

Randomized Double-Blind, Placebo Controlled Study to Evaluate NV-5138/SPN-820 (NV-5138), an mTORC1 Activator, by Quantitative EEG (qEEG) in Healthy Volunteers, Supports Target Engagement and Translational Strategy

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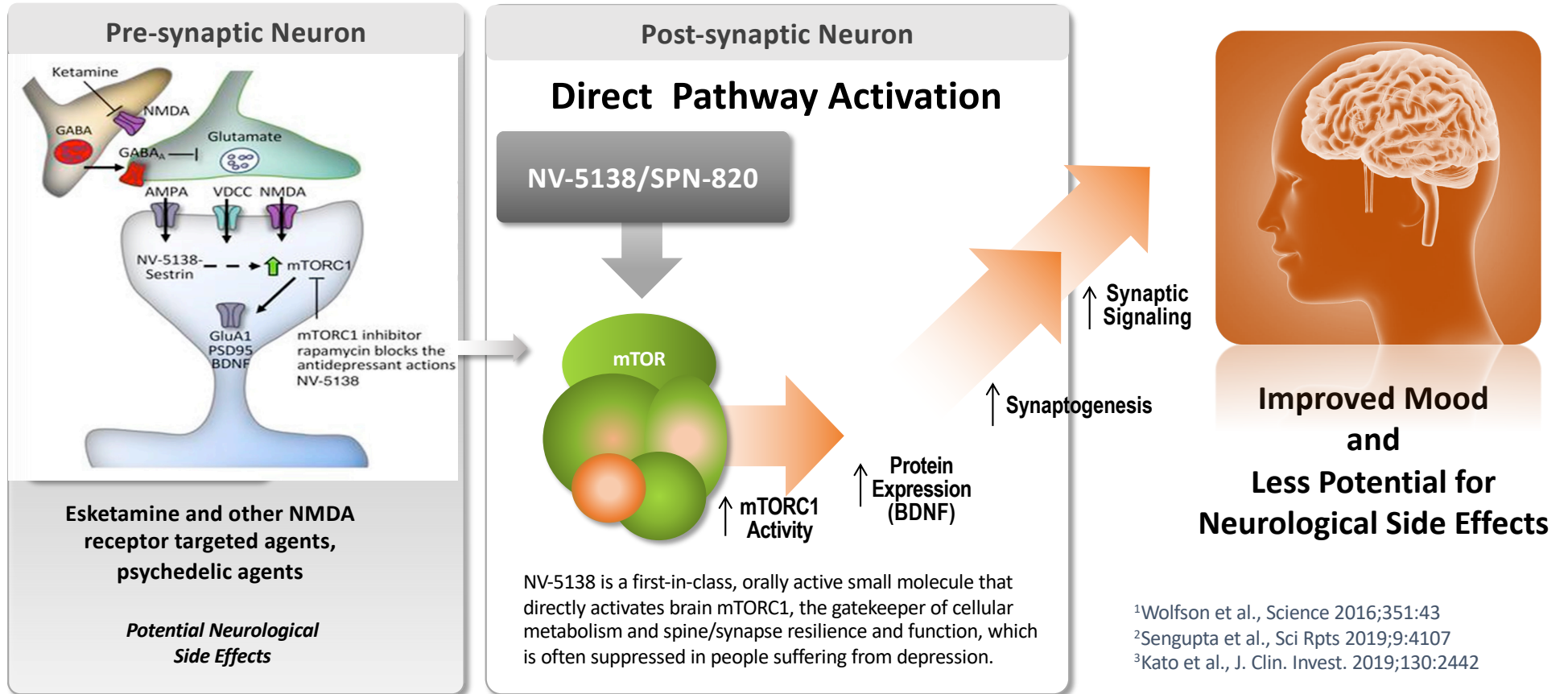


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NV-5138/SPN-820 Directly Activates Postsynaptic mTORC1 Pathway Signaling, Increasing Synaptic Signaling and Normalizing Mood



Background

- NV-5138, a direct mTORC1 activator demonstrates rapid and long-lasting 'antidepressant' effects comparable to ketamine in pre-clinical models.
 - A preliminary **qEEG study in rodents demonstrated that two sequential oral doses of NV-5138 vs vehicle (separated by 60 hours) produced significant spectral power changes** at frontal-frontal and frontal-parietal electrode derivations. Differences observed between dose 1 and dose 2. These data suggest that qEEG may be useful to further characterize the impact of NV-5138 on the central nervous system.
 - Early clinical data has shown that **single doses of NV-5138 up to 2400 mg were safe and well tolerated in healthy adults¹ and those with treatment-resistant depression (TRD in 32 subjects).² Significant improvement on HAM-D6 at 4-12 hours post dose, with sustained numerical separation from placebo past 24 hours.**
 - **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, PK, and tolerability were also evaluated.**
1. Leventer S, Gruener D, Schmalback T, et al. NV-5138 a Novel Direct Activator of the Mechanistic Target of Rapamycin Complex 1 (mTORC1): Safety, Tolerability and Pharmacokinetics (PK) in Plasma and Cerebrospinal Fluid (CSF) Following Oral Administration in Healthy Volunteers. Abstract presented at: American College of Neuropsychopharmacology (ACNP) 58th Annual Meeting; December 8-11, 2019; Orlando, FL.
 2. Targum S, Leventer S, Hughes T, Owen J, Vlasuk G. NV-5138 A Novel, Direct Activator of the Mechanistic Target of Rapamycin Complex 1 (mTORC1): A Phase 1b Randomized, Double-Blind, Placebo-Controlled Single Oral Dose Study in Subjects With Treatment-Resistant Depression (TRD). Abstract presented at: American College of Neuropsychopharmacology (ACNP) 58th Annual Meeting; December 8-11, 2019; Orlando, FL.

Background Rationale for qEEG Evaluation in Early Phase Studies

- **qEEG can be used to demonstrate brain penetration of a molecule, as well as provide evidence for proof of mechanism as a readout of brain activity in response to drug administration**
- **qEEG activity associated with psychiatric disorders such as MDD, most commonly, is an increase in lower frequency band amplitudes (delta and theta), and a decrease in alpha band amplitudes.**^{16,17}
- **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.**¹⁸
- **qEEG resting state measurements might have utility to evaluate treatment response in MDD.** A variety of measures have been evaluated: frontal alpha asymmetry¹⁹, prefrontal theta cordance,²⁰ pretreatment rostral anterior cingulate theta activity,²¹ antidepressant treatment response index,²² EEG functional connectivity,²³ and high frequency alpha.²⁴
- **Increased Alpha slow wave index (ASI) and decreased Theta/Beta ratio are typically linked to increasing arousal and cognitive processing and associated with antidepressant effects.**
- **qEEG coherence provides information about functional integration and connectivity between inter- and intra-hemispheric brain regions,²⁵ and coherence measures have been related to treatment response.**^{26, 27}

