A Randomized, Double-Blind, Placebo-Controlled Study of the Effects of Two Sequential Doses of NV-5138/SPN-820 on Quantitative Electroencephalography in Healthy Adult Males

Leonardo Jose Trejo, PhD[a]; Roman Rosipal, PhD[a,b]; Adrienne Moore, PhD[c]; Brendan Lujan, PhD[d]; Alisa R. Kosheleff, PhD[d]; Larry Ereshefsky, PharmD[e,f]; J. Randall Owen, MD[g]; George P. Vlasuk, PhD[g]

[a] *Pacific Development and Technology, LLC, Capitola, CA, USA*
[b] *Slovak Academy of Sciences, Bratislava, Slovakia*
[c] *Q-Metrx, Inc., Glendale, CA, USA*
[d] *Supernus Pharmaceuticals, Inc., Rockville, MD, USA*
[e] *APEX Innovative Sciences, Garden Grove, CA, USA*
[f] *University of Texas, Austin, TX, USA*
[g] *Navitor Pharmaceuticals, Inc., Cambridge, MA, USA*

Address correspondence to:
Leonardo Jose Trejo, PhD; 408 Hill Street, Capitola, CA, 95010, USA; phone: (831) 854-2521; ltrejo@pacdel.com

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Author Contributions:
Study design: LJT, RR, LE, GPV
Data collection: LJT, RR, AM
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Writing: LJT, BL, ARK
Reviewing: LJT, RR, AM, BL, ARK, LE, JRO, GPV

ABSTRACT

NV-5138 (or SPN-820) is a novel small molecule activator of the mechanistic target of rapamycin complex 1 (mTORC1) currently under development for use in treatment-resistant depression. This phase I study evaluated the safety, tolerability, and pharmacodynamics (as measured by quantitative electroencephalography, qEEG) of two sequential oral doses of NV-5138 in healthy adult males. Twenty-five participants were randomly assigned to double-blind treatment with a single dose of placebo or 2400 mg NV-5138 on Day 1, and a second dose of the same treatment on Day 3. The two doses of NV-5138 were safe and well tolerated, with no deaths, serious adverse events, or discontinuations due to adverse events. Spectral band amplitudes, derived frequency measures, and magnitude squared coherences were computed from qEEG recordings during resting state eyes-open and eyes-closed conditions at multiple timepoints. In the NV-5138 group only, significant changes in qEEG measures occurred at 1 hour post-dose on both days (near NV-5138 T_{max}), including decreases in low-frequency band amplitudes (theta) and increases in high-frequency EEG band amplitudes (high beta and gamma). These effects were mirrored by a decrease in the theta/beta ratio, a measure negatively associated with arousal and cognitive processing capability. Significantly increased high beta and gamma band coherences were also detected at several specific electrode pairs in both eyes-open and eye-closed conditions. NV-5138 actively modulated functional brain parameters consistent with positive effects on mood, cognition, and arousal. These results indicate that qEEG measures may be useful biomarkers of NV-5138 target engagement and related changes in neural activity.

Key words: treatment-resistant depression, qEEG, NV-5138, mTORC, biomarkers, pharmacodynamic

Highlights

- NV-5138 is a selective mTORC1 activator being developed for TRD.
- NV-5138 produces stereotyped qEEG responses following single doses in adult males.
- These changes are consistent with positive effects on mood, cognition, and arousal.
- qEEG is a reliable biomarker of NV-5138 central nervous system target engagement.

1. **INTRODUCTION**

Major depressive disorder (MDD) is a common neuropsychiatric condition, with a lifetime prevalence rate of ~13–17% in the United States (Hasin et al., 2005; Kessler et al., 2005). MDD is typically treated with antidepressants that target the monoamine signaling pathways, but efficacy with these compounds is limited as the temporal window to attain a therapeutic response is extensive (often weeks to months are necessary for symptomatic remission) (Fava, 2003), and frequent side effects (e.g., sexual dysfunction, weight gain) remain problematic for many patients (David & Gourion, 2016; Rush et al., 2004). Although available options for pharmacologic treatment of MDD have expanded significantly in the past 25 years, approximately 33–50% of patients do not respond satisfactorily to the first antidepressant prescribed, only 25% of the remaining patients respond to a second-step therapy, and up to 15% of patients do not respond to multiple interventions and will remain depressed, commonly referred to as treatment-resistant depression (TRD) (Cain, 2007; Rush et al., 2004; Trivedi et al., 2006; Wisniewski et al., 2007). Thus, there is currently an unmet therapeutic need for novel, fast-acting, and tolerable antidepressants with robust clinical efficacy.

Recent research into the mechanism of depression has suggested a critical role for the mechanistic target of rapamycin (mTOR) signaling pathway (Ignácio et al., 2016; Jernigan et al., 2011; Réus et al., 2015). Ketamine, an N-methyl-D-aspartate (NMDA) antagonist with downstream effects on mTOR complex 1 (mTORC1) (Harraz et al., 2016), has been shown to alleviate antidepressant behaviors in rodent models, effects which were subsequently blocked by the mTORC1 inhibitor rapamycin (Li et al., 2010). NV-5138/SPN-820 [(S)-2-amino-5,5-difluoro-4,4-dimethylpentanoic acid] is a novel, orally bioavailable, specific, small molecule which binds to sestrin and, via disinhibition, directly activates mTORC1 (half maximal effective concentration: 30–50 μM) in several brain regions, including the medial prefrontal cortex, hippocampus, striatum, and neocortex (Sengupta et al., 2019).

In preclinical rodent models, NV-5138 demonstrated rapid and long-lasting antidepressant effects comparable to those of ketamine (Kato et al., 2019). These behavioral effects after NV-5138 administration were dependent on the post-synaptic activation of mTORC1, and were associated with an increase in mTORC1 downstream signaling, synaptic protein expression (e.g., glutamate receptor 1

subunits and synapsin), and synaptic arborization in layer V pyramidal neurons in the medial prefrontal cortex of rats (Kato et al., 2019). Although the behavioral effects of both ketamine and NV-5138 appear to rely on mTORC1 activation, NV-5138 appears to exert its behavioral effects via a direct intracellular mechanism, bypassing extracellular targets such as NMDA receptors or monoaminergic targets. Quantitative electroencephalography (qEEG) can be used to demonstrate brain penetration and pharmacological target engagement.

qEEG can also be used to characterize the electrical brain activity associated with psychiatric disorders. When examining aberrant patterns of EEG activity among neuropsychiatric disorders, patients with depression were found to often exhibit increased delta, theta, and beta power (Coutin-Churchman et al., 2003; Newson & Thiagarajan, 2018), and decreased resting-state gamma bands (reviewed in Fitzgerald & Watson, 2018; Pizzagalli et al., 2006). Interestingly, low gamma band activity has been linked to cognitive processing in patients with depression (Roh et al., 2016; Siegle et al., 2010; Strelets et al., 2007), and meta-analyses indicate that cognitive impairment is commonly observed in MDD (Rock et al., 2014). qEEG characterization of the alpha rhythm (8–13 Hz) has been used as an index of cortical deactivation, where patients with MDD exhibit higher alpha power relative to controls (Jaworska et al., 2012). Additionally, qEEG resting state measurements have been used as a predictor of treatment response in MDD through assessment of frontal alpha asymmetry (Arns et al., 2016), prefrontal theta cordance (Bares et al., 2008; Bares et al., 2015), pretreatment rostral anterior cingulate theta activity (Pizzagalli et al., 2018), antidepressant treatment response index (Cook et al., 2020), and EEG functional connectivity (Ford et al., 1986; Rolle et al., 2020). qEEG has also been previously used to provide an acute functional readout of brain activity in response to drug administration (Keavy et al., 2016; Sanacora et al., 2014).

Early clinical data has shown that single doses of NV-5138 up to 2400 mg were safe and well tolerated in healthy adults (Leventer et al., 2019) and those with TRD (Targum et al., 2019). Among subjects with TRD, NV-5138 demonstrated rapid and sustained (up to the last measurement at 72 hours post-dose) antidepressant response relative to placebo as measured by the 6-item Hamilton Depression Scale (Targum et al., 2019). NV-5138 administration has also been shown to increase cerebrospinal fluid

concentrations of orotate (Leventer et al., 2019), a marker of mTORC1 activity (Ben-Sahra et al., 2013). The present study was designed to investigate the impact of two sequential doses of 2400 mg NV-5138 (separated by 2 days) on a full range of qEEG spectral amplitudes, derived frequency, and coherence measures, using a full scalp recording configuration in healthy adult males. Safety and tolerability were also evaluated.

