Electrophysiology Based Biomarkers as Outcome Measures, Demonstrate Signal Detection and Reliability in a Simulated Clinical Trial at an Experienced Clinical Research Center Evaluating Study Drug (Ketamine) and Placebo

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Introduction

We present study results sponsored by the ERP Biomarker Qualification Consortium (https://erpbiomarkers.org). Our study is designed to simulate a randomized placebo (Pbo) controlled clinical trial to detect differences in standardized Event Related Potential (ERP)/Quantitative (Q)EEG measures between study drug (Ketamine) and Pbo in healthy volunteers at an experienced early phase clinical research unit. Low ICCs are a critical limiting factor for the use of ERP/QEEG as biomarkers reducing, confidence in the results. For reliable study results ICCs≥ 0.5 (moderate) and preferably ICCs≥ 0.6 are used in many clinical trials. We report results for a study that tested participants twice under Ketamine administration vs placebo. The study had 3 main objectives:

- Measure the effect size (Cohen's d) of Ketamine-induced changes on ERP and QEEG measure collected and analyzed with standardized equipment and methods.
- Quantify the variability of the Ketamine effect on ERP and QEEG measures across two dosings [Intraclass correlation coefficient (ICC)]
- Investigate a possible "disordinal effect" of Ketamine on ERP and QEEG feature parameters, where the direction and magnitude of Ketamine-induced changes could be predicted by the baseline value.
- Our previously published study Validation of a Suite of ERP and QEEG Biomarkers, a pre-competitive, industry led study in subject with schizophrenia and healthy volunteers (EBS-A) provides supplemental data used in the disordinal effect analysis.

Methods

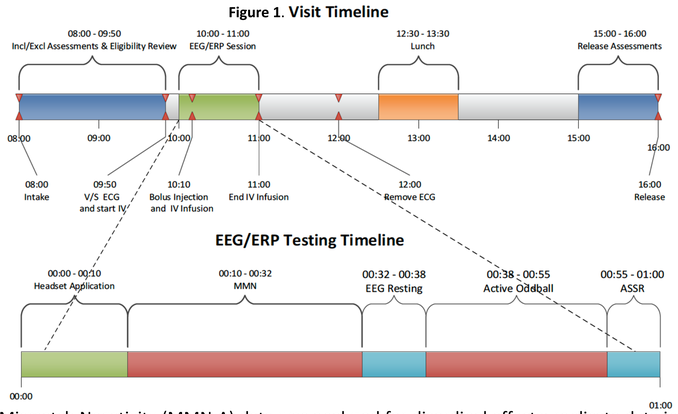
Study Design

The study was a randomized, double-blind, placebo-controlled, 3-arm, 3-period crossover design performed at one study site in the United States (CenExel HRI, NJ, USA). 31 subjects were enrolled to complete 24 study participants (administered Ketamine IV on two of the periods and Pbo on the remaining period. (See Table 1). The Ketamine dose was a 0.23 mg/kg bolus over 1 minute, followed by 0.58 mg/kg per hour for 30 minutes, and 0.29 mg/kg per hour for up to 29 minutes after that (GunduZ-Bruce et al., 2012). ERP and EEG data were collected during the infusions. (See Figure 1)

Table 1. Dosing Randomization Assignment Schedule

Arm 1	Placebo	Ketamine (K1)	Ketamine (K2)
Arm 2	Ketamine (K1)	Placebo	Ketamine (2)
Arm 3	Ketamine (1)	Ketamine (2)	Placebo

Figure 1. Visit Timeline



Mismatch Negativity (MMN-A) data was analyzed for disordinal effects: replicate data in stable patients with schizophrenia No TX (EBS-A, NCT04025502) was used for Regression to the Mean (RTM); current EBS-B's Pbo-KET Arm 1 (KET1: NCT04928703) was combined with Consortium provided data from 2 additional ketamine studies (KET2 and KET3).

Study Participants

EBS-B (this study) recruited volunteers. Detailed info can be found: www.clinicaltrials.gov (NCT04928703). Our prior Consortium EBS-A study (NCT04025502) used replicate evaluations in stable patients with Schizophrenia and was used, here, to demonstrate regression to the mean n=35 (Cecchi M et al. Schizophr Res. 2023 Apr; 254:178-189). Consortium data analyzed for disordinal effects were from this study (EBS-B KET1 n=8) and from KET2 n=19 (NCT05049343) and KET3 n=27 (Hamilton HK et al. Schizophr Res. 2018 Jan;191:87-94).

