*Lamontagne et al – Clinical, behavioral, and electrophysiological profiles along a continuum*

*of suicide risk: Evidence from an implicit association task*

**SUPPLEMENT**

**Supplemental Methods**

*Inclusion/Exclusion Criteria*

While participants were recruited on the basis of suicide risk, all participants in the current study were also assessed for psychiatric diagnoses using the Structured Clinical Interview for Axis I Diagnostic and Statistical Manual of Mental Disorders-Fifth edition (DSM-5) (SCID). Across groups, participants were excluded if they had current psychotic features or cognitive impairment, drug or alcohol dependence, or unstable medical conditions. All participants were maintained on their current psychiatric medication regimens during study participation. Participants in the minimal risk (MinR) group were excluded if they had current or past Axis I DSM-5 diagnoses, lifetime suicide attempt or ideations, or first-degree family history of psychiatric diagnosis or suicide attempt or death. Participants in the high risk (HR) group were excluded if they sustained an injury as a consequence of the suicide attempt that would preclude their ability to provide informed consent or understand and perform study procedures. In the event that participants were deemed ineligible for the study due to adverse effects of the suicide attempt, they were provided appropriate clinical stabilization until they could provide informed consent or be transferred to an appropriate treatment facility outside of the NIH. Participants in the HR group were also excluded if they posed a current homicidal risk or suicide risk that could not be managed in a voluntary inpatient setting. Additional precautions for research with individuals in acute suicide crises, such as checks of the physical environment and standardizing suicide-related language across research staff, are detailed in Ballard et al. (2020). Across groups, participants had normal or corrected-to-normal vision and fluency in written and spoken English.

For the open-label ketamine pilot study, inclusion was limited to only those individuals experiencing a suicide attempt or ideation with intent in the past two weeks, representing those at highest risk for imminent suicidal behavior. These participants were required to have at least minimal symptoms of anxiety or suicidal ideation (MADRS >10, HAM-A >7 and SSI >1). Suicidal ideation for this phase was not required in order to discourage participants from over-reporting suicidal thoughts in order to qualify for ketamine administration. Participants were excluded from this phase if they had medical contraindications to ketamine, including treatment with a monoamine oxidase inhibitor (MAOI) within two weeks prior to study participation.

*Magnetoencephalography (MEG) acquisition, preprocessing, and source localization*

Electrophysiological data were acquired in a magnetically shielded room (Vacuumschmelze, Hanau, Germany) using a 275-channel CTF MEG system with superconducting quantum interference device-based axial gradiometers (VSM MedTech Ltd., Coquitlam, BC, Canada). Data were collected at 1200 Hz with a bandwidth of 0-300 Hz. Synthetic third-order balancing was used for active noise cancellation. Each participant’s MEG data was co-registered to their MRI image using a T-1 weighted MRI scan (3T GE scanner; GE Signa, Milwaukee, WI) with MRI-visible fiducial markers placed on the head prior to scanning. MEG data were visually inspected offline, and trials with visible artifacts (e.g., head or muscle movements, eye blinks) and channels with excessive sensor noise were removed from analyses. Cleaned data were bandpass-filtered from 1 to 58 Hz and epoched from -100 to 1000 ms peristimulus time.

 MEG data were source-localized to a 5mm grid in the gamma frequency (30-58 Hz) using a linearly constrained minimum variance beamforming algorithm (synthetic aperture magnetometry; SAM). Sources were localized to a 1s window from target word onset. Following co-registration of sensor positions to each participant’s canonical brain image, activation maps were constructed without prior constraints on source localizations, and statistical maps of group activity were computed. Power estimates in the gamma frequency were calculated separately for self-life and self-death pairings. A linear mixed-effects model implemented in AFNI was used to evaluate main effects of group (HR, moderate risk (MoR), low risk (LR), MinR) and condition (self-life, self-death), as well as their interactions. The computational resources of the NIH HPC Biowulf cluster were used for these analyses ([http://hpc.nih.gov](http://hpc.nih.gov/)).

