Brain Stimulation xxx (2018) 1–13

Contents lists available at ScienceDirect

Brain Stimulation

journal homepage: http://www.journals.elsevier.com/brain-stimulation

Cerebellar transcranial direct current stimulation interacts with BDNF Val66Met in motor learning

Rick van der Vliet [a, b, *], Zeb D. Jonker [a, b, c], Suzanne C. Louwen [a, d], Marco Heuvelman [a], Linda de Vreede [a], Gerard M. Ribbers [b, c], Chris I. De Zeeuw [a, e], Opher Donchin [f], Ruud W. Selles [b, c, g], Jos N. van der Geest [a], Maarten A. Frens [a, h]

[a] Department of Neuroscience, Erasmus MC, Rotterdam, The Netherlands
[b] Department of Rehabilitation Medicine, Erasmus MC, Rotterdam, The Netherlands
[c] Rijndam Rehabilitation Center, Rotterdam, The Netherlands
[d] Department of Child and Adolescent Psychiatry, Erasmus MC, Rotterdam, The Netherlands
[e] The Netherlands Institute for Neuroscience, Royal Dutch Academy of Science, Amsterdam, The Netherlands
[f] Department of Biomedical Engineering and Zlotowski Center for Neuroscience, Ben Gurion University of the Negev, Be'er Sheva, Israel
[g] Department of Plastic and Reconstructive Surgery, Erasmus MC, Rotterdam, The Netherlands
[h] Erasmus University College, Rotterdam, The Netherlands

ARTICLE INFO

Article history:
Received 24 October 2017
Received in revised form
4 March 2018
Accepted 9 April 2018
Available online xxx

Keywords:
Motor learning
Motor adaptation
tDCS
Rehabilitation
Cerebellum
BDNF Val66Met

ABSTRACT

Background: Cerebellar transcranial direct current stimulation has been reported to enhance motor associative learning and motor adaptation, holding promise for clinical application in patients with movement disorders. However, behavioral benefits from cerebellar tDCS have been inconsistent.
Objective: Identifying determinants of treatment success is necessary. BDNF Val66Met is a candidate determinant, because the polymorphism is associated with motor skill learning and BDNF is thought to mediate tDCS effects.
Methods: We undertook two cerebellar tDCS studies in subjects genotyped for BDNF Val66Met. Subjects performed an eyeblink conditioning task and received sham, anodal or cathodal tDCS (N = 117, between-subjects design) or a vestibulo-ocular reflex adaptation task and received sham and anodal tDCS (N = 51 subjects, within-subjects design). Performance was quantified as a learning parameter from 0 to 100%. We investigated (1) the distribution of the learning parameter with mixture modeling presented as the mean (M), standard deviation (S) and proportion (P) of the groups, and (2) the role of BDNF Val66Met and cerebellar tDCS using linear regression presented as the regression coefficients (B) and odds ratios (OR) with equally-tailed intervals (ETIs).
Results: For the eyeblink conditioning task, we found distinct groups of learners ($M_{Learner} = 67.2\%$; $S_{Learner} = 14.7\%$; $P_{Learner} = 61.6\%$) and non-learners ($M_{Non\text{-}learner} = 14.2\%$; $S_{Non\text{-}learner} = 8.0\%$; $P_{Non\text{-}learner} = 38.4\%$). Carriers of the BDNF Val66Met polymorphism were more likely to be learners (OR = 2.7 [1.2 6.2]). Within the group of learners, anodal tDCS supported eyeblink conditioning in BDNF Val66Met non-carriers (B = 11.9% 95%ETI = [0.8 23.0]%), but not in carriers (B = 1.0% 95%ETI = [-10.2 12.1]%). For the vestibulo-ocular reflex adaptation task, we found no effect of BDNF Val66Met (B = −2.0% 95%ETI = [-8.7 4.7]%) or anodal tDCS in either carriers (B = 3.4% 95%ETI = [-3.2 9.5]%) or non-carriers (B = 0.6% 95% ETI = [-3.4 4.8]%). Finally, we performed additional saccade and visuomotor adaptation experiments (N = 72) to investigate the general role of BDNF Val66Met in cerebellum-dependent learning and found no difference between carriers and non-carriers for both saccade (B = 1.0% 95%ETI = [-8.6 10.6]%) and visuomotor adaptation (B = 2.7% 95%ETI = [-2.5 7.9]%).
Conclusions: The specific role for BDNF Val66Met in eyeblink conditioning, but not vestibulo-ocular reflex adaptation, saccade adaptation or visuomotor adaptation could be related to dominance of the role of simple spike suppression of cerebellar Purkinje cells with a high baseline firing frequency in eyeblink conditioning. Susceptibility of non-carriers to anodal tDCS in eyeblink conditioning might be

* Corresponding author. Erasmus MC, room Ee1477, Wytemaweg, 3015 CN, Rotterdam, The Netherlands.
E-mail address: r.vandervliet@erasmusmc.nl (R. van der Vliet).

https://doi.org/10.1016/j.brs.2018.04.009

explained by a relatively larger effect of tDCS-induced subthreshold depolarization in this group, which might increase the spontaneous firing frequency up to the level of that of the carriers.

Introduction

Over the past decade, cerebellar transcranial direct current stimulation (tDCS) has been reported to enhance motor associative learning [1] and motor adaptation [2–10] (see Ref. [11] for a review of the technical details), holding promise for patients with movement disorders [12]. However, cerebellar tDCS effects are inconsistent across the literature, as recent studies failed to replicate initial behavioral benefits [13–15]. This could mean that the behavioral gains reported in earlier studies result from chance and/or that determinants predicting successful tDCS are incompletely understood. Genetic differences between individuals might influence (1) the background performance level and therefore the potential to improve with tDCS [16] or (2) the susceptibility to tDCS. Therefore, to increase predictability of cerebellar tDCS effectiveness it is important to identify factors which modify treatment success [17], like genetic variants.

The common [18,19] BDNF Val66Met polymorphism, which decreases activity-dependent BDNF release [20], is a candidate determinant of cerebellar tDCS effectiveness, because (1) the polymorphism is associated with motor skill learning ability [21,22] and (2) BDNF is thought to mediate tDCS effects on synaptic plasticity and motor skill learning [22]. Since BDNF supports long-term potentiation [22,23] and formation of inhibitory synapses [24], Val66Met carriers have subtle behavioral alterations such as decreased memory [20], slowed motor skill learning [21,22] and more pronounced fear conditioning [25]. In addition, in mouse cortical brain slices, concurrent DCS and synaptic activation only leads to long-term potentiation when BDNF is not knocked out or blocked [22], suggesting that Val66Met carriers may benefit less from tDCS. However, whether BDNF Val66Met interacts with cerebellar tDCS in cerebellum-dependent motor learning has not yet been investigated.

Eyeblink conditioning and vestibulo-ocular reflex (VOR) adaptation are particularly well-characterized cerebellum-dependent learning tasks for which positive effects of cerebellar tDCS have been found. Eyeblinks are protective eyelid closures against damage to the cornea. They can be activated in response to predictive neutral cues such as auditory tones. This learned motor association is made in a relatively simple circuitry involving the interposed nucleus and lobule VI of the cerebellum [26–29] and extracerebellar areas in the hippocampus and amygdala [30–34]. Eyeblink conditioning is mediated by a sudden, carefully timed decrease in simple spike activity of cerebellar Purkinje cells that fire at a relatively high spontaneous firing frequency [28,35,36]. Zuchowski et al. found an increase in eyeblink conditioning with anodal tDCS and a decrease with cathodal tDCS [1], which is in line with the concept that in eyeblink conditioning Purkinje cells should operate at a sufficiently high simple spike firing frequency during spontaneous activity, because anodal tDCS is supposed to increase the baseline firing frequency of neurons [37–40]. The VOR generates eye movements opposite in direction, but with identical speed as head rotation to stabilize objects of interest on the retina. Changes in the environment or the body can make this relation inappropriate and result in retinal slip [41]. Retinal slip will recruit adaptive mechanisms in the cerebellar flocculus and downstream vestibular nuclei to increase (gain-increase adaptation) or decrease eye (gain-decrease adaptation) movement velocity and regain foveal stabilization [42–48]. VOR gain-decrease adaptation, which will be studied in this paper, is mediated by decreased velocity sensitivity of neurons in vestibular nuclei, at least partially induced by plasticity mechanism involving floccular Purkinje cells [44–47]. Recently, anodal cerebellar DCS during VOR adaptation was found to enhance learning rate of a gain-decrease paradigm in mice [9]. Therefore, eyeblink conditioning and VOR adaptation are two cerebellum-dependent, but fundamentally different tasks, which entail different cellular mechanisms, and which concern conceptually different paradigms in that conditioning implies learning new associations, whereas adaptation involves recalibrating and optimizing existing behavior.

The primary aim of this study was to investigate the interaction between BDNF Val66Met and cerebellar tDCS in eyeblink conditioning and VOR adaptation. To this end, we undertook two studies in genotyped subjects who received cerebellar tDCS and performed either an eyeblink conditioning task ($N = 117$, between-subjects design) or a VOR adaptation task ($N = 51$, within-subjects design). Based on motor skill learning studies [21,22], we expected faster learning for non-carriers in both tasks and therefore a more pronounced effect of cerebellar tDCS in carriers. Based on fear conditioning studies [25], we expected faster learning for carriers in the eyeblink conditioning task, which depends on the amygdala as well as the cerebellum, but not in the VOR adaptation task and therefore a more pronounced effect of cerebellar tDCS on eyeblink conditioning in non-carriers. In addition, we performed control experiments to evaluate the role of BDNF Val66Met in saccade and visuomotor adaptation.

Materials and methods

Subjects

Healthy right-handed, defined as having an Edinburgh handedness inventory score [49] larger than zero, individuals participated in the eyeblink conditioning (genetic analysis failed in 3/120 subjects leaving 117 for analysis) and VOR adaptation studies (genetic analysis failed in 4/55 subjects leaving 51 for analysis) (see Table 1). 9/51 subjects dropped out before the second VOR session but the available data of the first session was included in the analysis. The experiments were approved by the Erasmus MC medical ethics committee and performed in accordance with the Declaration of Helsinki.

Cerebellar tDCS

Cerebellar tDCS was delivered through two saline-soaked 5×5cm sponge electrodes (DC stimulator, NeuroConn GmbH, Ilmenau, Germany) placed on the right side of the scalp, 3 cm lateral to the inion (target electrode) and on the ipsilateral buccinator muscle (reference electrode). This electrode configuration is the standard for cerebellar tDCS in motor learning tasks [1–5,10] and is supported by electrophysiological [50] and modeling studies [51]. In the active conditions, we applied 2 mA anodal or cathodal tDCS during 20 min for the eyeblink conditioning experiment (similar to: [14]) and 2 mA anodal tDCS during 15 min for the VOR

Please cite this article in press as: van der Vliet R, et al., Cerebellar transcranial direct current stimulation interacts with BDNF Val66Met in motor learning, Brain Stimulation (2018), https://doi.org/10.1016/j.brs.2018.04.009

Table 1
Subject characteristics for the eyeblink conditioning, VOR adaptation, and saccade and visuomotor adaptation tasks. M = mean; S = standard deviation.

