Biological Psychiatry: CNNI

## Reply to: Two Methodologies in "Amygdala Activation and Connectivity to Emotional Processing Distinguishes Asymptomatic Patients With Bipolar Disorders and Unipolar Depression" That Can Produce False-Positive Results and Some Statistical Recommendations

### To the Editor:

Vandekar (1) has criticized the use of two methodological procedures that have been prone to high false-positive rates and producing biased results in our recent publication (2). As Vandekar has pointed out, there has been considerable debate on the possible inflation of type 1 errors resulting from use of these approaches (3–5). The recommendations to avoid these approaches are commendable. While we acknowledge the general limitations of the two procedures, we outline below the extent of their impact on the findings and interpretations of our study.

1. Gaussian random field–based spatial extent inference: We used a liberal cluster-forming threshold ($p < .05$), which has been regarded to inflate type 1 error rates (3,4). It is important to note that our study was hypothesis driven and designed based on strong previous evidence that has consistently shown the emotional reactivity of the amygdala to be differential in bipolar versus unipolar depression (6). In our study, we evaluated if this difference in amygdala reactivity persists beyond current acute mood states by testing patients in remission. We re-evaluated our data using a more conservative voxel-level familywise error correction as implemented in SPM8. All our activation findings and some of the key connectivity findings remained significant at familywise error corrected $p < .05$ (Tables 1 and 2). The results, particularly for connectivity during the supraliminal condition, that failed to reach significance should be interpreted with caution. However, as noted in our article, the overlap with previous evidence provides some confidence and suggests a likely power issue in seeing significance at the set familywise error threshold. We had acknowledged the limitation of the available sample size and our use of a liberal cluster-corrected statistical threshold in our article.
2. Circularity errors owing to subsequent testing of significant variables in a prediction analysis: We first tested for the significant activation and connectivity variables distinguishing the two disorders in a logistic regression analysis to construct a model of the most contributing features. We then tested for generalization of this model using a leave-one-out cross-validation procedure using the same dataset. Classification accuracy estimates from cross-validation procedures done using the same cohort are bound to be inflated compared with estimates obtained from using an independent cohort (5), possibly also contributed by the heterogeneity of patients within disorders. We acknowledge the pitfall of this approach to estimate clinical diagnostic value and recognize the importance of needing replication in an independent cohort—both points were recognized as limitations in our study limitations. Although our approach may not be an adequate test of generalizability of the classification model, it does inform the most promising features that could potentially classify the two disorders and remains to be validated in an independent sample.

While the criticism by Vandekar is valid regarding the possibly inflated type 1 error rates of these approaches in general, these need to be considered in the context of the study, especially when there is a strong hypothesis based on previous evidence. Whereas not all of our study results

**Table 1. Differences in Amygdala Activation for Both Supraliminal and Subliminal Emotional Face Processing for Euthymic Bipolar Disorder and Remitted Major Depressive Disorder Groups With Familywise Error Corrected *p* Values Included**

| Emotion | Direction | Region of Increased Activation | MNI Space | | | Cluster Size | *z* Score | *p* Value | $p_{FWE}$ Value |
|---|---|---|---|---|---|---|---|---|---|
| | | | X | Y | Z | | | | |
| Supraliminal | | | | | | | | | |
| All emotions | Main effect (BP < MDD) | L amygdala | −24 | −2 | −28 | 31 | 2.81 | .003 | .035 |
| Threat | Main effect (BP < MDD) | L amygdala | −24 | 0 | −28 | 32 | 2.67 | .004 | .05 |
| Sad | BP < MDD | L amygdala | −24 | 0 | −28 | 34 | 2.73 | .003 | .048 |
| Happy | BP vs. MDD | NS | NS | NS | NS | NS | NS | NS | NS |
| Neutral | BP < MDD | L amygdala | −24 | 0 | −28 | 50 | 3.02 | .001 | .022 |
| Subliminal | | | | | | | | | |
| All emotions | Main effect (BP < MDD) | L amygdala | −22 | −6 | −18 | 193 | 3.74 | < .001 | .002 |
| Threat | Main effect (BP < MDD) | L amygdala | −22 | −4 | −18 | 197 | 4.08 | < .001 | .001 |
| Sad | BP < MDD | L amygdala | −20 | −4 | −18 | 196 | 3.51 | < .001 | .005 |
| Happy | BP < MDD | L amygdala | −22 | −4 | −18 | 163 | 3.23 | .001 | .013 |
| Neutral | BP < MDD | L amygdala | −22 | −4 | −18 | 160 | 3.13 | .001 | .017 |