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Methods

- 25 healthy male subjects randomly assigned to a single dose of either placebo or 2400 mg NV-5138 on Day 1, and the same treatment on Day 3. The in-house period of the study was 8 days/7 nights, and up 7-day follow-up period after discharge.
- Tolerability and PK were evaluated.
- Baseline (Day -1) and Days 1 and 3 had time matched qEEGs recorded (5 minutes each eyes closed (EC); eyes open (EO)) corresponding to 1 hour pre-dose and 1-, 4-, and 8- hours post-dose.
- Recording: Compumedics Grael 4k V2 EEG amplifiers, Curry 8E Software, 23 electrodes (International 10-20 system). Pre-processing of 2 second segments uses Irregular-Resampling Auto-Spectral Analysis (IRASA), and a fast Fourier transformation on the oscillatory component (up to 25 Hz, Beta3), to separate from the fractal components (delta through gamma3 bands).
- Spectral band amplitudes (μV), frequency-derived measures, and magnitude squared coherence were assessed
 - 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) and gamma 3 (40.0 - 50.0 Hz)}.
 - Band amplitudes for thirteen spatial regions were estimated by averaging band amplitudes : Frontal: left (FP1, F3, F7), right (FP2, F4, F8), midline (Fz); Central: left (C3), right (C4), midline (Cz); Temporal: left (T3, T5), right (T4, T6); Parietal: left (P3), right (P4), midline (Pz); Occipital: left (O1), right (O2). .
- **Derived Frequency Measures: alpha slow-wave index (ASI), theta/beta ratio (TBR), and dominant frequency** (individual alpha frequency, IAF).
- **Value Differences** are used to quantify changes in each QEEG measure relative to baselines and are defined as the **normalized adjusted value difference**. Mean value differences and Value Ratios are portrayed on 'heat maps'
- **Value Ratios** are a secondary measure used to confirm patterns of change seen in value differences. **The value ratio is the ratio of a given measure at a post-dose timepoint to its value at the corresponding baseline.**

Methods – Statistical Analysis Plan

- **Salient changes in end points for drug vs placebo on Days 1 and 3, were classified as small, medium or large, confirmed by the ANCOVA model implemented as the Mixed Model Repeated Measures (MMRM) and using absolute post-dose values as dependent variable and the pre-dose baseline time point was used as a covariate.**
- Experience over many clinical trials has shown that changes in group mean normalized baseline-adjusted qEEG band amplitudes or derived measures with absolute values exceeding 0.1 ($\pm 10\%$ contrast) are likely to lead to significant and meaningful results in formal statistical analyses.
- In addition, the consistency of changes and their pattern across spatial regions can help to distinguish a potential effect of the experimental conditions from random variations. For example, isolated changes at a single spatial region or timepoint are not likely to be meaningful in small samples. Certain regions, including frontal and temporal, are susceptible to EOG, EMG or motion artifact, especially at lateral locations. When these locations disagree with central locations, artifact is likely to be present.
- **Using such heuristics, the pivot charts were reviewed to identify potential effects of treatment. Each endpoint was reviewed for salient changes that met the 10% change criterion and exhibited physiologically plausible patterns.**

Results

56 subjects were screened, with a total of 25 randomized (13 placebo/12 drug). 24 subjects completed the study (1 participant withdrew consent on Day 1 (placebo)).

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)

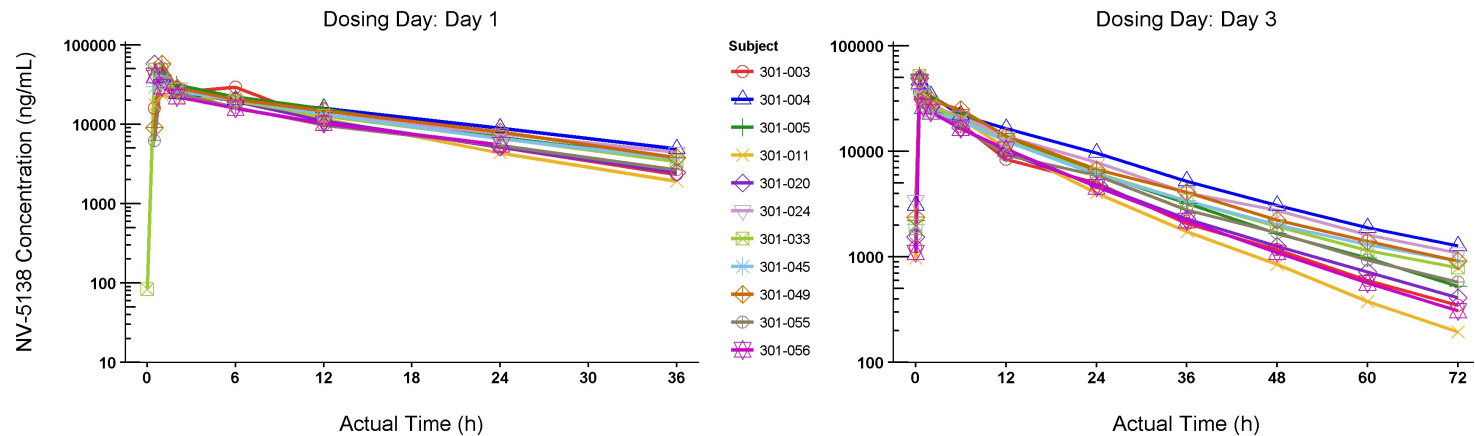
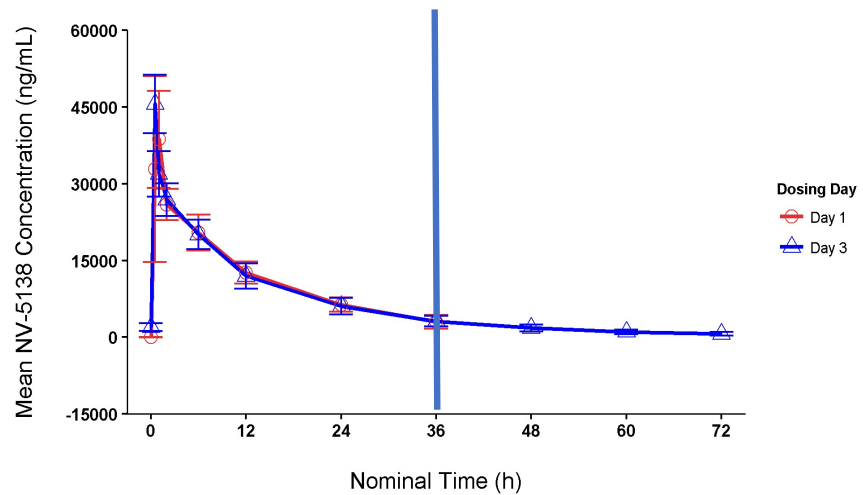
The two sequential doses of NV-5138 were well tolerated.