Paradigm	Group	Gender (%Male)	Age (M ± S)	Ethnicity (%Western-European)	Edinburgh handedness inventory (M ± S)
Eyeblink conditioning (N = 117)	Sham (N = 39)	41	21.5 ± 2.8	85	79.4 ± 20.2
	Carriers (N = 16)	31	21.3 ± 2.4	81	83.0 ± 18.0
	Non-carriers (N = 23)	48	21.7 ± 3.2	87	76.5 ± 21.8
	Anodal (N = 40)	40	20.6 ± 2.5	85	76.8 ± 19.5
	Carriers (N = 17)	35	20.8 ± 2.9	82	69.5 ± 22.7
	Non-carriers (N = 23)	43	20.4 ± 2.2	87	80.7 ± 16.5
	Cathodal (N = 38)	42	20.9 ± 2.4	82	75.3 ± 17.1
	Carriers (N = 14)	57	21.4 ± 2.5	79	72.3 ± 19.8
	Non-carriers (N = 24)	33	20.6 ± 2.4	83	77.0 ± 16.3
VOR adaptation (N = 51)	Carriers (N = 18)	11	21.8 ± 3.1	89	77.5 ± 24.5
	Non-carriers (N = 33)	42	21.7 ± 2.7	82	74.8 ± 15.1
Saccade adaptation and visuomotor adaptation (N = 72)	Carriers (N = 25)	40	21.6 ± 2.0	87	82.2 ± 15.6
	Non-Carriers (N = 47)	38	21.1 ± 2.5	91	79.0 ± 17.9

adaptation experiment (most commonly used duration: [52]). In the sham condition, 2 mA anodal or cathodal tDCS was delivered for only 30 s, which is an effective method for blinding subjects [53]. In both the active and sham condition, current amplitude was increased and decreased in a ramp-like fashion over 30 s according to a well-established protocol [2]. Experimenters were blinded using a list of stimulation codes corresponding with sham or active stimulation. This list was semi-randomized, balancing the number of subjects in each condition.

Genetics

The BDNF Val66Met polymorphism (rs6265) was genotyped using TaqMan assays as described before [54]. Subjects with at least one Met allele were termed “carriers”, others “non-carriers”.

Eyeblink conditioning

Eyeblink conditioning was studied by presenting an auditory tone (conditioned stimulus) shortly before applying an air puff to the eye (unconditioned stimulus) [55,56], similar to Zuchowski et al. [1]. Over trials, subjects learn to predict the air puff from the tone and close the eyelid before the puff reaches the cornea. We chose a between-subject design for this task, even though a within-subject design could have removed between-subject variability, because the motor memory in eyeblink conditioning is retained for a long time [57]. Furthermore, we included anodal as well as cathodal tDCS because both have been found to modulate eyeblink conditioning [1].

We used a SheBot system (Neurasmus, Rotterdam, The Netherlands [58]) controlled by a custom-built LabVIEW program (National Instruments Corporation, Austin, Texas, United States) to provide precisely timed (1) auditory tones via a headphone and (2) air-puffs via a nozzle placed 15 mm from the lateral corner of the eye. Eyelid closures were recorded with a small magnet on the upper eyelid and a sensor slightly below the eye [58]. During the experiment, subjects watched the movie “A Beautiful Mind” (Universal Pictures, 2005, Internet Movie DataBase #tt0268978) with audio but without subtitles.

The experiment consisted of unconditioned stimulus trials, conditioned stimulus trials and paired stimulus trials (see Fig. 1A). The experiment started with a baseline measurement (B) comprised of ten unconditioned stimulus trials and ten conditioned stimulus trials, followed by ten learning measurements (L1-L10) consisting of ten paired trials, one unconditioned stimulus trial and one conditioned stimulus trial (Fig. 1B). For each measurement, trial order and trial interval (ranging from 20 to 35 s) were pseudo-randomized. Cerebellar tDCS or sham stimulation started with L1.

Eyeblink data was automatically processed using a custom MATLAB program (The MathWorks Inc., Natick, Massachusetts, United States) (see Fig. 1E). First, trials were low-pass filtered with a zero-phase 6th order Butterworth filter using a 100 Hz cut-off frequency. Subsequently, trials were divided in four time windows: (1) a baseline window; (2) a short-latency response window; (3) a conditioned response window; and (4) an unconditioned response window. Peak time (ms) occurred at maximum eyelid closure in the conditioned or unconditioned response window. Peak amplitude (mV) was the difference between the eyelid signal at peak time and the mean eyelid signal in the baseline window. Eyeblink onset (ms) occurred at the last time point when the eyelid signal was smaller than 7.5% of the peak amplitude. The analysis was robust to small changes in the peak amplitude threshold. Trials were classified by the window that contained the eyeblink onset. Short-latency response and baseline responses were counted as invalid trials.

The learning parameter for this experiment was the percentage of conditioned responses in the last six learning blocks L5-L10. That is, the number of conditioned responses divided by the total number of conditioned and unconditioned responses in the 60 paired trials of the learning measurements L5-L10 multiplied by 100 (0 = no conditioning; 100 = complete conditioning). In addition, we investigated the short-latency responses as a percentage of the sum of conditioned responses, unconditioned responses and short-latency responses (short-latency response fraction).

VOR adaptation

VOR adaptation was studied by directly coupling head rotation to visual display rotation, which requires suppression of the reflex to minimize retinal slip [59–61], similar to an animal study performed by Das et al. [9]. In contrast to the eyeblink conditioning experiment, we chose a within-subject design as the motor memory is expected to last no more than three days [62–64]. Both stimulation sessions were separated by at least 7 days to ensure wash-out of the cerebellar tDCS [65,66] and VOR adaptation effects [62–64] of the first session. Furthermore, we did not include a cathodal condition to limit the number of experimental conditions for our subjects.

Subjects were seated in a rotational chair placed 224 cm in front of a wide translucent screen (235 cm × 170 cm). Head position was fixed relative to the chair with a bite-board (Dental Tecno Benelux, Ede, Netherlands). Chair rotation frequency was set to 0.295 Hz with an amplitude of 12° around the vertical axis, resulting in a peak angular velocity of 22.2°/s (similar to [67]). Two-dimensional binocular eye movements were recorded using infrared video-oculography (EyeLink I, SR Research, Ontario, Canada [68]). An

Please cite this article in press as: van der Vliet R, et al., Cerebellar transcranial direct current stimulation interacts with BDNF Val66Met in motor learning, Brain Stimulation (2018), https://doi.org/10.1016/j.brs.2018.04.009