*Dynamic Causal Modeling (DCM) Analysis*

A conductance-based neural mass model for DCM was used to estimate electrophysiological connectivity parameters based on the regions of interest identified in the source localization analysis. The canonical microcircuit (CMC) model implemented in SPM12 was used to model event-related potential (ERP) responses between these regions as previously described (see Gilbert & Moran, 2016). As shown in **Supplemental Figure S3A**, the CMC model includes excitatory and inhibitory connection parameters from four distinct cell layers: superficial and deep pyramidal cells (SPs/DPs), inhibitory interneurons (IIs), and spiny stellates (SSs). SPs carry feedforward connections to SSs and DPs, whereas DPs carry feedback connections to SPs and IIs. A Gaussian bump function was used in the analysis to model thalamic (stimulus-bound) input to the early visual cortex (EV).

 Four regions were selected to model forward and backward connections in the network: the EV (Talairach coordinates: -12, -97, -13), the left posterior cingulate cortex (PCC) (-14, -59, 15), the right insula (33, 3, 2), and the right orbitofrontal cortex (OBF) (26, 50, -8). These candidate regions of interest were selected based on our source-level analyses. As shown in **Supplemental Figure S3B**, two plausible models were constructed to account for connectivity between these regions of interest. In Model 1, forward connections carried signals from the EV to the PCC and insula, as well as from the PCC and insula to the OBF. Recurrent backward connections ensured reciprocal connectivity between these regions. Model 2 included the same architecture with the addition of lateral connections between the PCC and insula. Modulations by trial type (i.e., self-life, self-death) were examined on all region-to-region connections.

The negative free energy bound on the log-model evidence was used to adjudicate between the two model architectures, and the one with the highest log-model evidence was chosen for subsequent analyses. The model architecture **(Supplemental Fig. S3B)** was fitted to all participants, and parameter estimates were extracted to compare region-to-region connectivity and trial modulations on these connections.

*Data Analysis*

 Clinical measures and Life-Death Implicit Association Task (LD-IAT) D-scores were analyzed using one-way ANOVAs to examine group differences. Paired sample *t-*tests were used for the pilot study to compare treatment effects from baseline. As secondary analyses, D-scores for each group were compared to zero using a one-sample Wilcoxon signed-rank tests, and chi-square (χ2)tests were used to determine whether groups differed on the proportion of D-scores that were either positive (i.e., greater than zero, denoting a self-death bias) or negative (i.e., less than zero, denoting a self-life bias).

Due to the small sample size in the ketamine pilot study, Bayesian data analyses were computed (JASP v.0.17.3) (University of Amsterdam, The Netherlands: www.jasp-stats.org) as a secondary analysis to compare the effects of ketamine on insular gamma power. Bayes Factor (BF) analysis evaluates the extent to which one of the two hypotheses (null versus alternative) is better supported by the data. Given the absence of prior studies evaluating these parameters, we used a default Cauchy prior distribution with the effect size (δ) located at 0 and the width parameter (ω) set at 0.707. The BFs were interpreted based on convention (Lee & Wagenmakers, 2014): (1) evidence in favor of the alternative hypothesis (BF > 3), (2) inconclusive results (0.33 < BF < 3), and (3) evidence in favor of the null hypothesis (BF < 0.33).

For each group, Pearson correlations were used to examine whether LD-IAT D-scores were associated with (1) each clinical measure and (2) gamma-band activity in regions that yielded significant effects in the source-level analyses. For all analyses, pairwise comparisons were reported for significant interactions using Fisher’s least significant difference (LSD) test. Greenhouse Geisser corrections were used for violations of sphericity.

For MEG source-level analyses, a mixed-effects ANOVA was used to define source-localized cortical regions that showed a main effect of group (HR, MoR, LR, MinR) or condition (self-life, self-death), as well as group-by-condition interactions. Self-life and self-death trials were contrasted using a liberal criterion (p<0.05, uncorrected) in order to generate candidate regions of interest for the DCM analysis.

For the DCM parameters, Parametric Empirical Bayesian (PEB) analysis was used to identify the mixing of parameters contributing to both the average effect across all participants, as well as the difference between the HR group and the average effect across participants. Meaningful parameters were defined as those with a posterior probability (Pp) greater than 0.95. We specifically tested region-to-region connectivity parameters and their modulation by trial type.

**Supplemental Results**

 *LD-IAT D-score*

As a secondary analysis, group differences in the proportion of individuals with D-scores greater than zero (denoting a self-death bias) were examined. A chi-squared test revealed significantly different proportions of positive D-scores between groups [χ2(3, N=71)=11.44, *p*<0.01]. Seven scores in the HR group were greater than zero (7/15; 46.7%), which was twice the proportion of MinR scores (5/21; 23.8%) and approximately eight times the proportion of MoR (1/17; 5.9%) and LR (1/18; 5.6%) scores greater than zero. These analyses corroborate the finding that more participants in the HR group demonstrated a self-death bias compared to individuals in the MoR, LR, and MinR groups.