Category	NV-5138 2400 mg N = 12	Placebo N = 13
Participants with treatment-related AEs	0	2 (15.4%)
Number of treatment-related AEs	0	3
Investigations	1 (8.3%)	1 (7.7%)
Alanine amino transferase increased	0	1 (7.7%)
Aspartate amino transferase increased	0	1 (7.7%)
EEG bitemporal slowing and left-posterior temporal sharps*	1 (8.3%)	0
Gastrointestinal disorders	0	1 (7.7%)
Upper abdominal pain	0	1 (7.7%)

- No dissociative effects were observed, as evaluated with CADSS
- ***Subject baseline EEG was read WNL.** Following 'abnormal' study EEG Extended 6-hour safety EEG was obtained on Day 6
- Independent reviewer, reported the same EEG findings
- Conclusion, **20-minute safety EEGs might not pick up exclusionary abnormalities, whereas extended time matched safety evaluations approximating the duration of on drug procedures better identify pre-existing exclusionary conditions.**

Summary of plasma NV-5138 noncompartmental pharmacokinetic parameters by dosing day

Day	Statistic	C _{max} (ng/mL)	T _{max} (h)	AUC _{last} (h·ng/mL)	t _{1/2} (h)
Day 1	N	11	11	11	11
	Mean	45,100	NC	409,000	12.2
	SD	9,980	NC	60,200	1.62
	Arithmetic CV%	22.1	NC	14.7	13.3
	Geometric mean	43,900	NC	405,000	12.1
	Geometric CV%	26.1	NC	15.6	13.5
	Min	23,300	0.50	298,000	9.20
	Median	47,000	1.00	422,000	12.0
	Max	57,700	1.00	481,000	15.2
Day 3	N	11	11	11	11
	Mean	45,600	NC	462,000	15.6
	SD	5,700	NC	87,000	2.94
	CV%	12.5	NC	18.8	18.9
	Geometric mean	45,300	NC	455,000	15.3
	Geometric CV%	13.1	NC	18.5	19.0
	Min	35,700	0.50	369,000	10.9
	Median	47,400	0.50	460,000	15.6
	Max	52,300	0.50	632,000	21.1



Time Points for qEEG Assessment and Dosing

Day -1:

- pre-dose1 (time-matched pre-dose time on Day 1)
- pre-dose2 (+1 hr)
- pre-dose3 (+4 hr)
- pre-dose4 (+8 hr)

Day 1 dosing:

- **pre-dose5 (-1 hr prior pre-1st dose)**
- post-dose1 (+1 hr)
- post-dose2 (+4 hr)
- post-dose3 (+8 hr)

Day 2:

- post-dose4 (+23 hr)
- post-dose5 (+25 hr)
- post-dose6 (+28 hr)
- post-dose7 (+32 hr)

Day 3 dosing:

- **post-dose8 (-1 hr pre-2nd dose)**
- post-dose9 (+1 hr)
- post-dose10 (+4 hr)
- post-dose11 (+8 hr)

Day 4:

- post-dose12 (+23 hr)
- post-dose13 (+25 hr)
- post-dose14 (+28 hr)
- post-dose15 (+32 hr)

Day 5:

- post-dose16 (+47 hr)

Follow-Up:

- post-dose16

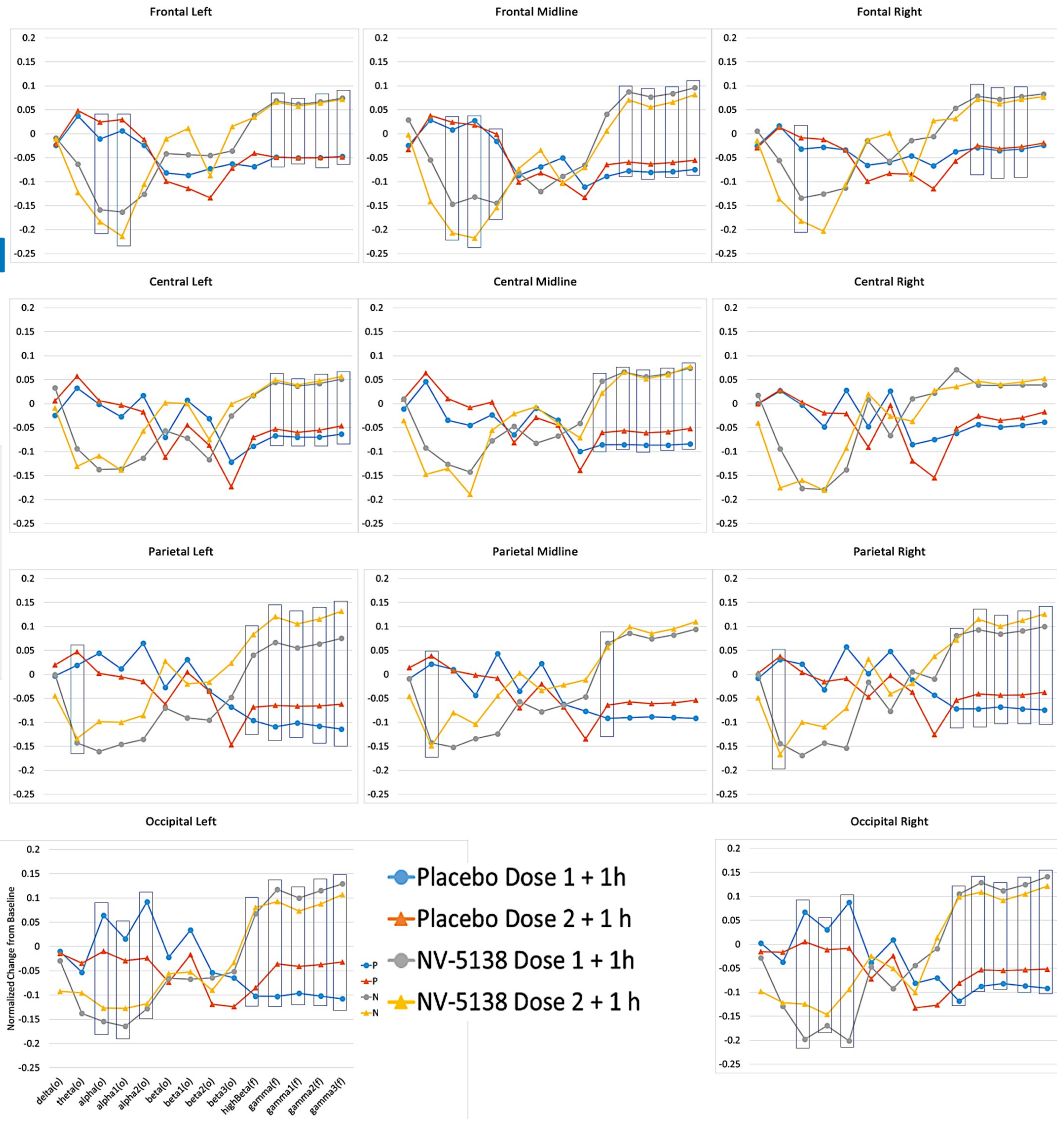
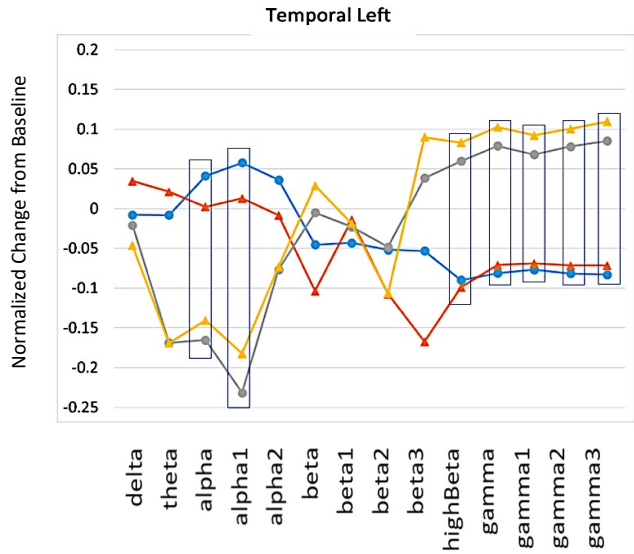
Dose	TimePoint	Day	Nominal Time	Time Match Day -1
0	PREDOSE1	-1	0800	
0	PREDOSE2	-1	1000	
0	PREDOSE3	-1	1300	
0	PREDOSE4	-1	1700	
0	PREDOSE5	1	0800	
1	POSTDOSE1	1	1000	1
1	POSTDOSE2	1	1300	4
1	POSTDOSE3	1	1700	8
1	POSTDOSE4	2	0800	-1
1	POSTDOSE5	2	1000	1
1	POSTDOSE6	2	1300	4
1	POSTDOSE7	2	1700	8
1	POSTDOSE8	3	0800	-1
1 + 2	POSTDOSE9	3	1000	1
1 + 2	POSTDOSE10	3	1300	4
1 + 2	POSTDOSE11	3	1700	8
1 + 2	POSTDOSE12	4	0800	-1
1 + 2	POSTDOSE13	4	1000	1
1 + 2	POSTDOSE14	4	1300	4
1 + 2	POSTDOSE15	4	1700	8
1 + 2	POSTDOSE16	5	0800	-1
1 + 2	POSTDOSE17	10	1700	8

