Epicranial direct current stimulation suppresses harmaline tremor in rats

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Abstract:

Introduction: Essential tremor (ET) is the most common neurologic movement disorder worldwide. It is characterized by a postural tremor, mostly in the upper extremities causing difficulties in daily activities which may lead to social exclusion. Some ET patients do not respond well to or do not tolerate medication. Thus, deep brain stimulation (DBS) can be offered. In a recent study we proposed a novel neuromodulation technique called epicranial current stimulation (ECS) that works in a minimally invasive way by placing the electrodes subcutaneously under the skin and directly over the skull. In the present study, we investigated the feasibility of using epicranial direct current stimulation (EDCS) to suppress tremor in rat harmaline ET model.

Methods: In experiment 1, seven Sprague Dawley rats were implanted with ECS electrodes placed over the motor cortex (MC) and the cerebellum to investigate if stimulating between them could suppress tremor. In experiments 2 and 3, eight rats were implanted with ECS electrodes placed over the MC, cerebellum and the rostral skull to separate the effects on tremor caused by stimulating each target. During each experiment rats were injected with harmaline which induced tremor that was quantified using an accelerometer. EDCS was then applied via the different electrode configurations to evaluate their tremor suppression effectiveness.

Results: Results from experiment 1 showed that $MC_{cathode}$-$Cerebellar_{anode}$ suppressed tremor compared to stimulation-OFF but MC_{anode}-$Cerebellar_{cathode}$ did not. Furthermore, experiments 2 and 3 showed that it was the cerebellar anodal electrode that was driving tremor suppression.

Conclusion: Cerebellar EDCS suppressed harmaline tremor in rat in a polarity dependent manner. EDCS could be a promising neuromodulation method for ET patients.

Introduction:

Essential tremor (ET) is the most common movement disorder. It is characterized by tremor in the upper extremities[1,2] and sometimes also affects other body parts[3,4]. The tremor is usually mild but can progress to a severe tremor, making it very challenging to independently carry out essential daily activities. This can affect patients' social interaction and quality of life. Pathological activity in the cerebello-thalamo-cortical circuit is believed to play the key role in the disease [5–8]. ET patients are usually treated with medication to suppress their tremor. However, some patients do not respond or cannot tolerate these pharmacological agents [2,9]. As a result, deep brain stimulation (DBS) has become an approved treatment option for ET. During DBS surgery, stimulation electrodes are implanted in the ventral intermediate nucleus of thalamus (Vim) through burr holes in the skull [1,10]. The electrodes are then used to deliver high frequency and continuous electric pulses to the Vim resulting in tremor supression. The mechanism by which DBS causes this therapeutic effect is not fully understood, but there is evidence that DBS works by interrupting a pathological oscillation in the cerebello-thalamo-cortical circuit [11–17].

MRI guided focused ultrasound has been used as a non-invasive technique to treat ET by inducing a lesion in the Vim[18,19]. Nevertheless, the effects induced by the thalamotomy are irreversible. As another alternative to Vim-DBS, two studies have investigated motor cortex stimulation using epidural electrodes [20,21]. Although the studies showed reduced tremor, this technique is still highly invasive as it requires a craniotomy to place the electrodes in close contact with the brain. On the other hand, transcranial magnetic stimulation (TMS) and transcranial electric stimulation (tES) are non-invasive brain stimulation techniques. It has been reported that TMS over the cerebellum has a therapeutic effect on ET, but these effects do not last for long after stimulation cessation[22,23] and delivering continuous, chronic TMS is not possible. In contrast, tES devices are compact and can be delivered in a home settingwith the two main tES modalities being the transcranial direct current stimulation (tDCS) and transcranial alternating current stimulation (tACS) [24,25]. However, tES therapeutic effects cannot

always be replicated[26] and may not always deliver robust neuromodulation effects due to the relatively weak electric field in the brain [27,28]. This is mainly due to the higher conductive nature of the skin compared to the skull causing most of the applied current to be shunted by the scalp[28].

In a recent study, we proposed epicranial current stimulation (ECS) as a novel minimally invasive neuromodulation method to stimulate the cortex [29]. ECS works by implanting subcutaneous electrodes directly over the skull. This can overcome the limitations of the other non-invasive brain stimulation techniques by inducing stronger electric fields in the brain with the possibility of delivering continuous stimulation. The gained advantages come at the cost of invasiveness where the technique requires an incision in the scalp to implant the subcutaneous electrodes[30]. However, it is a less invasive than DBS and epidural motor cortex stimulation.