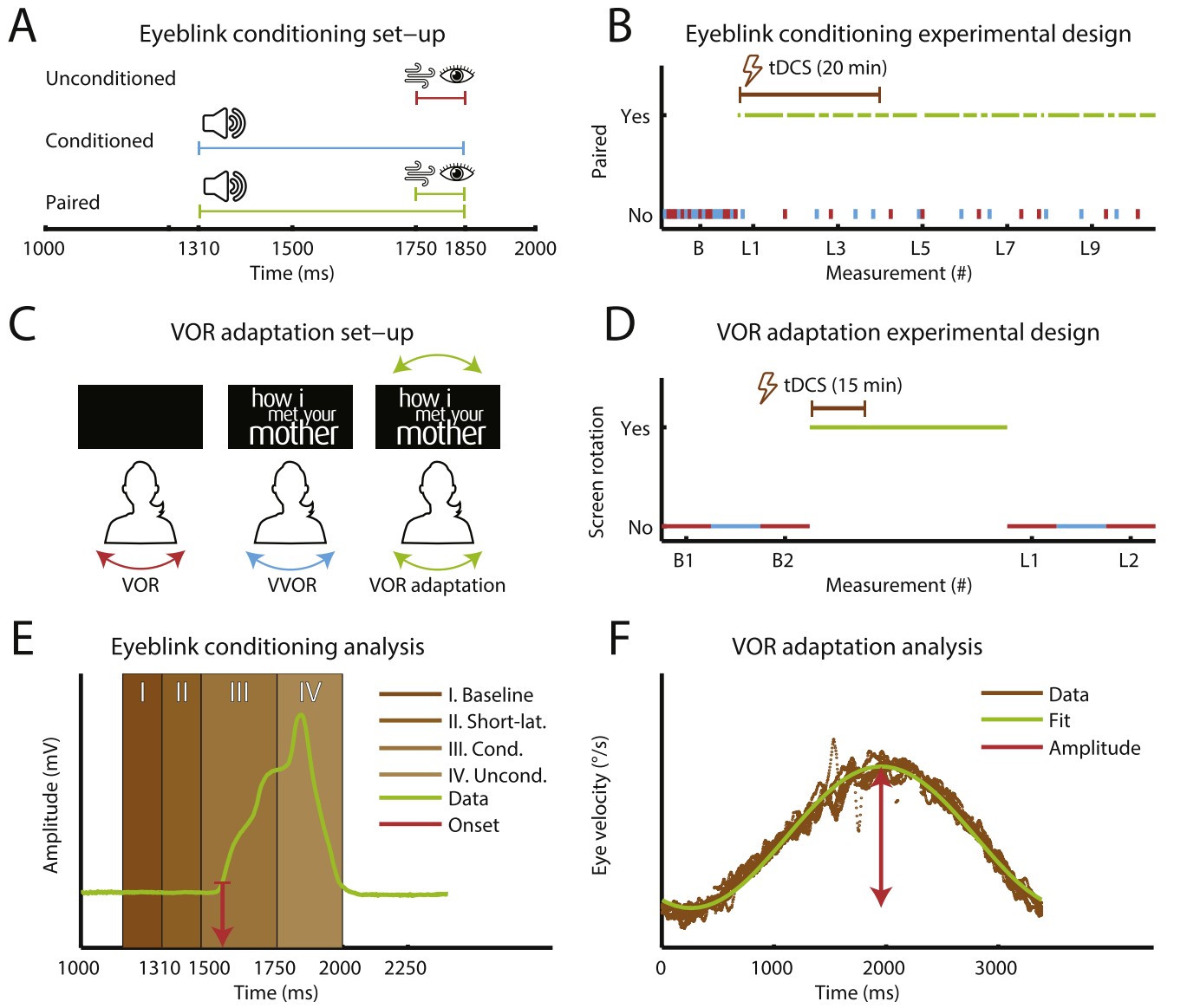

Fig. 1. Experimental procedures and data-analysis for eyeblink conditioning and VOR adaptation.
tDCS = transcranial direct current stimulation; VOR = vestibulo-ocular reflex; VVOR visually-enhanced vestibulo-ocular reflex.
A. Eyeblink conditioning set-up. The experiment consisted of unconditioned stimulus trials (red line), conditioned stimulus trials (blue line) and paired stimulus trials (green line). During each trial, eyelid movements were recorded for 3 s. For the unconditioned stimulus trials, an air puff (3 bar at source, 100 ms duration) was delivered from 1750 until 1850 ms after recording onset. For the conditioned stimulus trials, a tone (650 Hz, 75 dB, 540 ms duration) was played from 1310 ms until 1850 ms after recording onset. In paired stimulus trials, subjects received both the tone and the air puff, which overlapped for 100 ms. **B.** Eyeblink conditioning experimental design. The experiment started with a baseline measurement (B) comprised of ten unconditioned stimulus trials (red lines) and ten conditioned stimulus trials (blue lines), followed by ten learning measurements (L1-L10) consisting of ten paired trials (green lines), one unconditioned stimulus trial (red lines) and one conditioned stimulus trial (blue lines). **C.** VOR adaptation set-up. The experiment consisted of VOR trials (red line), VOR adaptation trials (blue lines) and VVOR trials (green line). During VOR measurements, subjects were asked to keep their eyes fixated at the middle of the screen during rotation in total darkness for 40 s. Rotation of the chair was paused for 30 s before each VOR measurement. During VVOR trials, subjects were rotated for 1 min while the movie was projected stationary on the middle of the screen. During VOR adaptation trials, the projection was rotated for 60 min with identical phase and amplitude as the chair. **D.** VOR adaptation experimental design. The experiment started with two baseline VOR trials (red lines, measurements B1 and B2), separated by a single VVOR trial (green line). Subsequently, subjects underwent a single VOR adaptation trial (blue line) and two VOR trials (red lines, measurements L1 and L2). **E.** Eyeblink conditioning analysis. Each trial (green line) was divided into (I) a baseline window of 150 ms before the start of the conditioned stimulus from $t_{start} = 1160$ ms until $t_{end} = 1310$ ms; (II) a short-latency response window of 150 ms after the start of the conditioned stimulus from $t_{start} = 1310$ ms until $t_{end} = 1460$ ms; (III) a conditioned response window of 290 ms before the start of the unconditioned stimulus from $t_{start} = 1460$ ms until $t_{end} = 1750$ ms; and (IV) an unconditioned response window of 250 ms after the start of the unconditioned stimulus from $t_{start} = 1750$ ms until $t_{end} = 2000$ ms. Trials were classified as the window that contained the eyeblink onset. **F.** VOR adaptation analysis. Forty-second eye velocity signals were cut into eleven rotations (brown line) of 3.39 s, aligned in time and fitted with a sine wave (green line) of the same frequency to extract the amplitude (red arrow).

episode of "How I Met Your Mother" with audio but without subtitles (Twentieth Century Fox Film Cooperation, 2005, Internet Movie DataBase #tt0460649) was back-projected (Infocus LP 335, Portland, Oregon, United States) onto the translucent screen (size 104 × 74 cm) via rotatable mirrors (model number 6900, Cambridge Technology, Cambridge, United Kingdom).

Trial types included VOR, visually-enhanced vestibulo-ocular reflex (VVOR) trials and VOR adaptation trials (see Fig. 1C). The experiment started with two baseline VOR trials (measurements B1 and B2), separated by a single VVOR trial. Subsequently, subjects underwent a single VOR adaptation trial and two VOR trials (measurements L1 and L2) (Fig. 1D).

Please cite this article in press as: van der Vliet R, et al., Cerebellar transcranial direct current stimulation interacts with BDNF Val66Met in motor learning, Brain Stimulation (2018), https://doi.org/10.1016/j.brs.2018.04.009

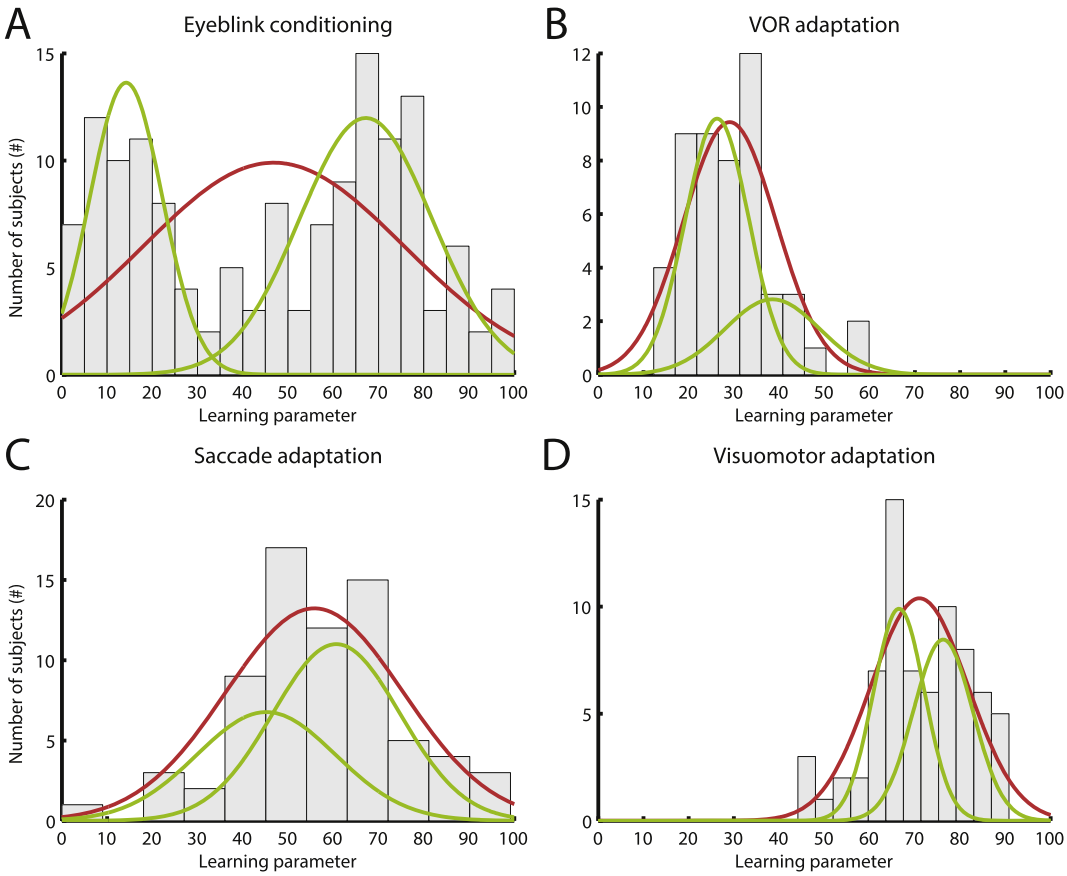

Fig. 2. Learning parameter distributions.
A-D. Histograms of the learning parameter for eyeblink conditioning (**A**), VOR adaptation (**B**), saccade adaptation (**C**) and visuomotor adaptation (**D**). The red Gaussians show the unimodal distributions. The green Gaussians the bimodal distribution.

Table 2
Mixture model results. The learning parameters for eyeblink conditioning, VOR adaptation, saccade adaptation and visuomotor adaptation were modeled with (1) a single normal distribution and (2) a learner/non-learner model composed of a mixture of two normal distributions. We compared model fit with the DIC, with lower DICs indicating better model fits. Eyeblink conditioning was best captured with a learner/non-learner model, whereas the adaptation paradigms were best described with a single group model. DIC = deviance information criterion, L = learner, M = mean, NL = non-learner, S = standard deviation.

	Single group			Learner/non-learner						
	DIC	M	S	DIC	P_{NL}	M_{NL}	S_{NL}	P_L	M_L	S_L
Eyeblink conditioning	1121	46.8	28.8	1067	38.4	14.2	8.0	61.6	67.2	14.7
VOR adaptation	384	29.1	10.3	416	69.5	26.3	7.0	30.5	38.5	10.5
Saccade adaptation	635	55.9	19.6	695	40.1	45.2	15.4	59.9	60.7	14.1
Visuomotor adaptation	548	71.0	10.7	603	50.2	66.5	5.7	49.8	77.8	6.6

Table 3
Linear and logistic regression models. VOR adaptation, saccade adaptation and visuomotor adaptation were best modeled as a single group (see Table 2) and therefore further analyzed with a linear regression of the learning parameter of all subjects. Eyeblink conditioning was best captured with a learner/non-learner model and therefore analyzed both with a logistic regression for the probability of being a learner and a linear regression for the learning parameter of the learner group. B = correlation coefficient, OR = odds ratio.

Paradigm	Model	Factor	OR	B
Eyeblink conditioning	Single group	Carrier	–	18.8 [2.3 35.3]
		$Anodal_{Carrier}$	–	−0.7 [-18.6 17.2]
		$Anodal_{Non\text{-}carrier}$	–	18.0 [2.6 33.3]
		$Cathodal_{Carrier}$	–	1.2 [-17.7 19.9]
		$Cathodal_{Non\text{-}carrier}$	–	2.3 [-13.0 17.5]
	Learner/non-learner	Carrier	4.2 [1.1 19.8]	2.9 [-8.5 14.5]
		$Anodal_{Carrier}$	0.8 [0.1 3.9]	1.0 [-10.2 12.1]
		$Anodal_{Non\text{-}carrier}$	2.5 [0.8 8.9]	11.9 [0.8 23.0]
		$Cathodal_{Carrier}$	1.2 [0.2 8.3]	−1.4 [-12.8 10.0]
		$Cathodal_{Non\text{-}carrier}$	1.3 [0.4 4.3]	−1.7 [-13.2 9.9]
VOR adaptation	Single group	Carrier	–	−2.0 [-8.7 4.7]
		$Anodal_{Carrier}$	–	3.4 [-3.2 9.5]
		$Anodal_{Non\text{-}carrier}$	–	0.6 [-3.4 4.8]
Saccade adaptation	Single group	Carrier	–	1.0 [-8.6 10.6]
Visuomotor adaptation	Single group	Carrier	–	2.7 [-2.5 7.9]

Please cite this article in press as: van der Vliet R, et al., Cerebellar transcranial direct current stimulation interacts with BDNF Val66Met in motor learning, Brain Stimulation (2018), https://doi.org/10.1016/j.brs.2018.04.009

Eye movement data was processed in MATLAB (The MathWorks Inc., Natick, Massachusetts, United States) (see Fig. 1F). Eye velocity gains were calculated per subject, eye and measurement (B1-2 and L1-2) according to the following procedure. First, saccades and eyeblinks were removed from the horizontal eye position using an internal Eyelink routine. Subsequently, the horizontal eye position was smoothened and differentiated with a Savitzky-Golay filter (third order polynomial, 10 Hz critical frequency) to obtain an eye movement velocity signal (°/s). Eleven rotations of 3.39 s from this 40-second velocity signal were aligned in time and fitted with a sine wave of the same frequency. Fitted velocity amplitudes (°/s) of left and right eye velocity signals were combined for each block by weighing with the number of recorded data points. Finally, all amplitudes were divided by the mean amplitude in B1 and B2 resulting in a normalized gain.

The learning parameter for this experiment was one minus the average amplitude of learning measurements L1 and L2 multiplied by 100 (0 = no adaptation; 100 = complete adaptation).

Saccade adaptation

Saccade adaptation was studied by relocating a target in an inward direction during a saccade to induce a post saccadic error [69–71]. Over trials, subjects learn to decrease the size of their saccades to compensate for these target jumps.

Subjects were seated in front of a monitor covered with a red filter (53 cm width, 1280 × 1024 pixel resolution) in a completely dark room. Steady head position was maintained using a chin rest at a fixed viewing distance of 82 cm. Eye movements were recorded binocularly at 250 Hz by means of video-oculography (EyeLink II, SR Research, Ontario, Canada).