Results – Post-dose qEEG at 1-Hour (NV-5138 Tmax) and Later Time Points

- Among the most robust changes in qEEG parameters occurred in the NV-5138 group, on both dosing days at **1-hour post-dose** (approximately at the time of NV-5138 Tmax).
- These assessments revealed **a decrease in low-frequency EEG bands (delta and theta) and an increase in high-frequency EEG bands (high beta, gamma), while alpha bands exhibited decreased amplitudes (or desynchronization).**
 - In the NV-5138 group only, significant changes in qEEG measures occurred at 1-hour post-dose on both days, including decreases in low-frequency band amplitudes (theta) and increases in high-frequency EEG band amplitudes (high beta and gamma) **with a resultant decrease in Theta/Beta ratio**
 - **Significantly increased high beta and gamma band inter- and intrahemispheric coherences were also detected at several specific electrode pairs in both eyes-open and eye-closed conditions. These met criteria for salient change ($\geq 10\%$)**
 - Salient changes observed in the fractal part of the EEG spectrum included increases in amplitudes for high beta, gamma, gamma 1, gamma 2, and gamma 3 bands (greatest change).
- **First dose @ 8 - 23 hours post-dose showed alpha 1, alpha 2 and beta 1 bands increased in amplitudes and increased Alpha Slow-wave Index (ASI) not observed at 1-hour Tmax** (suggestive of 'beneficial effects)
- **Second dose @ 1-hour post dose were similar to the first dose; including decrease in alpha amplitude vs. @ 4-8+ hours post-dose, alpha amplitude and ASI were increased.**
- **Changes in qEEG in the placebo group were minimal but did demonstrate changes over the day consistent with diurnal rhythm and activity.**

NV-5138 Decreases Low Frequency and Increases High Frequency Oscillatory and Fractal Spectral Band Amplitudes for thirteen spatial regions at 1-hour Post-dose

EO Spectral Band Amplitudes



Gamma 3 EC Heat Map Both Doses and Times

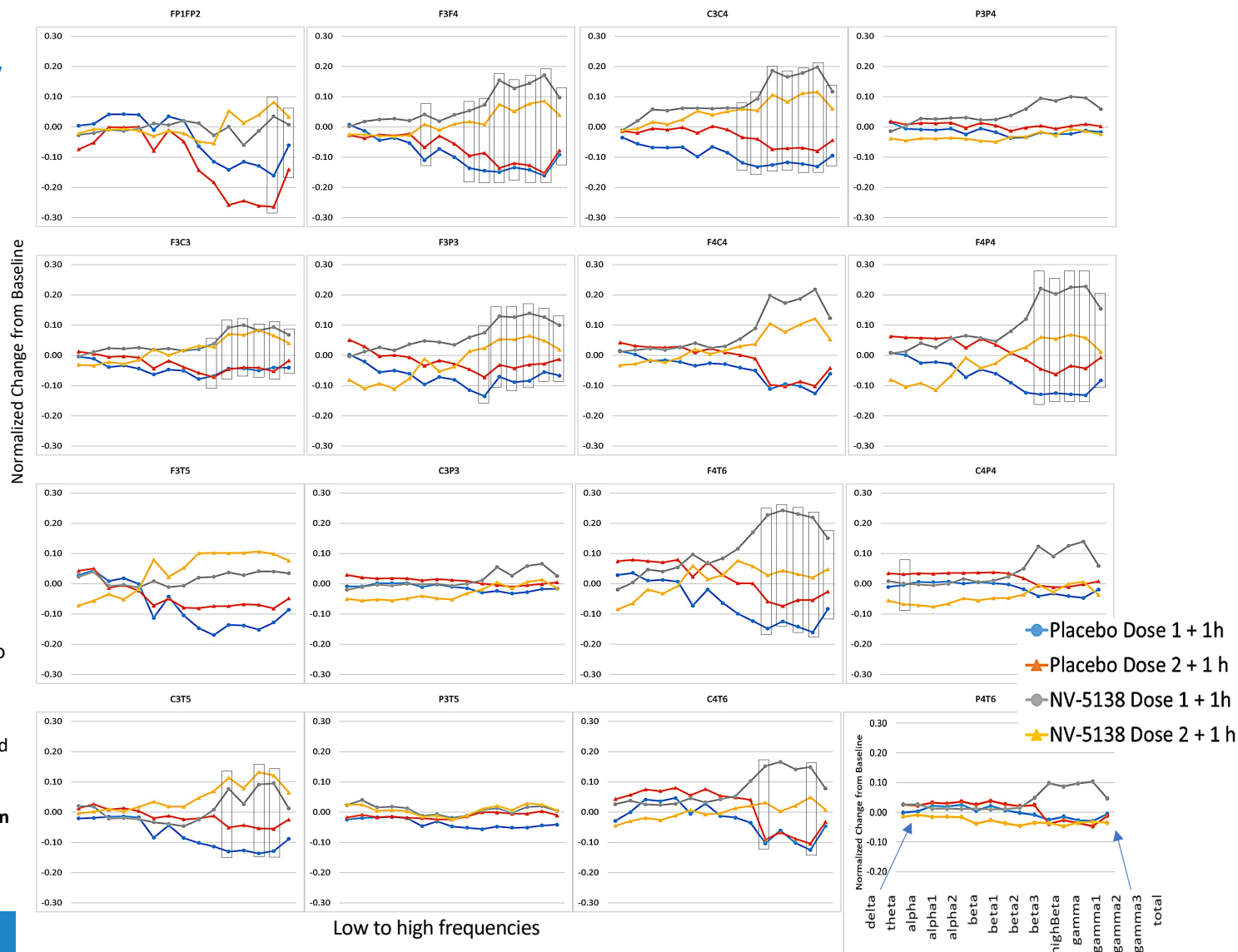
Dosing:

