Assessing the Neurophysiological Effects of Cannabinoids on Spasticity in Multiple Sclerosis

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# Equal contribution

Abstract

Background: Spasticity is a disabling symptom in Multiple Sclerosis (MS). Cannabinoids have been proven to reduce the subjective feeling of spasticity and thus have been suggested as an effective therapeutic option in MS. The neurophysiological mechanisms underlying their clinical efficacy, however, remain poorly understood.

Objective: We combined neurophysiological methods to test the effect of cannabinoids on altered motor function in MS patients suffering from spasticity. We hypothesized that cannabinoids exert their beneficial effects through changes in motor cortical or spinal excitability.

Methods: Eighteen cannabis-naïve secondary progressive MS patients with spasticity were included in a double-blind, placebo-controlled, crossover study. Patients were treated with either placebo or Cannabis Based Medicine Extract (CBME). They were assessed clinically, as well as using functional MRI (fMRI) and electrophysiological methods. Plasma levels of tetrahydrocannabinol (THC) and cannabidiol (CBD) were tested.

Results: CBME treatment did not produce significant benefits on spasticity when compared with placebo. No change in fMRI motor-evoked brain activation was observed. There was no difference in intracortical and spinal motor excitability between CBME and placebo. No correlation was found between plasma levels of THC or CBD and electrophysiological or imaging measures.

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Conclusions: Cannabinoids do not exert beneficial effects on MS-related spasticity through a direct action on the motor system as assessed by fMRI and electrophysiological methods.

Keywords: Multiple Sclerosis, Cannabinoids, Spasticity, Functional MRI, TMS

Introduction

Spasticity is a common symptom in Multiple Sclerosis (MS), produced by lesions of descending inhibitory motor pathways[1] and often associated with muscle spasms and limitation of mobility. Due to their ability to reduce the subjective feeling of spasticity, cannabinoids have been proposed as a therapeutic option in MS either in monotherapy or as add-on therapy for patients who do not respond completely to other antispastic treatments.[2-8] With this indication, Nabiximols has been approved in 10 different countries.[9,10]

The endogenous cannabinoid system is tonically active in the control of spasticity primarily through effects on the CB1 receptor.[11] Cannabinoids inducing a long-lasting activation of CB1 receptors can cause persistent inhibition of neurotransmitter release from terminals expressing CB1 receptors.[12-15] Evidence suggests that cannabinoids can modulate motor cortical excitability also through ion channel activity, independently from cannabinoid receptors.[12-15] Indeed, endocannabinoids have been demonstrated to modulate the functional properties of voltage-gated ion channels including Ca2+, Na+ and K+ channels, as well as ligand-gated ion channels such as 5-HT3 and nicotinic acetylcholine receptors.[16] Despite the experimental evidence supporting complex effects of cannabinoids on motor control[17,18] and clinical evidence suggesting their efficacy on spasticity, mechanisms underlying the clinical benefits of cannabinoids in MS have not been fully clarified.

Functional magnetic resonance imaging (fMRI) and transcranial magnetic stimulation (TMS) provide the opportunity of reliably quantifying cortical functional reorganization under pharmacological treatment.[19-21] Here, we use fMRI and electrophysiological methods to interrogate the motor system and assess the effect of Cannabis-Based Medicine Extract (CBME, Sativex, GW Pharma, Salisbury, UK)[22,23,6] on altered movement-related patterns of brain activation and cortical excitability in MS patients suffering from spasticity. If cannabinoid effects on spasticity are mediated by changes in motor cortical excitability, we would predict a reduction of spasticity in MS patients associated with measurable changes in activation and excitability of motor areas.

Materials and Methods

Patients

Right-handed secondary-progressive MS patients[24] with normal right hand function were included. Eligibility criteria were: baseline Expanded Disability Status Scale (EDSS)[25] score between 3.5 and 6.5; clinically stable disease for the preceding 30 days; spasticity in at least two muscle groups, defined as a score ≥ 2 on the Ashworth scale[26] for each muscle group; stable antispastic treatment in the preceding 4 weeks; no disease modifying therapies started in the preceding 6 months; no clinical condition precluding safe participation; no cannabinoid use or concomitant therapy with antidepressants, psychoactive drugs, corticosteroids prior to the study entry.

The Ethics Committee of Sapienza University of Rome approved the study. Participants gave written informed consent.