In this study we investigated the feasibility of using ECS to reduce tremor in rat harmaline ET model. This is the most commonly used model for ET [31,32]. Animal studies suggest that harmaline tremor originates in the inferior olive, which could be different from ET[33,34]. However, the oscillatory activity in the inferior olive induces an abnormal oscillation in the cerebello-thalamo-cortical network which is similar to that in ET patients [8,35]. We hypothesized that epicranial direct current stimulation (EDCS) would reduce tremor in a rat harmaline ET model by targeting the cerebellum and the motor cortex. To test this hypothesis, we applied EDCS over the motor cortex and the cerebellum and compared the tremor to a stimulation OFF condition. The results showed a significant decrease in tremor amplitude during EDCS. These effects were polarity dependent indicating that either cerebellar anodal stimulation and/or MC cathodal stimulation reduced harmaline tremor. Thus, we conducted another two experiments to investigate which was driving the observed effect by introducing a rostral electrode to allow stimulation between the rostral electrode and the motor cortex or between the rostral electrode and the cerebellum separately. These experiments showed that anodal EDCS of the cerebellum suppressed harmaline tremor.

Methods

Animals

For all experiments we used 15 male Sprague Dawley rats (Charles River Laboratories, France) in the weight range of 220 g to 389 g. They were housed at ~19 ˚C, 14/10 h light/dark cycle and had unrestricted access to food and water. The KU Leuven ethics committee for laboratory experimentation approved all procedures (project P228/2018).

Surgery and electrodes implantation

On surgery days, we anesthetized rats with a mixture of ketamine (45 mg/kg, Anestekin, Eurovet, Belgium) and medetomidine HCL (0.3 mg/kg, Narcostart, Kela Vetarinaria, Belgium) by injecting intra-peritoneally. We checked anesthesia depth using the toe pinch and added anesthesia as necessary. While they were in the stereotaxic frame, we made an incision in the scalp (on the upper part of the head) to expose the skull. We then built an electrode on the skull using the following procedure. First, we made sure the skull was dry. For every stimulation electrode we then drilled an outline into the skull. We erected a dental cement wall (~ 1mm) in this outline to demarcate the stimulation electrode. We filled the demarcated area on the skull with conductive paste (Ten20 conductive paste, weaver and company; MedCaT, the Netherlands). We placed a prefabricated bone screw electrode (Plastics One Inc, Roanoke, VA, USA) in the conductive paste. We then covered the conductive paste with more dental cement so that only an insulated metal wire stuck out of the constructed electrode. We constructed three such electrodes; one to target the cerebellum, another the motor cortex and a rostral electrode which did not target any brain region (see Fig. 1). During experiments this allowed us to stimulate with three distinct montages; namely MC-Cerebellar, MC-rostral, Cerebellar-rostral. For the precise coordinates and dimensions of the three electrodes see Figure 1.

At the end of surgery rats received a local anesthetic Xylocaine 2% (lidocaine HCl 20 mg/mL, AstraZeneca, UK) which was injected into the incision wound. A local antibacterial cream (Sodium fusidate 20 mg/g, Leo Pharma, Belgium) was applied to the surgery wound after which the scalp was sutured. Furthermore, they received a subcutaneous injection of a general analgesic Metacam (5 mg/mL, Boehringer Ingelheim). After surgery there were two days of post-operative care. This consisted of applying the aforementioned antibacterial cream and the general analgesic. Rats were given at least one week to recover from surgery before experiments started.

Harmaline injection and tremor measurement

On experiment days, rats received an intraperitoneal injection of harmaline solution (15 mg/kg) prepared by dissolving harmaline hydrochloride dehydrate (≥ 95%), (Sigma-Aldrich, St. Louis, MO, USA) in saline. Approximately 45 minutes after harmaline injection, the rats exhibited a whole body visible tremor that was recorded using a tri-axial accelerometer (ADXL353, Analog Devices, MA, USA). The accelerometer was mounted on the rats' back and secured with a hook-and-loop tape, which was sufficient to detect and measure the harmaline induced general body tremor. It was also a more comfortable and secure location as it does not affect the rats' movement and they cannot reach to bite it. The accelerometer data (three axes) were digitized using a digital analog converter (DAC, NI USB-6343, National Instruments, TX, USA) at 30 kHz sample rate, displayed and recorded for off-line analysis using a custom written MATLAB 2014a software (MathWorks, MA, USA).

When rats were laying still there was never tremor visible. Thus, for the experiment they were placed in a freely rotating wheel to ensure they were always moving, and the tremor was as consistent as possible.