*Group differences in gamma power for self-life and self-death trials*

As a secondary analysis, group differences in the proportion of individuals with higher self-death versus self-life gamma power in each region were examined. A significant chi-square was identified within the OBF [χ2(3, N=71)=12.71, *p*<0.01], revealing a larger proportion of participants in the HR group (87%) with higher self-death compared to self-life gamma power than the MinR group (53%). These proportions were not different between the HR and MoR (61%) groups or the MinR and LR (26%) groups, further highlighting a specific contrast between HR and MinR participants with respect to OBF gamma power for self-death trials.

*Association between LD-IAT D-scores and clinical measures/gamma power*

Across the full sample, D-scores positively correlated with Beck Depression Inventory-II (BDI-II) (*r*=0.25, *p*<0.05) and Beck Hopelessness Scale (BHS) (*r*=0.27, *p*<0.05) scores, such that higher severity of depressive symptoms and hopelessness was associated with a greater self-death bias. Although non-significant, positive correlations were also observed between D-scores and Montgomery-Asberg Depression Rating Scale (MADRS), Snaith-Hamilton Pleasure Scale (SHAPS), and Scale for Suicide Ideation (SSI) scores (*p*s>0.11). For the MoR and LR groups, D-scores were positively associated with BDI-II (*r*=0.53, *p*<0.05) and past SSI scores (*r*=0.54, *p*<0.05), respectively. Contrary to expectations, D-scores inversely correlated with both SHAPS and Barratt Impulsiveness Scale (BIS) scores for the MinR group (*p*s<0.05), such that higher anhedonia and impulsivity were associated with a greater self-life bias.

 Across brain regions, no significant correlations were observed in the full sample (*p*s>0.29). For the HR group, significant inverse correlations were observed between D-scores and gamma power in the OBF (*r*s>-0.50, *p*s<0.05), as well as non-significant inverse correlations in the PCC and insula (*p*s>0.11). This suggests that a greater self-death bias was associated with lower gamma power in these regions. The opposite pattern was observed in the MinR group, which showed significant *positive* correlations between D-scores and gamma power in the OBF (*r*s>0.53, *p*s<0.03) and insula (self-death trials only; *r*=0.65, *p*<0.01), as well as non-significant positive correlations in the PCC (*p*s>0.49). There were no significant correlations in the MoR or LR groups (*p*s>0.18).

*Ketamine’s effects on clinical measures and LD-IAT D-score*

 Ketamine administration did not alter LD-IAT D-scores compared to baseline (**Supplemental** **Table S4**); however, ketamine significantly decreased BDI-II and BHS scores relative to baseline (*p*s<0.05). Although ketamine decreased MADRS, SHAPS, and SSI scores relative to baseline, these effects did not reach statistical significance (*p*s>0.06). Notably, the Cohen’s *d* effect sizes were large for the SHAPS and SSI effects (*d*s>0.98).

An inverse correlation emerged between post-ketamine D-scores and self-death gamma power in the left insula (*r*=-0.89, *p*<0.05), such that lower D-scores (denoting a self-life bias) were associated with higher gamma power in this region (**Supplemental Table S5)**.

At baseline, higher MADRS scores were associated with lower gamma power in the right insula (*r*=-0.89, *p*=0.04). The same relationship was identified after ketamine administration (*r*=-0.93, *p*=0.02). Interestingly, baseline BDI-II scores correlated positively with post-ketamine left insular gamma power for self-life (*r*=0.94, *p*=0.02) and self-death (*r*=0.90, *p*=0.04) trials, perhaps suggesting that ketamine has more pronounced effects on insular gamma power in those with greater severity of depressive symptoms. No other effects emerged between clinical measures and D-scores (*p*s>0.08) or gamma power (*p*s>0.14) before and after ketamine administration.