Study design and clinical measurements

This was a 10-week, randomised, double blind, placebo-controlled, crossover trial (Figure. 01). At screening,
patients underwent clinical evaluation and MRI. At baseline EDSS, Ashworth Scale and the Numerical Rating Scale (NRS) of Spasticity were assessed. The Ashworth scale consists of a 5-point scale assessing the impairment resulting in spasticity. Each patient was assessed supine after resting for 15 min. For the Ashworth Scale, 10 muscle groups were assessed on each side. The NRS score is a subject-recorded scale in which 0 represents absence of, and 10 worst ever spasticity. Patients were randomly assigned to receive either CBME [Oromucosal whole plant cannabis medicine containing delta-9 tetrahydrocannabinol (THC) (27 mg/ml) and cannabidiol (CBD) (25 mg/ml)] or matched placebo (1:1 ratio). In this crossover study, each patient acted as his own control.

Figure 01 Study design. Laboratory tests included THC/CBD plasma level (at V2 and V4). Abbreviations: S=screening; V=visit.

Titration started off in clinic up to a maximum of 3 oromucosal sprays of CBME or placebo with an interval of 4 hours between each spray. Safety and tolerability were monitored and recorded. Patients were discharged after 8 hours of observation. Titration thereafter was determined on an individual basis as needed and as tolerated without exceeding the previous day’s dose by more than 50%. Subjects were also directed not to exceed 48 sprays per 24-hour interval. A three-week supply of study drug and a symptom diary were provided. Subjects were instructed to record the number of sprays of study drug that they used each day. During the 3-week treatment period, all patients reached either the optimal or the best tolerated, individualised dosage to subjectively relieve spasticity. After 3 weeks of treatment, patients underwent the fMRI scan, electrophysiological and clinical assessments, and THC and CBD level measurement. fMRI and electrophysiology were performed at drug steady state concentration, i.e., within 3 hours of the last CBME or placebo intake. After 2 weeks of washout, clinical evaluation was repeated, at the beginning of the subsequent 3-week study phase. After this period, patients repeated the clinical, laboratory, fMRI and electrophysiological assessments. The final assessment followed a 2-week washout period. A Treating Physician (E.O.) was responsible for drug administration and safety assessments. An Evaluating Physician (V.F.) performed clinical evaluations. A questionnaire testing
the success of masking was filled in at the end of each treatment phase.

Mean changes of spasticity scores for each treatment phase were quantified as greater or lower than 30% of the difference in pre vs. post-treatment scores. Ashworth Scale scores were compared using 2-way ANCOVA. The scores over the left and right side were summed within the upper and lower limbs yielding overall scores of 0-24 for upper and lower extremities. Treatment (number of sprays) and period effect (CBME vs. placebo) were included in the model as covariates. Changes in NRS were evaluated with a Wilcoxon analysis.

Plasma measurements

We measured plasma levels of THC and CBD using the LC-MS/MS spectrometric method of Valiveti and Stinchcomb[29] with slight modifications. Briefly, plasma samples were extracted with acetonitrile and then centrifuged at 10,000 g for 10 minutes. Supernatants were evaporated to dryness at 37°C under a gentle stream of nitrogen. The extract, reconstituted in 100 μl of acetonitrile, was injected into LC-MS-MS system. Quantitative analyses were performed using a PerkinElmer Micro Pump Series 200 interfaced with an API 2000 triple-quadrupole tandem mass spectrometer (Applied Biosystem). THC and CBD were separated on Reprosil-Pur Basic C18 column (150 x 4.6 mm, 5 μm) (Dr. Maish GmbH) and eluted isocratically with 2 mM ammonium formate - acetonitrile (20:80, v/v). Negative electrospray ionization was used and all analyses were performed in MRM mode. The transition were m/z 313→245 for Δ9-THC and CBD. The limit of quantitation (LOQ) was 0.1 ng/ml for Δ9-THC and CBD.

Spearman correlation assessed the relationship between THC/CBD levels and spasticity levels during CBME intake.

