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Cerebellar direct current stimulation modulates pain perception in humans

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Abstract.

Purpose: The cerebellum is involved in a wide number of integrative functions, but its role in pain experience and in the nociceptive information processing is poorly understood. In healthy volunteers we evaluated the effects of transcranial cerebellar direct current stimulation (tcDCS) by studying the changes in the perceptive threshold, pain intensity at given stimulation intensities (VAS:0-10) and laser evoked potentials (LEPs) variables (N1 and N2/P2 amplitudes and latencies).

Methods: Fifteen normal subjects were studied before and after anodal, cathodal and sham tcDCS. LEPs were obtained using a neodymium:yttrium–aluminium–perovskite (Nd:YAP) laser and recorded from the dorsum of the left hand. VAS was evaluated by delivering laser pulses at two different intensities, respectively two and three times the perceptive threshold.

Results: Cathodal polarization dampened significantly the perceptive threshold and increased the VAS score, while the anodal one had opposite effects. Cathodal tcDCS increased significantly the N1 and N2/P2 amplitudes and decreased their latencies, whereas anodal tcDCS elicited opposite effects. Motor thresholds assessed through transcranial magnetic stimulation were not affected by cerebellar stimulation.

Conclusions: tcDCS modulates pain perception and its cortical correlates. Since it is effective on both N1 and N2/P2 components, we speculate that the cerebellum engagement in pain processing modulates the activity of both somatosensory and cingulate cortices. Present findings prompt investigation of the cerebellar direct current polarization as a possible novel and safe therapeutic tool in chronic pain patients.

Keywords: Pain cerebellum, cerebellar direct current stimulation, tDCS, laser evoked potentials, pain modulation

1. Introduction

The cerebellum is involved in a wide number of integrative functions, ranging from working memory and associative learning to motor control (Schmahmann, 1991; Ito, 2006; Stoodley & Schmahmann,

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2009; Strick et al., 2009; Balsters et al., 2013). It is also involved in the sensory, cognitive (Borsook et al., 2008) and affective dimensions of pain (Ploghaus et al., 1999). In addition, the cerebellum plays a role in the sensory-motor integration aimed at antinociceptive behaviour (Bingel et al., 2002; Strigo et al., 2003; Borsook et al., 2008), as well as in salience-related affective and behavioral responses to nociceptive stimulation (Duerden & Albanese, 2013). In fact, although it is not known how nociceptive information is encoded in the cerebellum, it has been proposed that the cerebellum may integrate multiple effector systems including affective processing, pain modulation and sensorimotor control.

Afferent inputs from nociceptors reach the cerebellum through two different and segregated pathways, the spino-ponto-cerebellar and the spino-olivo-cerebellar route (Ekerot et al., 1987a, 1987b; Ekerot et al., 1991a), and the cerebellar influence on pain processing closely resembles the inhibitory tone exerted by Purkinje cells over the primary motor cortex (M1), a phenomenon referred as cerebellum-brain inhibition (Kelly & Strick, 2003).

Non-invasive brain stimulation (NIBS) techniques, such as repetitive Transcranial Magnetic Stimulation (rTMS) and transcranial Direct Current Stimulation (tDCS) have recently emerged as interesting, effective and promising tools for modulating pain experience (Antal & Paulus, 2010; Zaghi et al., 2011). In fact, a sufficient body of evidence shows analgesic effects of high-frequency rTMS of the primary motor cortex (M1) (Lefaucheur et al., 2014), with effects likely arising from the restoration of defective intracortical inhibitory processes (Lefaucheur et al., 2006). Among NIBS technique, tDCS applied either over the motor (Fregni et al., 2007; Mendonca et al., 2011; Dasilva et al., 2012; Reidler et al., 2012) or the prefrontal cortex (Boggio et al., 2008, 2009; Mylius et al., 2012) was also effective in pain modulation.

Only one study has assessed the effects of cerebellar rTMS, suggesting that changes in pain perception were not specific for cerebellar stimulation (Zunhammer et al., 2011). However, no study has investigated to date the role of transcranial cerebellar direct current stimulation (tcDCS), a new and well-tolerated technique for modulating cerebellar excitability, in modifying pain perception in humans (Ferrucci et al., 2008, 2012; Galea et al., 2009, 2011; Grimaldi et al., 2014; Priori et al., 2014). Notably, despite some inter-individual differences, recent modelling researches have revealed that, during tcDCS, the current spread to other structures outside the cerebellum is negligible and unlikely to produce functional effects (Fig. 1) (Parazzini et al., 2013, 2014a, 2014b).

The aim of our study was to evaluate the effects of tcDCS on pain perception and on its cortical correlates. We studied the changes in pain scores and in laser evoked potentials (LEPs) variables (perceptive threshold, N1 and N2/P2 amplitudes and latencies) in participants undergoing direct current polarization applied over the cerebellum.

2. Materials and methods

2.1. Subjects

Fifteen healthy volunteers (mean age ± SD: 25.8 ± 5.9 years, 7 women) with no history of neurological disorders were enrolled in the study. Women were studied in the second week after their last menses (Smith, et al. 1999). No subject had been under medication in the month preceding the experimental session which was scheduled at least 48 hours after the last alcohol and caffeine consumption. Written informed consent was obtained from all participants before enrollment in the study, which was approved by the local ethical Committee and followed the tenets of the Declaration of Helsinki.