ERP and EEG Testing

ERP and EEG data were collected using the COGNISION® System for EBS-B/KET1, KET2, and EBS-A (No Tx), and Neuroscan System for KET3

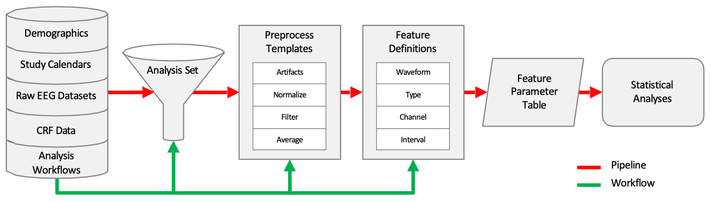
Each session for Consortium EBS-B study included 4 tests:

- Duration-deviant mismatch negativity: 1000Hz standard 50 msec; deviant 100 msec
- Eyes-closed resting state EEG
- Auditory One-deviant active oddball
- 40Hz auditory steady-state response

All data was evaluated for quality against predefined objective metrics

Data and Statistical Analysis

Figure 2. Data preprocessing and extraction of ERP and QEEG parameters performed through a predefined data and statistical analysis pipeline



EBS-B Analysis: Group differences between Pbo and mean Ketamine [(K1+K2/)2] for ERP/QEEG endpoints were analyzed using a two-tailed t-test. Effect size was estimated as Cohen's d. Variability of the Ketamine effect across the two dosing sessions was calculated as intra-class correlation coefficients (ICCs). ICCs were estimated for the two Ketamine sessions (K1, K2) and for the differences K1-Pbo and K2-Pbo.

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Disordinal Analysis: MMN-A data from each study were separately Z-score normalized for Ketamine with respect to mean (SD) of pbo/baseline data. Slope by linear regression and confidence Intervals were calculated for MMN baseline/Pbo vs. change with Ketamine. The data sets were combined, and additional statistical testing are further described in our prior presentation (QR code below). For EBS-B KET1, only ARM1 data was used, matching up to the baseline-Ketamine Rx sequences for KET 2 and KET3. The interaction effect for Pbo/baseline vs. ketamine was tested for slope differences; further evaluation by Johnson-Neyman analysis further characterizes the disordinal effect.

Mathalon D, ERP Consortia, Disordinal Effect Analysis. CNS Summit 2023

Results

EBS-B Primary Analysis: 31 subjects were enrolled to complete 24. Seven subjects discontinued due to CNS AEs related to ketamine. Age was 33.8 (SEM 1.07) years, gender distribution Male were 79% and Females were 21% of the population. Mean education was 12th grade. BMI ranged from 18.5 to 30.0. For the primary analyses all 24 subjects are included.

Disordinal effect analyses used a subset of subjects from EBS-A (No Tx) and EBS-B Arm 1: PBO-KET1 sequence was included to control for possible carryover effects. No Tx, KET1, KET2 and KET3 demographics are shown in Table 2.

Table 2: Demographics for Disordinal and RTM Analyses

Study	*No TX (EBS-A)*	*KET1 (Arm 1 EBS-B)*	*KET2*	*KET3*
Sample Size	35 (Schizophenia)	8 (Volunteers)	19 (Volunteers)	27 (Volunteers)
Age[1]	38.11 (1.91)	35.25 (1.82)	42.56 (2.06)	25.78 (1.2)
Gender				
Male[2]	17 (48.6%)	6 (75%)	13 (68.4%)	15 (55.6%)
Female[2]	18 (51.4%)	2 (25%)	6 (31.6%)	12 (44.4%)
Race				
White[2]	8 (22.9%)	2 (25%)	8 (42.1%)	21 (77.8%)
African American[2]	4 (11.4%)	5 (62.5%)	11 (57.9%)	1 (3.7%)
Other[2]	23 (65.7%)	1 (12.5%)	0 (0%)	5 (18.5%)
Education[1]	13.83 (0.32)	12		15.52 (0.51)
BMI[1]		26.99 (0.92)	27.21 (0.5)	23.88 (0.96)

Abbreviations: BMI = Body Mass Index. Notes: *1.* Mean (±SEM). 2.Total (% of Total).