Frequency Band	Spatial Location	Placebo Time Point																	NV-5138 Time Point																	Scale Max	Center	Scale Min																	
		PREDOSE1	PREDOSE2	PREDOSE3	PREDOSE4	PREDOSE5	POSTDOSE1	POSTDOSE2	POSTDOSE3	POSTDOSE4	POSTDOSE5	POSTDOSE6	POSTDOSE7	POSTDOSE8	POSTDOSE9	POSTDOSE10	POSTDOSE11	POSTDOSE12	POSTDOSE13	POSTDOSE14	POSTDOSE15	POSTDOSE16	POSTDOSE17	PREDOSE1	PREDOSE2	PREDOSE3	PREDOSE4	PREDOSE5	POSTDOSE1	POSTDOSE2	POSTDOSE3	POSTDOSE4	POSTDOSE5	POSTDOSE6	POSTDOSE7				POSTDOSE8	POSTDOSE9	POSTDOSE10	POSTDOSE11	POSTDOSE12	POSTDOSE13	POSTDOSE14	POSTDOSE15	POSTDOSE16	POSTDOSE17							
Gamma3	Frontal left	0.27	0.30	0.27	0.27	0.24	0.27	0.28	0.27	0.25	0.27	0.29	0.27	0.26	0.27	0.29	0.32	0.26	0.29	0.29	0.28	0.26	0.24	0.27	0.26	0.28	0.28	0.25	0.33	0.31	0.29	0.25	0.30	0.26	0.32	0.27	0.33	0.29	0.28	0.26	0.28	0.30	0.26	0.26	0.26	0.34	0.29	0.24							
Gamma3	Frontal midline	0.25	0.29	0.25	0.25	0.22	0.26	0.26	0.25	0.22	0.26	0.27	0.27	0.26	0.26	0.24	0.25	0.28	0.30	0.26	0.29	0.27	0.28	0.24	0.26	0.25	0.28	0.27	0.26	0.32	0.30	0.27	0.25	0.29	0.26	0.32	0.26	0.33	0.29	0.28	0.26	0.28	0.30	0.26	0.24	0.26	0.34	0.29	0.24						
Gamma3	Frontal right	0.25	0.30	0.25	0.26	0.23	0.27	0.27	0.27	0.23	0.26	0.27	0.27	0.25	0.26	0.29	0.31	0.26	0.29	0.29	0.28	0.24	0.31	0.28	0.27	0.31	0.29	0.27	0.33	0.32	0.33	0.28	0.31	0.27	0.34	0.29	0.30	0.33	0.30	0.27	0.29	0.31	0.28	0.27	0.27	0.34	0.29	0.24							
Gamma3	Temporal left	0.25	0.31	0.28	0.28	0.24	0.28	0.30	0.29	0.25	0.28	0.32	0.28	0.24	0.28	0.33	0.32	0.29	0.32	0.30	0.29	0.27	0.32	0.27	0.29	0.29	0.31	0.27	0.35	0.33	0.31	0.26	0.30	0.28	0.37	0.27	0.37	0.31	0.29	0.28	0.31	0.33	0.32	0.26	0.31	0.34	0.29	0.24							
Gamma3	Temporal right	0.27	0.32	0.26	0.27	0.26	0.30	0.30	0.29	0.24	0.26	0.28	0.28	0.23	0.26	0.32	0.32	0.27	0.33	0.31	0.29	0.25	0.31	0.26	0.28	0.30	0.30	0.28	0.36	0.34	0.31	0.29	0.27	0.30	0.28	0.33	0.29	0.36	0.31	0.31	0.29	0.29	0.33	0.32	0.25	0.25	0.34	0.29	0.24						
Gamma3	Central left	0.29	0.32	0.30	0.28	0.25	0.27	0.29	0.29	0.27	0.27	0.28	0.27	0.25	0.28	0.32	0.31	0.26	0.29	0.29	0.28	0.25	0.31	0.27	0.29	0.29	0.29	0.24	0.34	0.34	0.28	0.25	0.29	0.27	0.32	0.27	0.31	0.33	0.28	0.26	0.28	0.31	0.28	0.23	0.28	0.34	0.29	0.24							
Gamma3	Central midline	0.27	0.31	0.28	0.27	0.23	0.27	0.28	0.27	0.24	0.27	0.28	0.28	0.25	0.27	0.32	0.32	0.28	0.31	0.29	0.29	0.25	0.32	0.27	0.27	0.29	0.29	0.25	0.32	0.32	0.28	0.26	0.30	0.27	0.32	0.27	0.34	0.30	0.29	0.27	0.31	0.28	0.25	0.28	0.34	0.29	0.24								
Gamma3	Central right	0.30	0.32	0.28	0.28	0.25	0.28	0.30	0.28	0.25	0.26	0.28	0.29	0.27	0.31	0.32	0.28	0.31	0.31	0.29	0.26	0.31	0.28	0.28	0.31	0.28	0.26	0.34	0.33	0.29	0.27	0.31	0.26	0.30	0.28	0.32	0.33	0.30	0.27	0.28	0.31	0.28	0.25	0.26	0.34	0.29	0.24								
Gamma3	Parietal left	0.25	0.32	0.29	0.27	0.24	0.28	0.29	0.28	0.24	0.27	0.29	0.28	0.24	0.27	0.31	0.31	0.29	0.32	0.30	0.28	0.26	0.32	0.26	0.27	0.29	0.30	0.24	0.33	0.30	0.29	0.24	0.29	0.28	0.30	0.28	0.30	0.29	0.31	0.34	0.31	0.26	0.28	0.31	0.30	0.24	0.28	0.34	0.29	0.24					
Gamma3	Parietal midline	0.26	0.32	0.28	0.27	0.23	0.27	0.28	0.28	0.24	0.27	0.28	0.28	0.24	0.27	0.32	0.32	0.28	0.32	0.29	0.30	0.26	0.32	0.25	0.26	0.28	0.28	0.26	0.32	0.31	0.28	0.24	0.29	0.26	0.31	0.27	0.33	0.30	0.30	0.26	0.29	0.30	0.29	0.24	0.28	0.34	0.29	0.24							
Gamma3	Parietal right	0.28	0.31	0.29	0.28	0.24	0.28	0.29	0.29	0.25	0.27	0.29	0.29	0.25	0.27	0.31	0.31	0.28	0.33	0.31	0.30	0.27	0.32	0.26	0.27	0.29	0.28	0.27	0.33	0.32	0.29	0.25	0.29	0.27	0.32	0.28	0.36	0.31	0.31	0.27	0.30	0.31	0.30	0.24	0.25	0.34	0.29	0.24							
Gamma3	Occipital left	0.27	0.31	0.32	0.32	0.25	0.31	0.30	0.34	0.29	0.29	0.33	0.31	0.28	0.32	0.36	0.36	0.32	0.34	0.34	0.30	0.32	0.31	0.29	0.31	0.35	0.32	0.29	0.38	0.33	0.34	0.28	0.33	0.33	0.30	0.28	0.35	0.33	0.32	0.32	0.31	0.30	0.33	0.30	0.34	0.34	0.29	0.24							
Gamma3	Occipital right	0.28	0.31	0.31	0.31	0.28	0.32	0.34	0.33	0.32	0.33	0.33	0.31	0.28	0.32	0.36	0.36	0.34	0.34	0.30	0.32	0.31	0.29	0.29	0.31	0.30	0.26	0.38	0.33	0.33	0.28	0.33	0.33	0.32	0.31	0.29	0.33	0.32	0.31	0.31	0.30	0.31	0.34	0.27	0.30	0.34	0.29	0.24							
std Value Difference	Gamma3 Frontal left					-0.04	0.02	-0.01	-0.03	-0.05	0.04	-0.01	-0.02	-0.05	0.05	0.07	0.00	-0.01	0.05	0.02	-0.03	0.13																												0.15	0.00	-0.15			
Value differences	Gamma3 Frontal midline					-0.04	0.01	0.00	-0.05	-0.05	0.02	0.01	-0.01	-0.06	0.05	0.07	0.03	0.00	0.04	0.03	-0.02	0.11																															0.15	0.00	-0.15
Value Ratios	Gamma3 Frontal right					-0.04	0.05	0.01	-0.04	-0.07	0.05	0.02	-0.01	-0.05	0.09	0.07	0.02	-0.01	0.08	0.03	-0.03	0.19																															1.35	1.00	0.65
Gamma3 Temporal left						-0.06	0.02	0.02	-0.02	-0.08	0.05	-0.02	-0.03	-0.07	0.09	0.06	0.06	-0.01	0.03	0.01	0.02	0.12																															1.35	1.00	0.65
Gamma3 Temporal right						-0.04	0.07	0.03	-0.06	-0.10	0.05	0.01	-0.08	-0.10	0.12	0.09	0.00	0.00	0.09	0.04	-0.04	0.10																															1.35	1.00	0.65
Gamma3 Central left						-0.06	0.00	0.02	-0.04	-0.08	-0.02	-0.02	-0.05	-0.09	0.05	0.09	0.01	-0.04	0.00	0.00	-0.06	0.11																															1.35	1.00	0.65
Gamma3 Central midline						-0.06	0.00	0.00	-0.05	-0.07	0.00	0.00	-0.03	-0.08	0.07	0.07	0.02	-0.01	0.02	0.03	-0.03	0.09																															1.35	1.00	0.65
Gamma3 Central right						-0.04	0.04	-0.01	-0.09	-0.10	0.01	0.01	-0.07	-0.08	0.07	0.04	-0.03	-0.02	0.05	0.02	-0.08	0.05																															1.35	1.00	0.65
Gamma3 Parietal left						-0.06	0.01	0.02	-0.03	-0.09	0.01	0.01	-0.02	-0.08	0.09	0.09	0.05	-0.01	0.02	0.00	0.00	0.12																															1.35	1.00	0.65
Gamma3 Parietal midline						-0.07	0.00	0.02	-0.04	-0.09	0.01	0.01	-0.03	-0.08	0.07	0.09	0.03	-0.01	0.02	0.04	-0.01	0.10																															1.35	1.00	0.65
Gamma3 Parietal right						-0.07	0.02	0.02	-0.06	-0.09	0.01	0.01	-0.05	-0.09	0.08	0.09	0.00	0.00	0.05	0.04	-0.03	0.07																															1.35	1.00	0.65
Gamma3 Occipital left						-0.09	0.01	0.04	0.02	-0.09	0.03	0.00	-0.01	-0.05	0.12	0.04	0.07	-0.02	0.05	0.00	0.06	0.12																															1.35	1.00	0.65
Gamma3 Occipital right						-0.11	0.04	0.02	0.01	-0.08	0.05	-0.01	-0.04	-0.09	0.11	0.08	0.04	-0.04	0.06	0.07	0.03	0.11																															1.35	1.00	0.65
Gamma3 Frontal left						0.95	1.10	1.04	0.96	0.93	1.13	1.00	0.99	0.92	1.14	1.23	1.02	0.99	1.14	1.07	0.98	1.29																														1.35	1.00	0.65	
Gamma3 Frontal midline						0.94	1.07	1.06	0.91	0.91	1.09	1.05	0.99	0.90	1.15	1.23	1.09	1.03	1.14	1.11	0.99	1.25																															1.35	1.00	0.65
Gamma3 Frontal right						0.96	1.17	1.10	0.94	0.90	1.13	1.07	0.99	0.92	1.26	1.23	1.07	1.01	1.25	1.10	0.97	1.31																															1.35	1.00	0.65
Gamma3 Temporal left						0.90	1.18	1.09	1.01	0.88	1.22	1.00	0.99	0.90	1.28	1.18	1.18	1.02	1.22	1.06	1.09	1.17																															1.35	1.00	0.65
Gamma3 Temporal right																																																							