MRI data

Brain PD/T2-weighted images (WI) (TR/TE 2.000/20, 90 ms, matrix 256x256, FOV 24 cm, slice thickness 4 mm, no gap, 40 axial slices) and T1-WI (TR/TE 600/15 ms, matrix 256x256, FOV 24 cm, slice thickness 4 mm, no gap, 40 axial slices) were acquired on a 1.5T scanner (Philips Gyroscan NT 15, Netherlands) before and after the injection of a single dose (0.1 mg/kg) of gadolinium (Gd).

fMRI scans were performed using echo planar T2*-WI (TR 3000 ms, TE 50 ms, 90° flip angle, one excitation, matrix 64x64, FOV 24 cm, slice thickness 4 mm, no gap, 25 axial slices). During a block-design acquisition, patients performed with their right hands 7 blocks of acoustically cued active motor task alternated with 7 blocks of rest, followed by 7 blocks of passive motor task alternated with 7 blocks of rest. Both tasks consisted of simultaneous four-finger flexion-extension of the metacarpal-phalangeal joints. In the passive task a researcher guided the movement at a rate of 1 Hz.

Hyperintense T2 lesion load (LL) was calculated using Jim 3.0 (Xinapse System Ltd, 1 Aldwincle Road, Thorpe Waterville, NN14 3ED, UK).

fMRI data were analyzed using SPM2 (Wellcome Department of Cognitive Neuroscience, Institute of Neurology, University College of London, UK). Images were realigned, normalized and spatially smoothed using a Gaussian kernel of 8 mm. The time series from each subject was analyzed using the principles of the general linear model extended to allow the analysis of fMRI data.[22,23] Individual subject data were modelled using a boxcar design, convolved with the hemodynamic response function chosen to represent the relationship between neuronal activation and blood flow changes. Regions of task-related signal changes were thresholded on the basis of the amplitude (corrected p<0.05) and extent (corrected p<0.05) of the activation[30,31] For each subject task-related activations under CBME and placebo were tested during both active and passive conditions. Sec-
level analysis used paired t-test to determine changes in either active or passive motor activation, under either CBME or placebo. We tested for differences between fMRI changes induced by different treatments, as well as correlations between CBME-related changes during active or passive movements and clinical, paraclinical and electrophysiological measures. Within each region of statistical significance, local maxima of signal change (corrected $p<0.05$) were expressed in terms of x, y, z coordinates.\[32\]

**Electrophysiology**

Single-pulse TMS was delivered through a magnetic stimulator (Magstim 200 stimulator - The Magstim Company Ltd, Whitland, South West Wales, UK) connected to a circular coil over the vertex and over the cervical region to calculate the central motor conduction time (CMCT) as a subtraction of the mean motor evoked potential (MEP) latencies. Single- and paired-pulse TMS were also delivered over the left primary motor cortex with a figure-of-eight coil held tangent to the scalp with the handle pointing back and away from the midline at 45° over the optimal position for evoking an MEP in the contra-lateral first dorsal interosseous (FDI) muscle. Single TMS stimuli were delivered at rest and during muscle contraction (30% of the maximal effort, monitored through an audio-visual feedback, Tektronic 5103N oscilloscope) at intensity of 120% of resting motor threshold (RMT) (i.e., the lowest intensity able to evoke a MEP of 50 μV in at least 5/10 consecutive trials in the FDI muscle). We evaluated the peak-to-peak amplitude and latency of the MEPs in the trials at rest, and the duration of the cortical silent period (CSP) in trials performed during voluntary contraction.\[13\] Paired stimuli were delivered through two Magstim 200 stimulators connected by a Y cable to a figure-of-eight coil.\[34\] The short-interval intracortical inhibition (SICI) at 3 ms inter-stimulus interval (ISI) and intra-cortical facilitation (ICF) at 10 ms ISI were studied. The intensity of the conditioned stimulus was set at 80% of RMT and the test stimulus at 120% of RMT. Paired-pulses and single unconditioned stimuli were randomly delivered and 20 trials were performed for each condition. The peak-to-peak amplitude of the conditioned MEPs was expressed as a percentage of the unconditioned responses.

Electromyography (EMG) activity was recorded through surface electrodes placed over the right FDI muscle and filtered with a Digitimer D360 (bandwidth 20 Hz to 1 kHz, sampling rate 2 kHz) through a 1401 plus A/D laboratory interface (Cambridge Electronic Design, UK).