Data Quality Review (Table 3)

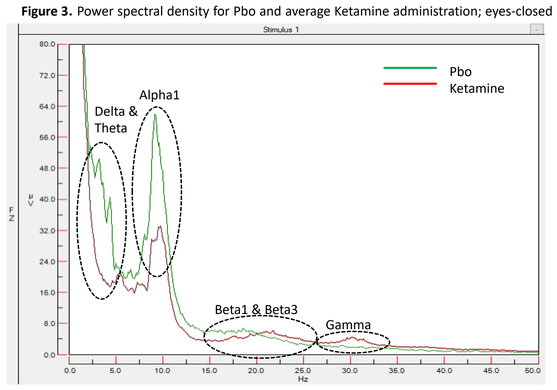
Only 6 out of 300 tests (2%) did not meet Quality Review criteria and were deemed "Not Valid" and were excluded from all analyses.

Paradigm	*Tests*	*Valid*	*Not Valid*	*Other*	*Comments*
Passive DD-MMN	75	73	1	1	Data-Set 34757 (subject 0022, Dosing 1) has no EEG recorded from channel P4. Data-Set 33790 (subject 0006, Dosing 1) failed QC; subject 0006 was not part of the Analysis Set.
Resting-State EEG	75	74	0	1	Data-Set 34757 (subject 0022, Dosing 1) has no EEG recorded from channel F4.
Active Oddball	75	71	4	0	Data-Set 33561 (subject 0003, Dosing 1), Data-Set 34155 (subject 0010, Dosing 3), Data-Set 34757; subject 0022, Dosing 1), and Data-Set 35360 (subject 0033, Dosing 1) failed QC.
40Hz ASSR	75	73	1	1	Data-Set 34757 (subject 0022, Dosing 1) has no EEG recorded from channel F3. Data-set 33616 (subject 0004, Dosing 1) failed QC; subject 0004 was not part of the Analysis Set.

Abbreviations: DD-MMN = Duration-Deviant Mismatch Negativity; ASSR = Auditory Steady-State Response.

Resting-State EEG

Figure 3. Power spectral density for Pbo and average Ketamine administration; eyes-closed resting state EEG. Data is from Fz electrode.



Findings for Resting-State EEG

- ICCs were good-to-excellent across most measures. See Table 4.
- Several features showed significant changes after Ketamine consistent with the literature including:
  - Decreases in Delta and Theta power
  - Increases in Beta 3 and Gamma power
  - Power spectra for Ketamine crosses over (>) Pbo in Beta2 range
  - Demonstrate expected activation
  - Increases in Peak Alpha Frequency are associated with clinical effects on depression

Table 4. QEEG feature parameters: significance, effect sizes and intraclass correlation coefficients