Task design was similar to Avila et al. [6], but with smaller amplitude saccades (10° rather than 20°) to reduce the occurrence of two-step saccades. The trial types were unperturbed and perturbed trials (see Fig. 6A). The experiment included baseline measurements of 50 unperturbed trials (measurements B1-50), followed by learning measurements of 150 perturbed trials (measurements L1-150) (see Fig. 6B).

Saccade amplitudes were calculated using an internal Eyelink routine. All amplitudes were divided by 10° to calculate normalized gains and corrected for an offset by subtracting the median amplitude of the baseline measurements. The learning parameter was defined as the quotient of 1 minus the median of L150-200, and the perturbation size 0.3.

Visuomotor adaptation

Reaching movement adaptation to visual mismatches was studied with visuomotor adaptation, wherein visual feedback of hand location is rotated with respect to actual reaching movement [72–74]. Subjects adjust their movement based on this visual mismatch by changing the angle of their reaches.

Subjects were seated in front of a vertical monitor (48 cm width, 1280 × 1024 pixel resolution, distanced 60 cm from the subjects) while holding a robotic handle in their right hand (custom-made, see Ref. [75]) which recorded hand position and velocity. To remove direct visual feedback of hand position, subjects wore an apron that was attached to the table around their neck.

Task design was similar to Galea et al. [2]. The trial types were unperturbed trials and perturbed trials (see Fig. 6C). The experiment design included baseline measurements of unperturbed trials (measurements B1-192) and learning measurements of perturbed trials (measurements L1-200) (see Fig. 6D). Order of the visuomotor and saccade adaptation experiments was counterbalanced across subjects.

Visuomotor adaptation data was processed using MATLAB (The MathWorks Inc., Natick, Massachusetts, United States). From each trial, we extracted movement start, defined as the time point when movement velocity exceeded 0.03 m/s, and movement end, defined as the moment when displacement from origin was equal to or larger than 9.5 cm. Aiming direction was calculated as the signed (+or -) angle in degrees between the vector connecting origin and target and the vector connecting the positions of the manipulandum at movement start and movement end. The clockwise direction was defined as positive. Aiming directions more than 30° away from the median of an epoch of 8 trials across all subjects, were removed from further analysis.

The learning parameter for this experiment was the negative average of L9 through L88 divided by the perturbation size of 30° (similar to Galea et al. [2]).

Statistics

We used a two-step approach to data-analysis of the learning parameter.

First, we investigated whether the distribution of the learning parameter was best captured by either a single normal distribution (unimodal) or a mixture of two normal distributions (bimodal). The latter distribution could arise if one group of subjects is able to learn the task (learners) whereas the other group is not (non-learners). For this analysis, we used a Bayesian Gaussian mixture model fitting one or two normal distributions to the learning parameter (averaged across stimulation conditions for the VOR adaptation experiment), with a beta prior for the probability of being a learner or a non-learner. We set the lower limit on the prior probability of being a learner or non-learner to 0.15 and the upper limit to 0.85 to neglect clusters smaller than 15% of the total population. Quality of the two models was compared for each paradigm with the deviance information criterion (DIC) according to [76], which rewards high likelihood and penalizes model complexity.

Second, in case the learning parameter was best captured by a unimodal distribution, the learning parameters of all subjects were studied with a 'single group' Bayesian linear regression model (independent variables described below). However, if the learning parameter was best captured by a bimodal distribution, we performed a 'learner/non-learner' regression analysis as the main analysis, and reported the 'single group' analysis for transparency purposes. For the 'learner/non-learner' analysis, we labeled the subjects as "learners" and "non-learners" based on the group the subjects were assigned to most in the mixture model and calculated (1) a Bayesian logistic regression model for the probability of being a learner, and (2) a Bayesian linear regression model for the learning parameter of the "learners" only (independent variables described below). For eyeblink conditioning (between-subjects), the regression model contained the independent variables "carrier", "$anodal_{Carrier}$", "$anodal_{Non\text{-}carrier}$", "$cathodal_{Carrier}$" and "$cathodal_{Non\text{-}carrier}$". For VOR adaptation (within-subjects), the regression model contained the independent variables "carrier", "$anodal_{Carrier}$" and "$anodal_{Non\text{-}carrier}$".

The short-latency response fraction was analyzed by fitting beta distributions to the carriers and non-carriers and calculating the difference in group means. It was necessary to use beta distributions because short-latency response fraction was heavily skewed towards zero.

Analysis for the saccade and visuomotor adaptation studies was similar to the eyeblink conditioning and VOR adaptation experiments with "carrier" as the independent variable.

Results for the linear and logistic regressions are reported as the mean regression coefficient with 95% equally-tailed intervals (ETIs). Results for the direct comparison of beta distributions are

Please cite this article in press as: van der Vliet R, et al., Cerebellar transcranial direct current stimulation interacts with BDNF Val66Met in motor learning, Brain Stimulation (2018), https://doi.org/10.1016/j.brs.2018.04.009

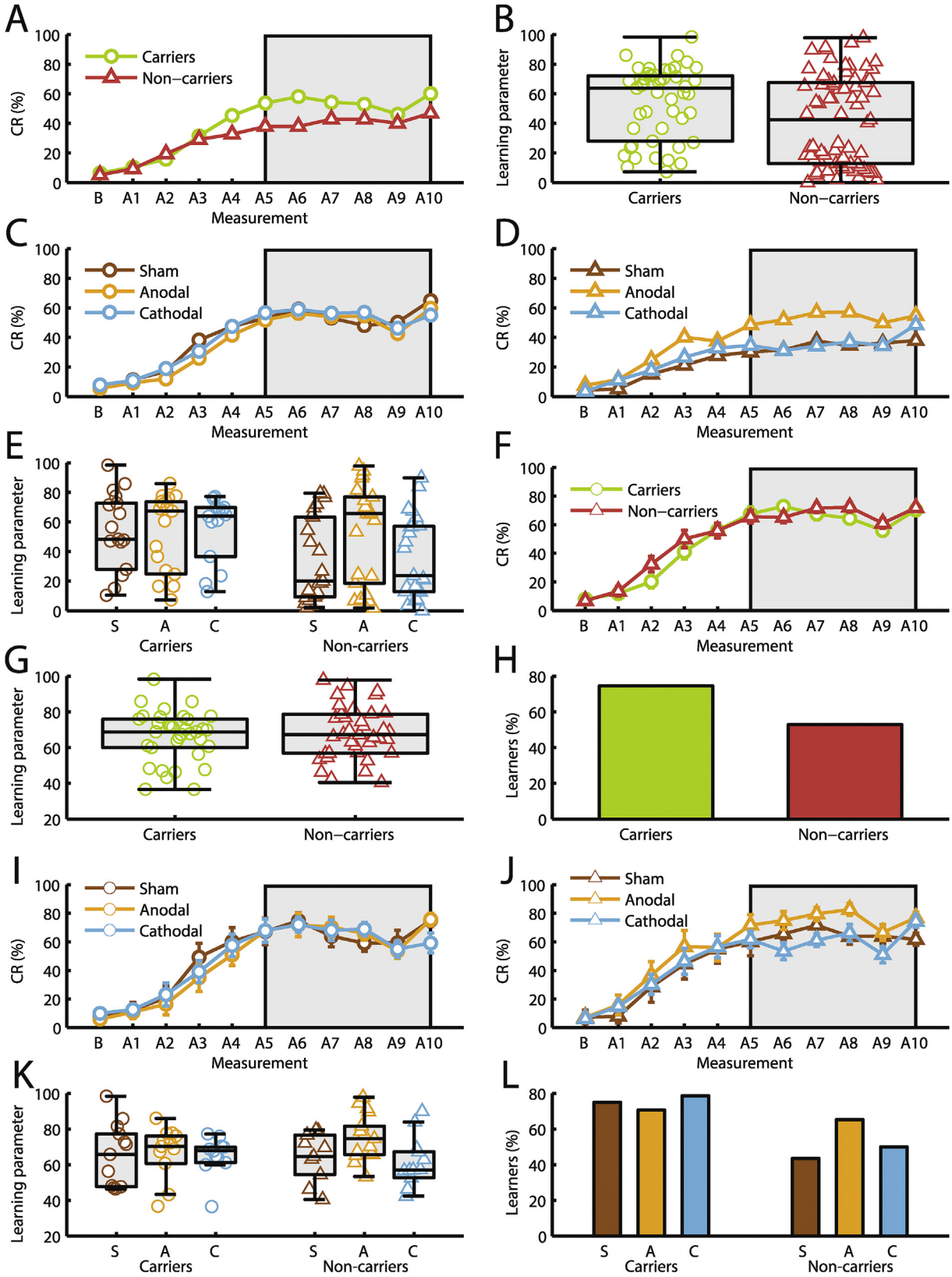

Fig. 3. Role of BDNF Val66Met and cerebellar tDCS in eyeblink conditioning.
Carriers are displayed in green, non-carriers in red. Sham tDCS is shown in brown, anodal tDCS in orange and cathodal tDCS in blue. Circles indicate carriers. Triangles indicate non-carriers. Error bars represent the standard error of the mean. S = sham; A = anodal; C = cathodal.
A-E. Overall plots showing learners and non-learners combined.
A. Overall learning curves for carriers (n = 47) and non-carriers (n = 70). **B.** Overall whisker plots of the learning parameter for carriers (n = 47) and non-carriers (n = 70). **C.** Overall learning curves for carriers receiving sham (n = 16), anodal (n = 17) or cathodal tDCS (n = 14). **D.** Overall learning curves for non-carriers receiving sham (n = 23), anodal (n = 23) or

Please cite this article in press as: van der Vliet R, et al., Cerebellar transcranial direct current stimulation interacts with BDNF Val66Met in motor learning, Brain Stimulation (2018), https://doi.org/10.1016/j.brs.2018.04.009

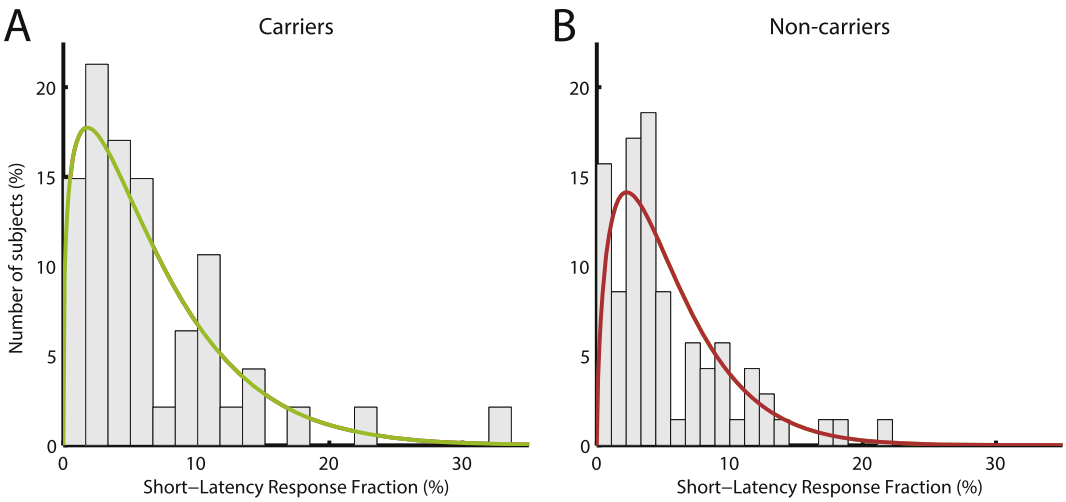

Fig. 4. Role of BDNF Val66Met in the short-latency response fraction.
A-B Histograms for the short-latency response fraction in carriers (A) and non-carriers (B).

presented as the mean difference between carriers and non-carriers with 95%ETIs. An effect size is considered significant if the ETI does not overlap with zero. All analyses were performed using three chains with 50,000 samples each and 20,000 burn-in samples in Openbugs version 3.2.3 (Openbugs foundation). Missing values are automatically handled by the Bayesian analysis and do not contribute to the posterior estimates of the model parameters [77]. Group results are described with medians and interquartile ranges.