Peripheral nerve stimulation used a Digitimer D160 stimulator (Digitimer Ltd, Welyn, Herts, UK) with bipolar electrical stimuli to the right tibial nerve at the popliteal fossa\[35,36\] to deliver electrical stimuli at intensity able to produce a maximal M wave in the soleus muscle. The soleus H reflex was collected using a 1 ms rectangular pulse with a constant current. The stimulator was randomly triggered about every 20 s. The intensity was set in order to evoke the maximal H-reflex amplitude. Ten trials for each condition were collected. The baseline-peak M wave amplitude and the peak-to-peak H reflex amplitude were expressed as H reflex/M wave ratio.

RMT, MEP latency and amplitude, CSP duration, CMCT and H reflex/M wave ratio data were analyzed using a one-way ANOVA with drug (CBME vs. placebo) as the main factor. Data obtained in the paired pulse study were analyzed using a two-way ANOVA with drug and ISI (3 and 10 ms) as the main factors.

**RESULTS**

Eighteen out of 20 screened patients completed the study [12 women, median (range) age 51 years (37-59), median (range) disease duration 21.5 (5-35) years, median (range) EDSS 6.0 (6.0-6.5), baseline median (range) Ashworth Scale 12 (4-22), median (range) NRS 7 (3-9)]. At baseline, 5 patients were taking immunomodulatory or immunosuppressant agents and 10 were in stable treatment for relieving spasticity. All patients
maintained stable drug intake. CBME was well tolerated with minor adverse events such as fatigue, nausea and dizziness.

Clinical results and correlation with drug levels

In the pre-CBME assessment, the mean±SD Ashworth Scale score was 1.6±2.3 for the upper limbs and 11.3±5.5 for the lower limbs. In the post-CBME evaluation, the mean score was 1.6±2.0 for the upper limbs and 10.5±6.3 for the lower limbs. In the pre-placebo assessment, the mean±SD Ashworth Scale score was 1.3±2.2 for the upper limbs and 11.3±6.1 for the lower limbs. In the post-placebo evaluation, the mean score was 0.9±1.6 for the upper limbs and 10.7±6.1 for the lower limbs. The mean±SD values for the NRS were 6.7±2.0 and 5.9±2.5 before and after CBME, and 6.3±1.9 and 6.2±2.4 before and after placebo. Table. 01 reports changes in spasticity during CBME and placebo treatment.

Mean±SD change (post vs. pre) in total Ashworth scale was -0.66±3.34 for CBME and -0.83±2.50 for placebo (p=0.48). Sub-scores for upper-body muscle groups were 0.00±1.68 during CBME and -0.44±1.89 during placebo (p=0.60); for lower-body muscle groups, CBME-related changes were -0.67±2.09 and placebo-related changes were -0.39±2.06 (p=0.75). Mean±SD change in NRS scores (post vs. pre) was -0.78±2.67 during CBME and -0.06±2.71 during placebo (p=0.64).

Over the study period the median number of daily sprays was 7.4 (range 2.7 to 12.5) for CBME and 16.1 (range 6.7-26) for placebo (p<0.001). Mean±SD plasma concentration was 1.84±2.19 ng/ml (range 0.08-9.17) for THC and 2.13±2.39 ng/ml (range 0.25-11.23) for CBD at the end of the CBME period. At the end of the placebo period, CBD and THC plasma concentrations were undetectable. There was no correlation between THC and CBD levels and changes in spasticity during CBME period. No difference in THC and CBD plasma levels was found between patients improving and patients unchanged or worsened during CBME administration.

<table>
<thead>
<tr>
<th>Changes (%)</th>
<th>Ashworth UE</th>
<th>Ashworth LE</th>
<th>NRS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CBME</td>
<td>Placebo</td>
<td>CBME</td>
</tr>
<tr>
<td>Improvement ≥ 30%</td>
<td>1</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Improvement &lt; 30%</td>
<td>0</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>No change</td>
<td>12</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td>Worsening &lt; 30%</td>
<td>3</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Worsening ≥ 30%</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Table. 01 Changes in the Ashworth Scale and the Numerical Rating Scale (NRS) testing spasticity during the CBME and placebo treatments measured as differences between pre vs. post treatment period. The table reports the number of patients who show improvements, no change or worsening during CBME or placebo. UE: upper extremities; LE: lower extremities.