Electrode montage and stimulation

To deliver electric stimulation a DC signal was sent from the aforementioned Matlab software to the DAC. The DAC sent a voltage signal to a stimulation unit. The stimulation unit consisted of two current sources, an AM 2200 analog current source (AM Systems, Sequim, WA, USA) and a Stimulus Isolation Unit SIU A100 (Invilog Research, Finland) that were connected in parallel to ensure a sufficient current source compliance voltage. To determine stimulation intensity current amplitude was increased from 0 mA in steps of 0.25 mA until the rat showed behavioral response indicating it could feel the stimulation (typically a small twitching response). The current was then reduced by 0.25 mA below this level for the experiment. This procedure was repeated for all electrode montages and the one with the lowest threshold amplitude was selected. On average the stimulation amplitude was 2.33 ± 0.34 mA for all rats across all experiments.

Experimental design

In experiment 1, the DC stimulation was applied between the MC electrode and the cerebellar electrode (Fig.1). Changing the location of the anode and cathode resulted in two stimulation conditions: MC_{anode}-$Cerebellar_{cathode}$ and $MC_{cathode}$-$Cerebellar_{anode}$. In this experiment, one complete recording session consisted of a 3-min recording of each condition, in addition to a 3-min recording with stimulation OFF with a 2 min of rest in between two consecutive conditions. This stimulation duration was determined in pilot experiments where 3 minutes showed a good balance between longer data collection and avoiding rat fatigue. The order of the stimulation conditions was randomized. Each rat had at least two complete recording sessions during one experiment day. Rats can quickly build up a tolerance to harmaline[36,37]. Thus, one week after the first experiment day, we tested if rats again responded to harmaline by expressing a visible tremor. If they did, a second recording day was performed by repeating the same sessions in the first recording day (i.e. two stimulation conditions and one OFF condition) but in a newly randomized order. If they did not respond, there was no second recording day.

In experiment 2, the DC stimulation was applied between the MC electrode and the rostral electrode (experiment 2) or between the cerebellar electrode and the rostral electrode (experiment 2). This resulted in two stimulation conditions in experiment 2 (MC_{anode}-$Rostral_{cathode}$ and $MC_{cathode}$-$Rostral_{anode}$) and two in experiment 3 ($Cerebellar_{anode}$-$MC_{cathode}$ and $Cerebellar_{cathode}$-MC_{anode}). Experiments 2 and 3 were structured in the same way as experiment 1, including a second experiment day when possible.

Tremor quantification and data analysis

After each experiment, the raw acceleration data were band pass filtered between 1 and 30 Hz by applying a second-order Butterworth filter in Matlab. Then, the first principal component of the three displacement axes was calculated using principal component analysis. This was followed by calculating the Fast Fourier Transform of each 3-min recording. During experiments, activity of rats varied and tremor amplitude varied with it. Thus, using the tremor power alone for the analysis would only be indicative on how active was the rat. To compensate for the variability in rat motion activity during different conditions, the well-established tremor power ratio (TPR) was calculated by dividing the power in the tremor frequency range (8-14 Hz) by the total motion power (1-20 Hz) [8,31,35,38–40]. To evaluate the effect of stimulation on tremor in each experiment, the TPR of all three conditions were compared.

Statistics

For each rat and for each condition, the average TPR value for all sessions was calculated. Then, Friedman test was first applied to test if there was an effect of condition. If significant, a Wilcoxon signed rank test was followed to test if either of the stimulation conditions were significantly different from the stimulation-OFF condition. To correct for the multiple testing, Bonferroni correction was applied with n = 2.

Results

EDCS between the MC and the cerebellum reduces tremor amplitude

The aim of experiment 1 was to test whether EDCS applied between electrodes placed over the MC and the cerebellum would affect harmaline tremor. Fi g.2A shows an example of one rat's normalized frequency power calculated from the accelerometer data obtained from one rat. When no stimulation was applied, the graph shows a very high peak in the tremor frequency band (8-14 Hz) indicating strong tremor. However, during the $MC_{cathode}$-$Cerebellar_{anode}$ condition, this peak was attenuated. In contrast, the MC_{anode}-$Cerebellar_{cathode}$ condition shows a similar tremor peak amplitude to stimulation-OFF. At group level, the mean frequency power averaged for all 7 rats in experiment 1 shows similar results (Fig.2B). To quantify that, the TPR was calculated for all 7 rats and was compared between the different conditions (Fig.2C). A Friedman test shows a significant difference in the TPR values between the different conditions ($p = 0.021$, $\chi^2(2) = 7.71$). Compared to stimulation-OFF, a post hoc Wilcoxon signed rank analysis of the results (Bonferroni correction, n=2) shows a significant decrease in the TPR value during $MC_{cathode}$-$Cerebellar_{anode}$ ($p = 0.031$, Cohen's effect size values $d = 1.5$ suggesting a very high practical significance) but not during MC_{anode}-$Cerebellar_{cathode}$ ($p > 0.99$). This indicates that EDCS stimulating the MC and/or the cerebellum suppresses the tremor in a polarity specific way.