2.2. Experimental design

As shown in Fig. 2, at the beginning of each session, before cerebellar tDCS and immediately afterwards, the laser Perceptive Threshold (PT), corresponding to the lowest intensity at which subjects perceived at least 50% of the stimuli (Cruccu et al., 1999; Agostino et al., 2000), was determined. In order to minimize the number of nociceptive stimuli, the nociceptive perception threshold was not assessed. A range of 10–40 stimuli (mean, SD; 25 ± 5) was used to assess the perceptive threshold before and after transcranial cerebellar stimulation. Less than 10 minutes were spent to determine PT, in line with previous reports (Truini et al., 2011).

After the PT assessment, participants were instructed to pay attention to incoming laser nociceptive stimuli in order to verbally rate the perceived intensity about 2-3 seconds after each laser stimulation, which was performed before tcDCS (T_0), immediately after its termination (T_1) and 60 min later (T_2).

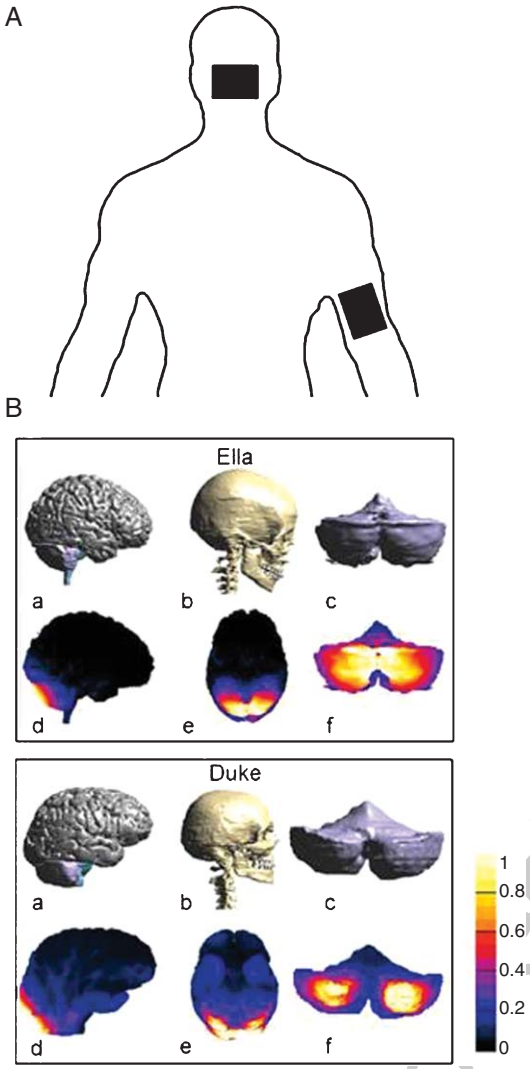

Fig. 1. - Current density generated by cerebellar transcranial direct current stimulation (cerebellar tDCS) in humans. *A*. Top panel shows (viewed from the back) the electrode positions for cerebellar tDCS. *B*. Examples of segmented tissues in two human realistic Virtual Family models (Ella and Duke) undergoing cerebellar tDCS. Simulations were conducted using the simulation platform SEMCAD X (by SPEAG, Schmid & Partner Engineering, AG, Zurich, Switzerland); a, lateral view of cerebellum, pons, midbrain, medulla; b, lateral view of the skull; c, back view of the cerebellum; d and e, lateral and inferior views of normalized current density amplitude field distributions over cortical, subcortical and brainstem regions; f, back view of normalized current density amplitude field distributions over the cerebellum. Values are normalized with respect to the maximum of the current density amplitude in the cerebellum. The spread of the current density (J) over the occipital cortex - quantified as the percentage of occipital volume where the amplitude of J-field is greater than 70% of the peak of J in the cerebellum - was only 4% for "Duke" and much less than 1% for "Ella" (modified from Priori et al. (2014), with permission).

Participants were blinded to the tcDCS polarity; anodal, cathodal and sham tcDCS stimulations were administered in three different sessions and separated by at least 1 week to avoid possible carry-over effects. The order of interventions was randomized and balanced across subjects. Laser stimuli of intensity two and three times the PT intensity (I_1, I_2) were delivered by an experimenter (A.T.), whereas the evaluation of electrophysiological parameters was done by F.S., both blinded to the tcDCS polarity; B. V. settled the tcDCS polarity.

2.2.1. *Subjective experience*

The perceived sensation was rated on the 0–10 Visual Analogue Scale (where 0 = no sensation and 10 = unbearable pain; the intermediate levels being: 1 = barely perceived; 2 = lightly pricking, not painful; 3 = clearly pricking, not painful; 4 = barely painful; 5 = painful, prompting to rub the skin; 6 = very painful and distressing; 7 and more: strongly unpleasant pain). VAS was studied in each subject after 10 nociceptive laser I_1 and I_2 stimuli (VAS $_1$, VAS_2). In each participant individual VAS values were averaged for each Time.

Laser Evoked Potentials were obtained by stimuli corresponding to two times the Perceptive value, according with previous literature and guidelines (Truini et al., 2005, 2010).