There was an association between the ongoing treatment phase and the Evaluating Physician’s assessment on whether the patient was on placebo or on CBME (p<0.01). According to the Evaluating Physician’s assess-
ment, 88.8% (n=16) of the CBME group and 11.1% (n=2) of the placebo group were on active treatment. Similarly, there was an association between the actual treatment and the patients’ perception of the received treatment (p<0.01). According to patients’ perception, 88.8% (n=16) of the CBME treatment and 27.7% (n=5) of the placebo treatment were allocated to the CBME group.

MRI results

Median T2-hyperintense LL was 10546.47 mm³ (range 468.42-42545.73). Four patients showed brain Gd-enhancing lesions (Gd-LL: 26.56 mm³, range 10.4-331.43).

There was no between-treatment difference in the movement rate during active (mean±SD CBME 0.93±0.13; mean±SD placebo 0.99±0.02, p=ns) and passive tasks (mean±SD CBME 0.95±0.10; mean±SD placebo 1.00±0.02, p=ns).

Foci of activation under placebo and CBME during task conditions are reported in Supplementary Tables. S01 and S02, and in Figure. 02

The active movement engaged the left primary motor cortex, right cerebellum and supplementary motor area (SMA) bilaterally both under placebo and CBME. Patients activated the left premotor and the right inferior frontal gyrus only under placebo. The left primary sensory cortex and the right insula were activated only under CBME. The passive movement activated the left primary sensory and premotor cortices, right cerebellum and SMA bilaterally both under placebo and under CBME. In addition, under CBME patients also activated the left superior temporal gyrus and insula bilaterally.

The average number of CBME sprays, as well as THC and CBD plasma concentrations did not correlate with activations in the CBME or in the placebo phases.
Electrophysiological results

Seventeen out of 18 patients underwent the electrophysiological measurements. One patient did not give his consent to the electrophysiological procedure. The TMS study was completed in 14 out 17 patients, as 3 patients complained of discomfort during the experimental procedure.

There was no difference in the RMT between the CBME and placebo groups ($F_{(1,13)}=0.16; p=0.69$). MEP amplitude ($F_{(1,13)}=1.13, p=0.30$), MEP latency ($F_{(1,13)}=0.18, p=0.68$), CMCT ($F_{(1,13)}=0.11, p=0.75$) and CSP duration ($F_{(1,13)}=0.40, p=0.45$) values were not different between treatment groups. Two-way ANOVA showed an effect of factor ISI ($F_{(2,26)}=10.86, p=0.0004$), but no effect of factor drug ($F_{(1,13)}=1.09, p=0.31$) and no interaction between factor drug and ISI ($F_{(2,26)}=0.14, p=0.86$) on the ICI and ICF values. Post-hoc analysis showed that conditioned MEP was inhibited at 3ms ISI and facilitated at 10ms ISI to a similar extent with CBME and placebo (Table. 02).

<table>
<thead>
<tr>
<th>Placebo</th>
<th>CBME</th>
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<tbody>
<tr>
<td>RMT (%) stimulator output</td>
<td>57.9±2.5</td>
</tr>
<tr>
<td>MEP latency (ms)</td>
<td>28.2±1.1</td>
</tr>
<tr>
<td>MEP amplitude (mV)</td>
<td>0.2±0.02</td>
</tr>
<tr>
<td>CMCT (ms)</td>
<td>12.1±1.0</td>
</tr>
<tr>
<td>ICI (%)</td>
<td>84.0±23.0</td>
</tr>
<tr>
<td>ICF (%)</td>
<td>212.0±30.0</td>
</tr>
<tr>
<td>CSP duration (ms)</td>
<td>119.0±7.3</td>
</tr>
</tbody>
</table>

Table. 02 Measures of intracortical excitability in MS patients after placebo and CBME treatments (n= 14). Values are indicated as mean±SD. Abbreviations: RMT: Resting Motor Threshold; MEP: Motor Evoked Potential; CMCT: Central Motor Conduction Time; ICI: Intra-cortical Inhibition; ICF: Intra-cortical Facilitation; CSP: Cortical Silent Period.

ANOVA analysis on tibial nerve stimulation showed no effect of main factor drug (CBME vs. placebo) on the H reflex/M wave ratio (placebo: 0.51±0.09; CBME: 0.52±0.07) ($F_{(1,16)}=0.02; p=0.86$).

There was no significant correlation between THC or CBD levels and electrophysiological measures (i.e., H/M ratio).