To study the time course effect of stimulation, the TPR value was calculated for each 1-sec period of data during each condition. Fig.2D shows the results obtained from the same rat as in Fig.2A. $MC_{Cathode}$-$Cerebellar_{anode}$ shows a variable TPR value that was mostly lower than the other conditions.

Stimulation amplitude effect

To investigate the effect of stimulation amplitude on tremor suppression, the TPR was calculated for one rat while increasing the stimulation amplitude from 2 mA to 2.5 mA and 3 mA respectively. The results show a bigger decrease in TPR as the stimulation amplitude is increased (Fig.3). This indicates that increasing stimulation amplitudes resulted in stronger tremor suppression.

Disentangling the region of effect

We found that the $MC_{cathode}$-$Cerebellar_{anode}$ condition reduced tremor but the MC_{anode}-$Cerebellar_{cathode}$ did not. Thus, either cerebellar anodal stimulation or MC cathodal stimulation reduced harmaline tremor. To investigate which was driving the observed effect, we introduced a rostral electrode which allowed us to stimulate between the rostral electrode and the motor cortex or between the rostral electrode and the cerebellum separately.

Anodal EDCS of the cerebellum suppresses tremor

In experiment 2, EDCS was applied between the cerebellar and the rostral electrodes to test the effect of cerebellar stimulation on the tremor. Fig.4 summarizes the results obtained from all 8 rats in this experiment. The grand average of the frequency power is higher during stimulation-OFF as compared to $Cerebellar_{anode}$- $Rostral_{cathode}$ (left panel). In contrast there is no difference between stimulation-OFF and $Cerebellar_{cathode}$- $Rostral_{anode}$. This was presented in the TPR values (right panel) where the Friedman test shows a significant effect of condition ($p = 0.0076$, $\chi^2(2) = 9.75$). This was followed by a Wilcoxon signed rank post hoc test (Bonferroni correction, $n = 2$), which shows a significantly lower tremor amplitude during $Cerebellar_{anode}$-$Rostral_{cathode}$ as compared to stimulation-OFF ($p = 0.0156$, Cohen's effect size values $d = 2$ suggesting a very high practical significance).

EDCS of the motor cortex does not reduce tremor

We tested whether MC stimulation could suppress tremor. To do this, we applied EDCS between the MC and the rostral electrode. We found no effect (Fig.5) for both stimulation conditions MC_{anode}-$Rostral_{cathode}$ and $MC_{cathode}$-$Rostral_{anode}$ as compared to the stimulation-OFF condition, Friedman test ($p = 0.641$, $\chi^2(2) = 0.89$).

Discussion and Conclusion

The main goal of this study was to investigate if EDCS can suppress harmaline induced tremor in a rat model. In the first experiment, stimulation electrodes were placed above the MC and the cerebellum to test if applying EDCS between them could affect tremor amplitude. The results from this experiment showed that tremor amplitude during $MC_{cathode}$-$Cerebellar_{anode}$ was significantly lower than during stimulation-OFF. This shows that harmaline tremor can be reduced by applying EDCS over the cerebellum, the motor cortex or a combination of both. Invasive patient and animal studies show that stimulating this network with electric pulses can suppress tremor [10,20,41]. There are two distinct differences with our study. The main advantage of the ECS approach is its minimally invasiveness, requiring no craniotomy or direct contact with brain tissue, potentially reducing perioperative risks of brain infection or injury.

To test the contribution of MC and cerebellar stimulation separately, experiments 2 and 3 were carried out. The results from these experiments specifically showed that only anodal cerebellar stimulation reduced tremor. Anodal cerebellar stimulation is believed to increase the excitability of Purkinje cells in the cerebellum which leads to an increase in the inhibitory inputs to deep cerebellar nuclei and thus a decrease in excitatory inputs to the thalamo-cortical circuit [42–45]. This mechanism could explain our results. In fact, deep cerebellar nuclei stimulation using electric pulses has already been shown to reduce harmaline tremor[46] and non-harmaline tremor in animals[47,48], as well as in human patients with cerebellar disorders[49]. Yet, in our study we are achieving tremor suppression effects in a less invasive manner.