**Supplemental References**

Ballard, E. D., Waldman, L., Yarrington, J. S., Gerlus, N., Newman, L. E., Lee, L., ... & Zarate Jr, C. A. (2020). Neurobiological research with suicidal participants: A framework for investigators. *General Hospital Psychiatry*, *62*, 43-48. doi: <https://doi.org/10.1016/j.genhosppsych.2019.11.007>

Gilbert, J. R., & Moran, R. J. (2016). Inputs to prefrontal cortex support visual recognition in the aging brain. *Scientific Reports*, *6*(1), 1-9. doi: https://doi.org/10.1038/srep31943

Lee, M. D., & Wagenmakers, E. J. (2014). *Bayesian Cognitive Modeling: A Practical Course*. Cambridge: Cambridge University Press.

**Supplemental Figure Legends**

**Supplemental Figure S1.** The Life-Death Implicit Association Task (LD-IAT). During the four training blocks (top panel), participants are shown single-category stimuli and asked to indicate whether the target word was associated with life or death (half of trials) or self (“me”) or other (“not me”) (half of trials). During the four critical blocks (bottom panel), participants are asked to categorize life/death with self/other. Target stimuli were presented for 1.5s with an intertrial interval of 1.5-2.5s.

**Supplemental Figure S2.** Main effect of self-life/death word pairings and gamma power. Across the entire sample, enhanced gamma power for self-death compared to self-life conditions was identified within the posterior cingulate cortex (PCC).

**Supplemental Figure S3.** Dynamic causal modeling (DCM) for the Life-Death Implicit Association Task (LD-IAT). **(A)** Canonical microcircuit (CMC) model. The CMC model includes excitatory and inhibitory connection parameters from four distinct cell layers: superficial and deep pyramidal cells (SPs/DPs), inhibitory interneurons (IIs), and spiny stellates (SSs). SPs carry feedforward prediction errors to SSs and DPs, whereas DPs carry feedback predictions to SPs and IIs (image adapted from Gilbert & Moran, 2016). **(B)** Model architecture. Two plausible models were constructed to account for interconnections between early visual cortex (EV), posterior cingulate cortex (PCC), insula (INS), and orbitofrontal cortex (OBF). **(C)** Bayesian model selection. Among the two models, results from Bayesian model selection showed strongest evidence for Model 2, which consisted of fully reciprocated forward and backward connections between the EV and PCC, PCC and OBF, OBF and INS, INS and EV, as well as lateral connections between the PCC and INS.

**Supplemental Table S1.** **Dynamic Causal Modeling (DCM) parameters mediating region-to-region connectivity and the difference between trial types on the Life-Death Implicit Association Task (LD-IAT).**

|  |  |  |
| --- | --- | --- |
| **Parameter** | **Parameter Estimate****(Ep)** | **Posterior Probability****(Pp)** |
| **Region-to-region Connectivity** |   |   |
| 1. Forward Connection (SP to SS) – EV to PCC  | -0.598 | 1 |
| 2. Forward Connection (SP to DP) – EV to PCC  | -0.3919 | 1 |
| 3. Forward Connection (SP to DP) – PCC to INS  | -0.1988 | 0.529 |
| 4. Backward Connection (DP to II) – PCC to EV  | 0.1705 | 0.502 |
| 5. Backward Connection (DP to II) – INS to PCC  | -0.1773 | 0.520 |
| **Trial Modulation** |  |  |
| 1. Forward Connections – EV to INS  | 0.7658 | 1 |
| 2. Forward Connections – PCC to INS  | -0.3059 | 0.536 |
| 3. Backward Connections – OBF to PCC  | -0.5604 | 1 |

Parametric empirical Bayesian analysis was used to identify the mixing of parameters contributing to both region-to-region connectivity and the difference between trial types (self-life versus self-death) in the LD-IAT. All effects compare the high risk (HR) group with the average across all groups. Meaningful parameters were defined as those with a posterior probability (Pp) greater than 0.95. Across trial types, significant effects were found on the forward connections between the early visual cortex (EV) and posterior cingulate cortex (PCC) carried by superficial pyramidal cells (SPs) to both spiny stellates (SSs) and deep pyramidal cells (DPs). Significant trial modulations were also found on the forward connections from the EV to the insula (INS) and backward connections from the orbitofrontal cortex (OBF) to the PCC. II: inhibitory interneurons.