Discussion

This study suggests that CBME does not produce changes in the amplitude and/or extent of fMRI motor activations nor does it induce changes in the excitability of motor areas respective to placebo. The lack of differences in fMRI activation between CBME and placebo may be explained by the fact that, while we interrogated the motor system, cannabinoids may exert their effects on spasticity through changes in systems other than motor. Consistently with the fMRI results, TMS showed no changes in motor cortical inter-neural activity mediated by GABAergic or glutamatergic effect$^{37,38}$ and in the central neural drive control as a result of CBME treatment. Notably, the H/M ratio did not change during CBME treatment, suggesting that the drug effects on spasticity are not directly or mainly mediated by a modulation of the motor system. Consistent with our findings, a previous study$^{39}$ failed to show an effect of CBME on spasticity and stretch reflex excitability (H-reflex, H/M ratio),

supporting the idea that spinal cord excitability does not change substantially during CBME treatment.

Clinical studies have demonstrated the therapeutic effects of cannabinoids on central pain in MS.\cite{22,36,40-43} We previously showed that CBME is effective in modulating the nociceptive system through an increase of the RIII reflex threshold and a decrease of the RIII reflex area, without changing the H/M ratio, a measure of lower motoneuron excitability.\cite{36} This finding supports the hypothesis that cannabinoids efficacy on spasticity does not rely significantly on the motor system, but instead is mediated, at least in part, by a modulation of the painful components of spasticity.

The median of daily sprays was higher in the placebo arm than in the active arm. Considering that cannabinoids may affect the patient's perception of spasticity and relieve the overall discomfort produced by the neurological impairment,\cite{44} without impairing cognition,\cite{45} our finding can be explained by the lack of perceived efficacy that, along with tolerability, guided patients in their titration.

There are strengths and limitations in this study. We failed to demonstrate a clinical effect of CBME in our cohort of patients, at least within the range of disabilities studied here. This may be due to the small effect size of the drug or to the small sample size of our cohort of patients. Indeed, CBME may not be effective in every patient with MS related spasticity, suggesting that enriched trials identifying clinical responders may be more powerful approaches also for neurophysiological investigations on the neural correlates of drug effects.\cite{6} Admittedly, there are inherent difficulties in predicting individual dose-response with cannabinoids and individual variation in dosages required to reach a clinical effect may constitute an important limitation. However, the median number of CBME daily sprays was comparable with that previously reported.\cite{23} Furthermore, mean THC plasma concentration was equal to 1.81 ng/ml, which is within the therapeutic range of 0.08-9.17 ng/ml.\cite{46} Despite the lack of significant clinical effects of CBME, our results are generated in a cohort whose size is comparable with other functional imaging studies. The combination of neurophysiological methods applied in this study and leading to converging results limits the impact of the methodological biases present in pharmacological imaging studies, in which physiological confounds may blur the true neural effects of the drug.\cite{47} We combined independent, yet complementary neurophysiological methods to probe the function of the motor system in MS.\cite{20} This combined approach of complementary methods strengthens our results since, although it does not allow us to exclude completely an effect of the drug on the motor system, it provides us with confidence that this effect, if present, is not the main or only one. We investigated the motor system probing regions involved in the function of the upper limb. While this limits the interpretability of our results to the upper limb disability, which admittedly may be less affected by spasticity than lower limbs, it probes part of a functional system, i.e. the one controlling upper limb movements, that is well-characterized in MS. The complex effects of THC on motor function represent another potential confounder. In rats, low doses of THC decrease locomotor activity by inhibiting the CB1 receptors, while higher doses stimulate movements and catalepsy emerges at the highest doses.\cite{15} Individual response to cannabinoids depending on the different distribution of CB1 receptors may be further enhanced by tissue damage or cannabinoid-induced vascular changes,\cite{48} which may influence the drug bioavailability. Notably, the levels of CBD and THC during the placebo periods were negligible, suggesting the absence of carry-over effects as potential confounder.

**Conclusions**

This study suggests that cannabinoid effects on spasticity are not directly or mainly mediated by a modulation of the motor system as measured by fMRI and electrophysiological methods. Sensory systems may be implicated in explaining the beneficial effects of cannabinoids on MS spasticity. Further investigations on the use of
cannabinoids for spasticity treatment in MS, therefore, may benefit from a careful selection of eligible patients.

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