Opportunities

In pharmacorefractory essential tremor, DBS can be offered to the patients. However, DBS is highly invasive requiring the introduction of external materials into the brain. On the other hand, ECS is minimally invasive requiring only an incision in the scalp, possibly done with just topical scalp

anesthesia. Therefore, as an alternative to DBS this would reduce risk to the patient. Furthermore, ET patients who do not meet DBS inclusion criteria, e.g., cardiovascular risk profile of the patient, could still be eligible for ECS. Earlier, we showed that ECS can efficiently stimulate brain tissue [29]. Combined this shows that ECS is not only promising in ET but also in other neurological disorders where electric stimulation may be beneficial. In fact, ECS is already undergoing clinical trials in epilepsy patients[30]. On the other hand, EDCS requires continuous direct current stimulation with amplitudes that may be higher than that in DBS. This could lead to faster battery depletion and the need for more replacement surgeries. However, with the introduction of rechargeable pulse generators, and depending on patient compliance, this might not be an issue[50].

In this study, an average stimulation amplitude of 2.33 mA was delivered to the rats. The rats did not show signs of being uncomfortable during stimulation. We opted for such stimulation amplitudes in order to guarantee achieving any possible therapeutic effects. Given the larger skull thickness in humans, higher stimulation amplitudes would most likely be needed[51]. However, in this experiment we didn't investigate whether a lower stimulation amplitude would have caused tremor reduction. In addition, a closed loop approach where stimulation amplitudes are dependent on tremor level may allow lower stimulation amplitudes. Despite that, it is still unclear whether a translated EDCS amplitude could be tolerated by humans. Evidence from computational modelling suggests that epicranial stimulation induces very limited electric field in the skin[29], allowing high tolerable amplitudes. These findings can be supported by the results from the ongoing clinical trials on epicranial stimulation in epilepsy patients[30].

Limitations

This study shows that cerebellar stimulation is a key structure in treatment of ET tremor. However, targeting the cerebellum may also stimulate deeper lying essential nuclei. This might be a potential limitation in applying this stimulation in human subjects[52–54]. However, it is possible to optimize electrode montage and amplitude to prevent or minimize stimulation of these nuclei[55]. Using

computational modelling and animal experiments, we have previously shown that ECS concentric electrodes leads to a more focused stimulation of the cortex[29]. This modelling study indicates that targeting the cerebellum is in principle possible without noticeably affecting deeper lying nuclei.

The effects of EDCS on tremor suppression in our study are weaker than that reported for DBS in clinic[56–58]. Nevertheless, this is a first study to investigate the feasibility of using EDCS to suppress tremor in rat harmaline ET model. Further experiments should be carried out to optimize EDCS parameters with the aim to improve its tremor suppression effects.

In conclusion, our study has shown that harmaline tremor in rats can be suppressed using EDCS in a polarity and montage dependent manner. If developed further this minimally invasive approach could become a less complex and risky alternative to DBS for ET.

Conflicts of interest

All authors confirm there are no conflicts of interest that could have influenced the outcome of this work.

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Figures:

