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TMS and EEG Pharmacodynamic Effects of a Selective Sphingosine-1-Phosphate Subtype 1 Receptor Agonist on Cortical Excitability in Healthy Subjects

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Current anti-epileptic drugs lack efficacy, cause many side effects and one third of all patients are treatment-resistant. Drugs targeting the sphingosine-1-phosphate receptor show potential anti-convulsant effects in animal models and decrease cortical excitability in patients with multiple sclerosis, but available compounds alter lymphocyte trafficking and cause immunosuppression, limiting their clinical anti-epileptic potential. TRV045 is a selective sphingosine-1-phosphate subtype 1 receptor agonist without effects on lymphocyte trafficking, demonstrating efficacy in animal models of epilepsy, with the potential to target abnormal cortical excitability. This randomized, double-blind, placebo-controlled, two-way cross-over, multiple-dose study evaluated the effects of TRV045 on cortical excitability in healthy male adults, measured by pharmaco-electroencephalography and transcranial magnetic stimulation (TMS). Subjects received TRV045 250 mg or placebo, once daily for 4 days, in randomized order. Endpoints were analyzed with a mixed effects model analysis of covariance. Twenty-five of the 27 subjects completed the study. There was a significant increase in alpha power with eyes open after treatment with TRV045 on Day 1, increasing after 4 days of dosing. Less pronounced significant effects in beta, gamma, and delta power were observed after 4 days. For TMS-Electromyography there was a non-significant decreased post-dose single-pulse peak-to-peak amplitude on Day 1 only, and there were no effects on paired-pulse parameters. Several significant TMS-Electroencephalography clusters were seen after 4 days of dosing. These findings show that TRV045 has central nervous system activity with evolving effects following repeated dosing. These data support further studies to elucidate the mechanism of action of TRV045 and its potential anti-epileptic effects.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ S1PR1 modulators may have anti-epileptic potential, but current non-selective S1PR modulators have a negative risk–benefit for this indication due to altered lymphocyte trafficking and immunosuppression.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ Whether TRV045 affects cortical excitability in male HV and whether it is a CNS penetrating and CNS active compound using pharmaco-EEG and TMS-EMG–EEG.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ This is the first study describing the effects of a S1PR1 modulator on pharmaco-EEG and TMS-EMG–EEG. We demonstrated CNS activity by an increase in higher frequencies and a decrease in the lower frequencies in pharmaco-EEG spectral power, effects that distinguish TRV045 from other standard AEDs.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ S1PR1 modulation potentially affects cortical excitability, which is relevant for the treatment of epilepsy, but requires further investigation. Both EEG spectral power and TMS-EMG–EEG are reliable PD biomarkers and can generate valuable data regarding the neurophysiological drug profile, which might be important when developing drugs with a novel MoA.

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Epilepsy is a central nervous system (CNS) disorder with abnormal brain activity causing unprovoked seizures, often requiring lifelong treatment.[1] To date, no disease-modifying therapies have been registered for the treatment of epilepsy. One third of the patients are therapy-resistant under current anti-epileptic drugs (AEDs) due to lack of efficacy or dose-limiting side effects.[2] There is an unmet need for effective AEDs with a different mechanism of action (MoA) from existing therapies. Sphingosine-1-phosphate subtype 1 receptors (S1PR1) are highly expressed in the CNS and regulate neuroinflammation,[3–5] a process involved in epileptogenesis. S1PR1s also influence neuronal function by regulating neurotransmitters such as glutamate.[5–8] Fingolimod, a market-approved drug for multiple sclerosis, is a non-selective S1PR modulator with high potency for S1PR1, but also shows activity at S1PR subtypes 3–5. Fingolimod demonstrated anti-epileptic effects in different preclinical epilepsy models, including the lithium-pilocarpine rat model,[9] pentylenetetrazol-induced kindling mouse model,[10] and supra-hippocampal kainate mouse model.[11] However, it also causes lymphopenia, which contributes to its efficacy in MS but makes it unfavourable for lifelong treatment of epilepsy.

TRV045 is a potent and selective S1PR1 agonist that has not shown lymphopenia in animal and early human studies. In the context of the NINDS Epilepsy Therapy Screening Program, TRV045 demonstrated seizure reduction in the corneal-kindled mouse model and the rat maximal electroshock seizure model of acute epilepsy.[12] Additionally, TRV045 improved seizure burden and seizure freedom in the post-kainic acid spontaneous recurrent seizure rat model of temporal lobe epilepsy,[12] and elevated seizure induction threshold, i.e., time to onset of generalized clonus, caused by the intravenous infusion of the chemoconvulsant pentylenetetrazol (Trevena, data on file), supporting the development of TRV045 as an AED.

Before studying TRV045's efficacy in epilepsy patients, we first wanted to demonstrate CNS penetrance and CNS-mediated pharmacodynamic (PD) effects in healthy volunteers (HV). Both quantitative resting-state encephalography (pharmaco-EEG)[13] and Transcranial Magnetic Stimulation-Electromyography-Electroencephalography (TMS-EMG–EEG)[14] have proven their usefulness as PD biomarkers for CNS target engagement in early-phase drug trials, and have translational value as both patients with generalized and focal epilepsy have abnormal brain activity measured with EEG and TMS.[15] Moreover, AEDs with different MoA modulate EEG and TMS signals differently, making both methods interesting tools for drug profiling novel therapeutic agents.[13,14,16–18] With this study we aimed to demonstrate CNS penetrance and to profile TRV045's drug effects by measuring CNS-mediated PD effects of S1PR1 modulation with pharmaco-EEG and TMS-EMG–EEG in male HV, to provide more information on TRV045's potential anti-epileptic effects.

MATERIAL AND METHODS

This study was approved by the Medical Ethics Committee, Foundation Beoordeling Ethiek Biomedisch Onderzoek (BEBO) and performed according to International Conference on Harmonisation guidelines on Good Clinical Practice. Written informed consent was obtained from all participants before study participation. The study was sponsored by Trevena and conducted at the Centre for Human Drug Research, Leiden, The Netherlands, from February 13, 2023 to June 05, 2023, registered under the European Union Clinical Trials Information System number 2022-502638-17-00.

Healthy males, aged 18–55 years, were selected after a medical screening following protocol-specific inclusion and exclusion criteria (**Table S1**). Females were excluded because of potential menstrual cycle-related confounding effects on cortical excitability.[19] Subjects with an increased risk of infection, TMS-related complications based on the TMS safety questionnaire,[20] and a resting motor threshold (rMT) higher than 75% of the maximum stimulator output (MSO) were excluded, as stimulation at 120% of this value would be very close to the MSO.

The study had a randomized, double-blind, placebo-controlled, two-period cross-over design with multiple dosing (**Figure 1**). Participants were admitted to the research center for 5 days during each visit, with a washout period of 1 week between dosing, which is appropriate as earlier pharmacokinetic (PK) evaluation at the top dose of 250 mg demonstrated a half-life of 14.9–19.7 hours after a single dose administration, and 10.9–14.8 hours after a 7-day multiple-dose administration (Trevena, data on file). The randomization was balanced to minimize first-order carry-over effects between visits. Safety assessments were performed, consisting of vital signs, electrocardiogram, physical and neurological examination, Columbia Suicide Severity Rating Scale, and laboratory assessments. Pharmaco-EEG and TMS–EMG–EEG, were performed pre-dose and 4 hours post-dose (expected T_{max}) on Day 1 and Day 4.