2.3. *Procedures*

2.3.1. *Laser evoked potentials (LEPs)*

The methods used for laser stimulation are reported in detail elsewhere (Truini et al., 2005, 2010). A neodymium:yttrium–aluminium–perovskite (Nd:YAP) laser was used (wavelength 1.04 μm, pulse duration 2–20 ms, maximum energy 7 J). The laser beam was transmitted from the generator to the stimulating probe via a 10 m length optical fibre; signals were then amplified, band pass filtered (0.1–200 Hz, time analysis 1000 ms) and fed to a computer for storage and analysis (Cruccu et al., 2008). The dorsum of the left hand was stimulated by laser pulses (individual variability: 3.89–15.75 J/cm^2) with short duration (5 ms) and small diameter spots (5 mm; Valeriani et al., 2012). Ten stimuli, whose intensity was established on the basis of the Perceptive Threshold assessed for each subject at T_0, T_1 and T_2, were delivered and the laser beam was shifted slightly between consecutive pulses to avoid skin lesions and reduce fatigue

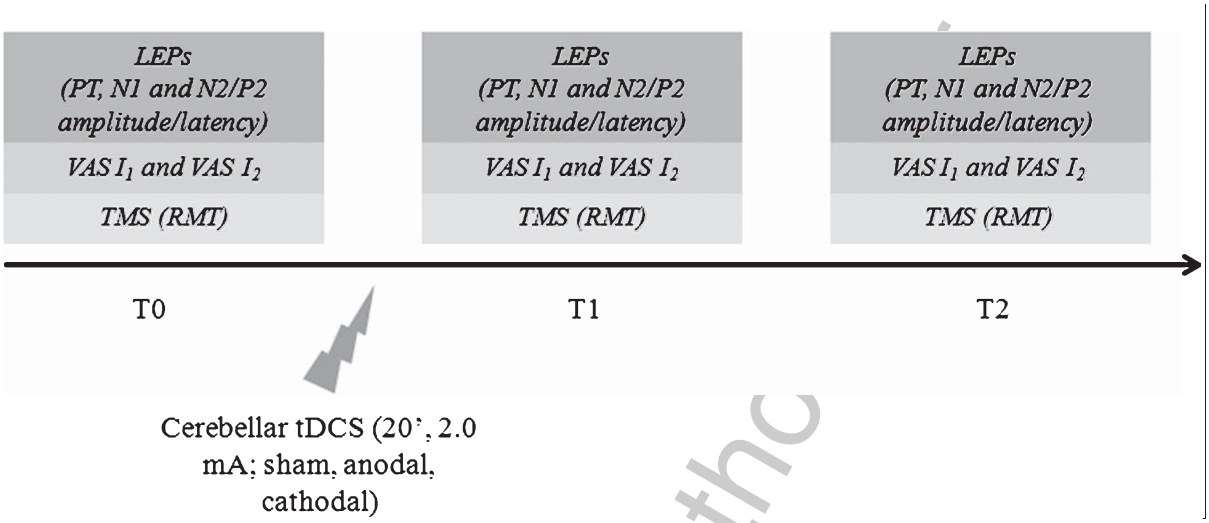

Fig. 2. – Experimental protocol. Psychophysical and electrophysiological variables evaluated at baseline (T_0) and at two different time points (T_1, T_2) following anodal, cathodal and sham tcDCS.

of peripheral nociceptors (Truini et al., 2005). The inter-stimulus interval was varied randomly (10–15 s). Participants were reclined on a couch and wore protective goggles. They were instructed to keep their eyes open and gaze slightly downwards; since the N2/P2 amplitude is enhanced by attention (Lorenz & Garcia-Larrea, 2003; Truini et al., 2005), they were requested to mentally count the number of stimuli. The main Aδ-LEP vertex complex, N2–P2, and the lateralised N1 component were recorded through standard disc, non-polarizable Ag/AgCl surface electrodes (diameter 10 mm; Biomed®, Florence, Italy). N2 and P2 components were recorded from the vertex (Cz) referenced to the earlobes; the N1 component was recorded from the temporal leads (T4) referenced to Fz (Cruccu et al., 2008). Blinks and saccades were recorded with an EOG electrode placed on the supero-lateral right canthus connected to the system reference. Ground was placed on the mid-forehead. Skin impedance was kept below 5 kΩ.

2.3.2. *Cerebellar transcutaneous direct current stimulation (tcDCS)*

tDCS was applied using a battery-driven constant current stimulator (HDCStim, Newronika, Italy) and a pair of electrodes in two saline-soaked synthetic sponges with a surface area of 25 cm^2. For cathodal stimulation the cathode was centered on the median line 2 cm below the inion, with its lateral borders about 1 cm medially to the mastoid apophysis, and the anode over the right shoulder (Ferrucci et al. 2008, 2012, 2013). For anodal stimulation, the current flow was reversed. In the real tcDCS conditions, direct current was transcranially applied for 20 minutes with an intensity of 2.0 mA, and constant current flow was measured by an ampere meter (current density $\approx$ 0.08 mA/cm^2). These values are similar to those previously reported for cerebellar stimulation (Ferrucci et al., 2008, 2013), are considered to be safe (Iyer et al., 2005) and are far below the threshold for tissue damage (Nitsche et al., 2003). Apart from occasional and short-lasting tingling and burning sensations below the electrodes, direct current stimulation strength remained below the sensory threshold throughout the experimental session. At the offset of tDCS, the current was decreased in a ramp-like manner, a method shown to achieve a good level of blinding among sessions (Gandiga et al., 2006; Galea, et al., 2009). For a sham tDCS, the current was turned on only for 5 seconds at the beginning of the sham session and then it was turned off in a ramp-shaped fashion, which induces initial skin sensations indistinguishable from real tDCS.