NV-5138 Increases High Frequency Coherence EO 1-Hour Post-dose

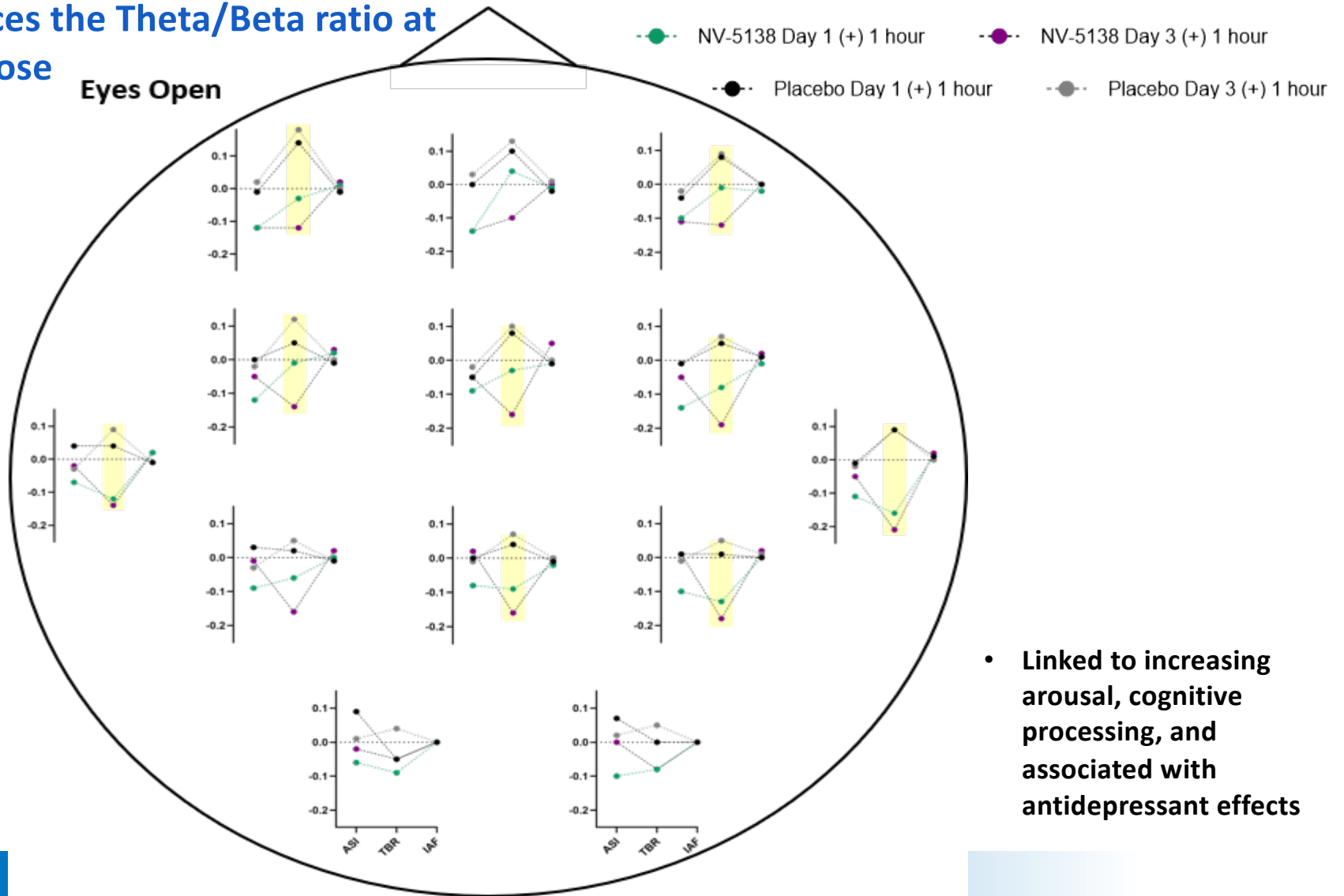
Endpoint	EC	
	NV-5138	Placebo
Delta (1.0 - 4.0 Hz)	-	-
Theta (4.0 - 8.0 Hz)	-	-
Alpha (8.0 - 12.0 Hz)	↑	-
Alpha 1 (8.0 - 10.0 Hz)	↑	-
Alpha 2 (10.0 - 12.0 Hz)	↑	-
Beta (12.0 - 25.0 Hz)	↑	-
Beta 1 (12.0 - 15.0 Hz)	↑	-
Beta 2 (15.0 - 18.0 Hz)	↑	-
Beta 3 (18.0 - 25.0 Hz)	↑↑	↓↑
Hi-Beta (25.0 - 30.0 Hz)	↑↑	↓↑
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)	↑↑↑	-
Total (1.0 - 50.0 Hz)	↑	-