The fed dose of TRV045 250 mg (**Figure 2**) administered once daily for 4 days was selected based on the PK data observed after a 7-day multiple dose administration, as described above, suggesting that steady state would be expected to have been reached around Day 4 of dosing. Based on the plasma concentrations seen at this dose, and the preclinical plasma protein binding data, it was estimated that administration of 250 mg for 4 days should achieve unbound plasma concentration consistent with the effective unbound concentrations observed in nonclinical seizure models (6–20 ng/mL) (Trevena, data on file). Protein binding for TRV045 was measured in mouse, rat, dog, monkey, and human plasma using high throughput dialysis methods, and is estimated at 99.84% in mice and 98.39% in humans. PK samples were collected pre-dose and 1, 2, 4, 6, and 8 hours post-dose on Day 1 and 4. On Days 2 and 3, only pre-dose samples were collected. TRV045 plasma concentrations were measured by a validated high-performance liquid chromatography with tandem mass spectrometry (LC–MS/MS), with the CV and lower limit of quantitation of 7.7% and 1.00 ng/mL respectively (data on file).

Pharmaco-EEG was recorded per IPEG guidelines, with 5-minute alternating periods of eyes open and closed every 64 seconds.[21] TMS was applied per current guidelines,[20] using a MagPro R30 with MagOption stimulator and an MCF-B65 butterfly coil (2 × 75 mm; both MagVenture GmbH, Hueckelhoven, Germany), targeting the motor hotspot of the dominant abductor digiti minimi (ADM) muscle, identified by the Edinburgh Handedness Questionnaire.[22] The coil was placed tangentially at a 45° angle to the skull midline. The rMT was determined,[23] followed by 75 single pulses (spTMS) at 120% rMT with intervals of 3.5–4.5 seconds. The paired-pulse TMS (ppTMS) included 75 pairs of pulses in randomized order with interstimulus intervals (ISI) of 2, 15, 100, and 300 milliseconds, using 120% rMT (80% for ISI 2 and 15 milliseconds conditioning pulses). Adapted noise masked auditory-evoked potentials.[24] EEG was continuously recorded using a 40-channel recording system (Refa-40, TMSi B.V., the Netherlands) with electrodes placed according to the international 10–20 system (32-lead cap, TMSi B.V., the Netherlands), replacing A1 and A2 with M1 and M2 (at the mastoids). Scalp electrode impedance was kept below 5 kΩ and ground electrode at AFz. Signals were recorded from 21 or 32 electrodes sampled at 1,024 Hz for pharmaco-EEG and 2,048 Hz for TMS. Electrooculograms (EOG) were recorded to detect ocular artefacts, and ADM muscle activity was recorded using Ag/AgCl electrodes in a belly-tendon montage.

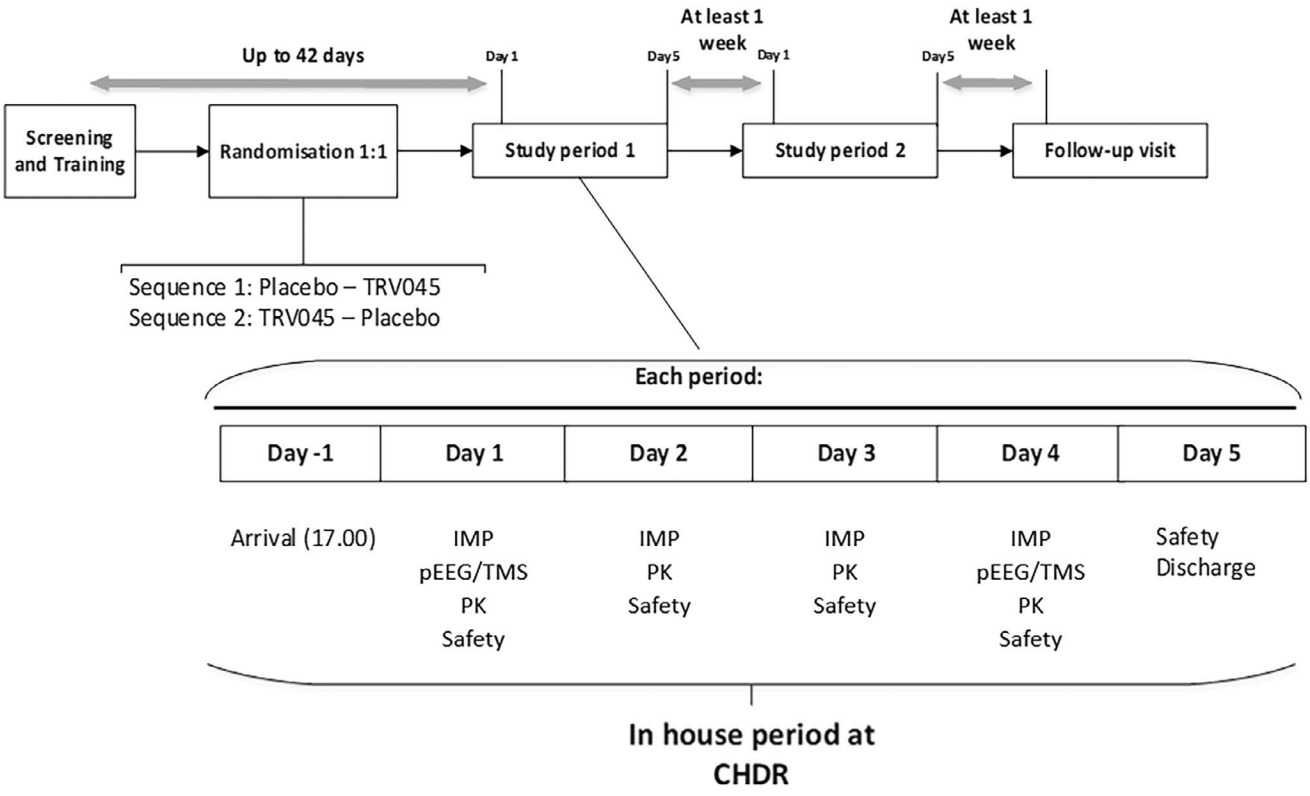


Figure 1 Study design. Schematic detail of treatment periods. Each subject has two identical treatment periods, in which treatments are administered in randomized order. IMP, investigational medicinal product; pEEG, Pharmaco-EEG; TMS, Transcranial magnetic stimulation; PK, pharmacokinetics blood sample.

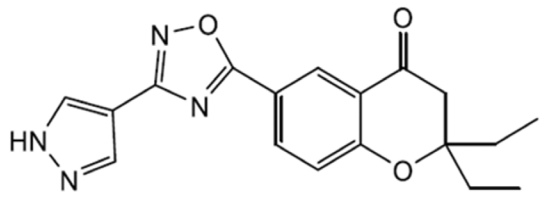
Chemical Name	6-(3-(1-H-pyrazol-4-yl)-1,2,4-oxadiazol-5-yl)-2,2-diethylchroman- 4-one
Structural Formula	

Figure 2 Chemical name and structure. International Union of Pure and Applied Chemistry (IUPAC) chemical name and structural formula of TRV045.