2. METHODS

This study was performed in accordance with applicable regulatory requirements, the International Council for Harmonisation guideline for Good Clinical Practice, and the ethical principles that have their origins in the Declaration of Helsinki. The protocol, informed consent form, and Investigator's Brochure were reviewed by a properly constituted Institutional Review Board operating in accordance with Code of Federal Regulations, Title 21 (21 CFR), Part 56, before implementation at the study site (Hassman Research Institute, Berlin, NJ, USA). Informed consent was obtained from all individual participants included in the study. Patients signed informed consent regarding publishing their data.

2.1 Study Design

This was a randomized, double-blind, placebo-controlled study of two doses, 48 hours ± 30 minutes apart, of NV-5138 in healthy adult male participants. The study included a 28-day screening period, an in-house period of 8 days/7 nights during which NV-5138 or placebo was administered, and a 3- to 7-day follow-up period after discharge. Twenty-five healthy male participants were randomly assigned (approximately 1:1) to double-blind treatment with either placebo or NV-5138. Each participant received one oral dose of either placebo or 2400 mg NV-5138 (both administered as oral solutions) on Day 1 and a second oral dose of the same treatment 48 hours later (± 30 minutes) on Day 3.

2.2 Study Participants

This study enrolled healthy men, age 18–55 years, who were fluent in English and had a body mass index (BMI) of 19–30 kg/m^2. Exclusion criteria included recent clinically significant illness, medical/surgical

procedure, or trauma; a clinically significant laboratory or systemic abnormality, including clinically significant vital sign, electrocardiogram (ECG), or EEG abnormalities; clinically significant abnormal physical or neurological examination findings; a history of seizure, loss of consciousness for an unknown reason, or any other known neurological disorder placing the participant at risk for seizures; significant recent weight loss; a diagnosis of intellectual disability; a history of psychiatric disorder, suicidality or suicidal behavior, clinically significant head trauma, use of prohibited substances, or participation in another study within the specified time periods pre-dose; or any condition or activity that might increase the risk to a participant, compromise the results of the study, or make the participant unsuitable for the study.

2.3 Assessments

2.3.1 qEEG Measures

Pharmacodynamic endpoints for analysis included spectral band amplitudes, frequency-derived measures, and magnitude squared coherence (MSC) assessed by qEEG. EEGs were recorded using Compumedics Grael V2 EEG amplifiers with Curry 8E Software from 23 electrodes placed according to the International 10–20 electrode placement system (Klem et al., 1999) with a sampling rate of 1024 Hz. For qEEG, 5-minute segments of eyes closed (EC) and eyes open (EO) EEG data were collected, as it is well accepted that the EO recording condition suppresses some spectra band activity, especially alpha, relative to the EC condition (Barry et al., 2007). On Days 1, 2, 3, and 4, EC and EO EEGs were recorded at the same times corresponding to 1 hour pre-dose, and 1, 4, and 8 hours post-dose (approximately 0800, 1000, 1300, 1700). Four baseline EEG recordings in both EC and EO condition were collected on Day −1 at times matching (± 30 minutes) those scheduled for the Day 1 pre-dose and 1-, 4-, and 8-hour post-dose timepoints. The post-dose intervals at which the EEG evaluations were performed following the second dose matched within ± 30 minutes the post-dose intervals of the EEG evaluations after the first dose. EC and EO EEG recordings were repeated on Day 5 at the 1-hour pre-dose timepoint and at follow-up on Day 10 at the 8-hour post-dose timepoint.

2.3.2 Safety and Tolerability

Safety and tolerability were assessed based on spontaneously reported adverse events (AEs), scheduled physical, neurological, and psychiatric safety examinations (including the Brief Psychiatric Rating Scale–Positive Symptom Subscale [BPRS (+)] and Clinician-Administered Dissociative States Scale [CADSS]), clinical laboratory test results, vital signs, oral temperature, weight, ECGs; EEGs; and the Columbia−Suicide Severity Rating Scale (C-SSRS).

2.4 Analysis and Statistical Methods

Standard pre-processing was applied to EEG recordings to band-pass filter (0.5 Hz to 70 Hz), re-reference to a linked-ears reference, reject ocular artifacts, and remove segments containing electromyogram (EMG) artifacts as needed. Segments of 2.0 seconds duration with no overlap were processed using Irregular-Resampling Auto-Spectral Analysis (IRASA) to separate oscillatory and fractal components (Wen & Liu, 2016). The IRASA toolbox for MATLAB, freely available from the authors, was used. The code was modified by setting possible negative spectral amplitudes of the oscillatory part estimate to zero, using the Hann window, and setting the number of subsets for the amplitude spectral density estimate to ten.

2.4.1 qEEG Spectral Analysis

Band amplitudes were estimated separately for both oscillatory and fractal EEG spectral components. Spectral amplitudes (μV/Hz) were computed in the range of 0.5 to 50 Hz and separated into clinically relevant bands: delta (1.0–4.0 Hz), theta (4.0–8.0 Hz), alpha (8.0–12.0 Hz), alpha 1 (8.0–10.0 Hz), alpha 2 (10.0–12.0 Hz), beta (12.0–25.0 Hz), beta 1 (12.0–15.0 Hz), beta 2 (15.0–18.0 Hz), beta 3 (18.0–25.0 Hz), high beta (25.0–30.0 Hz), gamma (30.0–50.0 Hz), gamma 1 (30.0–35.0 Hz), gamma 2 (35.0–40.0 Hz), gamma 3 (40.0–50.0 Hz), and total spectrum (1.0–50.0 Hz). Band amplitudes for 13 spatial regions were estimated by averaging band amplitudes for individual electrodes: Frontal: left (FP1, F3, F7), right (FP2, F4, F8), and midline (Fz); Central: left (C3), right (C4), and midline (Cz); Temporal: left (T3, T5) and right (T4, T6); Parietal: left (P3), right (P4), and midline (Pz); Occipital: left (O1) and right (O2). Frequency bands presented in figures are arranged from low to high frequency in the following order: delta, theta,

alpha, alpha 1, alpha 2, beta, beta 1, beta 2, beta 3, high beta, gamma, gamma 1, gamma 2, and gamma 3.

2.4.2 Derived Frequency Measures

The following derived frequency measures were computed from qEEG spectral analysis: alpha slow-wave index (ASI), theta/beta ratio (TBR), and dominant frequency (called individual alpha frequency, IAF). ASI was defined as the ratio of band amplitude sums: (alpha 1+alpha 2)/(delta+theta). TBR was defined as the ratio of band amplitude sums: theta/(beta 1+beta 2). IAF was defined as the frequency with maximal amplitude in the frequency band 8.0 to < 12.0 Hz.

2.4.3 qEEG Magnitude Squared Coherence

The MSC was computed using the *mscohere* function in MATLAB with a Hann window. Sixteen pairs of electrodes were used, including interhemispheric coherences for electrode pairs: FP1-FP2, F3-F4, C3-C4, P3-P4, and F3-C3, and intrahemispheric electrode pairs: F3-P3, F3-T5, C3-P3, C3-T5, P3-T5, F4-C4, F4-P4, F4-T6, C4-P4, C4-T6, and P4-T6.

Absolute endpoint values were analyzed using mixed models repeated measures (MMRM) with a covariate. For MMRM, a critical alpha level of $p < 0.05$ was selected as the significance criterion. MMRM was employed to test the effects of NV-5138 as compared to placebo treatment. The model was implemented using the PROC MIXED procedure of the SAS® Enterprise Guide 6.1 software. Dependent measures included band amplitude, derived measures, and coherence measures for the EC and EO conditions. Independent factors included in the model were treatment group (placebo or NV-5138) and time (qEEG measurement timepoints), with the interaction also considered. The autoregressive AR(1) covariance type to specify covariance structures for repeated measurements on subjects was used. As the analyses were considered exploratory in nature, no corrections for multiple comparisons were applied across model tests for different endpoints.

The present analyses were restricted to 1-hour post-dose timepoints for two reasons. Firstly, previous studies have shown that rapid-acting antidepressant drug candidates may show acute effects on EEG

within 90 minutes post-dose (Keavy et al., 2016; Sanacora et al., 2014). Secondly, NV-5138 time to maximal concentration in plasma is known to range from 0.5 to 1 hour (data on file). For these reasons, the present analyses focused exclusively on absolute values of qEEG measures at 1-hour post-dose on Day 1 and Day 3. Pre-dose Day 1 values of each measure served as the covariate.

3. RESULTS

3.1 Participant Disposition and Demographics

Fifty-six participants were screened. A total of 25 participants were randomized and received study drug (safety population); 13 participants received placebo and 12 participants received NV-5138. Of the 25 participants, 24 (96.0%) completed the study per the protocol (analysis population). The remaining participant withdrew consent after receiving the initial dose (placebo) on Day 1.