- Coherence endpoints. Symbols indicate either no salient changes (-), small to large increases (↑, ↑↑, ↑↑↑), small to large decreases (↓, ↓↓, ↓↓↓) or mixed decreases and increases (↓↑) in mean value differences. A change in mean normalized value difference indicates a decrease or increase in a QEEG parameter relative to baseline. **Changes that were confirmed to be significant treatment effects in formal MMR models are highlighted in red.**
- Related to functional integration and connectivity between inter- and intra-hemispheric brain regions



NV-5138 reduces the Theta/Beta ratio at 1- hour post-dose

Eyes Open



EC Alpha Heat Map Both Doses and Times

Dosing:



Value			Placebo Time Point																	NV-5138 Time Point																													
Condition	Frequency Band	Spatial Location	PREDOSE1	PREDOSE2	PREDOSE3	PREDOSE4	PREDOSE5	POSTDOSE 1	POSTDOSE 2	POSTDOSE 3	POSTDOSE 4	POSTDOSE 5	POSTDOSE 6	POSTDOSE 7	POSTDOSE 8	POSTDOSE 9	POSTDOSE 10	POSTDOSE 11	POSTDOSE 12	POSTDOSE 13	POSTDOSE 14	POSTDOSE 15	POSTDOSE 16	POSTDOSE 17	PREDOSE1	PREDOSE2	PREDOSE3	PREDOSE4	PREDOSE5	POSTDOSE 1	POSTDOSE 2	POSTDOSE 3	POSTDOSE 4	POSTDOSE 5	POSTDOSE 6	POSTDOSE 7	POSTDOSE 8	POSTDOSE 9	POSTDOSE 10	POSTDOSE 11	POSTDOSE 12	POSTDOSE 13	POSTDOSE 14	POSTDOSE 15	POSTDOSE 16	POSTDOSE 17	Scale Max	Scale Min	
EO Alpha	Frontal left		0.16	0.20	0.18	0.21	0.20	0.21	0.23	0.19	0.19	0.21	0.20	0.19	0.16	0.20	0.21	0.19	0.18	0.20	0.19	0.20	0.17	0.20	0.22	0.26	0.28	0.24	0.22	0.21	0.22	0.21	0.27	0.26	0.28	0.27	0.19	0.30	0.23	0.24	0.32	0.31	0.27	0.24	0.26	Scale Max	0.46		
EO Alpha	Frontal midline		0.22	0.27	0.25	0.28	0.28	0.29	0.31	0.25	0.24	0.29	0.27	0.27	0.22	0.28	0.29	0.27	0.24	0.27	0.28	0.28	0.23	0.29	0.32	0.36	0.39	0.36	0.31	0.28	0.30	0.30	0.35	0.36	0.38	0.37	0.36	0.24	0.33	0.31	0.33	0.42	0.43	0.36	0.34	0.37	Scale Center	0.33	
EO Alpha	Frontal right		0.16	0.21	0.19	0.21	0.21	0.22	0.24	0.19	0.19	0.22	0.20	0.21	0.17	0.22	0.21	0.20	0.18	0.21	0.21	0.19	0.21	0.23	0.26	0.28	0.25	0.23	0.22	0.23	0.22	0.27	0.27	0.28	0.29	0.29	0.19	0.29	0.24	0.25	0.31	0.32	0.26	0.24	0.27	Scale Min	0.19		
EO Alpha	Temporal left		0.21	0.26	0.25	0.26	0.26	0.30	0.31	0.25	0.24	0.30	0.28	0.26	0.22	0.26	0.25	0.23	0.23	0.27	0.27	0.26	0.23	0.28	0.26	0.29	0.32	0.26	0.25	0.22	0.27	0.25	0.31	0.30	0.33	0.32	0.32	0.23	0.35	0.31	0.29	0.35	0.34	0.31	0.28	0.32			
EO Alpha	Temporal right		0.21	0.26	0.27	0.28	0.27	0.31	0.32	0.27	0.26	0.31	0.27	0.27	0.23	0.27	0.28	0.27	0.25	0.29	0.25	0.27	0.27	0.32	0.33	0.31	0.26	0.24	0.29	0.28	0.32	0.33	0.34	0.33	0.31	0.23	0.37	0.31	0.30	0.38	0.37	0.30	0.29	0.33					
EO Alpha	Central left		0.24	0.32	0.29	0.31	0.32	0.34	0.33	0.31	0.28	0.32	0.31	0.29	0.28	0.31	0.31	0.27	0.27	0.31	0.28	0.29	0.23	0.30	0.30	0.30	0.35	0.29	0.28	0.25	0.28	0.27	0.40	0.39	0.39	0.38	0.37	0.26	0.38	0.31	0.37	0.42	0.41	0.35	0.32	0.38			
EO Alpha	Central midline		0.25	0.32	0.29	0.31	0.30	0.32	0.35	0.31	0.28	0.32	0.31	0.32	0.27	0.32	0.34	0.32	0.29	0.33	0.30	0.33	0.28	0.35	0.35	0.37	0.41	0.37	0.35	0.32	0.34	0.35	0.41	0.41	0.43	0.43	0.41	0.29	0.39	0.35	0.42	0.48	0.48	0.39	0.39	0.43			
EO Alpha	Central right		0.32	0.32	0.30	0.32	0.32	0.34	0.37	0.34	0.30	0.35	0.32	0.33	0.28	0.34	0.35	0.31	0.29	0.33	0.31	0.33	0.26	0.33	0.31	0.34	0.36	0.32	0.29	0.26	0.31	0.31	0.38	0.40	0.40	0.40	0.35	0.25	0.38	0.30	0.37	0.44	0.43	0.34	0.32	0.39			
EO Alpha	Parietal left		0.33	0.38	0.40	0.42	0.43	0.44	0.44	0.42	0.39	0.44	0.43	0.39	0.35	0.39	0.43	0.39	0.38	0.41	0.38	0.40	0.34	0.44	0.41	0.46	0.50	0.40	0.39	0.33	0.39	0.40	0.48	0.49	0.50	0.45	0.53	0.40	0.46	0.50	0.55	0.55	0.45	0.46	0.53				
EO Alpha	Parietal midline		0.34	0.39	0.41	0.41	0.44	0.43	0.47	0.45	0.42	0.47	0.46	0.43	0.37	0.43	0.44	0.44	0.43	0.45	0.42	0.46	0.38	0.52	0.47	0.49	0.54	0.45	0.46	0.38	0.45	0.46	0.55	0.59	0.56	0.53	0.57	0.44	0.51	0.48	0.54	0.59	0.58	0.48	0.53	0.57			
EO Alpha	Parietal right		0.32	0.38	0.40	0.42	0.41	0.43	0.47	0.43	0.39	0.46	0.44	0.41	0.35	0.41	0.44	0.41	0.40	0.43	0.39	0.41	0.36	0.47	0.43	0.46	0.49	0.42	0.41	0.36	0.42	0.43	0.50	0.54	0.53	0.48	0.52	0.40	0.50	0.46	0.48	0.56	0.55	0.45	0.48	0.54			
EO Alpha	Occipital left		0.27	0.30	0.31	0.33	0.36	0.39	0.43	0.35	0.35	0.39	0.36	0.32	0.29	0.33	0.36	0.34	0.32	0.35	0.34	0.37	0.32	0.39	0.34	0.41	0.44	0.35	0.32	0.28	0.34	0.33	0.37	0.41	0.41	0.38	0.44	0.31	0.40	0.39	0.37	0.45	0.44	0.35	0.39	0.43			