Pharmaco-EEG data included: total power per frequency band, per electrode, per eye state, e.g., alpha power in Fz-Cz with eyes open. TMS-EMG parameters included: mean single-pulse peak-to-peak MEP amplitude (μV); paired-pulse short intracortical inhibition at ISIs of 2 milliseconds ($SICI_2$), intracortical facilitation at ISI of 15 milliseconds (ICF_{15}) and long intracortical inhibition at ISIs of 100 and 300 milliseconds ($LICI_{100}$ and $LICI_{300}$). A comprehensive explanation of the pharmaco-EEG and TMS-EMG–EEG data synthesis is provided in Supplementary Text S1.

The sample size calculation was based on the MEP amplitude from Ruijs et al.,[14] with 24 subjects providing 80% power to detect a −300 μV difference, assuming a standard deviation of 500 μV and a two-sided significance level of 0.05. The study was double-blinded, randomization was generated digitally by an independent statistician using SAS 9.4, and subjects were enrolled sequentially (1–27) by the research physician. Medication was prepared by an unblinded pharmacist. Pharmaco-EEG data were log-transformed for analysis and back-transformed for interpretation as a percentage change. Treatment effects were analyzed using ANCOVA with treatment, time, period, and their interactions as fixed factors, and subject-related factors as random effects. Baseline measurement served as a covariate. The Kenward-Roger approximation estimated degrees of freedom, and parameters were estimated with restricted maximum likelihood.[26] Variance components were used for random effects, and treatment effects were reported with 95% CI, least square means (LSM), and *P*-value. Graphs display LSM estimates and change from baseline.

The TEP response following the test pulse was analyzed for all EEG leads, in the full-time sample of 0–300 milliseconds and at specific

periods of interest (TOIs) around the characteristic TEP components[27] (N15: 0–20 milliseconds; P30: 20–40 milliseconds; N45: 40–55 milliseconds; P60: 55–80 milliseconds; N100: 80–130 milliseconds; P180: 130–230 milliseconds). To compare treatment and placebo single and paired-pulse TEPs, cluster-based permutation analysis was used,[28] a nonparametric method suited for multidimensional TMS-EEG data. Dependent samples *t*-tests were used for electrode and time comparisons, clustering *t*-values with $P < 0.05$. Significant clusters were determined using permutation testing (1,500 permutations), and results were reported if less than 5% of the summed *t*-values obtained by permutation were larger than the cluster value observed in the original data. *P*-values of significant clusters are reported.

An exploratory post hoc PK/PD analysis was performed on the relationship between drug plasma concentrations and pharmaco-EEG and TMS-EMG parameters that showed the most promising results in the planned statistical analysis, namely alpha power in Fz-Cz, Pz-O1, and Pz-O2 with eyes open, and MEP amplitude. See Supplementary Text S2 for a detailed description. In summary, each parameter was modeled with an intercept only and with an additive and proportional effect, being a linear or non-linear (E_{MAX}) concentration-effect relationship, using a mixed-effects model. A day covariate was added to the models to investigate potential between-day differences.

RESULTS

Twenty-five out of 27 subjects completed all study periods with two early terminations unrelated to safety or tolerability. Demographics are given in **Table S2**. TRV045 was well tolerated without any severe or serious adverse events (AEs), or AE-related discontinuations. All AEs resolved. Overall, the incidence of AEs was similar between groups, except for headache, fatigue, and dizziness, which were reported more often during treatment with TRV045 (**Table S3**). Mean (± SD) TRV045 plasma concentrations increased over time and peaked at approximately 6 hours at concentrations of 690.5 ± 138.13 ng/mL on Day 1 and 893.80 ± 209.09 ng/mL on Day 4, corresponding with an estimated unbound concentration of 11.1 and 14.4 ng/mL, respectively, being within the range of efficacious unbound concentrations estimated in nonclinical seizure models.

For an overview of all pharmaco-EEG results, see **Table 1**. Overall treatment effects of TRV045 vs. placebo, TRV045 significantly increased alpha power in the parieto-occipital regions (Pz-O1 estimated difference (ED): 29.0%, 95%-CI: 6.5%;56.2%; Pz-O2 ED: 28.2%, 95%-CI: 7.4%;53.0%) and increased beta power in the frontocentral region (Fz-Cz ED: 18.5%, 95%-CI: 2.1%;37.6%) with eyes open. There was a non-significant decrease in delta power in the frontocentral region with eyes closed. Effects increased with multiple days of dosing. On Day 1, alpha power was significantly increased with eyes open, with a more localized effect (Pz-O2 ED: 26.9%, 95%-CI: 1.3%;58.9%), and delta power was significantly decreased in the frontocentral region with eyes closed (Fz-Cz ED: 14.7%, 95%-CI: −25.4%;−2.4%). On Day 4, not only was alpha power increased in all leads with eyes open (Fz-Cz ED: 40.7%, 95%-CI: 6.8%;85.3%; Pz-O1 ED: 38.8%, 95%-CI: 11.2%;73.1%; Pz-O2 ED 26.6%, 95%-CI: 0.9%;58.9%) (**Figure S1**), but beta power and gamma power were also increased in the frontocentral region (respectively Fz-Cz ED: 23.1%, 95%-CI: 3.0%;47.1%; and Fz-Cz ED: 21.3%, 95%-CI: 1.5%;45.1%). In addition to delta power being significantly decreased in the frontocentral region with eyes closed (Fz-Cz ED: −14.0%, 95%-CI; −24.9%;−1.4%), delta power also decreased in the parieto-occipital region with eyes open (Pz-O2 ED: −15.2%, 95% CI: −27.6%;−0.5%).

For an overview of all sp- and ppTMS-EMG parameters, see **Table 2**. The spTMS-EMG MEP amplitude did not significantly change after administration of TRV045 in the overall treatment period (ED: 130.850 µV, 95%-CI: −433.4;171.7), but there was a trend toward a decrease in MEP amplitude post-dose on Day 1 (ED: −304.136 µV, 95%-CI: −688.2;79.9) (**Figure 3**) that was not observed on Day 4, nor when combining Day1 + 4 treatment effects. No statistically significant effects were observed on rMT or any of the ppTMS-EMG parameters. A complete overview of the TMS-EEG results and corresponding topo-plots can be found in **Tables S4–S6** and **Figure 4**. No significant clusters were found for spTMS-EEG. For ppTMS-EEG, two significant clusters were observed on Day 4 following TRV045 treatment, one pre-dose and one post-dose, both at an ISI of 300 milliseconds and in the ipsilateral centro-parietal region. Pre-dose, we observed a significantly more negative P30 TEP amplitude in this cluster, but this difference was not significant when analyzing the entire time period. Post-dose, we observed a significantly more positive P60 TEP amplitude in this cluster; however, this difference was not significant when analyzing the entire time period.