Participants ranged in age from 19 to 52 years (mean, 39 years; median, 41 years) and were predominantly black or African American (80.0%), with the remainder white (8.0%) or “other” (12.0%). Four (16.0%) of the participants were Hispanic or Latino. BMI ranged from 21.0 to 29.9 kg/m^2 (mean, 26.1 kg/m^2; median, 25.8 kg/m^2). There were no major differences between treatment groups in demographic or baseline characteristics (**Table 1**).

3.2 Quantitative Electroencephalography

3.2.1 General Observations

Most qEEG changes for spectral band amplitudes, derived frequency measures, and MSC were seen in the NV-5138 group, with the placebo group showing fewer changes, and these generally opposed the changes seen in the NV-5138 group. Relative to a time-matched pre-dose baseline, participants administered NV-5138 demonstrated decreases in low-frequency EEG band amplitudes (delta, theta, and alpha) and increases in high-frequency EEG bands (high beta and gamma) one hour after dosing on both

Days 1 and 3. The NV-5138 effects on derived frequency band measures included a marked decrease in the TBR as compared to an increase in the placebo group.

In the EC condition (**Figure 1**), the effects of treatment on the oscillatory part of the EEG spectrum in the NV-5138 group included decreased delta and theta band amplitudes, slightly increased ASI, and greatly decreased TBR (**Figure 2**). Placebo subjects showed small increases in ASI and TBR. In the fractal part of the EEG spectrum, changes in the NV-5138 group included decreased delta and theta band amplitudes and TBR, and increased amplitudes for high beta, gamma, gamma 1, gamma 2, and gamma 3 bands. Placebo subjects showed slightly decreased amplitudes in the high beta, gamma, gamma 2, and gamma 3 bands, in opposition to the increases seen in the NV-5138 group. In the NV-5138 group, MSC decreased for lower frequencies (alpha to beta 2) and increased for high frequencies (beta 3 and above) at several specific electrode pairs. In the placebo group, MSC was largely unchanged with small decreases observed in beta 3 and high beta, which opposed increases seen in the NV-5138 group (**Figure 3)**.

In the EO condition (**Figure 1**), the effects of treatment in the NV-5138 group for the oscillatory spectrum included slightly decreased delta, moderately decreased theta, greatly decreased alpha, small decreases in beta 1 and beta 2, increased beta 3, decreased ASI, greatly decreased TBR (**Figure 2**), and slightly increased IAF. The placebo group showed small decreases in beta, beta 1, and beta 2. The placebo group also showed a moderate decrease in beta 3, which opposed the increase seen in the NV-5138 group. Although later timepoints are not formally tested here, we note that alpha band amplitudes in the NV-5138 group increased relative to baseline from 23 hours to 32 hours after the first dose and second dose, with the clearest increases seen in the alpha 2 band (**Supplemental Figure 2**). This pattern was not seen with placebo treatment. In the fractal EEG spectrum, changes in the NV-5138 group included increased amplitudes in the high beta, gamma, gamma 1, gamma 2, and gamma 3 bands, where the increases were greatest in the gamma 3 band. Placebo-treated subjects showed the exact opposite, a pattern of decreased amplitudes in all these bands.

Coherence changes in the EC condition (**Figure 3**) in the NV-5138 group showed increased MSC in high frequency bands. The greatest increases occurred in the gamma bands. For example, on Day 1, left

fronto-parietal (F3-P3) MSC increased more than 15%[1] for gamma 1 and gamma 2 and ranged from 13% to 16% over high beta and gamma bands. On Day 3, a similar pattern was seen for F3-P3, but MSC ranged lower, from 4% to 13%, over high beta and gamma bands with a maximum change of 13% in gamma 1. Right fronto-temporal coherence (F4-T6) showed a similar pattern with greater changes in MSC on Day 1 than Day 3 and ranging from 13% to 24% over the beta 3 through gamma 3 bands with a maximum of 24% in gamma 1. Similar changes were seen in other long-range intrahemispheric coherences in each hemisphere (F3-P3, F4-P4, and F3-T5); however most interhemispheric coherences (FP1-FP2, F3-F4, C3-C4, and P3-P4) and short-range intrahemispheric coherences (F4-C4, F3-C3, C3-T5, and C4-T6) showed either no changes or small changes (10% to 14%). Generally, changes in MSC for lower frequency bands were fewer and smaller than for high frequency bands. In the NV-5138 group, decreased MSC was seen at C4-P4 in alpha, alpha 1, alpha 2, beta 1, and beta 2 ranging from −9% to −11% on Day 3 but not on Day 1. A similar pattern was seen at F3-P3. Placebo-treated subjects showed few changes, with no increases on either day, but had slightly decreased MSC in beta 3 and high beta at C3-T5 on Day 1 only.

The pattern of MSC changes in the EO condition (**Figure 3**) in the NV-5138 group was similar to that seen for the EC condition but with greater and more widespread increases in high-frequency MSC. For example, on Day 1, left fronto-parietal (F3-P3) MSC ranged from 13% to 14% over high beta and gamma bands. For right fronto-central (F4-C4) MSC, increases were seen on both days in all gamma bands, ranging from 17% to 22%. There were few changes in MSC for placebo-treated subjects. All of these changes were relatively small and opposite in direction to those of the NV-5138 group. For example, at F4-C4, placebo-treated subjects showed decreased MSC ranging from −10% to −13% over the gamma bands, with a minimum of −13% in gamma 3. Other electrode pairs also showed decreased MSC, mostly within the mid-beta and gamma bands.

[1] % represents the value of normalized change from baseline, which ranges from −1 to +1.

3.2.2 Mixed Model Repeated Measures Analysis

The preceding general observations were supplemented by formal hypothesis testing using the MMRM analysis procedure described above (Section 2.4.3). Endpoint analysis was restricted to the 1-hour post-dose timepoint to coincide with maximum NV-5138 plasma concentrations, consistent with previous studies demonstrating that rapid-acting antidepressant drug candidates may show acute effects on EEG within 90 minutes post-dose (Keavy et al., 2016; Sanacora et al., 2014). The null hypothesis was that mean endpoint differences from baseline at 1 h post-dose were equal with placebo and NV-5138 treatment. Endpoints for which there were significant treatment effects are listed in **Table 2** and further described in sections 3.2.2.1–3.2.2.3.

3.2.2.1 Oscillatory Endpoints

MMRM least-squares mean estimate (LSME) amplitudes indicated the following significant changes with NV-5138 treatment relative to placebo at 1 h post-dose. These changes were marked by decreases in EC and EO theta at temporal and central regions, decreases in EC and EO TBR at central regions, decreased frontal EO alpha 2, increased parietal and temporal EO beta 3, and decreased frontal and occipital EO IAF.

- Temporal left and temporal right EC theta were reduced.
- Central left, central midline, and temporal left EO theta were reduced.
- Central midline and central right EC TBR were reduced.
- Central left EO TBR was reduced.
- Frontal midline EO alpha 2 was reduced.
- Parietal right and temporal right EO beta 3 were increased.
- Frontal right and occipital right EO IAF were reduced.

3.2.2.2 Fractal Endpoints

MMRM LSME amplitudes indicated the following significant changes with NV-5138 treatment relative to placebo at 1 h post-dose. These changes were marked by decreased parietal and temporal EC theta; decreased parietal and temporal EC and EO TBR; decreased central, temporal, and parietal EC alpha 1;

increased EO beta in multiple bands over a broad range of regions; increased EC and EO gamma in multiple bands over a broad range of regions; and increased EC temporal total spectrum amplitude. The gamma increases included many at midline, central, and parietal regions, which are less prone to EMG artifacts than frontal, temporal, and occipital regions.

- Parietal left, parietal midline, parietal right, and temporal left EC theta were reduced.
- Frontal right, parietal left, temporal left, and temporal right EC TBR were reduced.
- Parietal right, temporal left, and temporal right EO TBR were reduced.
- Central left, parietal left, parietal midline, parietal right, and temporal left EC alpha 1 were reduced.
- Temporal right EO beta was increased.
- Temporal right EO beta 3 was increased.
- Central right, frontal right, parietal right, temporal left, and temporal right EC high beta were increased.
- Central right, frontal right, occipital left, occipital right, parietal left, parietal midline, parietal right, temporal left, and temporal right EO high beta were increased.
- Central left, central right, frontal left, frontal midline, frontal right, parietal midline, parietal right, temporal left, and temporal right EC gamma were increased.
- Frontal left, frontal right, temporal left, and temporal right EC gamma 1 were increased.
- Central left, frontal left, frontal right, parietal midline, parietal right, temporal left, and temporal right EC gamma 2 were increased.
- Central left, central midline, central right, frontal left, frontal midline, frontal right, parietal left, parietal midline, parietal right, temporal left, and temporal right EC gamma 3 were increased.
- Central midline, central right, frontal midline, occipital left, occipital right, parietal left, parietal midline, parietal right, temporal left, and temporal right EO gamma were increased.
- Occipital left, occipital right, parietal left, parietal midline, parietal right, temporal left, and temporal right EO gamma 1 were increased.