Sample size calculation

We powered the eyeblink conditioning and VOR adaptation studies to find a positive effect of anodal cerebellar tDCS in the smaller non-carrier group (estimated as 30% of the population [18,19]). Based on [21,22], BDNF Val66Met carriers were predicted to learn 50% less than non-carriers. All power analyses included a drop-out rate of 10%. For eyeblink conditioning, tDCS effect sizes were based on [1] ($B_{Anodal,\ carrier}$=30%, $B_{Cathodal,\ carrier}$=-30%; population standard deviation of 20%). We estimated 35 subjects per group would give >90% power and included 40 subjects per group. For VOR adaptation, tDCS effect size was based on [2,61] ($B_{Anodal,\ carrier}$=15%, within-subject standard deviation of 15%). We estimated a group size of 50 subjects would give >90% power and included 55 subjects. The saccade and visuomotor adaptation studies were powered to find a $B_{Carrier} = 10\%$ given a population standard deviation of 10% and included 75 subjects.

Results

Eyeblink conditioning

We found that eyeblink conditioning was best captured with a bimodal distribution of the learning parameter (see Fig. 2A and Table 2), which is line with a recent study [78]. The main statistical analysis was therefore based on the 'Learner/non-learner model' and the results are presented in Table 3 ('Learner/non-learner model') and Fig. 3F–L. We found that whereas the learning parameter was similar for carriers and non-carriers (see Fig. 3F–G and Table 3), the percentage of learners was higher for carriers than for non-carriers (see Fig. 3H and Table 3). In the carrier group, neither anodal tDCS nor cathodal tDCS affected the learning parameter compared with sham (see Fig. 3I and K and Table 3). Similarly, neither anodal tDCS nor cathodal tDCS affected the percentage of learners compared with sham (see Fig. 3L). In the non-carrier group, anodal tDCS increased the learning parameter (see Fig. 3J–K and Table 3) compared with sham, but not the percentage of learners compared with sham (see Fig. 3L and Table 3). Cathodal tDCS did not affect the learning parameter nor the percentage of learners (see Fig. 3J–L and Table 3).

To give full data transparency, results of the 'single group' analysis are also presented in Table 3 ('Single group model') and Fig. 3A–E. In line with the 'Learner/non-learner' analysis, we found (1) an increase in the learning parameter for carriers compared to non-carriers (see Fig. 3A–B and Table 3), (2) no effect of cerebellar tDCS on the learning rate for carriers (see Fig. 3C,E and Table 3) and (3) an increase in the learning parameter with anodal stimulation for non-carriers (see Fig. 3D–E and Table 3).

There was no significant difference in the short latency response fraction between non carriers and carriers ($M_{Non\text{-}carrier}$ - $M_{Carrier} = -1.2\%$ 95%ETI = [-3.3–0.6]%) (see Fig. 4).

VOR adaptation

The learning parameter for VOR adaptation was best described by a unimodal distribution (see Fig. 2B and Table 2). We therefore performed the statistical analysis based on the 'single group' model only (see Fig. 5 and Table 3).

cathodal tDCS (n = 24). **E.** Overall whisker plots of the learning parameter for carriers and non-carriers receiving sham, anodal or cathodal tDCS.
F-L. Plots showing learners only.
F. Learning curves for carriers (n = 35) and non-carriers (n = 37) who were classified as learners. **G.** Whisker plots of the learning parameter for carriers and non-carriers who were classified as learners. **H.** Bar graphs of the proportion of learners in the carrier and non-carrier group. **I.** Learning curves for carriers who were classified as learners and received sham (n = 12), anodal (n = 12) or cathodal tDCS (n = 11). **J.** Learning curves for non-carriers who were classified as learners and received sham (n = 10), anodal (n = 15) or cathodal tDCS (n = 12). **K.** Whisker plots of the learning parameter for carriers and non-carriers receiving sham, anodal or cathodal tDCS who were classified as learners. **L.** Bar graphs of the proportion of learners for carriers and non-carriers receiving sham, anodal or cathodal tDCS.

Please cite this article in press as: van der Vliet R, et al., Cerebellar transcranial direct current stimulation interacts with BDNF Val66Met in motor learning, Brain Stimulation (2018), https://doi.org/10.1016/j.brs.2018.04.009

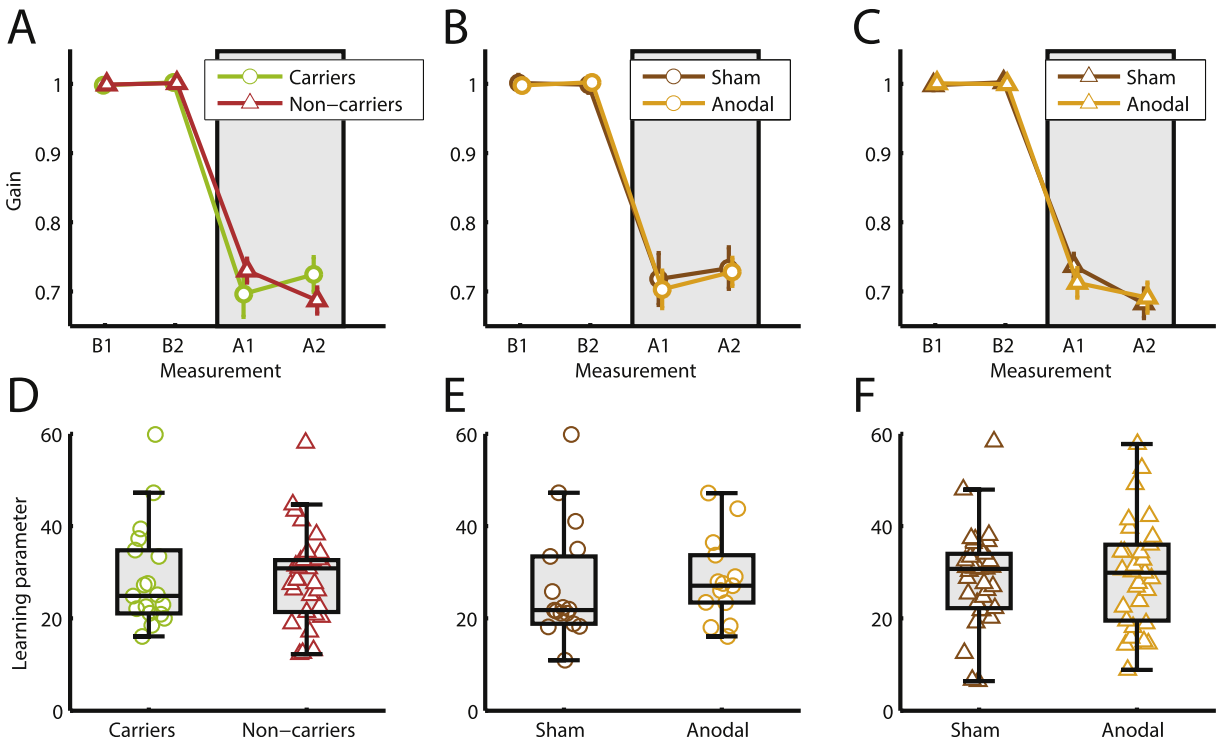

Fig. 5. Role of BDNF Val66Met and cerebellar tDCS in VOR adaptation.
A-C. Learning curves for (**A**) carriers and non-carriers, averaged over the two tDCS conditions, (**B**) carriers receiving sham and anodal tDCS and (**C**) non-carriers receiving sham and anodal tDCS. **D-F.** Learning parameters for (**D**) carriers and non-carriers, averaged over the two tDCS conditions, (**E**) carriers receiving sham and anodal tDCS and (**F**) non-carriers receiving sham and anodal tDCS.

The learning parameter was similar for carriers and non-carriers (see Fig. 5A and C and Table 3). In carriers, no effect of anodal tDCS was found compared with sham (see Fig. 5B and E and Table 3). Similarly, in non-carriers, no effect of anodal tDCS was found compared with sham (see Fig. 5C and F and Table 3).

Saccade adaptation and visuomotor adaptation

To further investigate whether a role for BDNF Val66Met is absent in cerebellum-dependent motor adaptation, we performed additional saccade and visuomotor adaptation tasks. Genetic analysis failed in 3/75 individuals leaving 72 for analysis.

The learning parameters of saccade and visuomotor adaptation were best described by a unimodal distribution (see Fig. 2C–D and Table 2) and therefore analyzed with the 'single group' model only. For saccade adaptation, no difference was found for the learning parameter between carriers and non-carriers (see Fig. 6 E–F and Table 3). Similarly, for visuomotor adaptation, no difference was found for the learning parameter between carriers and non-carriers (see Fig. 6 G–H and Table 3).

Discussion

Role of BDNF Val66Met in cerebellum-dependent learning

The higher proportion of eyeblink conditioning learners in carriers compared to non-carriers could depend on modulation of cerebellar activity. Within the cerebellum, BDNF released from mossy fibers [24] may control the response of both granule cells and Purkinje cells to GABA [79] and thereby keep baseline simple spike firing frequency and the potential for conditioning within normal limits. Carriers of the BDNF Val66Met polymorphism on the other hand are expected to have an altered granule and Purkinje cell response to GABA, which may increase baseline simple spike firing frequency allowing for stronger disinhibition of cerebellar nuclei neurons and faster eyeblink conditioning [80]. Why then does the polymorphism not affect adaptation? Learning mechanisms for gain-decrease VOR adaptation, gain-decrease saccade adaptation and visuomotor adaptation are believed to depend more on synaptic plasticity in cerebellar and vestibular nuclei rather than the cerebellar cortex, and might be less directly related to baseline simple spike firing frequencies [44–48,81,82].

Alternatively, BDNF Val66Met might also influence other brain regions that are involved in eyeblink conditioning, like the amygdala [30–34] and the hippocampus [30]. We did not find a difference in short latency responses between carriers and non-carriers, which makes a direct effect of the amygdala unlikely [31]. However, it has been suggested that the amygdala can enhance eyeblink conditioning indirectly, by modulating the saliency of the conditioned stimulus [34]. In contrast, the hippocampus is believed to inhibit eyeblink conditioning [30]. Indeed, lower BDNF concentrations in the mouse hippocampus have been associated with faster eyeblink conditioning [83]. Furthermore, BDNF Val66Met carriers show stronger cued fear conditioning, with decreased activity in the hippocampus and increased activity in the amygdala [25]. This extracerebellar hypothesis is also compatible with the null effect of BDNF Val66Met in the adaptation tasks, which do not depend on the hippocampus or amygdala [41,84].