PK/PD relationship results are presented in **Figure 5** and **Figure S2**. For alpha power, we only found a significant concentration-effect relationship when estimating separate coefficients for each day (**Figure S2**). For Fz-Cz, this resulted in a significant additive linear relationship for Day 4 only ($P = 0.03$), where a small increase was seen with increasing concentrations. For Pz-O1 ($P = 0.0002$) and Pz-O2 ($P = 0.0007$) proportional linear relationships were estimated, where values decrease on Day 1 but increase on Day 4 with increasing concentrations. The MEP amplitude demonstrated a proportional linear concentration-effect relationship ($P < 0.0001$) and significantly improved when separate coefficients were estimated per day ($P = 0.03$), see **Figure 5**.

DISCUSSION

This study evaluated the PD effects of S1PR1 modulation by TRV045 on spontaneous electrical brain activity and cortical excitability, measured by pharmaco-EEG and TMS-EMG–EEG. TRV045 significantly increased power in the higher frequency (alpha, beta, and gamma) bands and decreased power in the lower (delta) frequencies in various brain regions, with an increasing effect over multiple days of dosing. The data provide pharmacodynamic evidence of CNS penetrance and CNS activity of TRV045, without accompanying clinical signs of sedation, as the incidence of somnolence was comparable between groups (**Table S3**). A non-significant decrease in MEP amplitude was observed post-dose on Day 1, possibly indicating an acute decrease in cortical excitability following initial dosing with TRV045, which, however, was not present on Day 4. Two significant ppTMS-EEG clusters were found at ISI of 300 milliseconds in the ipsilateral centro-parietal region after 4 days of dosing with TRV045, with a more negative P30 TEP amplitude pre-dose and a more positive post-dose P60 TEP amplitude, but these differences were not confirmed after analyzing the entire time period. The PK/PD relationship analysis identified day-dependent linear effects for TRV045 plasma

Table 1 Summary of results for pharmaco-EEG

TRV045 250 mg vs. Placebo							
Eyes closed				Eyes open			
Overall	Individual contrasts			Overall	Individual contrasts		
All post-dose timepoints[a]	Day 1 post-dose	Day 1+4 post-dose	Day 4 post-dose	All post-dose timepoints[a]	Day 1 post-dose	Day 1+4 post-dose	Day 4 post-dose
EEG alpha power Fz-Cz							
8.8	−9.3	1.4	13.3	27.2	−5.7	15.2	40.7
(−4.3, 23.7)	(−23.4, 7.5)	(−11.8, 16.5)	(−4.6, 34.4)	(−0.5, 62.6)	(−28.4, 24.2)	(−10.5, 48.4)	(6.8, 85.3)
P=0.1840	*P*=0.2552	*P*=0.8411	*P*=0.1502	*P*=0.0543	*P*=0.6697	*P*=0.2609	***P*=0.0164**
EEG alpha power Pz-O1							
1.0	−4.3	0.8	6.2	29.0	10.6	23.9	38.8
(−11.9, 15.9)	(−19.8, 14.1)	(−13.0, 16.8)	(−11.0, 26.7)	(6.5, 56.2)	(−11.4, 37.9)	(1.5, 51.1)	(11.2, 73.1)
P=0.8784	*P*=0.6178	*P*=0.9150	*P*=0.5009	***P*=0.0113**	*P*=0.3645	***P*=0.0358**	***P*=0.0047**
EEG alpha power Pz-O2							
−1.1	−2.8	0.1	3.1	28.2	26.9	26.8	26.6
(−13.6, 13.2)	(−19.0, 16.6)	(−13.6, 16.1)	(−14.3, 24.0)	(7.4, 53.0)	(1.3, 58.9)	(4.9, 53.2)	(0.9, 58.9)
P=0.8632	*P*=0.7565	*P*=0.9871	*P*=0.7400	***P*=0.0079**	***P*=0.0386**	***P*=0.0158**	***P*=0.0418**
EEG beta power Fz-Cz							
10.4	5.8	7.9	10.1	18.5	3.6	12.9	23.1
(−4.9, 28.1)	(−11.0, 25.8)	(−7.6, 26.1)	(−7.5, 31.1)	(2.1, 37.6)	(−13.3, 23.8)	(−3.5, 32.0)	(3.0, 47.1)
P=0.1846	*P*=0.5149	*P*=0.3227	*P*=0.2722	***P*=0.0270**	*P*=0.6943	*P*=0.1242	***P*=0.0235**
EEG beta power Pz-O1							
9.1	6.5	8.2	9.8	8.9	−0.5	5.7	12.3
(−8.6, 30.2)	(−13.2, 30.8)	(−10.0, 30.0)	(−10.5, 34.8)	(−10.7, 32.7)	(−20.7, 24.9)	(−13.9, 29.8)	(−10.5, 41.0)
P=0.3198	*P*=0.5375	*P*=0.3897	*P*=0.3611	*P*=0.3765	*P*=0.9636	*P*=0.5771	*P*=0.3044
EEG beta power Pz-O2							
7.7	8.8	6.0	3.3	13.3	6.4	8.2	10.0
(−8.4, 26.5)	(−9.6, 31.0)	(−10.4, 25.4)	(−14.3, 24.5)	(−6.7, 37.6)	(−14.4, 32.4)	(−11.4, 32.2)	(−11.7, 36.9)
P=0.3544	*P*=0.3642	*P*=0.4821	*P*=0.7281	*P*=0.1941	*P*=0.5645	*P*=0.4248	*P*=0.3852
EEG delta power Fz-Cz							
−6.3	−14.7	−14.3	−14.0	−6.6	−5.8	−9.6	−13.2
(−15.0, 3.2)	(−25.4, −2.4)	(−23.1, −4.6)	(−24.9, −1.4)	(−17.9, 6.3)	(−20.1, 11.2)	(−21.1, 3.7)	(−26.5, 2.5)
P=0.1771	***P*=0.0214**	***P*=0.0061**	***P*=0.0306**	*P*=0.2639	*P*=0.4710	*P*=0.1391	*P*=0.0919
EEG delta power Pz-O1							
−2.2	−2.4	−2.6	−2.8	−7.1	−6.7	−9.4	−12.1
(−10.6, 7.0)	(−15.0, 12.0)	(−12.1, 8.0)	(−15.4, 11.7)	(−18.2, 5.5)	(−20.3, 9.3)	(−20.8, 3.7)	(−24.9, 3.0)
P=0.6077	*P*=0.7233	*P*=0.6031	*P*=0.6845	*P*=0.2403	*P*=0.3842	*P*=0.1451	*P*=0.1091
EEG delta power Pz-O2							
−1.9	4.9	−1.1	−6.7	−6.0	0.2	−7.8	−15.2
(−8.9, 5.5)	(−8.7, 20.5)	(−9.9, 8.6)	(−18.9, 7.3)	(−16.2, 5.5)	(−14.4, 17.2)	(−18.7, 4.6)	(−27.6, −0.5)
P=0.5837	*P*=0.4956	*P*=0.8138	*P*=0.3265	*P*=0.2699	*P*=0.9838	*P*=0.1949	***P*=0.0432**
EEG gamma power Fz-Cz							
4.7	−11.1	0.0	12.5	12.4	−3.9	8.0	21.3
(−6.1, 16.8)	(−24.3, 4.5)	(−11.6, 13.2)	(−4.5, 32.4)	(−0.2, 26.6)	(−19.6, 14.9)	(−5.7, 23.6)	(1.5, 45.1)
P=0.3914	*P*=0.1508	*P*=0.9996	*P*=0.1554	*P*=0.0529	*P*=0.6573	*P*=0.2583	***P*=0.0343**