For all the electrophysiological recordings we chose the left side to avoid interference from the return electrode placed over the contralateral shoulder. At experimental debriefing, subjects were not able to discriminate between the applied anodal, cathodal and sham tDCS.

Table 1
Row data (expressed as mean value ± 1 standard deviation; *a* = anodal stimulation; *c* = cathodal stimulation; sh = sham condition). Both psychophysical and electrophysiological data for each subject are fully available, as supplementary electronic material, at http:www.enricasantarcangelo.com/publications

		aT0	*aT1*	*aT2*	*cT0*	*cT1*	*cT2*	*shT0*	*shT1*	*shT2*
PT	mean	4.62	6.07	6.09	4.85	3.76	3.68	4.72	4.66	4.89
	SD	0.80	0.95	0.92	0.86	0.62	0.67	0.98	0.62	0.81
VAS I_1	mean	3.89	2.55	2.65	3.67	4.93	4.67	3.87	3.93	3.87
	SD	0.84	0.57	0.62	0.82	0.96	0.82	0.74	0.70	0.92
VAS I_2	mean	5.40	4.02	4.03	5.24	6.73	6.65	5.33	5.49	5.30
	SD	0.63	0.82	0.71	0.48	0.47	0.49	0.78	0.69	0.64
N1 amplitude (μV)	mean	12.92	8.48	8.01	11.04	14.96	14.94	11.01	11.11	11.21
	SD	3.18	2.98	2.58	2.65	2.58	3.33	2.50	2.67	2.83
N1 latency (ms)	mean	124.19	161.46	157.10	127.04	107.15	104.05	128.17	128.67	130.66
	SD	10.90	13.38	13.68	10.75	6.75	9.12	13.20	12.71	12.09
N2P2 amplitude(μV)	mean	11.14	7.38	7.57	10.52	14.53	13.75	11.14	11.25	11.47
	SD	2.62	2.37	2.33	2.65	2.96	3.29	2.72	2.69	2.16
N2P2 latency (ms)	mean	151.57	189.32	187.26	148.78	126.73	132.30	153.90	151.08	155.51
	SD	13.12	17.49	21.39	22.01	18.49	18.70	14.33	15.07	16.75

2.3.3. Transcranial magnetic stimulation (TMS)

Changes in Resting Motor Threshold (RMT) were evaluated at different intervals before and after the completion of tcDCS. A Magstim Super Rapid Transcranial Magnetic Stimulator (Magstim Company, Dyfed, UK, 2.2 T maximum field output) connected to a standard eight-shaped focal coil with wing diameters of 70 mm was used. The handle of the eight-shaped focal coil was pointed backwards and rotated about 45 deg to the mid-sagittal line, to induce a tissue current perpendicular to the motor strip in the precentral sulcus (Rossi et al., 2009; Groppa et al., 2012). RMT was defined as the minimum stimulator output that induces motor evoked potentials (MEPs) of more than 50 μV in at least five out of 10 trials when first digital interosseus (FDI) muscle was completely relaxed (Ni et al., 2007). The motor "hot spot" for the targeted muscle was identified by single pulses of TMS delivered at a slightly suprathreshold stimulus intensity and the magnetic stimuli induced monophasic pulses. The coil was placed over the right motor cortex (centered on C4 according with the 10–20 EEG International System) and electromyographic recordings were made by two standard non-polarizable Ag/AgCl surface electrodes (diameter 10 mm; Biomed®, Florence, Italy), one placed over the belly of the contralateral FDI muscle, and the other on the skin overlying the first metacarpophalangeal joint of the first finger of the left hand. RMT was evaluated to exclude possible cerebellar stimulation spread out inducing motor cortex activation.

2.4. Variables and statistical analysis

We studied the subjective experience - perceptive threshold (PT) and pain intensity perceived after laser I1 and I2 (VAS1, VAS2) - and electrophysiological variables, that is the peak-to-peak amplitude of the N1 wave and N2/P2 complex, the peak latency of N1 and N2, as reported in previous papers using Nd:YAG laser (Lefaucheur et al., 2001, 2002).

Analyses were performed through SPSS.15 statistical Package. Psychophysical (PT, VAS_1, VAS_2) and electrophysiological variables (mean values of ten traces: N1amplitude and latency, N2/P2 amplitude and latency) as well as Resting Motor Thresholds (RMT) were analysed following a 3 Stimulation conditions (anodal, cathodal, sham) × 3 Times (T_0, T_1, T_2) design. The Greenhouse-Geisser ε correction for non sphericity was applied when necessary. Contrast analysis between Times (F values) and paired *t* tests between stimulations were alternatively used for *post-hoc* comparisons, when appropriate. After Bonferroni correction, significance level was set at $p < 0.007$.