- Central midline, frontal midline, occipital left, occipital right, parietal left, parietal midline, parietal right, temporal left, and temporal right EO gamma 2 were increased.
- Central midline, central right, frontal midline, occipital left, occipital right, parietal left, parietal midline, parietal right, temporal left, and temporal right EO gamma 3 were increased.
- Temporal right EC total spectrum was increased.

3.2.2.3 Coherences

A large number of significant differences in coherence between NV-5138 and placebo treatment were observed (**Table 2**). Instead of listing these here, they are summarized as follows:

- For most bands and electrode pairs, coherences increased with NV-5138 treatment as compared to placebo. These included EO alpha and alpha 2; EC alpha 2; and EO and EC beta, beta 1, beta 2, beta 3, and high beta. In the gamma and total spectrum bands, increases occurred only in the EO condition.
- The magnitude of coherence increases was progressively greater with band frequency. The mean increases within bands, over EC and EO and electrode pairs (from **Table 2**), were: alpha: 0.05, beta: 0.06, beta 1: 0.06, beta 2: 0.06, beta 3: 0.07, high beta: 0.10, and gamma (all bands): 0.13.
- The distribution of increases was mostly intrahemispheric, with right hemisphere preponderance. Overall, 39 increases were intrahemispheric and, of these, 25 were in the right hemisphere and 14 were in the left hemisphere. A smaller number of increases (eight) were central interhemispheric and these were limited only to the C3-C4 electrode pair.
- Considering where and for which bands the increases were most pronounced, the F4-C4 electrode pair, which included increases in the beta 3, high beta, gamma (all bands), and total spectrum, stood out from other electrode pairs. For this electrode pair, the average increase was 0.12. The second most pronounced pair was F4-P4, which included increases in alpha 2, beta 1, beta 3, high beta, all gamma bands, and total spectrum, and had an average increase of 0.10. Average increases for all other electrode pairs ranged from 0.06 to 0.08.

- Overall, these results indicate that NV-5138 induced the greatest increases in long-range, fronto-central, and fronto-parietal intrahemispheric right hemisphere coherence, which was mostly driven by higher beta and gamma band effects.
- Only one electrode pair showed decreased coherences, P4-T6, and these were limited to the beta bands (beta, beta 1, beta 2, and beta 3). The average magnitude of these decreases was −0.06.

3.2.3 Comparisons of Changes on Day 1 and Day 3

To determine whether a second dose of NV-5138 amplified any of the EC qEEG changes seen with the first dose, we also compared and statistically tested (*t* test, a critical alpha level of $p < 0.05$) differences between Day 1 and Day 3 LSME values of MMRM. None of these tests indicated greater effects of a second dose as compared to the first dose. Although the LSME differences were not significant, the decrease of EC TBR was clearly greater on Day 3 than Day 1 at multiple spatial regions (**Figure 2**). Regarding changes in high frequency band amplitudes, the LSME differences between Day 1 and Day 3 were not significant. Inspection of the group means (not LSME), however, shows that Day 1 and Day 3 increases in beta through gamma EC band amplitudes were nearly equal, with slightly greater overall increases on Day 3 than on Day 1 (**Figure 1**).

To determine whether a second dose of NV-5138 amplified any of the EO qEEG changes seen with the first dose, we also compared and statistically tested (*t* test, a critical alpha level of $p < 0.05$) differences between Day 1 and Day 3 LSME of the MMRM. In the NV-5138 group, the LSME for oscillatory EO TBR significantly decreased ($p = 0.039$) more on Day 3 than on Day 1 at the central left region. The group means also show that TBR decreased more on Day 3 than Day 1 at all regions except for left and right occipital, where decreases in TBR were generally smaller than other regions (**Figure 2**). No other LSME differences were significant. However, both the LSME and group means (**Figure 2**) showed that decreases in theta, alpha, alpha 1, and alpha 2 over frontal regions were greater on Day 3 than Day 1. As for the EC condition, these comparisons indicate an increased effect of NV-5138 at low frequencies with a second dose, as seen in significantly greater decreases in TBR and greater mean decreases in theta and

alpha band amplitudes on Day 3 than Day 1. In the EO condition, the greater TBR reduction one hour after dose two vs. dose one was significant.

There were no significant differences between Day 1 and Day 3 in the changes from baselines in EC or EO LSME of MSC. However, the group means indicate that, for the electrode pairs that had large increases in MSC in the high frequency bands, these increases were generally greater on Day 1 than Day 3 (**Figure 3**). Although these differences were not significant, the trend runs counter to the notion that all EEG effects were greater overall after dose two than dose one, with high-frequency MSC changes showing the opposite pattern. However, the aforementioned decreases in MSC at the C4-P4 electrode pair, which spanned alpha and beta bands, were generally greater on Day 3 than Day 1 (**Figure 3**). This observation is in line with the relatively greater LSME decreases in alpha band amplitudes on Day 3 vs. Day 1 and agrees with the notion that EEG amplitude changes were greater after dose two than dose one.

3.3 Safety and Tolerability

Two sequential doses of NV-5138 (2400 mg) separated by 48 hours were safe and well tolerated. Treatment-related AEs (**Table 3**) were mild, with no deaths, no serious AEs, or discontinuations due to AEs. The number of treatment-related AEs was higher in the placebo group (n = 3) than in the NV-5138 group (n = 0), and the three treatment-related AEs that occurred in two participants in the placebo group were resolved without treatment. All treatment-related AEs were mild. Although one participant treated with NV-5138 had two treatment-emergent AEs related to EEG findings on Days 1 and 3 at the 8-hour post-dose timepoint, these were later judged by an independent neurologist to be unrelated to the study drug. No dissociative effects were reported, and there were no clinically meaningful abnormalities in laboratory test results; vital signs; ECG data; safety EEG data; BPRS (+), C-SSRS, or CADSS scores; or neurological or physical examination findings. There was no evidence of proconvulsant activity on safety EEGs.

4. DISCUSSION

4.1 Overview

In this exploratory study of the effect of two sequential 2400 mg oral doses of NV-5138 on qEEG parameters in healthy participants, NV-5138 demonstrated substantial effects on spectral band amplitudes, frequency-derived measures, and MSC with no significant impacts on safety. The strongest NV-5138-dependent effects occurred 1 h post-dose, and approximately coincided with the time of maximal NV-5138 plasma concentrations (i.e., T_{max}, **Supplemental Table S1**). Nearly all qEEG changes related to time after dosing were confined to the NV-5138 group, with the placebo group showing little or no evidence of an effect of time after dosing on any measure.

Spectral analysis revealed that NV-5138 consistently produced decreases in low-frequency EEG bands (delta, theta, and alpha) and increases in high-frequency EEG bands (gamma). In alpha bands, there were sharp decreases in amplitudes (or desynchronization) near T_{max} on both dose days. These effects are all signs of functional changes in EEG activity, and they did not occur in the placebo group. Clinically, increased arousal, vigilance, and alertness are associated with these functional changes in the EEG spectra during activated states (e.g., mental effort), which are characterized by desynchronized alpha waves (low alpha amplitude) and relative absence of delta and theta waves. The EEG under such conditions may also show increased amplitudes in high-frequency bands (higher beta and gamma bands) (Sander et al., 2015). The observed phenotypes on functional qEEG parameters in the present study are consistent with EEG signatures that have been associated with increased alertness and arousal. Functional changes in EEG activity in the present study were also evidenced by increases in beta and gamma band amplitudes. Increased beta-gamma band amplitudes have been linked to higher levels of perceptual or cognitive processing (Başar et al., 1999; Fitzgibbon et al., 2004; Kaiser & Lutzenberger, 2005). Moreover, NV-5138 consistently produced increases in qEEG coherences for most electrode pairs, with the magnitude being progressively greater with increasing band frequency. Spatially, the observed increased coherence was noted in intrahemispheric brain regions, occurring most prominently in fronto-central, and fronto-parietal regions.

4.2 Impact of Antidepressants on qEEG

Although studies reporting the impact of antidepressants on qEEG parameters are relatively uncommon, a few such reports exist for novel compounds approved for the treatment of depression. For example, ketamine (whose s-enantiomer derivative esketamine is approved for the treatment of TRD) has been shown to produce significant changes in EEG band amplitudes and coherence (Páleníček et al., 2011), though the pattern of effects is variable. In a group of 30 male and female healthy adults, ketamine increased theta power, decreased alpha power, and shifted the spectral edge to lower frequencies in a dose-dependent manner (Kochs et al., 1996). However, in another study of healthy young adult males, sub-anesthetic doses of ketamine decreased delta, theta, and alpha power while increasing gamma power on an acute timescale (de la Salle et al., 2016). Our results are in partial agreement with those findings of the latter study, as both ketamine and NV-5138 decreased low-frequency EEG power (delta and theta) and increased high-frequency EEG band power (gamma). Another study in healthy volunteers used magnetoencephalography to test the effects of ketamine on brain oscillatory sources (Muthukumaraswamy et al., 2015). The authors found decreased alpha band power and increased high gamma band power within 90 minutes of ketamine infusion. Our results are also in partial agreement with these findings, as NV-5138 similarly decreased low frequency band amplitudes and increased high-frequency band amplitudes on an acute timescale. Whether these similarities are due to a convergent action on the mTOR signaling cascade or another mechanism is yet to be elucidated.