Table is updated from Table 2 in Korgaonkar *et al.* (2).
BP, bipolar disorder; FWE, familywise error; L, left; MDD, major depressive disorder; MNI, Montreal Neurological Institute; NS, not significant.

https://doi.org/10.1016/j.bpsc.2018.12.006
ISSN: 2451-9022

**Table 2. Differences in Left Amygdala Connectivity in Negative and Positive Affect Networks for Both Supraliminal and Subliminal Emotional Face Processing for Euthymic Bipolar Disorder and Remitted Major Depressive Disorder Groups With Familywise Error Corrected *p* Values Included**

| Emotion | Direction | Region | MNI Space | | | Cluster Size | *z* Score | *p* Value | $p_{FWE}$ Value |
|---|---|---|---|---|---|---|---|---|---|
| | | | X | Y | Z | | | | |
| Supraliminal | | | | | | | | | |
| Negative Affect Network | | | | | | | | | |
| Negative affect | Main effect (BP > MDD) | sgACC | 4 | 26 | −14 | 81 | 2.33 | .01 | .112 |
| | Main effect (BP > MDD) | R amygdala | 28 | 0 | −18 | 137 | 2.78 | .003 | .045 |
| | Main effect (BP < MDD) | R hippocampus | 30 | −24 | −8 | 174 | 2.26 | .012 | .373 |
| | Interaction | L insula | −36 | −2 | 4 | 158 | 2.27 | .012 | .518 |
| Threat | Main effect (BP < MDD) | R hippocampus | 34 | −24 | −8 | 95 | 2.35 | .009 | .323 |
| | Interaction | L insula | −32 | 16 | −18 | 75 | 2.41 | .008 | .426 |
| Sad | BP > MDD | R insula | 44 | 22 | 0 | 369 | 2.63 | .004 | .287 |
| | BP < MDD | L hippocampus | −30 | −20 | −14 | 150 | 2.68 | .004 | .171 |
| | BP < MDD | R hippocampus | 24 | −16 | −12 | 114 | 2.28 | .011 | .366 |
| Neutral | BP < MDD | R amygdala | 28 | −2 | −18 | 194 | 3.26 | .001 | .013 |
| | BP < MDD | R hippocampus | 28 | −6 | −18 | 223 | 2.77 | .003 | .149 |
| Positive Affect Network | | | | | | | | | |
| Happy | BP < MDD | mOFC | 0 | 56 | −2 | 256 | 2.86 | .002 | .083 |
| | BP < MDD | L caudate | −12 | 4 | 20 | 119 | 2.64 | .004 | .171 |
| | BP < MDD | L putamen | −26 | −6 | −8 | 110 | 2.66 | .005 | .415 |
| Subliminal | | | | | | | | | |
| Negative Affect Network | | | | | | | | | |
| Negative affect | Main effect (BP < MDD) | pgACC | −2 | 48 | 4 | 241 | 2.72 | .003 | .048 |
| | Main effect (BP < MDD) | sgACC | 2 | 24 | −8 | 90 | 1.95 | .026 | .223 |
| | Main effect (BP < MDD) | L insula | −38 | 20 | 2 | 302 | 2.15 | .016 | .605 |
| | Main effect (BP < MDD) | R insula | 40 | 10 | 6 | 77 | 2.2 | .014 | .557 |
| | Main effect (BP < MDD) | R amygdala | 26 | 0 | −16 | 95 | 2.07 | .019 | .206 |
| | Main effect (BP < MDD) | R hippocampus | 38 | −30 | −8 | 305 | 2.97 | .002 | .088 |
| | Interaction | pgACC | 0 | 48 | 8 | 138 | 2.53 | .006 | .074 |
| | Interaction | L insula | −36 | 4 | −6 | 879 | 3.44 | < .001 | .038 |
| | Interaction | R insula | 36 | 14 | −6 | 872 | 3.92 | < .001 | .008 |
| | Interaction | R hippocampus | 32 | −20 | −8 | 184 | 3.44 | < .001 | .023 |
| Threat | Main effect (BP < MDD) | L insula | −38 | 18 | 2 | 690 | 2.99 | .001 | .138 |
| | Main effect (BP < MDD) | R insula | 40 | 10 | 6 | 652 | 3.28 | .001 | .061 |
| | Main effect (BP < MDD) | R hippocampus | 28 | −20 | −10 | 435 | 3.2 | .001 | .048 |
| | Interaction | L insula | −38 | −4 | −2 | 519 | 2.8 | .003 | .212 |
| | Interaction | R insula | 34 | 18 | −6 | 244 | 2.93 | .002 | .175 |
| Sad | BP > MDD | L insula | −30 | 10 | −20 | 80 | 3.23 | .001 | .123 |
| | BP < MDD | R amygdala | 28 | −2 | −22 | 92 | 2.9 | .002 | .035 |
| Neutral | BP < MDD | R hippocampus | 22 | −36 | 4 | 129 | 2.44 | .007 | .279 |
| Positive Affect Network | | | | | | | | | |
| Happy | BP < MDD | mOFC | 2 | 48 | 0 | 82 | 2.16 | .015 | .316 |