The changes of all variable in T_1 and T_2 were expressed as ratio between post and pre stimulation values (T_1/T_0, T_2/T_0) and compared between each other according to a 2 Stimulation (anodal, cathodal) × 2 Times (T_1/To, T_2/T_0) design.

Table 2
Contrast analyses: all comparisons were highly significant ($p < 0.0001$)

		anodal	cathodal	sham
PT	time	df F = 44.30	F = 18.67	ns
	T0 vs Tl	2,28 F = 77.669)	F = 27.523	
	T0 vs T2	1,14 F = 78.745	F = 27.827	
	Tl vs T2	1,14 ns	ns	
		anodal vs sham	cathodal vs sham	
	T0	1,14 ns	ns	
	Tl	1,14 t = 5.069	t = 6.991	
	T2	1,14 t = 3.709	t = 5.849	
VAS		anodal	cathodal	sham
	time	2,28 F = 41.954	F = 31.448	ns
	T0 vs Tl	1,14 F = 56.968	F = 48.596	
	T0 vs T2	1,14 F = 52.289	F = 52.5	
	Tl vs T2	ns	ns	
		anodal vs sham	cathodal vs sham	
	T0	1,14 ns	ns	
	Tl	1,14 t = 6.44	t = 5.916	
	T2	1.14 t = 5.294	t = 5.82	

3. Results

Row data (mean, SD) are shown in Table 1. Baseline values were in line with those reported by earlier studies performed by using Nd:YAG laser (Lefaucheur et al., 2001). Indeed, only one study described a longer latency of the N2 wave (Cruccu et al., 2008). The sham stimulation did not modulate any psychophysical and electrophysiological variable (Table 2). Since no pre-post difference was found for sham polarity, this condition was not included in the comparison between Stimulations and Times.

3.1. Psychophysics

PT exhibited a significant Stimulation effect ($F_{(2,28)} = 35.055$, $p < 0.0001$, $\eta^2 = 0.715$) and a significant Stimulation × Time interaction ($F_{(4,56)} = 39.464$, $p < 0.0001$, $\eta^2 = 0.738$). Decomposition of the latter (Table 2) revealed that: a) PT was higher for the anodal and lower for the cathodal stimulation conditions compared with the sham stimulation for both T_1 and T_2; b) with respect to T_0, PT increased in T_1 and T_2 in the anodal condition and decreased in the cathodal condition, while no significant difference was observed between T_1 and T_2 (Fig. 3A).

Significantly different VAS_1 and VAS_2 were observed for the two stimulation intensities ($F_{(1,14)} = 54.262$, $p < 0.0001$) and the three Stimulation conditions ($F_{(2,18)} = 88.882$, $p < 0.0001$). Decomposition of the significant Stimulation × Time interaction ($F_{(4,56)} = 115.96$, $p < 0.0001$) revealed that the reported pain intensity for both stimulation intensities (VAS1 and VAS2) was higher for the cathodal and lower for the anodal stimulation compared to the sham stimulation (Table 2). It increased in T_1 and decreased in T_2 with respect to T_0, whereas no significant difference was found between T_1 and T_2 (Fig. 3-B).

3.2. Laser evoked potentials

Figure 4-A shows the LEPs recorded in all experimental conditions in a representative subject. Both N1 and N2/P2 amplitude (N1, $F_{(4,56)} = 106.95$, $p < 0.0001$, $\eta^2 = 0.884$; N2/P2, $F_{(4,56)} = 86.864$, $p < 0.0001$, $\eta^2 = 0.861$) and latency (N1, $F_{(4,56)} = 110.869$, $p < 0.0001$, $\eta^2 = 0.888$; N2/P2, $F_{(4,56)} = 36.60$, $p < 0.0001$, $\eta^2 = 0.723$) exhibited a significant Time × Stimulation interaction. Its decomposition (Table 3) showed that both amplitudes increased and both latencies decreased for cathodal stimulation in T_1 and T_2 with respect to T_0; the opposite occurred for the anodal stimulation. Both stimulations induced responses significantly different from the sham condition (Fig. 4-B). The responses obtained after cathodal stimulation were significantly improved (higher amplitudes, lower latencies) than those produced by the anodal one (N1 amplitude: $F_{(1,14)} = 413.45$, $p < 0.0001$; N1 latency: $F_{(1,14)} = 496.228$, $p < 0.0001$; N2/P2 amplitude: ($F_{(1,14)} = 445.37$, $p < 0.0001$; N2/P2 latency: $F_{(1,14)} = 119.056$, $p < 0.0001$).

3.3. Resting motor thresholds

RMT values at baseline did not differ among experimental conditions (mean ± SD; sham: 50.8 ± 8.3%; anodal: 49.1 ± 6.2%; cathodal: 50.3 ± 6.3%). ANOVA did not reveal any significant Stimulation ($F_{(2,28)} = 0.882$, $p = 0.425$, $\eta^2 = 0.059$), Time ($F_{(2,28)} = 0.212$, $p = 0.810$, $\eta^2 = 0.015$) and Stimulation × Time effect ($F_{(4,56)} = 0.339$, $p = 0.851$, $\eta^2 = 0.024$) for RMT (Fig. 5).