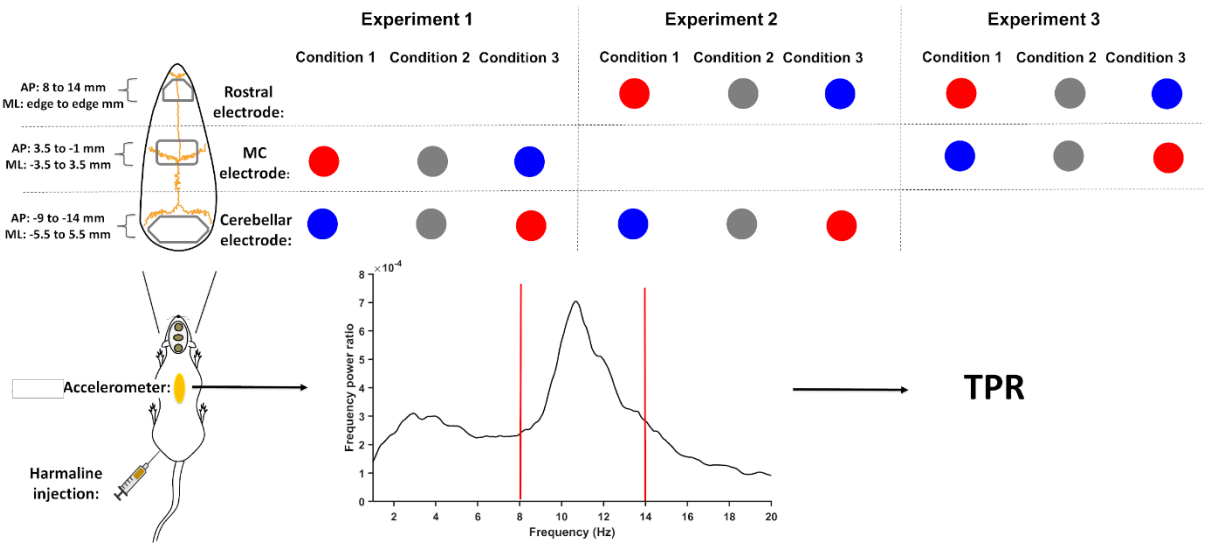

Figure 1: Schematic diagram showing the rat experimental setup with the different stimulation conditions and the tremor outcome variable calculated using the accelerometer data. After electrode implantation and harmaline injection, epicranial direct current stimulation (EDCS) was applied between the different electrodes while the tremor was measured using an accelerometer. In experiment 1, EDCS was applied between the motor cortex (MC) and the cerebellum. In experiment 2 EDCS was applied between the cerebellum and the rostral electrode (notice that this electrode, given the co-ordinates, does not cover any brain region). In experiment 3 the stimulation was applied between the MC and the rostral electrode. For each experiment, two stimulation conditions were applied by switching the anode and the cathode polarities between the two used electrodes. To quantify the tremor amplitude, the tremor power ratio (TPR) was calculated as the sum of the frequency power ratio (denoted by black plot; stimulation OFF condition in the provided example) in the tremor frequency range (denoted by vertical red lines). To test the effect of stimulation, this value was calculated for each stimulation condition and was compared to that during stimulation-OFF. Note that the stimulation conditions and the stimulation-OFF condition were presented in a random order during the experiment.