4.3 Relevance of EEG Spectra to Mood, Cognition, and Arousal

Specific changes in EEG spectra have been linked to the regulation of mood in MDD. The most common changes in EEG power of MDD patients are increases in both theta and beta bands (Newson & Thiagarajan, 2018), and melancholic MDD patients exhibit an even more pronounced increase in beta power (Pizzagalli et al., 2002). Additionally, increased or decreased alpha power density has been linked to mood and is a functional manifestation of patients exhibiting MDD (Itil, 1983; Ulrich et al., 1984). Notably, hemispheric power symmetry also seems to be compromised in male MDD patients compared to healthy participants, where MDD patients may exhibit increased interhemispheric alpha asymmetry (Knott et al., 2001). NV-5138 decreased theta band amplitudes, decreased alpha band amplitudes, and

increased both interhemispheric and intrahemispheric high-frequency coherence in the present study, which may be indicative of a positive impact on mood regulation. Specifically, in the present study, the coherence effects were somewhat asymmetric, being more pronounced in the right hemisphere, and intrahemispheric coherence changes were far more prevalent and larger than interhemispheric coherence changes. In addition, increased coherence was noted at many electrode pairs for beta and gamma bands, and decreases were seen only for right central-parietal electrode pairs (**Figure 3**) and in alpha and lower beta bands. Other studies have shown that patients with MDD tend to have increased coherence, particularly in beta bands, and, to a lesser extent, in theta and alpha bands, as compared to healthy controls (Leuchter, 2012). Our results suggest that in healthy male participants, NV-5138 has the opposite effect of reducing coherence in lower frequency bands (i.e., theta, alpha, and low beta) and increasing coherence in higher frequency bands (i.e., high beta and gamma), which may be a provisional indication of therapeutic efficacy.

Definite changes in EEG spectra amplitudes have been linked to cognitive processing. Specifically, in healthy humans, high amplitude EEG alpha activity is usually observed during periods of relaxed wakefulness that are associated with low cognitive load, and reduced alpha activity is correlated with the functional cortical activity of those specific brain regions involved in cognitive processing tasks, including short-term and long-term working memory processes (Klimesch, 1997). Conversely, increased alpha activity has been observed in those brain regions not involved in the execution of specific cognitive or motor tasks, which may even represent active inhibition of task-irrelevant brain circuits (Klimesch, 1997). Moreover, gamma band activity has been linked to the encoding and subsequent recall of information. Namely, increased gamma band activity has been observed in distinct assays to probe cognitive processing in learning and memory paradigms (Sederberg et al., 2003; Tallon-Baudry et al., 1998). Indeed, other studies have hypothesized that the TBR is an inverse biomarker of cognitive processing capacity (Clarke et al., 2019). In the current study NV-5138 produced decreases in alpha band amplitudes (desynchronization), decreases in TBR, and increases in gamma band activity, all of which imply up-regulation of cognitive processing.

Stereotyped EEG responses have been used as an indicator for arousal, with definitive vigilance stages ranging from high wakefulness to sleep onset, which correspond to functional brain activity (Olbrich et al., 2009). This is notable because the degree of brain arousal is thought to underlie motivated behaviors, which may be disrupted in a number of neuropsychiatric diseases including MDD (Hegerl et al., 2012). For example, in a study of 30 unmedicated depressed patients, a significantly more stable pattern of vigilance was observed (relative to 30 healthy controls) using a recently developed algorithm to characterize vigilance by EEG responses (Hegerl et al., 2012). A signature of this study and others was an increase in alpha power (Ulke et al., 2017; Ulke et al., 2019). In a separate study, destabilizing vigilance (e.g., sleep deprivation) produced an improvement in MDD symptoms in 50% of the patients (Benedetti & Colombo, 2011). Notably, in the current study, NV-5138 acutely decreased alpha band power, which also suggests up-regulation of arousal.

4.4 Limitations

The present results should be interpreted in the context of some limitations. Although gender differences in EEG spectra of healthy adults have been noted previously (Kaneda et al., 1996), the present study was comprised entirely of healthy adult males, therefore precluding any conclusions about the effects of NV-5138 on qEEG measures in children, adolescents, or adult females, as well as any conclusions about the effects in patients with depression. Further, although psychiatric medications are typically administered at low daily doses chronically over a period of several weeks or more, NV-5138 was administered as two acute sequential doses over 48 hours; whether the results reported here might generalize to daily, long-term NV-5138 dosing is unknown. Importantly, although NV-5138 produced robust changes in several qEEG parameters known to be associated with mood, cognition, or arousal, such measures were not included in this study; whether the changes in brain activity reported here are associated with functional changes in behavior, cognition, arousal, or mood state remain to be elucidated. Finally, with 80% of participants identifying as Black or African American, the study population was not representative of the broader United States population, and thus may limit generalizability to other racial groups.

4.5 Conclusion

In this exploratory double-blind, placebo-controlled study, two sequential doses of NV-5138 (2400 mg) were safe and well tolerated, with no evidence of dissociative effects. Substantial changes on several measures of qEEG activity were observed in the NV-5138 group (but not in the placebo group) and by association with plasma concentration curves, were consistent with a pharmacodynamic effect on brain activity. The nature of the qEEG changes in the present study also indicated a functional effect consistent with transiently increased arousal, vigilance, and cognitive capacity. Furthermore, dose-related coherence changes followed a pattern opposite to those that characterize coherence patterns in MDD patients. Interestingly, increased coherence was most prominently seen with the frontal cortex, an area whose dysfunction has been well-documented in clinical depression (Goodwin, 1997). The observation that the effects were restricted to the NV-5138 group suggest that qEEG may provide utility as a physiological biomarker of NV-5138 central nervous system engagement. Our data show clearly that single 2400 mg doses of NV-5138 produce rapid and quantifiable effects on brain activity, which are consistent with previously reported qEEG signatures associated with up-regulation of mood, cognition, and arousal.

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Table 1. Demographic characteristics

Characteristic	NV-5138 2400 mg (N = 12)	Placebo (N = 13)	All Participants (N = 25)
Age			
Year, mean (SD)	35.3 (7.6)	42.5 (9.4)	39.0 (9.2)
Median (min, max)	36 (23, 48)	46 (19, 52)	41 (19, 52)
Gender, n (%)			
Male	12 (100%)	13 (100%)	25 (100%)
Race, n (%)			
Black or African American	9 (75.0%)	11 (84.6%)	20 (80.0%)
White	1 (8.3%)	1 (7.7%)	2 (8.0%)
Other	2 (16.7%)	1 (7.7%)	3 (12.0%)
Ethnicity, n (%)			
Not Hispanic or Latino	9 (75.0%)	12 (92.3%)	21 (84.0%)
Hispanic or Latino	3 (25.0%)	1 (7.7%)	4 (16.0%)
Height, cm			
Mean (SD)	181 (7.5)	176 (6.3)	179 (7.2)
Median (min, max)	182 (165, 194)	179 (167, 188)	180 (165, 194)
Weight, kg			
Mean (SD)	88.4 (12.8)	78.9 (9.2)	83.4 (11.9)
Median (min, max)	87.4 (68.4, 112.0)	76.8 (66.7, 97.8)	81.5 (66.7, 112.0)
BMI, kg/m^2			
Mean (SD)	26.8 (2.7)	25.4 (2.6)	26.1 (2.7)
Median (min, max)	27.0 (22.1, 29.9)	24.9 (21.0, 29.8)	25.8 (21.0, 29.9)

Abbreviations: BMI, body mass index; max, maximum; min, minimum; N, number of subjects; SD, standard deviation

Table 2

Table 2. Endpoints for which the difference (least-squares mean estimate) between NV-5138 and placebo treatment conditions was significant at 1 h post-dose