4. Discussion

Our study shows that cerebellar direct current polarization modulates nociceptive perception and its cortical correlates in healthy humans. Specifically, cathodal tcDCS increases pain perception, increases amplitudes and decreases LEPs latencies, likely though

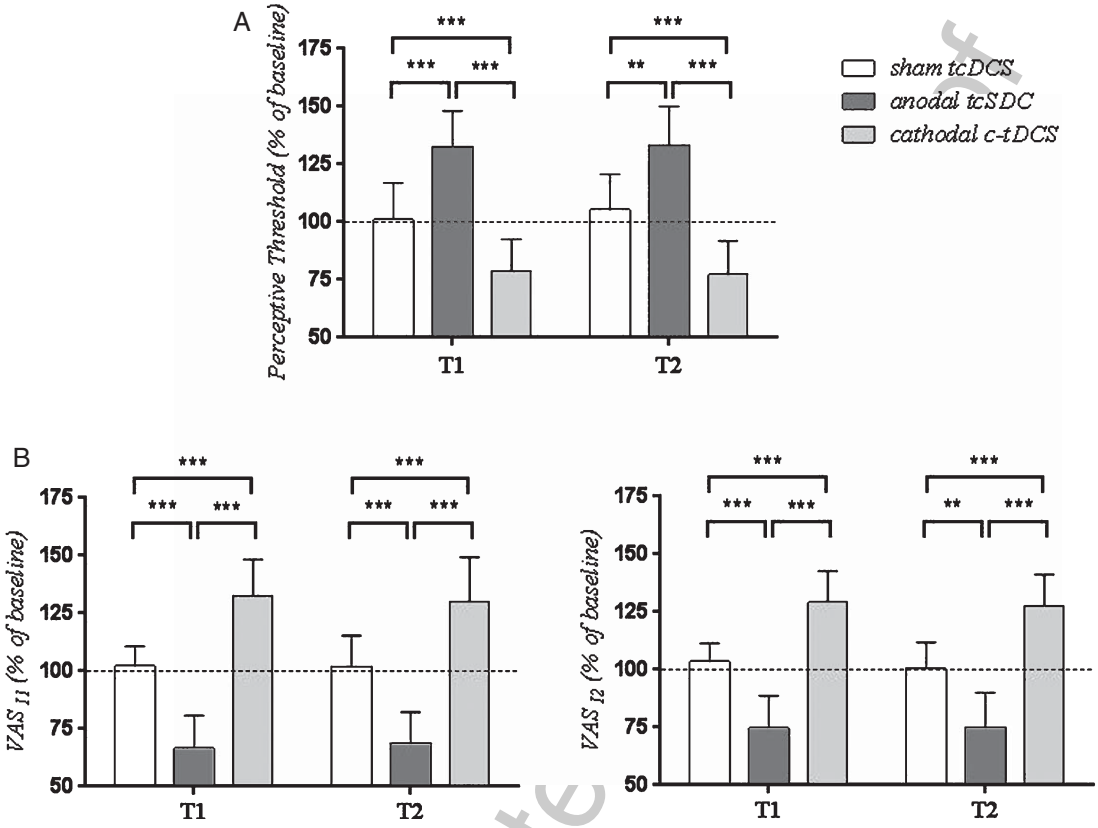

Fig. 3. - A. Perceptive Threshold. Changes (mean ± S.D) at T_1 and T_2 with respect to baseline values (T_1/T_0, T_2/T_0), following sham (black), anodal (white) and cathodal (grey) tcDCS. (**$p < 0.001$; ***$p < 0.0001$). *B.* Changes in visual analogue scale (VAS) scores over time. VAS scores at two different stimulus intensity, respectively two (*A*, left) and three (*B*, right) times higher than the PT. (**$p < 0.001$; ***$p < 0.0001$).