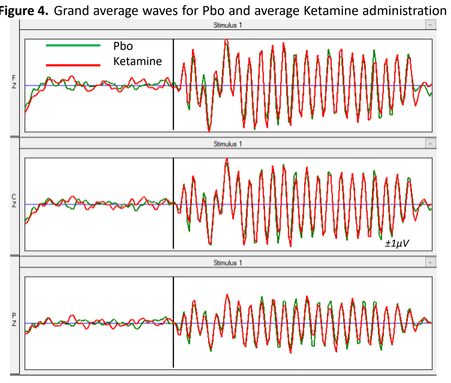
Feature[1]	Channel	Frequency	Measure	Pbo[2]	Mean Ketamine (K1+K2/)2[3]	Cohen's d	ICC K1, K2	ICC K1-P, K2-P
Delta-Absolute	AVG	[1.5-6]	Power (μV²/Hz)	98.874 (9.308)	71.031 (6.715)*	0.49	0.8554	0.9491
Delta-Relative	AVG	[1.5-6]	n/a	0.356 (0.029)	0.385 (0.029)	0.215	0.799	0.7814
Theta-Absolute	AVG	[6-8.5]	Power (μV²/Hz)	34.406 (4.481)	24.469 (2.385)*	0.565	0.7849	0.8989
Theta-Relative	AVG	[6-8.5]	n/a	0.112 (0.010)	0.127 (0.010)	0.33	0.6577	0.5688
Alpha1-Absolute	AVG	[8.5-10.5]	Power (μV²/Hz)	81.538 (16.15)	38.954 (8.874)**	0.586	0.7599	0.907
Alpha1-Relative	AVG	[8.5-10.5]	n/a	0.217 (0.025)	0.152 (0.02)**	0.673	0.6479	0.6369
Alpha2-Absolute	AVG	[10.5-12.5]	Power (μV²/Hz)	45.324 (8.629)	26.611 (4.718)*	0.529	0.8875	0.9504
Alpha2-Relative	AVG	[10.5-12.5]	n/a	0.13 (0.018)	0.114 (0.014)	0.169	0.8493	0.9158
Beta1-Absolute	AVG	[12.5-18.5]	Power (μV²/Hz)	28.479 (3.862)	17.730 (2.675)**	0.66	0.7122	0.8036
Beta1-Relative	AVG	[12.5-18.5]	n/a	0.093 (0.005)	0.086 (0.006)	0.273	0.6671	0.5817
Beta2-Absolute	AVG	[18.5-21]	Power (μV²/Hz)	10.099 (1.661)	7.932 (1.923)*	0.474	0.7673	0.2831
Beta2-Relative	AVG	[18.5-21]	n/a	0.033 (0.004)	0.035 (0.004)	0.118	0.6267	0.02658
Beta3-Absolute	AVG	[21-30]	Power (μV²/Hz)	17.203 (2.628)	22.66 (5.820)	0.272	0.8829	0.7763
Beta3-Relative	AVG	[21-30]	n/a	0.059 (0.006)	0.101 (0.012)**	0.879	0.5946	0.4652
Total Power	AVG	[1.5-30]	Power (μV²/Hz)	315.922 (38.708)	209.386 (23.567)**	0.638	0.8999	0.9509
Gamma-Absolute	AVG	[30-40]	Power (μV²/Hz)	8.123 (1.030)	16.398 (2.785)**	0.733	0.6245	0.4948
Alpha Peak Frequency	AVG	[6-12.5]	Frequency (Hz)	10.012 (0.141)	10.461 (0.196)**	1.148	0.8543	0.5672
Slow Wave Index	AVG		n/a	0.988 (0.131)	0.734 (0.116)~	0.412	0.6823	0.7213
Theta/Beta Ratio	AVG		n/a	1.017 (0.131)	1.294 (0.166)*	0.448	0.7516	0.6071

Abbreviations: ICC K1, K2 = Intraclass Correlation Coefficients (ICC) for first Ketamine administration and second Ketamine administration; ICC K1-P, K2-P = ICC for first Ketamine administration minus Placebo, and second Ketamine administration minus Placebo; AVG = Average of all electrode locations.

1. The frequency bands selected for Pharmaco-EEG analysis were taken from an International Pharmaco-EEG Society (IPEG) guidance document intended to standardize Pharmaco-EEG analysis methods for the pharmaceutical industry (Jobert et al., 2012).
2. Values are mean (±SEM).
3. *p < 0.05, **p < 0.01, and ~p < 0.1 compared to Placebo.

40Hz Auditory Steady State Response (ASSR)

Figure 4. Grand average waves for Pbo and average Ketamine administration from 40Hz ASSR test. Data is shown at midline electrodes.



Findings for the 40Hz ASSR:

- Data showed high signal to noise ratio and excellent entrainment, morphological concordance. See Figure 4
- Ketamine had a minimal effect on ITC, consistent with Sivarao, 2016. See Table 5.
- Assessment of ITC 'later in time' during a ketamine infusion (perhaps also sequence effect of prior testing) reduces ITC response to ketamine.
- The increase in 40Hz power (EP and TP) after Ketamine was mostly driven by non-time-locked increase in Gamma power, consistent with Resting-State EEG.