Condition	Band	Spatial Region	LSME Difference (NV-5138 − Placebo)	SEM	df	t	p value
Oscillatory Endpoints							
EC	theta	Temporal Left	−0.03	0.01	21.20	−2.71	0.0130
EC	theta	Temporal Right	−0.04	0.02	20.95	−2.09	0.0488
EC	TBR	Central Midline	−1.35	0.61	21.53	−2.21	0.0384
EC	TBR	Central Right	−0.83	0.39	21.40	−2.12	0.0455
EO	theta	Central Left	−0.03	0.01	20.71	−2.50	0.0211
EO	theta	Central Midline	−0.04	0.02	20.91	−2.29	0.0328
EO	theta	Temporal Left	−0.03	0.01	21.27	−2.37	0.0273
EO	alpha 2	Frontal Midline	−0.04	0.02	21.05	−2.43	0.0241
EO	beta 3	Parietal Right	0.04	0.01	21.49	2.67	0.0141
EO	beta 3	Temporal Right	0.03	0.02	20.55	2.10	0.0482
EO	TBR	Central Left	−0.26	0.12	21.38	−2.21	0.0377
EO	IAF	Frontal Right	−0.40	0.16	20.77	−2.56	0.0184
EO	IAF	Occipital Right	−0.61	0.23	20.56	−2.70	0.0134
Fractal Endpoints							
EC	theta	Parietal Left	−0.10	0.03	21.32	−2.80	0.0106
EC	theta	Parietal Midline	−0.11	0.04	21.31	−2.81	0.0103
EC	theta	Parietal Right	−0.08	0.03	20.83	−2.39	0.0262
EC	theta	Temporal Left	−0.07	0.03	20.87	−2.64	0.0155
EC	alpha 1	Central Left	−0.08	0.04	20.93	−2.17	0.0416
EC	alpha 1	Parietal Left	−0.08	0.03	21.13	−2.62	0.0160
EC	alpha 1	Parietal Midline	−0.08	0.03	21.14	−2.19	0.0395
EC	alpha 1	Parietal Right	−0.06	0.03	20.89	−2.08	0.0500
EC	alpha 1	Temporal Left	−0.06	0.02	21.08	−2.41	0.0252
EC	high beta	Central Right	0.10	0.04	20.97	2.58	0.0175
EC	high beta	Frontal Right	0.09	0.03	20.97	2.86	0.0093
EC	high beta	Parietal Right	0.07	0.03	21.26	2.28	0.0334
EC	high beta	Temporal Left	0.06	0.03	21.00	2.32	0.0306
EC	high beta	Temporal Right	0.08	0.03	20.10	2.76	0.0121
EC	gamma	Central Left	0.05	0.02	11.87	2.35	0.0372
EC	gamma	Central Right	0.05	0.02	18.84	2.14	0.0461
EC	gamma	Frontal Left	0.06	0.02	21.54	2.60	0.0164
EC	gamma	Frontal Midline	0.05	0.02	21.05	2.12	0.0465
EC	gamma	Frontal Right	0.06	0.02	20.87	2.55	0.0186
EC	gamma	Parietal Midline	0.05	0.02	20.74	2.51	0.0203
EC	gamma	Parietal Right	0.05	0.02	20.37	2.40	0.0260
EC	gamma	Temporal Left	0.06	0.01	20.99	4.18	0.0004
EC	gamma	Temporal Right	0.07	0.02	21.07	3.88	0.0009
EC	gamma 1	Frontal Left	0.06	0.02	21.41	2.33	0.0297
EC	gamma 1	Frontal Right	0.05	0.02	21.06	2.17	0.0419

EC	gamma 1	Temporal Left	0.05	0.01	20.89	3.66	0.0015
EC	gamma 1	Temporal Right	0.06	0.02	20.95	3.30	0.0034
EC	gamma 2	Central Left	0.05	0.02	12.61	2.24	0.0436
EC	gamma 2	Frontal Left	0.06	0.02	21.53	2.44	0.0236
EC	gamma 2	Frontal Right	0.05	0.02	20.93	2.39	0.0263
EC	gamma 2	Parietal Midline	0.05	0.02	20.89	2.30	0.0317
EC	gamma 2	Parietal Right	0.05	0.02	20.56	2.22	0.0376
EC	gamma 2	Temporal Left	0.06	0.01	20.99	4.15	0.0005
EC	gamma 2	Temporal Right	0.07	0.02	21.05	3.67	0.0014
EC	gamma 3	Central Left	0.06	0.02	12.04	2.47	0.0292
EC	gamma 3	Central Midline	0.05	0.02	20.20	2.40	0.0263
EC	gamma 3	Central Right	0.05	0.02	18.68	2.48	0.0228
EC	gamma 3	Frontal Left	0.06	0.02	21.58	2.78	0.0109
EC	gamma 3	Frontal Midline	0.05	0.02	21.03	2.32	0.0307
EC	gamma 3	Frontal Right	0.06	0.02	20.73	2.81	0.0105
EC	gamma 3	Parietal Left	0.04	0.02	20.41	2.16	0.0426
EC	gamma 3	Parietal Midline	0.05	0.02	20.30	2.95	0.0079
EC	gamma 3	Parietal Right	0.06	0.02	19.98	2.71	0.0133
EC	gamma 3	Temporal Left	0.07	0.02	21.01	4.35	0.0003
EC	gamma 3	Temporal Right	0.07	0.02	21.09	4.33	0.0003
EC	TBR	Frontal Right	−0.12	0.06	21.46	−2.13	0.0445
EC	TBR	Parietal Left	−0.08	0.04	20.91	−2.09	0.0489
EC	TBR	Temporal Left	−0.07	0.03	21.37	−2.18	0.0407
EC	TBR	Temporal Right	−0.09	0.04	21.41	−2.17	0.0413
EC	total	Temporal Right	0.04	0.02	21.08	2.15	0.0430
EO	beta	Temporal Right	0.08	0.03	20.38	2.37	0.0277
EO	beta 3	Temporal Right	0.10	0.04	20.34	2.81	0.0108
EO	high beta	Central Right	0.13	0.05	20.76	2.52	0.0199
EO	high beta	Frontal Right	0.09	0.04	20.75	2.21	0.0385
EO	high beta	Occipital Left	0.10	0.04	21.16	2.45	0.0231
EO	high beta	Occipital Right	0.11	0.04	20.75	2.84	0.0098
EO	high beta	Parietal Left	0.11	0.04	20.76	2.54	0.0191
EO	high beta	Parietal Midline	0.09	0.04	20.68	2.42	0.0250
EO	high beta	Parietal Right	0.10	0.04	20.58	2.63	0.0159
EO	high beta	Temporal Left	0.13	0.05	20.75	2.49	0.0212
EO	high beta	Temporal Right	0.14	0.04	20.26	3.43	0.0026
EO	gamma	Central Midline	0.05	0.02	21.05	2.59	0.0169
EO	gamma	Central Right	0.04	0.02	20.64	2.11	0.0470
EO	gamma	Frontal Midline	0.05	0.02	21.24	2.58	0.0173
EO	gamma	Occipital Left	0.06	0.02	11.83	2.69	0.0198
EO	gamma	Occipital Right	0.05	0.02	13.46	3.49	0.0038
EO	gamma	Parietal Left	0.07	0.02	21.38	3.08	0.0056
EO	gamma	Parietal Midline	0.06	0.02	20.72	3.58	0.0018
EO	gamma	Parietal Right	0.06	0.02	20.64	2.70	0.0136
EO	gamma	Temporal Left	0.07	0.02	19.44	2.89	0.0092
EO	gamma	Temporal Right	0.06	0.02	20.73	3.09	0.0056
EO	gamma 1	Occipital Left	0.05	0.02	10.78	2.28	0.0438