(Continued)

Table 1 (Continued)

TRV045 250 mg vs. Placebo							
Eyes closed				Eyes open			
Overall	Individual contrasts			Overall	Individual contrasts		
All post-dose timepoints[a]	Day 1 post-dose	Day 1+4 post-dose	Day 4 post-dose	All post-dose timepoints[a]	Day 1 post-dose	Day 1+4 post-dose	Day 4 post-dose
EEG gamma power Pz-O1							
13.7	7.8	13.3	19.1	11.9	−1.1	10.4	23.1
(−18.4, 58.4)	(−25.8, 56.5)	(−19.6, 59.5)	(−18.0, 72.9)	(−21.8, 60.1)	(−34.2, 48.9)	(−23.8, 59.9)	(−18.2, 85.3)
P=0.4299	*P*=0.6859	*P*=0.4589	*P*=0.3482	*P*=0.5160	*P*=0.9580	*P*=0.5848	*P*=0.3072
EEG gamma power Pz-O2							
13.1	5.2	7.0	8.7	20.5	11.8	12.6	13.5
(−13.2, 47.4)	(−21.1, 40.3)	(−18.4, 40.2)	(−18.6, 45.2)	(−8.5, 58.7)	(−17.3, 51.2)	(−15.1, 49.4)	(−16.2, 53.7)
P=0.3440	*P*=0.7198	*P*=0.6117	*P*=0.5590	*P*=0.1746	*P*=0.4568	*P*=0.3936	*P*=0.4035
EEG theta power Fz-Cz							
−0.1	−9.9	−9.2	−8.5	14.4	3.0	4.5	6.0
(−13.2, 15.0)	(−24.3, 7.2)	(−21.9, 5.4)	(−23.3, 9.0)	(−2.7, 34.6)	(−14.7, 24.4)	(−11.7, 23.7)	(−12.3, 28.1)
P=0.9872	*P*=0.2327	*P*=0.1961	*P*=0.3128	*P*=0.0983	*P*=0.7529	*P*=0.5974	*P*=0.5371
EEG theta power Pz-O1							
4.0	0.0	2.0	4.1	13.0	2.7	5.1	7.6
(−9.4, 19.5)	(−17.0, 20.5)	(−12.3, 18.7)	(−13.6, 25.4)	(−5.6, 35.3)	(−17.3, 27.4)	(−13.0, 26.9)	(−13.3, 33.5)
P=0.5600	*P*=0.9998	*P*=0.7901	*P*=0.6704	*P*=0.1712	*P*=0.8058	*P*=0.5913	*P*=0.4985
EEG theta power Pz-O2							
1.8	3.3	0.8	−1.6	14.9	17.2	12.0	7.1
(−13.7, 20.2)	(−16.5, 27.8)	(−15.6, 20.5)	(−20.6, 22.0)	(−4.4, 38.1)	(−6.5, 46.9)	(−7.8, 36.0)	(−14.7, 34.4)
P=0.8218	*P*=0.7620	*P*=0.9268	*P*=0.8807	*P*=0.1285	*P*=0.1645	*P*=0.2402	*P*=0.5484

Estimated difference of TRV045 250 mg vs. placebo of pharmaco-EEG parameters in %, with 95% confidence interval (95%-CI) and *P*-values of the total power ($(\mu V)^2$), per frequency band, per lead, per eye state. Frequency per band: alpha (α): 8.5 < 12.5 Hz; beta (β): 12.5 < 30.0 Hz; delta (δ): 1.5 < 6.0 Hz; gamma (γ): 30.0 < 40.0 Hz; and theta (θ) 6.0 < 8.5 Hz. Statistically significant ($P < 0.05$) results are marked in bold. Significant increases in power are highlighted in green. Significant decreases in power are highlighted in yellow. EEG, electroencephalography; vs., versus. [a]All post-dose timepoints include: Day 1+4 hours post-dose, Day 4 pre-dose, Day 4+4 hours post-dose.

concentrations and alpha power with eyes open as well as MEP amplitude. Interestingly, for alpha power with eyes open, values remained unaltered (Fz-Cz) or decreased (Pz-O1 and Pz-O2) with increasing TRV045 concentrations on Day 1, but increased on Day 4 (all). For MEP amplitude, a larger concentration-dependent decrease was found for Day 1 vs. Day 4.

To our knowledge, there is no literature available on the effects of S1PR modulators on the EEG spectral power in animals or humans. A systematic review aiming to profile EEG effects of AEDs with different MoAs provides an overview of studies performed in epilepsy patients and HV. The most common findings were EEG slowing, with increased delta and theta activity and decreased activity in higher frequency ranges.[16] EEG slowing, in particular, the attenuation of the posterior dominant alpha rhythm, correlates with cognitive impairment, a frequently reported AE of AEDs in HV studies with ion channel blockers, including gabapentin, carbamazepine, and phenytoin.[29–31] Interestingly, milacemide, a glycine prodrug with anti-convulsant effects by increasing GABA and endogenous glycine levels, showed an effect opposite to other AEDs, but in line with our study. It reduced delta power and increased alpha and beta power at lower dosages while improving cognitive functions,[32] which might be attributed to milacemide's MoA, as glycine is a co-agonist of glutamate for activation of NMDA receptors[33,34] and could (indirectly) affect glutamate neurotransmission. This, together with the results from S1PR1 animal models[5–8] and a TMS-EMG study performed in relapsing–remitting multiple sclerosis (RRMS) patients showing fingolimod decreases ICF,[35] a marker which is mainly mediated by glutamate,[36–38] further support the hypothesis that S1PR1 modulators may influence glutamatergic neurotransmission, thereby reducing cortical excitability. Thus, the observed EEG changes following TRV045 treatment may suggest that reduced glutamate activity (in)directly underlies these effects, indicating potential anti-convulsant activity without sedative side effects.

Although TRV045 did not significantly affect spTMS-EMG parameters, there was a trend toward a decreased MEP amplitude after the first dose with an effect size (**Figure 3**) comparable to those observed after a single dose of other AEDs,[14] possibly indicating an early decrease in cortical excitability following a single dose of TRV045. Moreover, we found a proportional linear