Table 5. Feature parameters from the 40Hz ASSR: significance, effect sizes and intraclass correlation coefficients

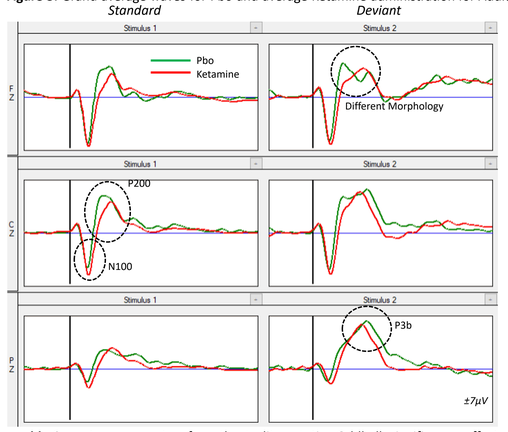
Feature	Channel	Frequency	Interval ms	Measure	Placebo[1]	Mean Ketamine[2] (K1+K2)/2	Cohen's d	ICC K1, K2	ICC K1-P, K2-P
ITC000	Fz	38-42	-99-0	n/a	0.07 (0.008)	0.071 (0.005)	0.011	0.2152	0.7205
ITC100	Fz	38-42	1-100	n/a	0.182 (0.013)	0.169 (0.014)	0.182	0.2305	0.3016
ITC200	Fz	38-42	101-200	n/a	0.335 (0.026)	0.317 (0.028)	0.187	0.6122	0.3591
ITC300	Fz	38-42	201-300	n/a	0.409 (0.031)	0.39 (0.029)	0.148	0.7235	0.6697
ITC400	Fz	38-42	301-400	n/a	0.332 (0.032)	0.337 (0.028)	0.045	0.6385	0.4696
ITC500	Fz	38-42	401-500	n/a	0.318 (0.031)	0.32 (0.027)	0.019	0.6457	0.5091
ITC1500	Fz	38-42	1-500	n/a	0.44 (0.026)	0.419 (0.029)	0.21	0.734	0.5579
EP	Fz	38-42	1-500	μV²/Hz	0.156 (0.018)	0.189 (0.025)~	0.411	0.5537	0.2205
TP	Fz	38-42	1-500	μV²/Hz	0.559 (0.048)	0.788 (0.093)**	0.577	0.4927	0.7679

Abbreviations: ICC K1, K2 = Intraclass Correlation Coefficients (ICC) for first Ketamine administration and second Ketamine administration; ICC K1-P, K2-P = ICC for first Ketamine administration minus Placebo, and second Ketamine administration minus Placebo; ITC = Intertrial Coherence; EP = Evoked Power; TP = Total Power.

1. Values are mean (±SEM).
2. **p < 0.01, and ~p < 0.1 compared to Placebo.

Auditory Active Oddball

Figure 5. Grand average waves for Pbo and average Ketamine administration for Auditory Active Oddball. Data shown for midline electrodes.



Findings for Auditory Active Oddball:

- ERP waves showed (Figure 5) excellent morphology and large amplitude *(μV)*.
- ICC was good-to-excellent for most measures. See Table 6.
- Button-Press Accuracy was not different across conditions
  - Dose of ketamine selected to provide CNS effects with minimal impairment on behavioral tasks
- N100 and P200 demonstrated differences in amplitude and longer latencies on Ketamine.
- Subjects showed impaired cognitive processing based on reduced P3b amplitude on Ketamine.

Table 6. Feature parameters from the Auditory Active Oddball: significance, effect sizes and intraclass correlation coefficients