**Supplemental Table S2. Bivariate correlations between Life-Death Implicit Association Task (LD-IAT) D-score and clinical measures.**

|  |  |
| --- | --- |
|  | **D-Score** |
|  | HighRisk | Moderate Risk | LowRisk | Minimal Risk | Total(Full Sample) |
| BDI-II | .10 | **.53\*** | .19 | -.05 | **.25\*** |
| MADRS | .17 | .42 | -.03 | -.30 | .21 |
| HAM-A | .23 | .18 | -.20 | -.31 | .06 |
| BHS | -.30 | .39 | .18 | .12 | **.27\*** |
| BIS | -.24 | -.16 | .06 | **-.71\*** | -.17 |
| SHAPS | .15 | .25 | .43 | **-.78\*\*** | .17 |
| SSI Current | -.14 | .16 | .11 | N/A | .17 |
| SSI Past | .01 | .01 | **.54\*** | N/A | .17 |

Asterisks denote statistical significance, (\*) p<0.05, (\*\*) p<0.01. Values reflect bivariate correlations between the life-death implicit association task (LD-IAT) D-score and total scores on the following clinical measures: Beck Depression Inventory-II (BDI-II); Montgomery-Åsberg Depression Rating Scale (MADRS); Hamilton Anxiety Rating Scale (HAM-A); Beck Hopelessness Scale (BHS); Barratt Impulsiveness Scale (BIS); Snaith-Hamilton Pleasure Scale (SHAPS); Scale for Suicide Ideation (SSI).

**Supplemental Table S3. Bivariate correlations between LD-IAT D-score and gamma power.**

|  |  |
| --- | --- |
|  | **D-Score** |
|  | HighRisk | Moderate Risk | LowRisk | MinimalRisk | Total(Full Sample) |
| **PCC** |  |  |  |  |  |
|  Self-life Self-death | -.24-.23 | -.14-.24 | -.25-.28 | .07.18 | -.12-.11 |
| **Insula** |  |  |  |  |  |
|  Self-life Self-death | -.43-.29 | -.12-.16 | .27.26 | .43**.65\*\*** | .04.13 |
| **OBF** |  |  |  |  |  |
|  Self-life Self-death | **-.61\*\*****-.50\*** | -.23-.34 | .10.06 | **.53\*****.63\*\*** | -.07-.04 |

Asterisks denote statistical significance, (\*) p<0.05, (\*\*) p<0.01. Values reflect bivariate correlations between the Life-Death Implicit Association Task (LD-IAT) D-score and gamma power estimates for self-life and self-death trials in the posterior cingulate cortex (PCC), right insula, and orbitofrontal cortex (OBF).

**Supplemental Table S4.** **Effects of ketamine on LD-IAT D-score and clinical measures.**

|  |  |
| --- | --- |
|  |  |
|  | Delta Score(𝚫Post - Pre) | *t* value | *p* value | Cohen’s *d* |
| D-Score | 0.08(+0.12) | 0.62 | 0.57 | 0.28 |
| BDI-II | **-14.67\* (+4.33)** | **-3.39** | **0.02** | **1.38#** |
| MADRS | -3.67(+7.74) | -0.47 | 0.66 | 0.19 |
| BHS | **-6.00\*****(+1.98)** | **-3.03** | **0.03** | **1.24#** |
| SHAPS | -7.67(+3.19) | -2.40 | 0.06 | 0.98# |
| SSI Current | -1.40(+0.60) | -2.33 | 0.08 | 1.04# |

Delta scores reflect the mean (+SEM) difference between baseline and post-ketamine scores on the Life-Death Implicit Association Task (LD-IAT) D-score and clinical measures. (\*) denotes statistical significance, p<0.05. (#) denotes large effect sizes. BDI-II: Beck Depression Inventory-II; MADRS: Montgomery-Åsberg Depression Rating Scale; BHS: Beck Hopelessness Scale; SHAPS: Snaith-Hamilton Pleasure Scale; SSI: Scale for Suicide Ideation.

**Supplemental Table S5.** **Bivariate correlations between gamma power and LD-IAT D-score.**

|  |  |
| --- | --- |
|  |  |
|  | BaselineD-Score | Post-Ketamine D-score |
| **Left Insula** |  |  |
|  Self-life Self-death | -.20-.58 | -.78**-.89\*** |
| **Right Insula** |  |  |
|  Self-life Self-death | .19-.14 | -.55-.10 |

Asterisks denote statistical significance, (\*) p<0.05. Values reflect bivariate correlations between Life-Death Implicit Association Task (LD-IAT) D-score and gamma power estimates for self-life and self-death trials in the left and right insula.