Table 2 Summary of results for TMS-EMG

TRV045 250 mg vs. Placebo	Overall	Individual contrasts		
	All post-dose timepoints[a]	Day 1 post-dose	Day 1+4 post-dose	Day 4 post-dose
Single-pulse TMS-EMG parameters				
Peak-to-peak MEP amplitude (μV)	−130.9	−304.1	−178.9	−53.7
	(−433.4, 171.7)	(−688.2, 79.9)	(−502.5, 144.7)	(−437.8, 330.4)
	P=0.3793	*P*=0.1182	*P*=0.2678	*P*=0.7802
rMT (%)	−0.7	0.8	−0.1	−1.0
	(−1.9, 0.5)	(−1.2, 2.7)	(−1.5, 1.3)	(−2.9, 0.9)
	P=0.2531	*P*=0.4261	*P*=0.8797	*P*=0.3100
Paired-pulse TMS-EMG parameters				
$SICI_2$ (%)	−3.7	8.7	−1.8	−12.3
	(−16.2, 8.8)	(−8.9, 26.3)	(−15.8, 12.1)	(−30.1, 5.4)
	P=0.5435	*P*=0.3273	*P*=0.7920	*P*=0.1688
ICF_{15} (%)	−17.1	19.5	−4.2	−27.9
	(−51.0, 16.7)	(−24.2, 63.2)	(−40.8, 32.3)	(−71.6, 15.8)
	P=0.3052	*P*=0.3737	*P*=0.8160	*P*=0.2052
$LICI_{100}$ (%)	4.4	−0.3	1.4	3.1
	(−5.3, 14.)	(−12.2, 11.6)	(−8.8, 11.7)	(−8.8, 5.0)
	P=0.3614	*P*=0.9613	*P*=0.7816	*P*=0.6035
$LICI_{300}$ (%)	3.4	5.5	4.2	3.0
	(−3.5, 10.3)	(−7.3, 18.2)	(−4.4, 12.9)	(−9.7, 15.8)
	P=0.3131	*P*=0.3953	*P*=0.3270	*P*=0.6383

Estimated difference of TRV045 250 mg vs. placebo of single-pulse and paired-pulse TMS-EMG parameters, with 95% confidence interval (95%-CI) and *P*-value. ICF_{15}, intracortical facilitation at interstimulus-interval of 15 milliseconds; $LICI_{100}$, long intracortical inhibition at interstimulus interval of 100 milliseconds; $LICI_{300}$, long intracortical inhibition at interstimulus interval of 300 milliseconds; MEP, motor-evoked potential; rMT, resting motor threshold; $SICI_2$, short intracortical inhibition at interstimulus interval of 2 milliseconds; TMS-EMG, transcranial magnetic stimulation—electromyography; vs., versus. [a]All post-dose timepoints include: Day 1+4 hours post-dose, Day 4 pre-dose, Day 4+4 hours post-dose.

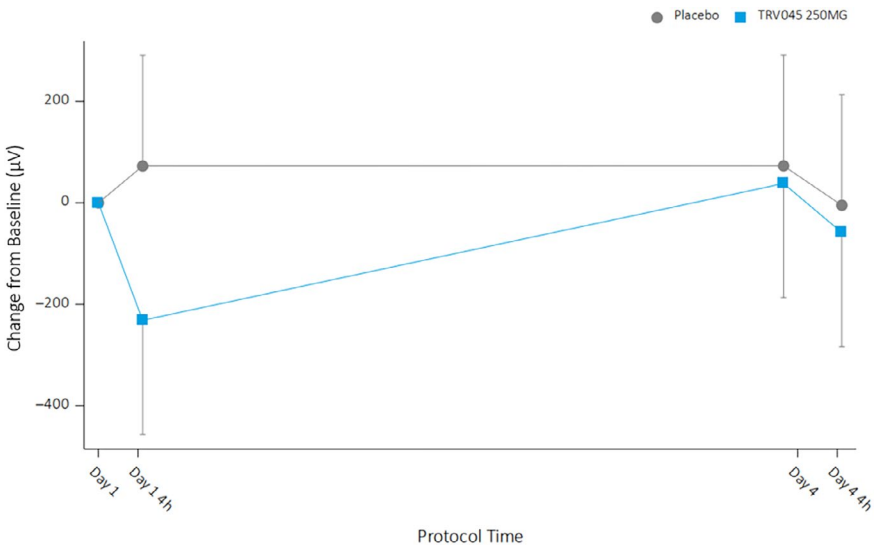


Figure 3 Peak-to-peak MEP Amplitude (LSM, 95%-CI). Change from baseline of the least square means (LSM) with 95% confidence interval (95% CI) as error bars of the motor-evoked potential (MEP) amplitude (μV), using single-pulse transcranial magnetic stimulation, for TRV045 and placebo. LSM, least square means; MEP, motor-evoked potential; 95% CI, 95% confidence interval.