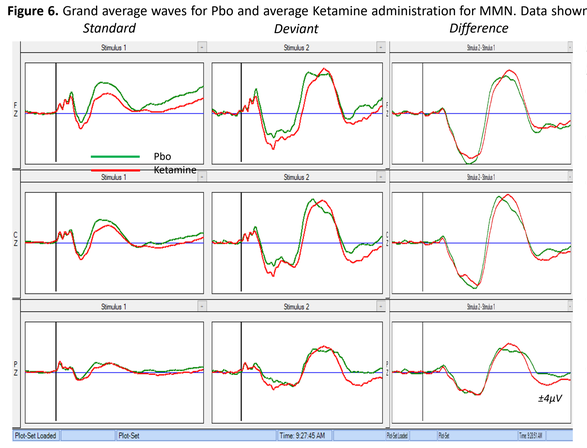
Feature	Channel	Interval 1 (ms)	Interval 2 (ms)	Measure	Placebo[1]	Avg Ketamine[2]	Cohen's d	ICC K1, K2	ICC K1-P, K2-P
BPA	n/a	n/a	n/a	%	89.841 (2.918)	89.087 (2.168)	0.075	0.6758	0.7282
FA	n/a	n/a	n/a	%	0.377 (0.095)	0.705 (0.184)*	0.493	0.607	0.436
MRT	n/a	n/a	n/a	ms	414.857 (23.717)	433.667 (21.730)	0.22	0.6974	0.5907
P50-Amp	Cz	28-76	24-80	mcV	1.536 (0.278)	1.541 (0.229)	0.005	0.8338	0.6
P50-Lat	Cz	28-76	24-80	ms	41.714 (2.183)	43.048 (2.377)	0.133	0.4429	0.265
N100-Amp	Cz	68-140	64-144	mcV	-5.190 (0.480)	-5.725 (0.430)*	0.463	0.8929	0.6747
N100-Lat	Cz	68-140	64-144	ms	92.571 (1.996)	97.429 (2.466)*	0.568	0.851	0.7663
P200-Amp	Cz	136-256	132-260	mcV	6.345 (0.535)	4.651 (0.396)**	0.814	0.7924	0.8573
P200-Lat	Cz	136-256	132-260	ms	195.81 (6.575)	214.286 (4.667)**	0.705	0.3382	0.473
N200-Amp	Cz	176-320	172-324	mcV	2.306 (0.582)	2.563 (0.47)	0.096	0.5869	0.5314
N200-Lat	Cz	176-320	172-324	ms	236.444 (9.439)	239.000 (7.082)	0.05	0.3305	0.6702
P3B-Amp	Pz	248-472	244-476	mcV	6.976 (0.588)	6.051 (0.577)*	0.466	0.6309	0.5437
P3B-Lat	Pz	248-472	244-476	ms	310.476 (10.204)	288.286 (5.170)~	0.425	0.2994	0.8778

Abbreviations: ICC K1, K2 = Intraclass Correlation Coefficients (ICC) for first and second Ketamine administration; ICC K1-P, K2-P = ICC for first Ketamine administration minus Placebo, and second Ketamine administration minus Placebo; BPA = Button Press Accuracy; FA = False Alarms; MRT = Median Reaction Time; Amp = Amplitude; Lat = Latency. :

1. Values are mean (±SEM).
2. **p < 0.01, and ~p < 0.1 compared to Placebo.

Duration-Deviant Mismatch Negativity - oddball stimulus was 50ms longer than the standard tone

Figure 6. Grand average waves for Pbo and average Ketamine administration for MMN. Data shown for midline electrodes.



Findings for the Duration-Deviant Mismatch Negativity:

- ERP waves showed excellent morphology. See Figure 6.
- ICC was good-to-excellent for most measures. See Table 7.
- Morphology for the ERP wave from the Standard stimulus was comparable to the Active Oddball, suggesting consistent results across testing paradigms.
- No group effects of Ketamine on the MMN ERP parameters were observed.
- Ketamine's effect on MMN-Amplitude (MMN-A) and Direction was dependent on the subject's baseline amplitude values.

Table 7. Feature parameters from the Mismatch Negativity: significance, effect sizes and intraclass correlation coefficients

Feature	Channel	Interval 1 (ms)	Interval 2 (ms)	Measure	Placebo[1]	Avg Ketamine	Cohen's d	ICC K1, K2	ICC K1-P, K2-P
P50-Amp	Cz	24-76	20-80	mcV	1.595 (0.249)	1.356 (0.226)	0.207	0.5187	0.6277
P50-Lat	Cz	24-76	20-80	ms	47.833 (2.587)	47.583 (2.102)	0.02	0.3098	0.5734
N100-Amp	Cz	64-132	60-136	mcV	-1.608 (.0251)	-2.089 (0.279)	0.287	0.6685	0.7652
N100-Lat	Cz	64-132	60-136	ms	93.667 (3.43)	93.583 (2.629)	0.005	0.3745	0.5759
P200-Amp	Cz	108-220	104-224	mcV	2.649 (0.307)	2.167 (0.27)	0.291	0.6836	0.7851
P200-Lat	Cz	108-220	104-224	ms	171.667 (4.121)	174.417 (4.449)	0.083	0.4319	0.7057
MMN-Amp	Fz	104-260	100-264	mcV	-5.099 (0.496)	-5.060 (0.386)	0.02	0.677	0.6799
MMN-Lat	Fz	104-260	100-264	ms	171.833 (4.8)	170.500 (4.865)	0.053	0.5134	0.55
P3A-Amp	Cz	224-352	220-356	mcV	4.143 (0.371)	4.503 (0.480)	0.152	0.8136	0.816
P3A-Lat	Cz	224-352	220-356	ms	279.167 (6.185)	287.083 (5.256)	0.247	0.5163	0.6581