EO	gamma 1	Occipital Right	0.05	0.02	13.03	3.03	0.0097
EO	gamma 1	Parietal Left	0.06	0.02	21.12	2.69	0.0137
EO	gamma 1	Parietal Midline	0.05	0.02	20.61	2.84	0.0099
EO	gamma 1	Parietal Right	0.05	0.02	19.89	2.25	0.0357
EO	gamma 1	Temporal Left	0.06	0.02	18.72	2.57	0.0190
EO	gamma 1	Temporal Right	0.06	0.02	20.67	2.92	0.0082
EO	gamma 2	Central Midline	0.04	0.02	20.96	2.31	0.0311
EO	gamma 2	Frontal Midline	0.04	0.02	21.10	2.34	0.0291
EO	gamma 2	Occipital Left	0.06	0.02	11.59	2.60	0.0239
EO	gamma 2	Occipital Right	0.05	0.02	13.36	3.36	0.0049
EO	gamma 2	Parietal Left	0.07	0.02	21.36	2.96	0.0073
EO	gamma 2	Parietal Midline	0.06	0.02	20.70	3.39	0.0028
EO	gamma 2	Parietal Right	0.06	0.02	20.49	2.59	0.0174
EO	gamma 2	Temporal Left	0.07	0.02	19.28	2.89	0.0094
EO	gamma 2	Temporal Right	0.06	0.02	20.67	3.06	0.0060
EO	gamma 3	Central Midline	0.05	0.02	21.17	3.16	0.0047
EO	gamma 3	Central Right	0.05	0.02	20.72	2.22	0.0379
EO	gamma 3	Frontal Midline	0.05	0.02	21.52	3.00	0.0067
EO	gamma 3	Occipital Left	0.07	0.02	12.69	2.95	0.0116
EO	gamma 3	Occipital Right	0.06	0.02	13.86	3.73	0.0023
EO	gamma 3	Parietal Left	0.08	0.02	21.51	3.27	0.0036
EO	gamma 3	Parietal Midline	0.06	0.02	20.79	4.01	0.0006
EO	gamma 3	Parietal Right	0.07	0.02	21.03	2.92	0.0081
EO	gamma 3	Temporal Left	0.07	0.02	19.74	3.01	0.0070
EO	gamma 3	Temporal Right	0.06	0.02	20.81	3.16	0.0048
EO	TBR	Parietal Right	−0.09	0.04	19.40	−2.55	0.0196
EO	TBR	Temporal Left	−0.10	0.04	19.80	−2.76	0.0120
EO	TBR	Temporal Right	−0.11	0.04	19.85	−2.93	0.0084
Coherence Endpoints							
EC	alpha 2	F4-P4	0.04	0.02	18.16	2.17	0.0433
EC	beta	F3-P3	0.06	0.02	19.97	2.61	0.0169
EC	beta	F3-T5	0.04	0.02	19.40	2.19	0.0412
EC	beta	F4-T6	0.05	0.02	17.98	2.23	0.0385
EC	beta 1	C3-C4	0.07	0.04	21.61	2.09	0.0484
EC	beta 1	F4-P4	0.04	0.02	17.69	2.15	0.0455
EC	beta 1	P4-T6	−0.05	0.02	18.86	−2.15	0.0452
EC	beta 2	C3-C4	0.07	0.03	20.65	2.25	0.0353
EC	beta 2	F3-P3	0.05	0.02	20.25	2.12	0.0466
EC	beta 2	P4-T6	−0.06	0.03	18.46	−2.40	0.0271
EC	beta 3	F3-P3	0.08	0.03	18.90	2.68	0.0149
EC	beta 3	F3-T5	0.05	0.02	18.58	2.11	0.0484
EC	beta 3	F4-T6	0.07	0.03	17.75	2.22	0.0395
EC	high beta	F3-P3	0.09	0.04	20.79	2.29	0.0326
EC	high beta	F3-T5	0.08	0.03	20.60	2.42	0.0248
EC	high beta	F4-T6	0.12	0.05	19.04	2.39	0.0272
EO	alpha	C3-C4	0.06	0.03	21.49	2.10	0.0474
EO	alpha 2	C3-C4	0.06	0.03	21.52	2.22	0.0370

EO	beta	C3-C4	0.07	0.03	21.74	2.46	0.0224
EO	beta	F3-P3	0.07	0.03	21.22	2.48	0.0214
EO	beta	F3-T5	0.05	0.02	21.21	2.80	0.0106
EO	beta	F4-T6	0.05	0.02	21.19	2.20	0.0390
EO	beta	P4-T6	−0.06	0.02	15.71	−2.61	0.0191
EO	beta 1	C3-C4	0.07	0.03	21.43	2.33	0.0297
EO	beta 1	P4-T6	−0.06	0.02	15.11	−2.77	0.0142
EO	beta 2	C3-C4	0.07	0.03	21.65	2.35	0.0283
EO	beta 2	F3-T5	0.04	0.02	21.04	2.27	0.0336
EO	beta 2	P4-T6	−0.06	0.02	15.70	−2.62	0.0188
EO	beta 3	C3-C4	0.08	0.03	21.50	2.36	0.0278
EO	beta 3	F3-P3	0.08	0.03	21.06	2.72	0.0127
EO	beta 3	F3-T5	0.06	0.02	21.02	2.92	0.0081
EO	beta 3	F4-C4	0.09	0.04	21.50	2.19	0.0395
EO	beta 3	F4-P4	0.09	0.04	21.18	2.38	0.0269
EO	beta 3	F4-T6	0.07	0.03	20.52	2.52	0.0202
EO	beta 3	P4-T6	−0.07	0.03	16.56	−2.39	0.0293
EO	high beta	F3-P3	0.10	0.04	21.41	2.31	0.0282
EO	high beta	F3-T5	0.08	0.03	20.83	2.96	0.0107
EO	high beta	F4-C4	0.11	0.04	21.12	2.42	0.0456
EO	high beta	F4-P4	0.12	0.04	20.59	2.84	0.0228
EO	high beta	F4-T6	0.10	0.03	20.01	2.99	0.0099
EO	gamma	F4-C4	0.14	0.06	18.46	2.38	0.0133
EO	gamma	F4-P4	0.13	0.05	16.14	2.89	0.0446
EO	gamma	F4-T6	0.09	0.04	16.71	2.16	0.0068
EO	gamma 1	F4-C4	0.14	0.05	18.26	2.49	0.0366
EO	gamma 1	F4-P4	0.13	0.04	17.13	2.90	0.0242
EO	gamma 1	F4-T6	0.11	0.04	15.38	2.80	0.0312
EO	gamma 2	F4-C4	0.13	0.06	18.46	2.15	0.0075
EO	gamma 2	F4-P4	0.14	0.04	15.86	3.11	0.0248
EO	gamma 3	F4-C4	0.14	0.06	18.47	2.25	0.0099
EO	gamma 3	F4-P4	0.13	0.05	15.85	2.49	0.0073
EO	total	F4-C4	0.10	0.04	18.64	2.76	0.0127
EO	total	F4-P4	0.08	0.04	16.48	2.15	0.0470
EO	total	F4-T6	0.06	0.03	15.99	2.25	0.0392

Abbreviations: C, central; df, degrees of freedom; EC, eyes closed; EO, eyes open; F, frontal; IAF, individual alpha frequency; LSME, least-squares mean estimate; P, parietal; SEM, standard error of the mean; t, t-statistic; T, temporal; TBR, theta/beta ratio

Table 3. Adverse events in healthy adult male participants

Parameter	NV-5138 N = 12	Placebo N = 13
Participants with at least one treatment-emergent AE	1 (8.3%)	2 (15.4%)
Participants with at least one treatment-related AE	0	2 (15.4%)
Number of treatment-related AEs	0	3
Number of treatment-emergent AEs	2	3
Alanine aminotransferase increased	0	1 (7.7%)
Aspartate aminotransferase increased	0	1 (7.7%)
Upper abdominal pain	0	1 (7.7%)
EEG abnormal (bitemporal slowing on EEG)[a]	1 (8.3%)	0
EEG abnormal (left posterior temporal sharps on EEG)[a]	1 (8.3%)	0

Values are reported as n (%). Abbreviations: AEs, adverse events; EEG, electroencephalography; N, number of subjects

[a] These events occurred concurrently in the EEGs of a single participant.

FIGURE CAPTIONS

Figure 1. NV-5138 decreases low frequency and increases high frequency spectral band amplitudes at 1 hour post-dose.

qEEG band amplitudes were measured from recordings in the eyes-closed (top) and eyes-open (bottom) conditions for placebo (Day 1: black circles; Day 3: gray diamonds) or NV-5138 (Day 1: green squares; Day 3: purple triangles), and are presented as normalized change from baseline on the y-axis, a measure defined as contrast = (post-dose−baseline)/(baseline+post-dose) ranging from −1 to 1. The x-axis denotes the EEG bands in which amplitudes were measured ranging from delta to gamma 3. Bands delta through beta 3 were measured in the oscillatory part of the spectrum. Bands high beta through gamma 3 were measured in the fractal part of the spectrum. Statistically significant changes are highlighted in yellow as determined by mixed model repeated measures analysis. Abbreviations: norm. CFB, normalized change from baseline; qEEG, quantitative electroencephalography

Figure 2. NV-5138 reduces the theta/beta ratio at 1 hour post-dose.

qEEG-derived measures were computed from recordings in the eyes-closed (top) and eyes-open (bottom) conditions for placebo (Day 1: black circles; Day 3: gray diamonds) or NV-5138 (Day 1: green squares; Day 3: purple triangles), and are presented as normalized change from baseline (see Figure 1). The x-axis denotes a subset of the derived qEEG measures that were analyzed, including oscillatory alpha slow wave index (ASI), oscillatory theta/beta ratio (TBR), and oscillatory individual alpha frequency (IAF). Statistically significant changes are highlighted in yellow as determined by mixed model repeated measures analysis. Abbreviations: norm. CFB, normalized change from baseline; qEEG, quantitative electroencephalography

Figure 3. NV-5138 decreases low frequency coherence and increases high frequency coherence of select electrode pairs at 1 hour post-dose.

qEEG coherences were computed from recordings in the eyes-closed (top) and eyes-open (bottom) conditions for placebo (Day 1: black circles; Day 3: gray diamonds) or NV-5138 (Day 1: green squares; Day 3: purple triangles), and are presented as normalized change from baseline (see Figure 1). The y-axis represents normalized change from baseline in magnitude squared coherence from the raw EEG spectrum (not decomposed with IRASA). The x-axis denotes the frequency bands analyzed ranging from delta to gamma 3. Statistically significant changes are highlighted in yellow as determined by mixed model repeated measures analysis. Abbreviations: C, central; F, frontal; norm. CFB, normalized change from baseline; IRASA, Irregular-Resampling Auto-Spectral Analysis; P, parietal; qEEG, quantitative electroencephalography; T, temporal

Figure 1

Figure 1. NV-5138 decreases low frequency and increases high frequency spectral band amplitudes at 1 hour post-dose.