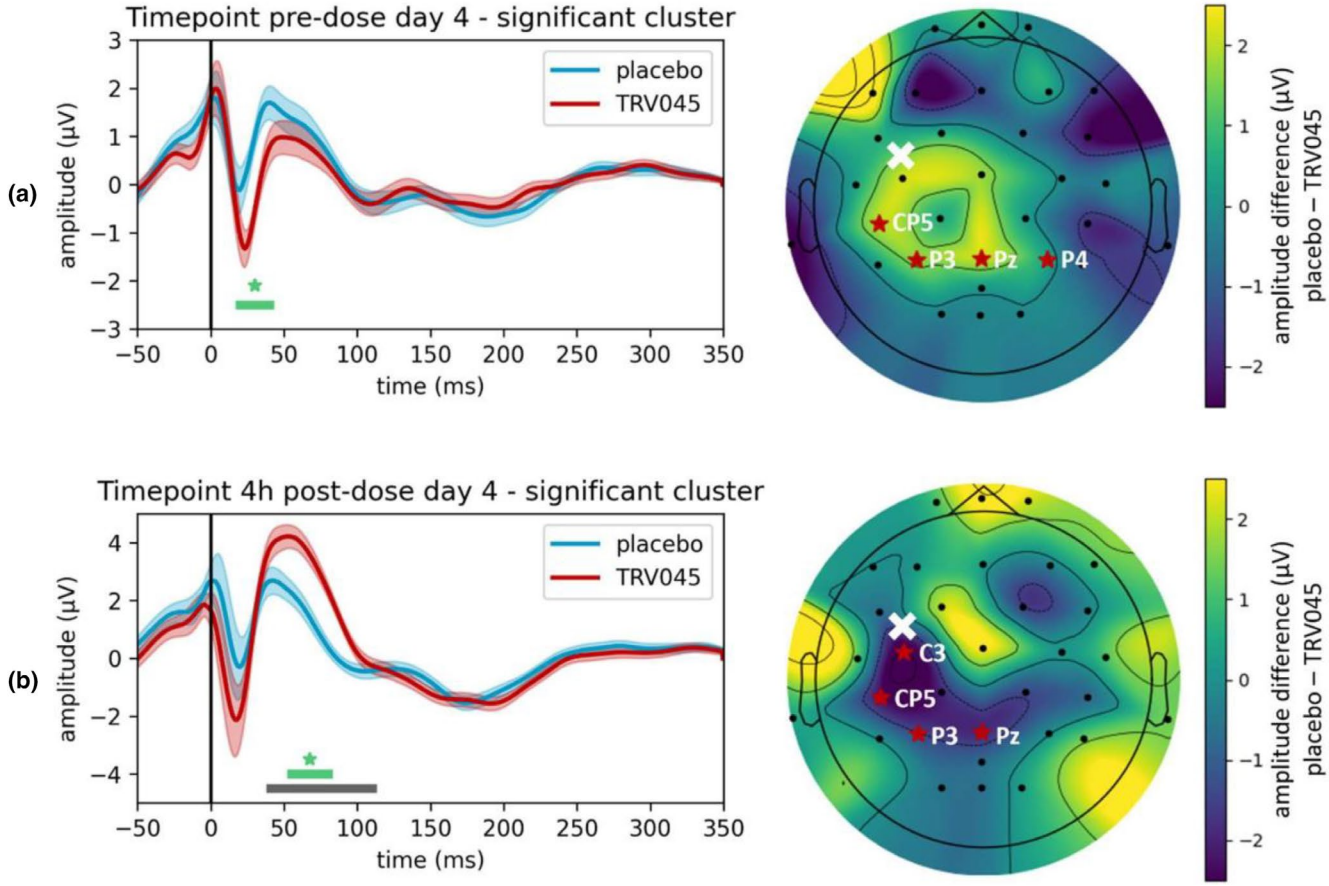


Figure 4 Grand average and topographical distribution of significant ppTMS-EEG clusters. (**a**) Overview of the significant cluster (P30; $P=0.02$) found pre-dose on day 4 when comparing TEPs of the placebo (in blue) and TRV045 (in red) conditions for ppTMS-EEG $LICI_{300}$. On the left side, the grand average (mean ± SEM) overall significant electrodes is presented. On the right side, the difference in topographical distribution (placebo—TRV045) at the time of the cluster (time window 27.5–32.5 ms) is presented. The thick green bar represents the time window (20–40 ms) of significant differences, the white cross the stimulation site, and the black dots the electrode positions with the significant electrodes as red stars. (**b**) Overview of the significant cluster (P60; $P<0.01$) found at post-dose on day 4 when comparing TEPs of the placebo (in blue) and TRV045 (in red) conditions for ppTMS-EEG $LICI_{300}$. On the left side, the grand average (mean ± SEM) overall significant electrodes is presented. On the right side, the difference in topographical distribution (placebo—TRV045) at the time of the cluster (time window 65–70 milliseconds) is presented. The thick green bar represents the time window (55–80 milliseconds) of significant differences, the thick grey bar the time window (41–110 milliseconds) of the entire cluster (including non-significant parts), the white cross the stimulation site, and the black dots the electrode positions with the significant electrodes as red stars. ppTMS-EEG, paired-pulse transcranial magnetic stimulation—encephalography; $LICI_{300}$, long intracortical inhibition at interstimulus interval of 300 milliseconds; SEM, standard error of the mean.

concentration-effect relationship between TRV045 plasma concentrations and MEP amplitude, with a larger concentration-dependent decrease on Day 1 vs. Day 4. A −400 µV reduction could theoretically be achieved at a concentration of 777 ng/mL on Day 1, so there was sufficient drug exposure to measure an effect (mean ± SD concentration 690.5 ± 138.1). Therefore, it should be considered that we had smaller statistical power to demonstrate significant effects after a single dose of TRV045 with one post-dose measurement on Day 1, as the study was powered on three post-dose measurements across 4 days of dosing, in contrast to our previous study, in which we performed 2 post-dose measurements after a single dose of AED.[14] Although the MEP amplitude observed pre-dose on Day 4 was comparable to the baseline MEP amplitude, it decreased again post-dose but with a smaller effect size compared to Day 1, which is confirmed by the difference in concentration-effect relationships for both days, indicating a potentially changing effect that is not only driven by plasma concentrations. Surprisingly, we did not find any significant effects of TRV045 on ppTMS-EMG parameters, although this was expected based on its hypothesized MoA, as ICF is thought to reflect excitatory transmission by glutamate.[36–38] A study conducted in RRMS patients reported a decrease in ICF at ISI of 9 milliseconds after 60 days of fingolimod treatment.[35] The most important differences between the fingolimod study and the current study are that we evaluated HV and only measured ICF at ISI 15 milliseconds, which is a longer interval than the ISIs evaluated in the fingolimod study. Although not significant, we did observe a decrease in ICF on Day 4 consistent with the fingolimod results, which raises the question of whether measuring a broader range of ISIs would have allowed us to demonstrate a significant effect of TRV045 on

	Parameter estimate	Residual Standard Error (%)
Baseline	853.262	0.65
Slope (Day 1)	-0.0006	19.7
Additive slope constant (Day 4) → Slope (Day 4)	0.0003 →-0.0003	43.8

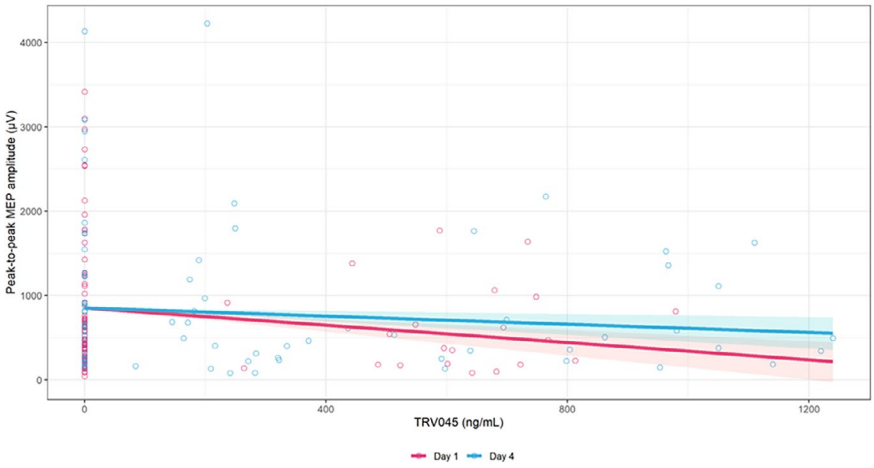


Figure 5 PK/PD relationship analysis of peak-to-peak MEP Amplitude. PK/PD relationship as determined for peak-to-peak MEP amplitude. Data were simulated 1,000 times based on random sampling from the variance–covariance matrix of estimated model parameters, and summarized to a mean (solid line) and 95% confidence interval (shaded area) while stratified per day. A proportional linear concentration-effect relationship with day covariate described the data best (*P*-value as compared to intercept model: <0.0001, *P*-value as compared to model without Day covariate: 0.03, drop in AIC as compared to additive model: −1,645). MEP, motor-evoked potential; PD, pharmacodynamic; PK, pharmacokinetic.

cortical excitability. On the contrary, time effects, such as disease progression, should be considered a possible confounder when interpreting the fingolimod study results, given its 60-day duration, patient population, and lack of control group.