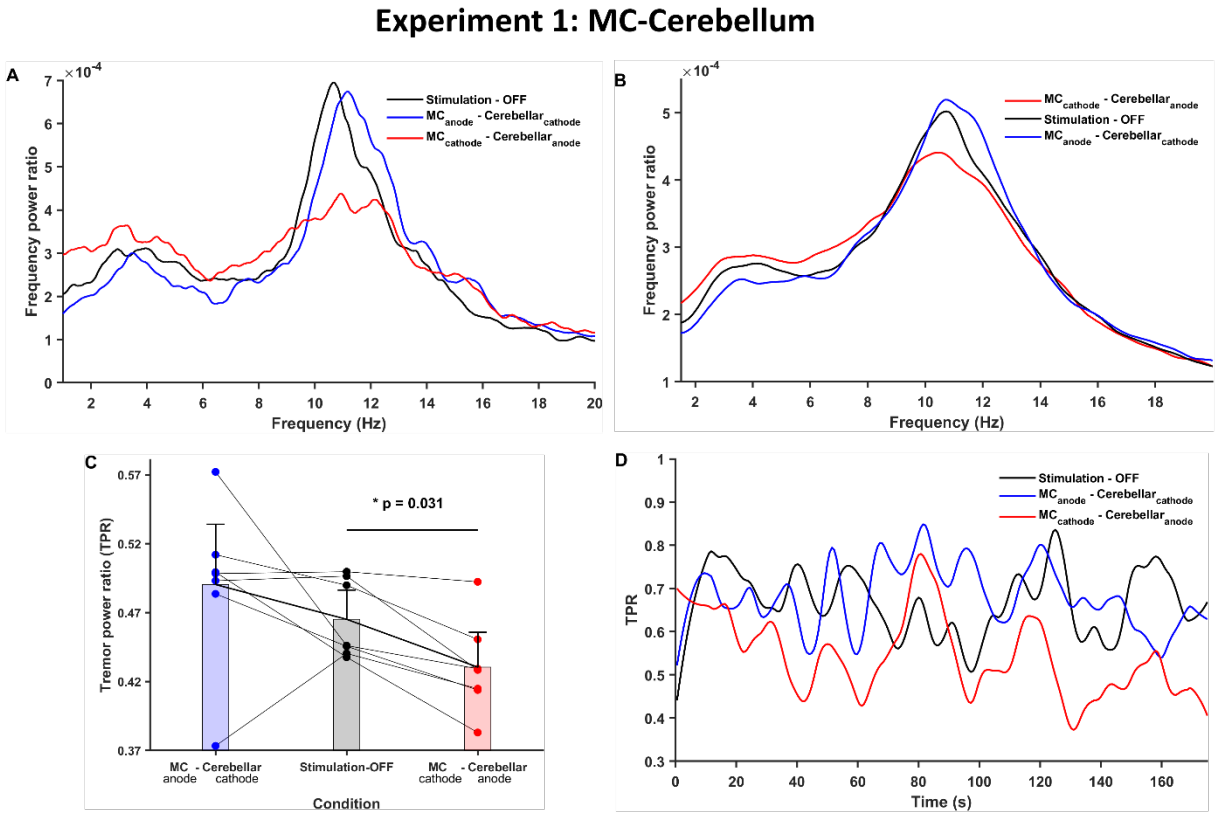

Figure 2: Results from experiment 1 showing the effect of stimulation between the MC and the cerebellum on harmaline tremor. (A) shows the mean frequency power of the stimulation and stimulation-OFF conditions for one rat in Experiment 1. When stimulation-OFF was applied, the accelerometer data shows high power in the tremor frequency band. However, the tremor power decreased when the $MC_{cathode}$-$Cerebellar_{anode}$ condition was applied. On the other hand, no effect on was obtained when switching the polarity of stimulation in condition MC_{anode}-$Cerebellar_{cathode}$. Similar results were shown when averaging along all 7 rats (B). (C) shows the tremor power rate (TPR) values at a group level. Each data point in this figure represents the TPR of one rat with the lines connecting data of the same rat during different conditions. The results show significant tremor suppression compared to stimulation-OFF for the condition $MC_{Cathode}$-$Cerebellar_{anode}$ (Wilcoxon signed rank test p = 0.031, corrected for multiple comparison with n=2) but not for condition MC_{anode}-$Cerebellar_{cathode}$ which shows no effects. This indicates that DC anodal stimulation of the cerebellum and/or cathodal stimulation of the MC suppresses the tremor. (D) shows TPR as a function of time for the same rat presented in (A). $MC_{Cathode}$-$Cerebellar_{anode}$ (red) shows a variable TPR value that was lower than that of the other conditions during most of the times.

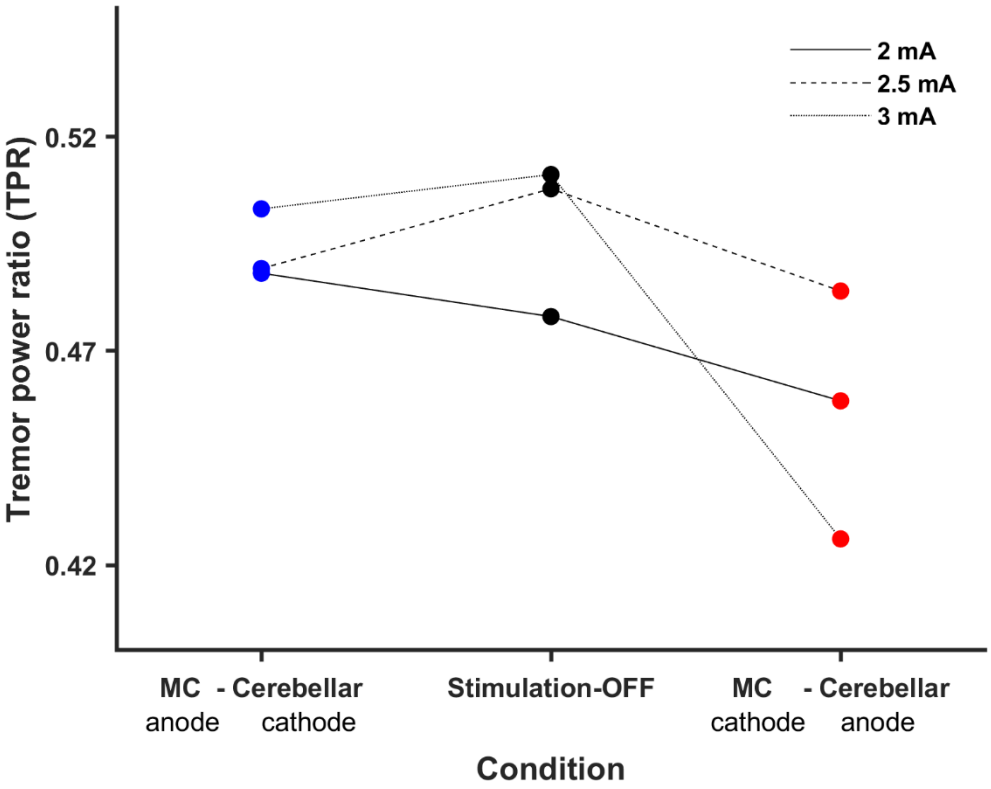

Figure 3: The effect of increasing stimulation amplitude on tremor suppression in one rat. The results shows a bigger decrease in the TPR for higher stimulation amplitudes. This indicates that increasing stimulation amplitude induces stronger tremor suppression.