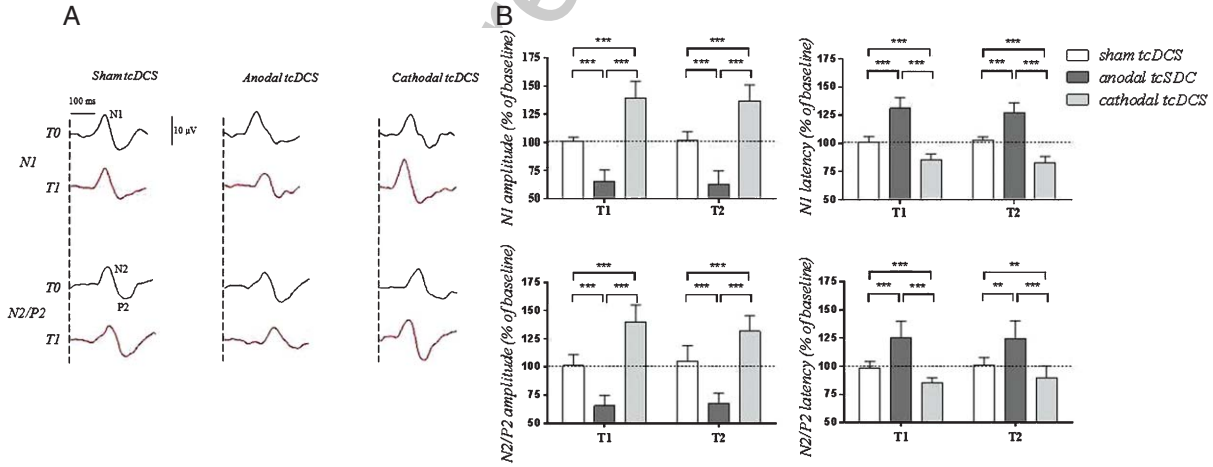

Fig. 4. – A. LEPs grand averaging: traces were recorded at baseline (T_0, black) and immediately after cerebellar polarization (T_1, red) due to sham (left column), anodal (middle) and cathodal (right) tcDCS. *B.* Histograms showing LEPs variables and VAS scores changes (mean ± S.D) after sham (black), anodal (white) or cathodal (grey) tcDCS with respect to baseline. Top panels: changes in N1 variables (amplitude and latency) over time; bottom panels: changes in N2/P2 complex (**$p < 0.001$; ***$p < 0.0001$).

Table 3
LEPs *post-hoc* analyses. $p < 0.0001$ for all comparison except when explicitly indicated: $^{**}p < 0.002$; *, $p < 0.005$

	N1	amplitude	latency	N2/P2	amplitude	latency	
		anodal			cathodal		sham
	df						
time	2,28	F = 67.152	F = 96.489		F = 134.912	F = 34.946	ns
T0 vs T1	1,14	F = 109.178	F = 188.15		F = 165,953,	F = 64.281	
T0 vs T2	1,14	F = 75.143	F = 167.697		F = 145.125	F = 37.818	
T1 vs T2	1,14	ns	ns		ns	ns	
		anodal			cathodal		
time	2,28	F = 102.281	F = 98.717		F = 65.77	F = 20.918	ns
T0 vs T1	1,14	F = 511.186	F = 104.027		F = 144.112	F = 103.864	
T0 vs T2	1,14	F = 96.329	F = 116.841		F = 105.183	F = 14.012**	
T1 vs T2	1,14	ns	ns		ns	ns	ns
		anodal vs sham			anodal vs sham		
T0	1,14	ns	ns		ns	ns	
T1	1,14	ns	t = 9.25		t = 6.01	6.262	
T2	1,14	ns	t = 8.128		t = 6.731	5.236	
		cathodal			cathodal vs sham		
T0	1,14	ns	ns		t = 3.281*	ns	
T1	1,14	t = 16.594	t = 8.029		t = 8.262	t = 5.20	
T2	1,14	t = 7.309	t = 12.669		t = 5.048	t = 5.301	

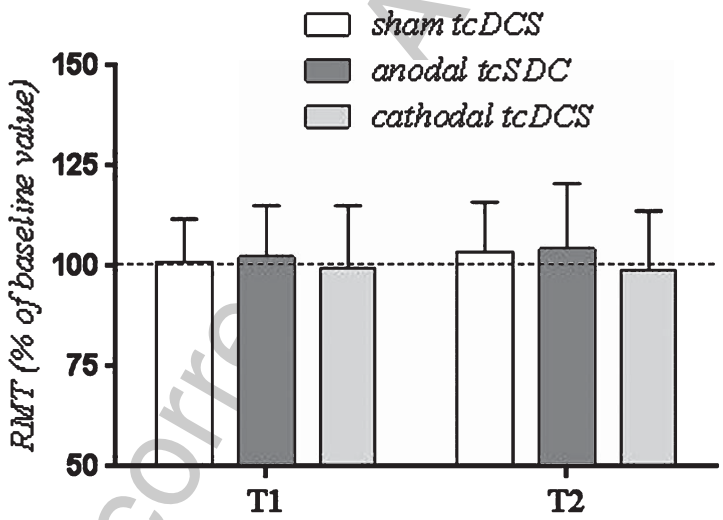

Fig. 5. - Resting Motor Thresholds. Changes (mean ± S.D) in Resting Motor threshold (RMT), expressed as percentage of the maximum stimulator output, after sham (black), anodal (white) and cathodal (grey) tcDCS with respect to baseline, marked as dotted line ($^{**}p < 0.001$; $^{***}p < 0.0001$).

reduction of the inhibitory tone exerted by the cerebellum on brain targets. Anodal polarization elicits opposite effects producing analgesia. Both findings support the role of the cerebellum in pain control; it is noticeable that cathodal cerebellar stimulation induces hyperalgesia as occurs in patients with cerebellar infarction (Ruscheweyh et al., 2014).

We would like to underline that, in the present study, LEPs were obtained at laser intensities depending on the perceptive threshold, which varied as a function of anodal and cathodal stimulation. This means that the cerebellar stimulation has not a selective analgesic effect, as it influences both non nociceptive and nociceptive perception. A pre-eminent analgesic cannot be assessed because the nociceptive threshold was not evaluated.