The relevance of BDNF Val66Met for eyeblink conditioning might extend to other cerebellum-dependent modalities of motor, emotional and cognitive associative learning [85] and pathologies of cerebellum-dependent associative learning such as schizophrenia [86,87].

Please cite this article in press as: van der Vliet R, et al., Cerebellar transcranial direct current stimulation interacts with BDNF Val66Met in motor learning, Brain Stimulation (2018), https://doi.org/10.1016/j.brs.2018.04.009

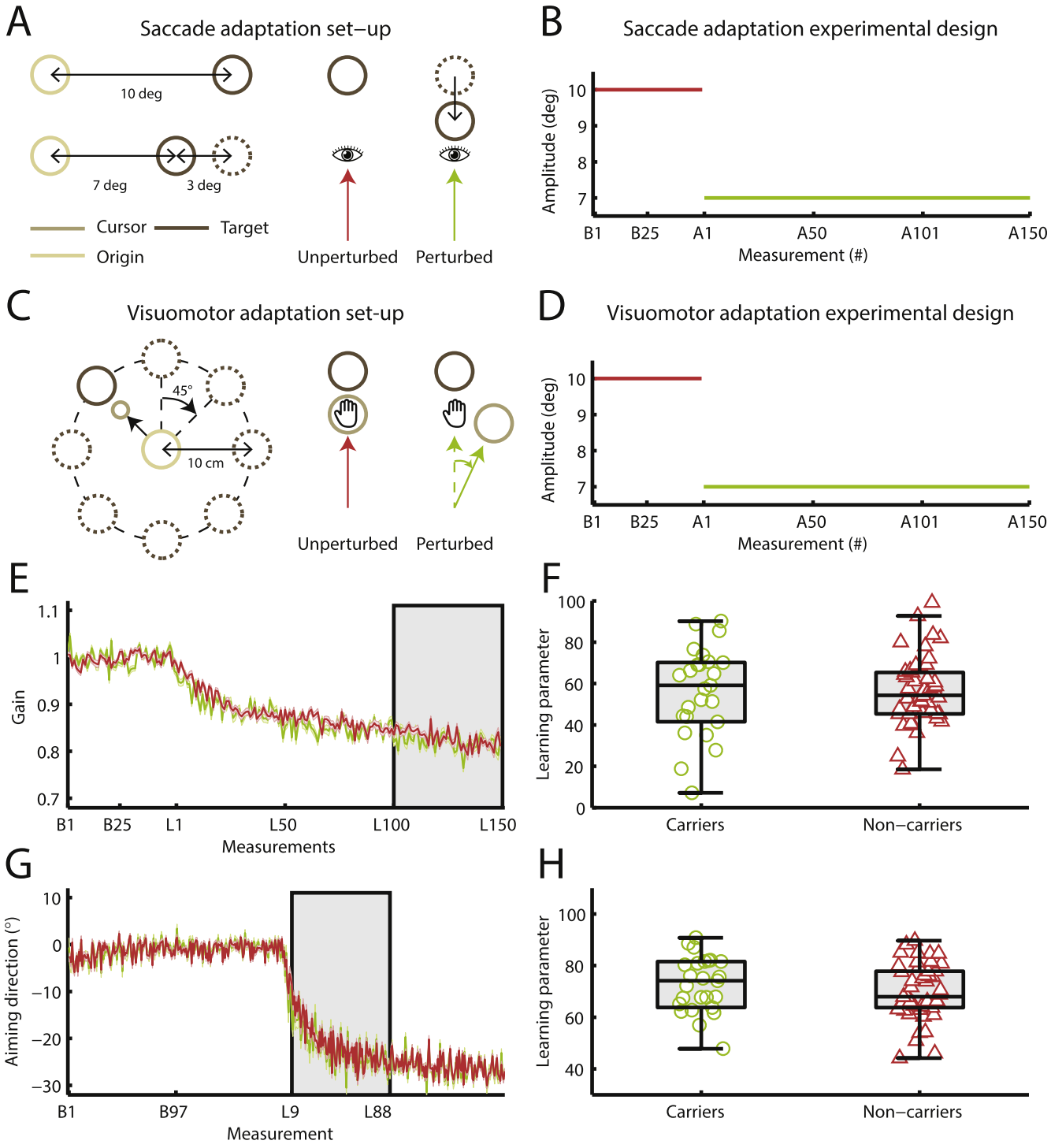

Fig. 6. Role of BDNF Val66Met in saccade and visuomotor adaptation.
A. Saccade adaptation set-up. Subjects were instructed to look at the origin (red circle 0.25° of visual angle radius) displayed on a black background, 5° of visual angle left of the center of the screen. After a uniformly distributed random delay between 400 and 1400 ms, the origin disappeared and a target (red circle 0.25° of visual angle radius) appeared 5° of visual angle right of the center of the screen. Saccades were detected online using a velocity threshold of 60°/s and a boundary threshold of 1.2° to the right of the fixation position. If no saccade was detected after 500 ms, the screen was blanked for 500 ms and the trial was restarted showing the origin. The duration of one trial was 3000 ms. In unperturbed trials (red line), the target was shown at a fixed location 10° to the right of the origin from presentation start until trial end. In perturbed trials (blue line), the target was displaced 3° of visual angle inward as soon as a saccade was detected i.e., during the saccade. **B.** Saccade adaptation experimental design. The experiment included baseline measurements of 50 unperturbed trials (red line, B1-50), followed by learning measurements of 150 perturbed trials (green line, L1-150). **C.** Visuomotor adaptation set-up. Subjects were instructed to make straight rapid shooting movements from the origin towards the target. A trial started when the cursor (position of robotic handle; green circle 2 mm radius) was within 0.5 cm of the origin (red circle 2 mm radius) for 1 s, with the appearance of the target (red circle 2 mm radius) at one of 8 positions. A trial ended when the robotic handle passed an (invisible) circle with 10 cm radius around the origin or trial duration exceeded 2 s. At this point, the cursor was shown at its last position until the start of the next trial and the movement was dampened. Color cues were given to keep movement velocity in a tight range (blue when too slow >600 ms; yellow when too fast <400 ms; green when correct 400–600 ms). The cursor reappeared at its measured position when located 0.5 cm from the origin. In unperturbed trials (red lines), the cursor was shown at the location of the robotic handle while in perturbed trials (green lines) cursor position was rotated 30° clockwise around the origin with respect to manipulandum position. **D.** Visuomotor adaptation experimental design. The experiment design included baseline measurements of unperturbed trials (red line, B1-192) and learning measurements of perturbed trials (green line, L1-200). **E.** Role of BDNF Val66Met in saccade adaptation. Learning curves (left column) and learning parameters (right column) for carriers of the BDNF Val66Met polymorphism (green) and non-carriers (red). **F.** Role of BDNF Val66Met in visuomotor adaptation. Learning curves (left column) and learning parameters (right column) for carriers of the BDNF Val66Met polymorphism (green) and non-carriers (red).

Please cite this article in press as: van der Vliet R, et al., Cerebellar transcranial direct current stimulation interacts with BDNF Val66Met in motor learning, Brain Stimulation (2018), https://doi.org/10.1016/j.brs.2018.04.009

Mechanisms of cerebellar tDCS

The interaction between cerebellar tDCS and BDNF Val66Met in eyeblink conditioning might point to a common effect on simple spike firing frequency. Anodal tDCS only increases eyeblink conditioning in non-carriers, who learn more slowly and have higher activity-dependent BDNF release. However, it seems unlikely that the effect of anodal tDCS in the cerebellum is mediated by BDNF release, as has been suggested for the motor cortex [22], because this would decrease rather than increase the eyeblink conditioning response. Rather, we expect anodal tDCS to directly modulate the baseline simple spike firing frequency of cerebellar neurons through subthreshold depolarization [36,37,39,40,88,89]. Carriers might be less sensitive to subthreshold depolarization, because baseline firing frequency is already increased (1) as a direct result of diminished BDNF release or (2) as a result of stronger excitation by the amygdala. In contrast, no effect of cerebellar tDCS on VOR adaptation was found for either carriers or non-carriers, which might be related to a minor role for simple spike firing in VOR adaptation compared to eyeblink conditioning [46,48]. Alternatively, the cerebellar flocculus, which is involved in VOR adaptation is located deeper in the cerebellum than Lobule VI, which is involved in eyeblink conditioning, and the local electric field strength [51] might therefore be insufficient for cerebellar tDCS to have an effect. Modeling-based approaches are necessary to further explore this open question [12].

The complex interaction between (1) cerebellar tDCS, (2) anatomical substrates and neurophysiological mechanisms of motor learning, and (3) genetic factors requires detailed animal studies combining electrophysiological and behavioral experiments to further develop cerebellar tDCS as a neuromodulatory technique.

Variable results of cerebellar tDCS

The interaction between BDNF Val66Met and anodal tDCS might explain some of the inconsistency in cerebellar tDCS literature. The null result for anodal tDCS found by Beyer et al. compared with increased eyeblink conditioning found by Zuchowski et al. [1] might have resulted from a higher proportion of carriers in the subject population of Beyer et al. [14]. However, decreased eyeblink conditioning with cathodal tDCS [1] could only be explained from our results by an uneven distribution of non-learners. In addition, since no interaction between cerebellar tDCS and BDNF Val66Met in VOR adaptation was found, as well as no direct effect of BDNF Val66Met on VOR adaptation, saccade adaptation and visuomotor adaptation, we do not think conflicting literature results in other tasks, such as visuomotor adaptation [2,4,10,13], can be explained by our results. Possibly, other individual determinants are important in these tasks.

Careful characterization of genetic and other individual factors will be necessary in future (pre)clinical studies of cerebellar tDCS to decrease response variability and identify non-learners who do not benefit from stimulation.

Declarations of interest

None.

Acknowledgements

This work was supported by ZonMw (project # 10-10400-98-008; MF, RS), ERC-adv (project # 294775; CDZ) and ERC-Poc (project # 768914; CDZ), ZonMw (project # 91210067; CDZ), and NWO-ALW (project # 854.10.004; CDZ).