Experiment 2: Cerebellum-Rostral

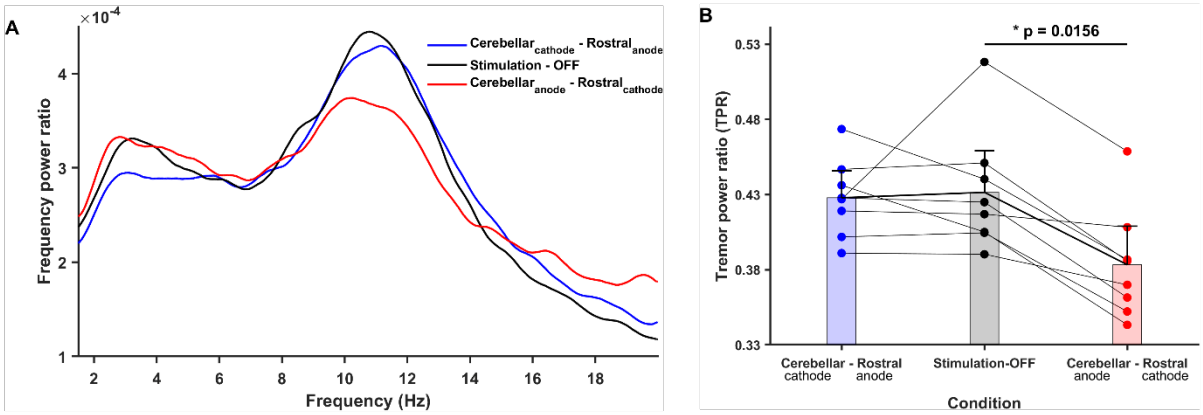

Figure 4: Results from experiment 2 showing the effect of stimulating the cerebellum on the tremor. (A) shows the mean frequency power of the stimulation and stimulation-OFF conditions averaged for all rats in experiment 2. The accelerometer data shows higher power in the tremor frequency band during stimulation-OFF condition compared to the Cerebellar$_{anode}$-Rostral$_{cathode}$ condition but not for the Cerebellar$_{cathode}$-Rostral$_{anode}$ condition. This is also demonstrated by the tremor power rate (B) which shows a significant tremor suppression for the condition Cerebellar$_{anode}$-Rostral$_{cathode}$ compared to stimulation-OFF (Wilcoxon signed rank test p = 0.0156, corrected for multiple comparison with n=2). However, the condition Cerebellar$_{cathode}$-Rostral$_{anode}$ had no effect. This indicates that DC anodal stimulation of the cerebellum could suppress the tremor.

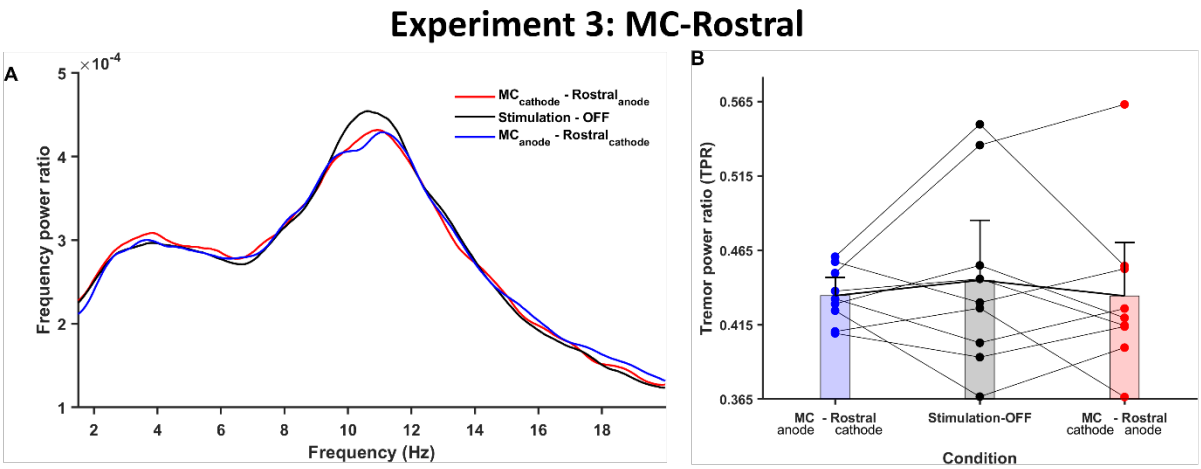

Figure 5: Results from experiment 3 showing the effect of stimulating the MC on the tremor. The mean frequency power averaged for all rats (A) shows no difference between any of the stimulation condition compared to stimulation-OFF. This is also shown by the tremor power rate (B). This indicates that DC stimulation of the MC has no effect on the tremor.