As tcDCS was effective on the modulation of both N1 and N2/P2 components and these responses are generated by parallel and partially segregated spinal pathways reaching different cortical targets

(Valeriani et al., 2007), we may suggest that the cerebellum is engaged in pain processing by modulating the activity of both somatosensory and cingulate cortices. Indeed, the cerebellum is involved in both the sensory-discriminative and emotional dimension of pain (Singer et al., 2004; Moriguchi et al., 2007), and non-invasive cerebellar current stimulation may modulate pain experience and the associated cortical activity through many, not alternative mechanisms. In particular, changes in N1 reflects the modulation of the sensory component of pain, while the vertex N2/P2 represents the neural correlate of affective aspects of pain experience (Garcia-Larrea et al., 1997; Valeriani et al., 2007). Notably, tcDCS may act not only on spinal nociceptive neurons, but also on wide-range cortical networks of the pain matrix (Singer et al., 2004), thus influencing LEPs and pain experience through both top-down and bottom-up mechanisms.

The present study does not allow to hypothesize how and where tcDCS influences the cerebellar activity. A main role of Purkinje cells has been suggested, as their activity modulation may affect the cerebellar inhibitory control of the cerebral cortex (Galea et al., 2009). This would be in line with the effects elicited by tDCS in the cerebral cortex which are observable after both short and long term delay, likely also interfering with long-term potentiation (LTP)-like phenomena (Hamada et al., 2012; Priori et al., 2014). Moreover, prolonged spiking activity in the cerebellar Golgi inhibitory neurons modulates the activity of the Purkinje cells and could partly account for the tcDCS after-effects (Hull et al., 2013).

The lack of changes in RMT indicates that the analgesic effects of anodal tcDCS are due to a specific modulation of the cerebellar activity and not to motor activation. On the other hand, tcDCS-induced cerebellar modulation (Purpura & McMurtry 1965) could be not sufficient *per se* to activate the cerebello – thalamo - cortical motor pathway (Galea et al., 2009); thus, the reported analgesia and its cortical correlates cannot be sustained by the motor cortex activation. This view is supported by the absence of any association between motor symptoms and pain perception in cerebellar patients (Ruscheweyh et al., 2014). In the same line, in healthy subjects it has been recently shown that motor task-induced increased cortical excitability and analgesia are not associated (Volz et al., 2012), Indeed, RMT is a highly sensitive marker of motor tract excitability, as it reflects activation of a small, low-threshold and slow-conducting core of pyramidal neurons (Hess et al., 1987; Rossini & Rossi, 2007); although RMT may reflect changes in the activity of different central nervous system structures, it has been satisfactorily used to assess motor cortex excitability also in cerebellar patients (Battaglia et al., 2006).

Another critical point is the possibility to modulate with tcDCS both neural correlates underlying nociceptive processing and pain perception. Previous studies using tDCS over motor cortex were inconsistent among each other: some works suggested that tDCS is able to modify pain perception (Boggio et al., 2008), while others showed divergent effects on psychophysical and neurophysiological outcome parameters (Luedtke et al., 2012; Ihle et al., 2014), likely due to a possible overestimation of the role of motor areas on pain processing (Antal et al., 2008).

Our findings cannot be compared to the results obtained by other Authors. In fact, the unique study focused on the analgesic effects of non-invasive cerebellar stimulation reported till now (Zunhammer et al., 2011) considered only subjective pain thresholds. In addition, it described similar analgesic effects of cerebellar and neck structures repetitive transcranial magnetic stimulation (rTMS), thus denying any cerebellar specificity in the observed effects and suggesting that the peripheral information passing through the cerebellum may be responsible for analgesia. The main difference between the two studies, possibly accounting for different results, consists of the neuromodulation techniques used.

4.1. Limitations of the study

The present study has a few limitations. First, our findings do not allow any hypothesis on the role of the cerebellum in chronic pain. The observations on patients with cerebellar damage (Ruscheweyh et al., 2014) suggest that their impaired inhibitory control mechanisms may be not associated with the development of chronic pain. Second, we cannot exclude the possibility that tcDCS could modulate not only the cerebellum, but also surrounding areas such as the periaqueductal gray. However, recent modelling researches have revealed that, during tcDCS, the current spread to other structures outside the cerebellum is negligible (Parazzini et al., 2013, 2014). Moreover, several studies have proved that in humans pain processing is encoded within posterior areas of each cerebellar hemisphere, specifically in the hemispheric lobule VI, Crus I and VIIb (Moulton et al., 2011), where the

tcDCS-induced electrical field is strongly concentrated (Parazzini et al., 2013). A further limitation is that we did not study the contribution of C-fibers, the main component of spino-ponto-cerebellar and spino-olivo-cerebellar pathways. In fact, ultra-late LEPs related to C-fibers activations have not yet been standardized for clinical application and their occurrence could be markedly influenced by high order, cognitive processes as they seem to be more affected by the level of consciousness and attention than A-delta responses (Qiu et al., 2002; Opsommer et al., 2003; Mouraux & Plaghki, 2006). Finally, we wish to emphasize that in neuropathic patients the effects of the cerebellar stimulation could be quite different from those described here, as both anatomical and functional connectivity are different from those observed in healthy participants (Rocca et al., 2010; Riedl et al., 2011; Absinta et al., 2012; Longo et al., 2012; Ceko et al., 2013).

5. Conclusions

Our findings indicate a cerebellar effect on pain experience and on its cortical correlates and prompt further investigation aimed at assessing whether the cerebellar direct current polarization could be used as a novel and safe therapeutic tool in chronic pain patients.

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