References

[1] Zuchowski ML, Timmann D, Gerwig M. Acquisition of conditioned eyeblink responses is modulated by cerebellar tDCS. Brain Stimul 2014;7:525–31. https://doi.org/10.1016/j.brs.2014.03.010.
[2] Galea JM, Vazquez A, Pasricha N, de Xivry JJ, Celnik P. Dissociating the roles of the cerebellum and motor cortex during adaptive learning: the motor cortex retains what the cerebellum learns. Cerebr Cortex 2011;21:1761–70. https://doi.org/10.1093/cercor/bhq246.
[3] Jayaram G, Tang B, Pallegadda R, Vasudevan EVL, Celnik P, Bastian A. Modulating locomotor adaptation with cerebellar stimulation. J Neurophysiol 2012;107:2950–7. https://doi.org/10.1152/jn.00645.2011.
[4] Block H, Celnik P. Stimulating the cerebellum affects visuomotor adaptation but not intermanual transfer of learning. Cerebellum 2013;12:781–93. https://doi.org/10.1007/s12311-013-0486-7.
[5] Herzfeld DJ, Pastor D, Haith AM, Rossetti Y, Shadmehr R, O'Shea J. Contributions of the cerebellum and the motor cortex to acquisition and retention of motor memories. Neuroimage 2014;98:147–58. https://doi.org/10.1016/j.neuroimage.2014.04.076.
[6] Avila E, Van Der Geest JN, Kamga SK, Verhage MC, Donchin O, Frens MA, et al. Cerebellar transcranial direct current stimulation effects on saccade adaptation. Neural Plast 2015;2015:1–9. https://doi.org/10.1155/2015/968970.
[7] Shah B, Nguyen TT, Madhavan S. Polarity independent effects of cerebellar tDCS on short term ankle visuomotor learning. Brain Stimul 2013;6:966–8. https://doi.org/10.1016/j.brs.2013.04.008.
[8] Panouilleres MTTN, Miall RC, Jenkinson N. The role of the posterior cerebellum in saccadic adaptation: a transcranial direct current stimulation study. J Neurosci 2015;35:5471–9. https://doi.org/10.1523/JNEUROSCI.4064-14.2015.
[9] Das S, Holland P, Schonewille M, de Zeeuw C, Frens MA, Donchin O. Polarity-dependent effects of trans-cranial direct current stimulation (tDCS) in cerebellar learning depends on the state of neuronal network. Brain Stimul 2014;7:e3. https://doi.org/10.1016/j.brs.2014.01.014.
[10] Hardwick RM, Celnik PA. Cerebellar direct current stimulation enhances motor learning in older adults. Neurobiol Aging 2014;35:2217–21. https://doi.org/10.1016/j.neurobiolaging.2014.03.030.
[11] van Dun K, Bodranghien FCAA, Mariën P, Manto MU. tDCS of the cerebellum: where do we stand in 2016? Technical issues and critical review of the literature. Front Hum Neurosci 2016;10:199. https://doi.org/10.3389/fnhum.2016.00199.
[12] Ferrucci R, Bocci T, Cortese F, Ruggiero F, Priori A. Cerebellar transcranial direct current stimulation in neurological disease. Cerebellum Ataxias 2016;3:16. https://doi.org/10.1186/s40673-016-0054-2.
[13] Jalali R, Miall RC, Galea JM. No consistent effect of cerebellar transcranial direct current stimulation (tDCS) on visuomotor adaptation. J Neurophysiol 2017. https://doi.org/10.1152/jn.00896.2016. jn.00896.2016.
[14] Beyer L, Batsikadze G, Timmann D, Gerwig M. Cerebellar tDCS effects on conditioned eyeblinks using different electrode placements and stimulation protocols. Front Hum Neurosci 2017;11:23. https://doi.org/10.3389/fnhum.2017.00023.
[15] Hulst T, John L, Küper M, van der Geest JN, Göricke SL, Donchin O, et al. Cerebellar patients do not benefit from cerebellar or M1 transcranial direct current stimulation during force-field reaching adaptation. J Neurophysiol 2017;118:732–48. https://doi.org/10.1152/jn.00808.2016.
[16] Furuya S, Klaus M, Nitsche MA, Paulus W, Altenmuller E. Ceiling effects prevent further improvement of transcranial stimulation in skilled musicians. J Neurosci 2014;34:13834–9. https://doi.org/10.1523/JNEUROSCI.1170-14.2014.
[17] Buch ER, Santarnecchi E, Antal A, Born J, Celnik PA, Classen J, et al. Effects of tDCS on motor learning and memory formation: a consensus and critical position paper. Clin Neurophysiol 2017;128:589–603. https://doi.org/10.1016/j.clinph.2017.01.004.
[18] Cargill M, Altshuler D, Ireland J, Sklar P, Ardlie K, Patil N, et al. Characterization of single-nucleotide polymorphisms in coding regions of human genes. Nat Genet 1999;22:231–8. https://doi.org/10.1038/10290.
[19] Shimizu E, Hashimoto K, Iyo M. Ethnic difference of the BDNF 196G/A (val66met) polymorphism frequencies: the possibility to explain ethnic mental traits. Am J Med Genet B Neuropsychiatr Genet 2004;126B:122–3. https://doi.org/10.1002/ajmg.b.20118.
[20] Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A, et al. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell 2003;112:257–69. doi:S0092-8674(03)00035-7.
[21] McHughen SA, Rodriguez PF, Kleim JA, Kleim ED, Marchal Crespo L, Procaccio V, et al. BDNF val66met polymorphism influences motor system function in the human brain. Cerebr Cortex 2010;20:1254–62. https://doi.org/10.1093/cercor/bhp189.
[22] Fritsch B, Reis J, Martinowich K, Schambra HM, Ji Y, Cohen LG, et al. Direct current stimulation promotes BDNF-dependent synaptic plasticity: potential implications for motor learning. Neuron 2010;66:198–204. https://doi.org/10.1016/j.neuron.2010.03.035.
[23] Park H, Poo M. Neurotrophin regulation of neural circuit development and function. Nat Rev Neurosci 2013;14:7–23. https://doi.org/10.1038/nrn3379.