Abbreviations: ICC K1, K2 = Intraclass Correlation Coefficients (ICC) for first Ketamine administration and second Ketamine administration; ICC K1-P, K2-P = ICC for first Ketamine administration minus Placebo, and second Ketamine administration minus Placebo; MMN = Mismatch Negativity; Amp = Amplitude; Lat = Latency.

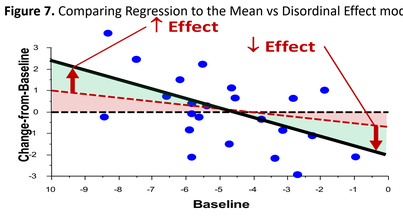
Notes:

1. Values are mean (±SEM).

Disordinal Effect or Regression to the Mean (RTM)

Linear regression for MMN-A Z-scores at Baseline (BL) Against Z-scores for Change MMN-A as Retest from BL

Figure 7. Comparing Regression to the Mean vs Disordinal Effect models



Findings for Disordinal Effects Analysis

- Test vs Retest Change-from-BL regression analysis performed for MMN-A with NO Tx EBS-A shows → RTM, with a shallow slope, Figure 8 (Z-Scores)
- Individual slopes KET1 (0.844), KET2 (1.244), KET3 (1.040) were not significantly different (p=0.502).
- The combined MMN-A regression for BL vs BL-KET retest shows a steeper slope (p<0.001) than RTM. See Figure 9 (Z-Scores)
- Subjects with a positive Z-score at baseline (low MMN-A) have a negative Z-score for change: as the peak goes more negative; MMN-A gets larger.
- Johnson-Neyman analysis is overlaid on Figure 9

Figure 8. No Tx Test-Retest Study (EBS-A): Regression showed shallow slope 0.234, R^2=0.176 (p=0.012). Consistent with an RTM model

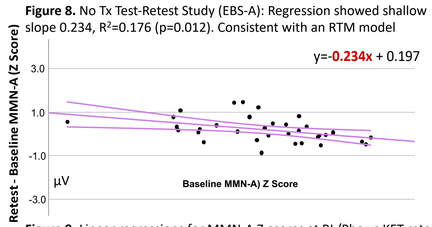
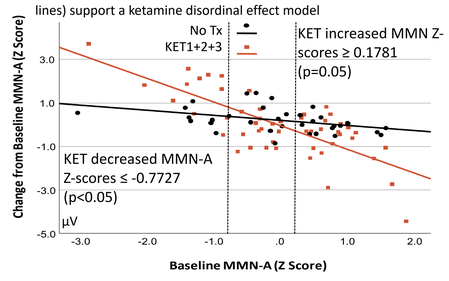


Figure 9. Linear regressions for MMN-A Z-scores at BL/Pbo vs KET retest. Change from BL for No Tx (EBS-A) slope is compared to the combined data from KET1/2/3 studies showed significant slope differences (p<0.001). Johnson-Neyman analysis (Z-score cutoffs – below vertical lines) support a ketamine disordinal effect model



Conclusions

- With standardized equipment and methods, high quality EEG/ERP biomarkers can be measured (good-excellent ICCs) in a Ketamine-challenge study, designed to emulate a clinical trial, at an experienced commercial site.
- Ketamine showed significant effects on various ERP and QEEG measures consistent with the literature.
- Ketamine produces a "disordinal" effect on MMN amplitude: inducing larger changes with smaller MMNs at baseline and smaller changes with larger MMNs at baseline, not simply RTM.
- Findings support a possible approach for precision medicine in the treatment of CNS disorders.