EO Alpha	Occipital right		0.28	0.30	0.32	0.34	0.36	0.39	0.45	0.36	0.35	0.38	0.36	0.32	0.28	0.36	0.36	0.32	0.33	0.36	0.33	0.39	0.33	0.38	0.36	0.43	0.46	0.39	0.32	0.28	0.34	0.34	0.39	0.44	0.42	0.41	0.46	0.32	0.42	0.40	0.38	0.48	0.45	0.37	0.38	0.46			
Adjusted Value Difference																																																	
EO Alpha	Frontal left							-0.01	0.06	-0.05	0.06	0.03	-0.01	-0.03	0.01	0.02	0.03	-0.03	0.04	-0.01	0.00	-0.02	0.02	0.10																							Scale Max	0.15	
EO Alpha	Frontal midline							0.01	0.07	-0.05	0.01	-0.02	-0.01	-0.04	-0.01	0.02	0.03	-0.02	0.03	-0.02	0.00	-0.02	0.00	0.11																							Scale Center	0.00	
EO Alpha	Frontal right							-0.03	0.07	-0.05	0.03	0.00	-0.02	-0.02	0.01	-0.01	0.00	-0.01	0.02	-0.06	0.01	0.00	0.02	0.10																							Scale Min	-0.15	
EO Alpha	Temporal left							0.04	0.05	0.02	0.00	0.02	-0.02	-0.01	0.00	0.00	-0.05	-0.03	-0.01	-0.02	-0.06	-0.02	-0.03	0.04																									
EO Alpha	Temporal right							0.02	0.05	0.03	0.04	0.00	-0.02	-0.04	0.00	0.02	0.00	-0.02	0.02	0.01	-0.01	-0.09	-0.01	0.00	0.05																								
EO Alpha	Central left							0.00	0.02	0.01	0.04	0.00	-0.03	-0.02	0.04	0.01	-0.01	-0.05	0.04	-0.04	-0.08	-0.05	-0.02	0.05																									
EO Alpha	Central midline							-0.03	0.04	-0.01	0.01	0.00	-0.01	0.00	-0.01	0.01	0.04	0.01	0.03	-0.02	-0.04	0.00	0.02	0.10																									
EO Alpha	Central right							0.00	0.07	0.03	0.04	0.00	0.00	0.00	0.03	0.00	0.03	0.02	0.03	-0.04	-0.07	-0.02	-0.05	0.09																									
EO Alpha	Parietal left							0.04	0.04	0.03	0.03	0.02	-0.01	-0.04	0.01	0.00	0.00	-0.02	0.03	0.00	-0.07	-0.05	-0.01	0.03																									
EO Alpha	Parietal midline							0.01	0.03	0.02	0.03	0.02	-0.01	-0.03	0.01	0.01	0.03	0.01	0.05	0.01	-0.05	-0.03	0.00	0.07																									
EO Alpha	Parietal right							0.02	0.06	0.04	0.03	0.02	0.00	-0.03	0.02	0.00	-0.03	0.03	0.04	0.01	-0.07	-0.02	-0.01	0.07																									
EO Alpha	Occipital left							0.06	0.11	0.03	0.07	0.05	0.02	-0.03	0.01	-0.01	0.04	0.02	0.04	0.01	-0.01	0.00	0.02	0.04																									
EO Alpha	Occipital right							0.07	0.13	0.04	0.08	0.05	-0.01	-0.06	-0.02	0.01	0.01	0.00	0.04	0.02	-0.03	0.00	0.01	0.05																									
Value Ratio																																																	
EO Alpha	Frontal left							1.02	1.20	0.97	1.24	1.12	1.04	0.96	1.08	1.10	1.12	1.04	1.15	1.06	1.04	1.00	1.14	1.00																							Scale Max	1.35	
EO Alpha	Frontal midline							1.06	1.22	0.95	1.11	1.09	1.04	0.95	1.02	1.11	1.11	1.04	1.14	1.03	1.09	1.00	1.10	1.03																							Scale Center	1.00	
EO Alpha	Frontal right							0.99	1.21	0.97	1.15	1.05	1.03	0.99	1.05	1.02	1.05	1.06	1.12	0.96	1.09	1.02	1.16	1.03																							Scale Min	0.65	
EO Alpha	Temporal left							1.14	1.15	1.10	1.08	1.14	1.05	1.07	1.05	1.11	0.96	1.13	1.05	1.10	1.00	1.09	1.01	1.10																									
EO Alpha	Temporal right							1.11	1.16	1.15	1.14	1.13	0.99	1.03	1.10	1.12	1.01	1.27	1.13	1.11	0.94	1.07	1.09	1.00																									
EO Alpha	Central left							1.04																																									

Conclusion

- NV-5138 was generally safe, well tolerated. PK comparable across doses and studies.
- NV-5138 penetrated the brain and actively modulated neural activity as measured by qEEG.
- At 1- hour post-dose the increased beta through gamma band amplitudes and coherences, suggest NV-5138 might increase perceptual and cognitive processing.
- The alpha amplitudes and ASI were reduced 1- hour post-drug; increase at later time points;
 - TRD pilot study showing HAM-D6 improvements at 4 and 12 hours post single-dose.
- Decreased Theta/Beta ratio observed are typically linked to increasing arousal and cognitive processing and associated with antidepressant effects
- The pattern of electrophysiology changes on drug was consistent with antidepressant effects.
- Time course of drug effects post-dose should extend out past Tmax, if downstream changes in cellular function, gene expression, etc. are expected.
- Limitations include small sample size and use of healthy male volunteers precluding conclusions in females or in patients with depression.
 - The use of *a priori* 'salience' and defining physiologically meaningful 'signal' helped to guide data analysis and reduce the number of repeated measures statistical analyses