-naive major depression patients. *Biological psychiatry* 71(7)**,** 611-617.