[24] Chen AI, Zang K, Masliah E, Reichardt LF, Chao MV, Rajagopal R, et al. Glutamatergic axon-derived BDNF controls GABAergic synaptic differentiation in the cerebellum. Sci Rep 2016;6:20201. https://doi.org/10.1038/srep20201.
[25] Soliman F, Glatt CE, Bath KG, Levita L, Jones RM, Pattwell SS, et al. A genetic variant BDNF polymorphism alters extinction learning in both mouse and human. Science (80-) 2010;327:863–6. https://doi.org/10.1126/science.1181886.
[26] Yeo CH, Hardiman MJ, Glickstein M. Classical conditioning of the nictitating membrane response of the rabbit. I. Lesions of the cerebellar nuclei. Exp Brain Res 1985;60:87–98. https://doi.org/10.1007/BF00237022.
[27] Yeo CH, Hardiman MJ, Glickstein M. Classical conditioning of the nictitating membrane response of the rabbit. II. Lesions of the cerebellar cortex. Exp Brain Res 1985;60:99–113. https://doi.org/10.1007/BF00237023.
[28] Jirenhed D-A, Bengtsson F, Hesslow G. Acquisition, extinction, and reacquisition of a cerebellar cortical memory trace. J Neurosci 2007;27:2493–502. https://doi.org/10.1523/JNEUROSCI.4202-06.2007.
[29] McCormick DA, Lavond DG, Clark GA, Kettner RE, Rising CE, Thompson RF. The engram found? Role of the cerebellum in classical conditioning of nictitating membrane and eyelid responses. Bull Psychonomic Soc 1981;18:103–5. https://doi.org/10.3758/BF03333573.
[30] Lee T, Kim JJ. Differential effects of cerebellar, amygdalar, and hippocampal lesions on classical eyeblink conditioning in rats. J Neurosci 2004;24. https://doi.org/10.1523/JNEUROSCI.5382-03.2004.
[31] Boele HJ, Koekkoek SKE, De Zeeuw CI. Cerebellar and extracerebellar involvement in mouse eyeblink conditioning: the ACDC model. Front Cell Neurosci 2010;3:19. https://doi.org/10.3389/neuro.03.019.2009.
[32] Siegel JJ, Taylor W, Gray R, Kalmbach B, Zemelman BV, Desai NS, et al. Trace eyeblink conditioning in mice is dependent upon the dorsal medial prefrontal cortex, cerebellum, and amygdala: behavioral characterization and functional Circuitry(1,2,3). eNeuro 2015;2. https://doi.org/10.1523/ENEURO.0051-14.2015.
[33] Sakamoto T, Endo S. Amygdala, deep cerebellar nuclei and red nucleus contribute to delay eyeblink conditioning in C57BL/6 mice. Eur J Neurosci 2010;32:1537–51. https://doi.org/10.1111/j.1460-9568.2010.07406.x.
[34] Farley SJ, Radley JJ, Freeman JH. Amygdala modulation of cerebellar learning. J Neurosci 2016;36:2190–201. https://doi.org/10.1523/JNEUROSCI.3361-15.2016.
[35] De Zeeuw CI, Ten Brinke MM. Learning and memory. In: Kandel ER, Dudai Y, Mayford M, editors. Learn. Mem. first ed. Cold Harbor Spring: Cold Harbor Spring Laboratories; 2016. p. 389.
[36] ten Brinke MM, Boele H-J, Spanke JK, Potters J-W, Kornysheva K, Wulff P, et al. Evolving models of pavlovian conditioning: cerebellar cortical dynamics in awake behaving mice. Cell Rep 2015;13:1977–88. https://doi.org/10.1016/j.celrep.2015.10.057.
[37] Chan CY, Hounsgaard J, Nicholson C. Effects of electric fields on transmembrane potential and excitability of turtle cerebellar Purkinje cells in vitro. J Physiol 1988;402:751–71. https://doi.org/10.1113/jphysiol.1988.sp017232.
[38] Bikson M, Inoue M, Akiyama H, Deans JK, Fox JE, Miyakawa H, et al. Effects of uniform extracellular DC electric fields on excitability in rat hippocampal slices in vitro. J Physiol 2004;557:175–90. https://doi.org/10.1113/jphysiol.2003.055772.
[39] Chan CY, Nicholson C. Modulation by applied electric fields of Purkinje and stellate cell activity in the isolated turtle cerebellum. J Physiol 1986;371:89–114. https://doi.org/10.1113/jphysiol.1986.sp015963.
[40] Reato D, Rahman A, Bikson M, Parra LC. Low-intensity electrical stimulation affects network dynamics by modulating population rate and spike timing. J Neurosci 2010;30:15067–79. https://doi.org/10.1523/JNEUROSCI.2059-10.2010.
[41] Zee RJLDS, Leigh RJ, Zee DS. The neurology of eye movements. fourth ed. New York: Oxford University Press; 2006. http://10.0.4.69/med/9780199969289.001.0001.
[42] Blazquez PM, Hirata Y, Heiney SA, Green AM, Highstein SM. Cerebellar signatures of vestibulo-ocular reflex motor learning. J Neurosci 2003;23:9742–51.
[43] Hirata Y, Highstein SM. Acute adaptation of the vestibuloocular reflex: signal processing by floccular and ventral paraflocculus Purkinje cells. J Neurophysiol 2001;85:2267–88.
[44] Lisberger SG, Pavelko TA, Bronte-Stewart HM, Stone LS. Neural basis for motor learning in the vestibuloocular reflex of primates. II. Changes in the responses of horizontal gaze velocity Purkinje cells in the cerebellar flocculus and ventral paraflocculus. J Neurophysiol 1994;72:954–73.
[45] Lisberger SG. Neural basis for motor learning in the vestibuloocular reflex of primates. III. Computational and behavioral analysis of the sites of learning. J Neurophysiol 1994;72:974–98.
[46] Carcaud J, França de Barros F, Idoux E, Eugène D, Reveret L, Moore LE, et al. Long-lasting visuo-vestibular mismatch in freely-behaving mice reduces the vestibulo-ocular reflex and leads to neural changes in the direct vestibular pathway. eNeuro 2017;4.
[47] Lisberger SG, Pavelko TA, Broussard DM. Neural basis for motor learning in the vestibuloocular reflex of primates. I. Changes in the responses of brain stem neurons. J Neurophysiol 1994;72:928–53.
[48] Voges K, Wu B, Post L, Schonewille M, De Zeeuw CI. Mechanisms underlying vestibulo-cerebellar motor learning in mice depend on movement direction. J Physiol 2017;595:5301–26. https://doi.org/10.1113/JP274346.
[49] Oldfield RC. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 1971;9:97–113.
[50] Galea JM, Jayaram G, Ajagbe L, Celnik P. Modulation of cerebellar excitability by polarity-specific noninvasive direct current stimulation. J Neurosci 2009;29:9115–22. https://doi.org/10.1523/JNEUROSCI.2184-09.2009.
[51] Rampersad SM, Janssen AM, Lucka F, Aydin Ü Lanfer B, Lew S, et al. Simulating transcranial direct current stimulation with a detailed anisotropic human head model. IEEE Trans Neural Syst Rehabil Eng 2014;22:441–52. https://doi.org/10.1109/TNSRE.2014.2308997.
[52] van Dun K, Bodranghien F, Manto M, Mariën P. Targeting the cerebellum by noninvasive neurostimulation: a review. Cerebellum 2016. https://doi.org/10.1007/s12311-016-0840-7.
[53] Gandiga PC, Hummel FC, Cohen LG. Transcranial DC stimulation (tDCS): a tool for double-blind sham-controlled clinical studies in brain stimulation. Clin Neurophysiol 2006;117:845–50. https://doi.org/10.1016/j.clinph.2005.12.003.
[54] van der Vliet R, Ribbers GM, Vandermeeren Y, Frens MA, Selles RW. BDNF Val66Met but not transcranial direct current stimulation affects motor learning after stroke. Brain Stimul 2017;10:882–92. https://doi.org/10.1016/j.brs.2017.07.004.
[55] Cason H. The conditioned eyelid reaction. J Exp Psychol 1922;5:153–96. https://doi.org/10.1037/h0074822.
[56] Smit AE, van der Geest JN, Vellema M, Koekkoek SKE, Willemsen R, Govaerts LCP, et al. Savings and extinction of conditioned eyeblink responses in fragile X syndrome. Gene Brain Behav 2008;7:770–7. https://doi.org/10.1111/j.1601-183X.2008.00417.x.
[57] Christian KM, Thompson RF. Neural substrates of eyeblink conditioning: acquisition and retention. Learn Mem 2003;10:427–55. https://doi.org/10.1101/lm.59603.
[58] Koekkoek SKE, Den Ouden WL, Perry G, Highstein SM, De Zeeuw CI. Monitoring kinetic and frequency-domain properties of eyelid responses in mice with magnetic distance measurement technique. J Neurophysiol 2002;88:2124–33.
[59] Tiliket C, Shelhamer M, Roberts D, Zee DS. Short-term vestibulo-ocular reflex adaptation in humans. I. Effect on the ocular motor velocity-to-position neural integrator. Exp Brain Res 1994;100:316–27. https://doi.org/10.1007/BF00227201.
[60] Shelhamer M, Tiliket C, Roberts D, Kramer PD, Zee DS. Short-term vestibulo-ocular reflex adaptation in humans. II. Error signals. Exp Brain Res 1994;100:328–36. https://doi.org/10.1007/BF00227202.
[61] Montfoort I, Van Der Geest JN, Slijper HP, De Zeeuw CI, Frens MA. Adaptation of the cervico- and vestibulo-ocular reflex in whiplash injury patients. J Neurotrauma 2008;25:687–93. https://doi.org/10.1089/neu.2007.0314.
[62] Schubert MC, Migliaccio AA, Minor LB, Clendaniel RA. Retention of VOR gain following short-term VOR adaptation. Exp Brain Res 2008;187:117–27. https://doi.org/10.1007/s00221-008-1289-9.
[63] Yakushin SB, Palla A, Haslwanter T, Bockisch CJ, Straumann D. Dependence of adaptation of the human vertical angular vestibulo-ocular reflex on gravity. Exp Brain Res 2003;152:137–42. https://doi.org/10.1007/s00221-003-1543-0.
[64] Yakushin SB, Bukharina SE, Raphan T, Buttner-Ennever J, Cohen B. Adaptive changes in the angular VOR: duration of gain changes and lack of effect of nodulo-uvulectomy. Ann N Y Acad Sci 2003;1004:78–93.
[65] Monte-Silva K, Kuo M-F, Hessenthaler S, Fresnoza S, Liebetanz D, Paulus W, et al. Induction of late LTP-like plasticity in the human motor cortex by repeated non-invasive brain stimulation. Brain Stimul 2013;6:424–32. https://doi.org/10.1016/j.brs.2012.04.011.
[66] Lefebvre S, Laloux P, Peeters A, Desfontaines P, Jamart J, Vandermeeren Y. Dual-tDCS enhances online motor skill learning and long-term retention In chronic stroke patients. Front Hum Neurosci 2012;6:343. https://doi.org/10.3389/fnhum.2012.00343.
[67] Watanabe S, Hattori K, Koizuka I. Flexibility of vestibulo-ocular reflex adaptation to modified visual input in human. Auris Nasus Larynx 2003;30:S29–34. https://doi.org/10.1016/S0385-8146(02)00134-7.
[68] van der Geest JN, Frens MA. Recording eye movements with video-oculography and scleral search coils: a direct comparison of two methods. J Neurosci Meth 2002;114:185–95.
[69] McLaughlin SC. Parametric adjustment in saccadic eye movements. Percept Psychophys 1967;2:359–62. https://doi.org/10.3758/BF03210071.
[70] Coesmans M, Röder C, Smit A, Koekkoek S, De Zeeuw C, Frens M, et al. Cerebellar motor learning deficits in medicated and medication-free men with recent-onset schizophrenia. J Psychiatry Neurosci 2014;39:E3–11. https://doi.org/10.1503/jpn.120205.
[71] Frens MA, van Opstal AJ. Transfer of short-term adaptation in human saccadic eye movements. Exp Brain Res 1994;100:293–306. https://doi.org/10.1007/BF00227199.
[72] Krakauer JW, Pine ZM, Ghilardi MF, Ghez C. Learning of visuomotor transformations for vectorial planning of reaching trajectories. J Neurosci 2000;20:8916–24.
[73] Tseng Y-WW, Diedrichsen J, Krakauer JW, Shadmehr R, Bastian AJ. Sensory prediction errors drive cerebellum-dependent adaptation of reaching. J Neurophysiol 2007;98:54–62. https://doi.org/10.1152/jn.00266.2007.
[74] Werner S, van Aken BC, Hulst T, Frens MA, van der Geest JN, Strüder HK, et al. Awareness of sensorimotor adaptation to visual rotations of different size. PLoS One 2015;10. https://doi.org/10.1371/journal.pone.0123321. e0123321.

Please cite this article in press as: van der Vliet R, et al., Cerebellar transcranial direct current stimulation interacts with BDNF Val66Met in motor learning, Brain Stimulation (2018), https://doi.org/10.1016/j.brs.2018.04.009

[75] Rabe K, Livne O, Gizewski ER, Aurich V, Beck A, Timmann D, et al. Adaptation to visuomotor rotation and force field perturbation is correlated to different brain areas in patients with cerebellar degeneration. J Neurophysiol 2009;101. https://doi.org/10.1152/jn.91069.2008.

[76] Celeux G, Forbes F, Robert CP, Titterington DM. Deviance information criteria for missing data models. Bayesian Anal 2006;1:651–73. https://doi.org/10.1214/06-BA122.

[77] Kruschke JK. Doing Bayesian data analysis. Academic Press; 2010. a tutorial with R, JAGS, and Stan.

[78] Löwgren K, Bååth R, Rasmussen A, Boele H-J, Koekkoek SKE, De Zeeuw CI, et al. Performance in eyeblink conditioning is age and sex dependent. PLoS One 2017;12. https://doi.org/10.1371/journal.pone.0177849. e0177849.

[79] Cheng Q, Yeh HH. PLCgamma signaling underlies BDNF potentiation of Purkinje cell responses to GABA. J Neurosci Res 2005;79:616–27. https://doi.org/10.1002/jnr.20397.

[80] De Zeeuw CI, Ten Brinke MM. Motor learning and the cerebellum. Cold Spring Harb Perspect Biol 2015;7. https://doi.org/10.1101/cshperspect.a021683. a021683.

[81] Robinson FR, Fuchs AF, Noto CT. Cerebellar influences on saccade plasticity. Ann N Y Acad Sci 2002;956:155–63. https://doi.org/10.1111/j.1749-6632.2002.tb02816.x.

[82] Donchin O, Rabe K, Diedrichsen J, Lally N, Schoch B, Gizewski ER, et al. Cerebellar regions involved in adaptation to force field and visuomotor perturbation. J Neurophysiol 2012;107:134–47. https://doi.org/10.1152/jn.00007.2011.

[83] Janke KL, Cominski TP, Kuzhikandathil EV, Servatius RJ, Pang KCH. Investigating the role of hippocampal BDNF in anxiety vulnerability using classical eyeblink conditioning. Front Psychiatr 2015;6:106. https://doi.org/10.3389/fpsyt.2015.00106.

[84] Krakauer JW, Ghilardi M-F, Mentis M, Barnes A, Veytsman M, Eidelberg D, et al. Differential cortical and subcortical activations in learning rotations and gains for reaching: a PET study. J Neurophysiol 2004;91. https://doi.org/10.1152/jn.00675.2003.

[85] Timmann D, Drepper J, Frings M, Maschke M, Richter S, Gerwig M, et al. The human cerebellum contributes to motor, emotional and cognitive associative learning. A review. Cortex 2010;46:845–57. https://doi.org/10.1016/j.cortex.2009.06.009.

[86] Andreasen NC, Pierson R. The role of the cerebellum in schizophrenia. Biol Psychiatr 2008;64:81–8. https://doi.org/10.1016/j.biopsych.2008.01.003.

[87] Yeganeh-Doost P, Gruber O, Falkai P, Schmitt A. The role of the cerebellum in schizophrenia: from cognition to molecular pathways. Clinics (Sao Paulo) 2011;66(Suppl 1):71–7. https://doi.org/10.1590/s1807-59322011001300009.

[88] Radman T, Ramos RL, Brumberg JC, Bikson M. Role of cortical cell type and morphology in subthreshold and suprathreshold uniform electric field stimulation in vitro. Brain Stimul 2009;2:215–28. https://doi.org/10.1016/j.brs.2009.03.007. 228.e1–3.

[89] Rahman A, Reato D, Arlotti M, Gasca F, Datta A, Parra LC, et al. Cellular effects of acute direct current stimulation: somatic and synaptic terminal effects. J Physiol 2013;591:2563–78. https://doi.org/10.1113/jphysiol.2012.247171.

Please cite this article in press as: van der Vliet R, et al., Cerebellar transcranial direct current stimulation interacts with BDNF Val66Met in motor learning, Brain Stimulation (2018), https://doi.org/10.1016/j.brs.2018.04.009