TRV045 had significant effects on the ppTMS-EEG at ISI of 300 milliseconds in the ipsilateral centro-parietal region on Day 4. Pre-dose on Day 4, TRV045 resulted in a significantly more negative P30 TEP amplitude and post-dose a more positive P60 TEP amplitude (**Tables S5 and S6**; **Figure 4**). In particular, the Day 4 pre-dose cluster was unexpected given that TRV045 plasma concentration was the lowest of all post-dose timepoints (**Table S7**). This, together with the fact that we did not find significant clusters on Day 1, indicates that the observed effect is not an acute effect. However, the lack of significant differences observed for spTMS-EEG and other ppTMS-EEG ISIs does raise the question of whether the significant differences observed at ISI 300 milliseconds are true pharmacological effects or a type I error (false positive). There are no previous TMS-EEG studies investigating the effects of S1PR modulators, and overall, there is limited data available on pharmacological effects on ppTMS-EEG TEP, making it difficult to directly relate our outcomes to the current literature. To our knowledge, there have only been two studies investigating the pharmacological effects of ppTMS-EEG TEP in healthy volunteers.[14,39] In these studies, ion channel blockers levetiracetam and valproic acid, and GABAergic drugs lorazepam, diazepam, and baclofen were investigated. Levetiracetam, lorazepam, baclofen, and diazepam all affected the paired-pulse TEP at ISIs >100 milliseconds,[14,39] but only affected later TEP components (N100 and P180), whereas TRV045 modulated earlier TEP components (P30 and P60). There is some evidence hinting that the P60 TEP component of the ppTMS-EEG might be mediated by glutamate/$GABA_B$ interaction, as a conditioning pulse delivered at a short ISI (SICI 2 milliseconds) attenuated the test pulse P60 TEP component, and at longer intervals (ICF 10 milliseconds and LICI 100 milliseconds) enhanced the P60 component.[39,40] However, as TEPs represent the sum of excitatory and inhibitory neural activity at a given time following the TMS pulse, it is difficult to translate the observed effects to an increase or decrease in cortical excitability, let alone an isolated mechanism of action.

Differences in effects between Day 1 and 4 measured with pharmaco-EEG and TMS-EMG–EEG may provide insights into the evolving effects of TRV045 with repeated dosing. The observed inverse PK/PD relationship on Day 1 for alpha power is remarkable, as TRV045 increased alpha power on Day 1 in the planned statistical analysis. This discrepancy could be explained by methodological differences between the two analyses: the PK/PD analysis does not account for potential effects in the placebo treatment as the mixed effects model analysis of covariance does, and Day 1 changes in alpha power in the placebo and active group moved in opposite directions (**Figure S1B,C**). Also, the slopes observed on Day 1 were very small, which raises the question whether the significant PK/PD relationship is actually due to a true pharmacological effect of TRV045. Moreover, less data was available to inform the concentration-effect relationship on Day 1 vs. Day 4, with fewer observations and a smaller range of drug exposures on Day 1. The limited amount of data prevented us from studying underlying dynamics such as placebo effects, rhythms, or indirect (i.e., delayed) effects, which may be necessary for distinguishing

the actual EEG effects. For MEP amplitude, we found a larger concentration-dependent decrease for Day 1 vs. Day 4, in line with the results from the planned statistical analysis, which could indicate other (compensating) mechanisms starting to play a role after multiple dosing when solely looking at direct cortical excitability. There is no literature available on whether the initial dose effects of AEDs on cortical excitability, as previously described for TMS-EMG–EEG,[14] persist with repeated dosing or evolve over time. Thus, it is hard to fully contextualize these observations relative to what is known about AEDs and their effects on EEG and TMS. The brain is a complex system of excitatory and inhibitory pathways, and drugs influencing cortical excitability could have different initial and repeat dose effects, which could also differ between HV and epilepsy patients. Based on the observed PD effects in this study and the current literature, we see sustained PD effects on Day 4 that could hypothetically be due to glutamatergic modulation by TRV045. Although TMS-EMG effects dissipated after Day 1, the EEG effects suggest potential anti-epileptic activity of TRV045 after multiple dosing at the tested 250 mg dose. How these biomarkers relate to clinical effects is not fully clear, therefore, subsequent studies should evaluate this dosing regimen in epilepsy patients, to add to the understanding of the underlying mechanisms and temporal evolution of TRV450's effects and its clinical efficacy in larger phase II-III trials. Here it is worth noting that AEDs could potentially have a greater effect in a state of pathologically increased cortical excitability.

This study has several limitations. As drug effects changed over time combining TMS measurements on Day 1 and 4 in the statistical analysis did not provide the same information as performing multiple measurements on a single day. The dense schedule of assessments did not allow for more than one post-dose PD measurement per day, so they were timed to coincide with T_{max} to measure maximum PD effects, assuming a direct concentration-effect relationship. Additionally, our power calculation was based on a single-dose study in which all post-dose measurements were performed on the same day,[14] and measuring TMS on different days introduced between-day-variability in the analysis. Although we used baseline correction to minimize variability as much as possible, our model does not correct for day-to-day variability. Therefore, we might have been underpowered to demonstrate a significant effect on the MEP amplitude. Another limitation was the administered dose, as higher TRV045 exposures could have had a larger and significant effect. However, we could not dose higher than 250 mg as this was the highest dose administered in humans in a multiple-dose fashion and pharmacokinetics were not dose proportional at this dose level, so dosing higher would not further increase plasma exposures. Another limitation is that C_{max} was reached slightly later than anticipated (6 hours), so PD measures were not performed at peak plasma levels (4 hours), although the impact is probably limited as exposures were comparable at these timepoints (plasma concentration mean ± SD: Day 1 + 4 hours 591.8 ± 177.5 ng/mL vs. Day 1 + 6 hours 652.1 ± 149.2 ng/mL and Day 4 + 4 hours 834.9 ± 228.0 ng/mL vs. Day 4 + 6 hours 849.3 ± 218.2 ng/mL). As the study was not primarily designed to perform PK/PD analysis, the data are less suitable for identifying PK/PD relationships. A full mechanism-based model requires more serial measurements and a broader dose range. Also, the limited number of data points with measurable TRV045 concentrations available per subject and dosing interval could have resulted in the observed differences between days. Thus, one should note that this data cannot be used, for example, simulations of longer-term dosing. Furthermore, only males were included in the study to avoid menstrual cycle-related confounding effects on cortical excitability,[19] limiting the translatability of the findings to females. Although the family-wise error rate was controlled using cluster-based permutation analysis, no further correction for multiple testing was performed due to the exploratory nature of the study. The cross-over nature of the study might present limitations; however, a 1-week washout period should prevent PK carry-over effects given TRV045's half-life. Furthermore, our statistical model corrects for differences between treatment periods and balanced randomization minimizes potential delayed PD carry-over effects.

With this study, we demonstrated that TRV045, a S1PR1 agonist, is a CNS-active compound, with an incremental effect on the pharmaco-EEG signal after multiple days of dosing with a distinct pattern that differs from registered AEDs. There appears to be an acute decrease in corticospinal excitability, but the reduction in MEP amplitude observed on Day 1 was not significant and was not sustained after multiple days of dosing. This is the first study describing the effects of a S1PR1 modulator on pharmaco-EEG and TMS-EMG–EEG. Further evaluation is required to elucidate the physiological underpinnings of TRV045's observed PD effects and whether these translate into anti-epileptic effects.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

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CONFLICT OF INTEREST

C.M.K.E.C., A.A.G., E.S.K., M.E.O., R.J.D., G.J.G., and J.A.A.C.H. are employees of the Centre for Human Drug Research, and declare no other competing interests; J.K., M.A.D., R.C. are employees of Trevena, Inc, and declare no other competing interests.

AUTHOR CONTRIBUTIONS

C.M.K.E.C., A.A.G., G.J.G., J.K., M.A.D., R.C., and J.A.A.C.H. wrote the manuscript. C.M.K.E.C., A.A.G., G.J.G., J.K., M.A.D., and J.A.A.C.H designed the research. C.M.K.E.C., G.J.G., J.A.A.C.H performed the research. A.A.G., E.S.K., M.E.O., and R.J.D. analyzed the data.

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