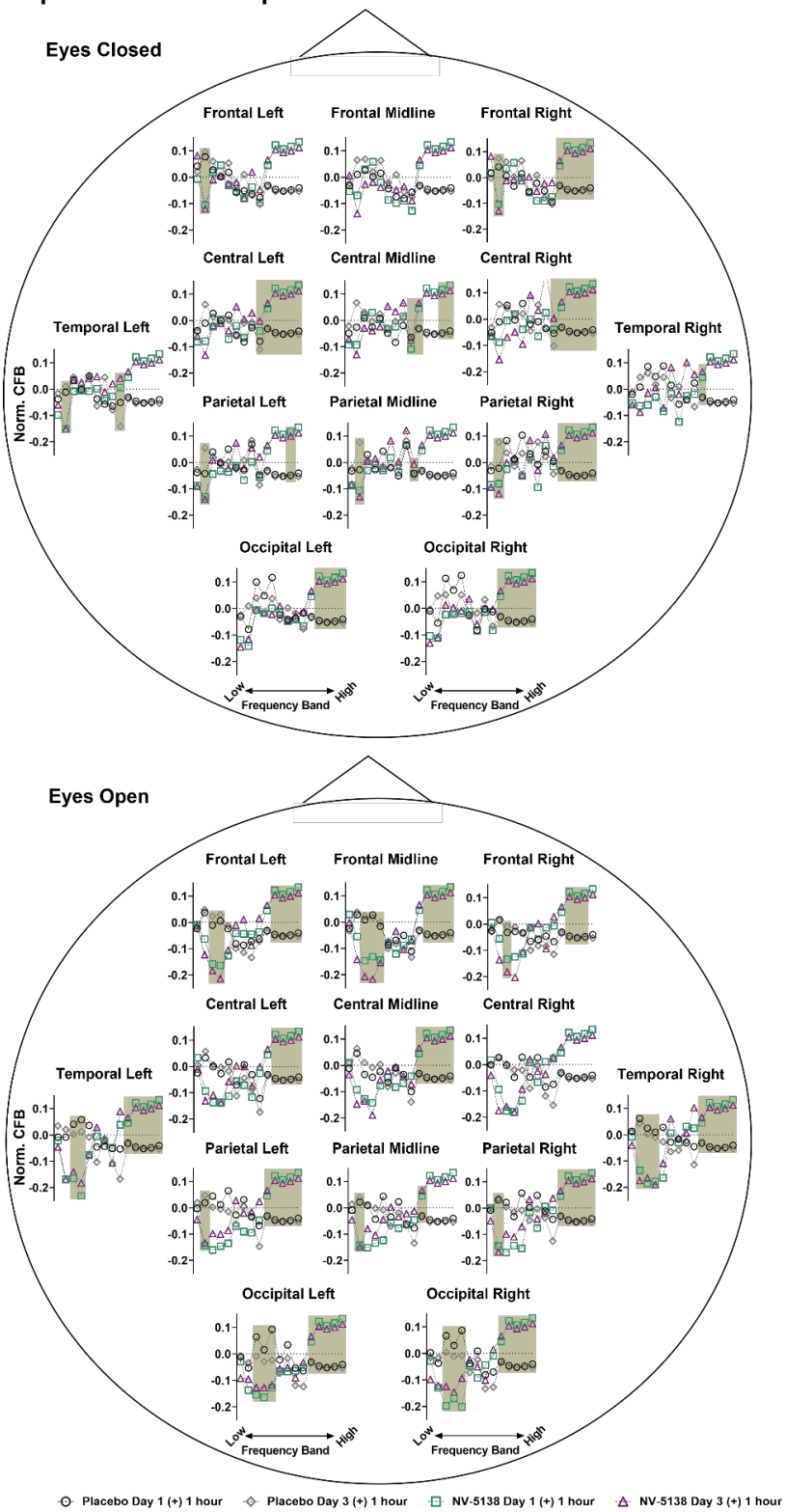

Figure 2

Figure 2. NV-5138 reduces the theta/beta ratio at 1 hour post-dose.

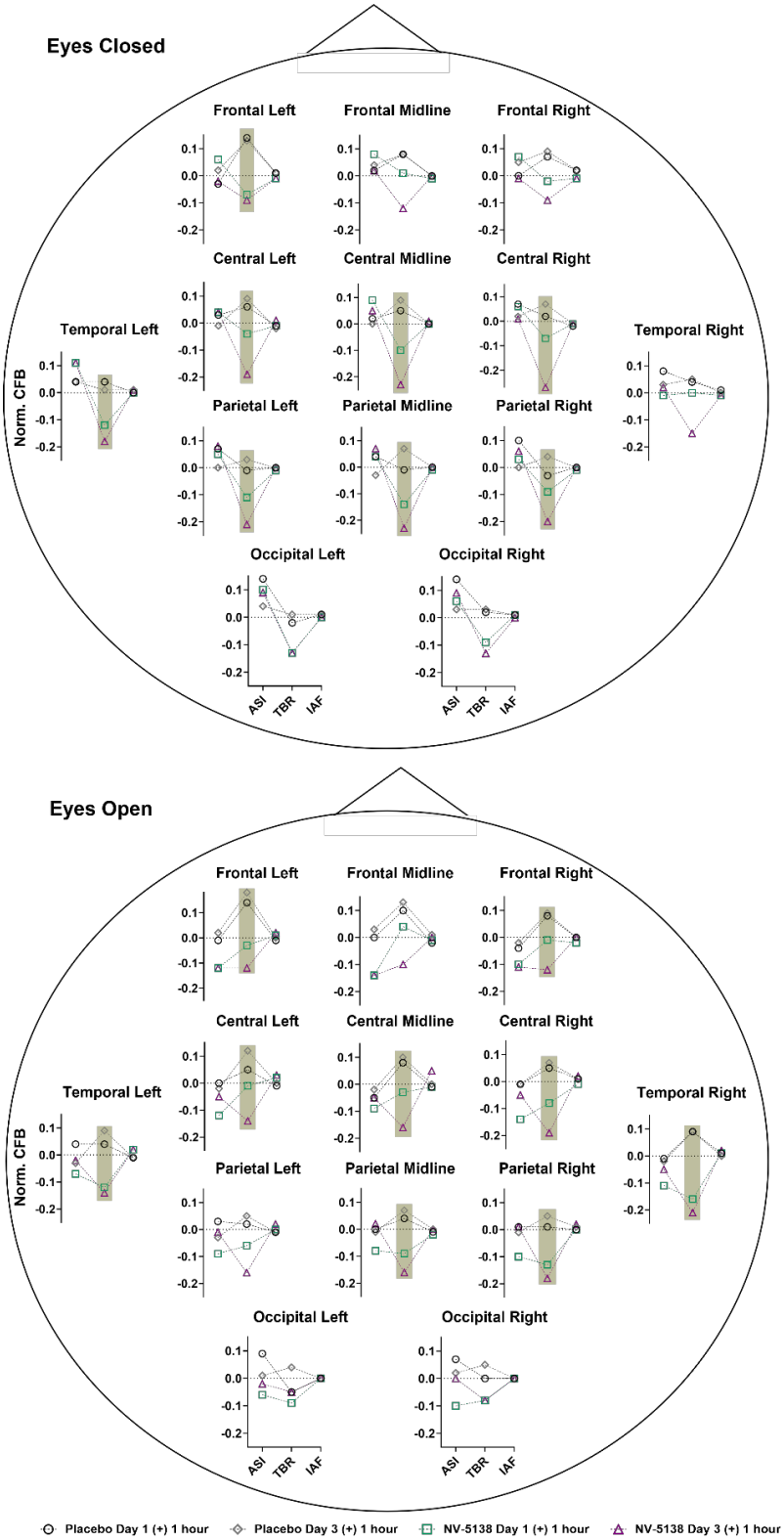

Figure 3. NV-5138 decreases low frequency coherence and increases high frequency coherence of select electrode pairs at 1 hour post-dose.