Table is updated from Table 3 in Korgaonkar *et al.* (2).

BP, bipolar disorder; FWE, familywise error; L, left; MDD, major depressive disorder; MNI, Montreal Neurological Institute; mOFC, medial orbitofrontal cortex; NS, not significant; R, right; pgACC, pregenual anterior cingulate cortex; sgACC, subgenual anterior cingulate cortex.

survived using a more stringent control of type 1 error rate, the use of the two methodological approaches does not impact the overall study interpretation. Our results are also largely consistent with previous observations comparing these disorders.

The overemphasis of the field to accept only results that meet a set statistical threshold means unfortunately that several potentially interesting trends are being missed. This issue is especially critical in neuroimaging studies, which necessitates stringent criteria to control for type 1 error rates owing to the inherent nature of several data points (or voxels) in imaging datasets. This is further exacerbated for studies that are generally low powered owing to difficulties in recruitment and data collection of specific patient

Biological Psychiatry: CNNI

cohorts. In these cases, consistency of findings (despite being low powered to meet set stringent statistical criteria) across studies provides some level of confidence, and such findings are a useful contribution in building the overall evidence base.

Mayuresh S. Korgaonkar

## Acknowledgments and Disclosures

The author reports no biomedical financial interests or potential conflicts of interest.

## Article Information

From the Brain Dynamics Centre, Westmead Institute for Medical Research, The University of Sydney, Westmead; and Discipline of Psychiatry, University of Sydney School of Medicine, Sydney, New South Wales, Australia.

Address correspondence to Mayuresh S. Korgaonkar, Ph.D., Brain Dynamics Centre, Westmead Institute for Medical Research, 176 Hawkesbury Road, Westmead, NSW 2145, Australia; E-mail: M.Korgaonkar@sydney.edu.au.

See also associated correspondence: https://doi.org/10.1016/j.bpsc.2018.12.007.

Received Dec 6, 2018; accepted Dec 7, 2018.

## References

1. Vandekar SN (2019): Two methodologies in "Amygdala activation and connectivity to emotional processing distinguishes asymptomatic patients with bipolar disorders and unipolar depression" that can produce false-positive results and some statistical recommendations. Biol Psychiatry Cogn Neurosci Neuroimaging 4:409–410.
2. Korgaonkar MS, Erlinger M, Breukelaar IA, Boyce P, Hazell P, Antees C, *et al.* (2019): Amygdala activation and connectivity to emotional processing distinguishes asymptomatic patients with bipolar disorders and unipolar depression. Biol Psychiatry Cogn Neurosci Neuroimaging 4:361–370.
3. Eklund A, Nichols TE, Knutsson H (2016): Cluster failure: Why fMRI inferences for spatial extent have inflated false-positive rates. Proc Natl Acad Sci U S A 113:7900–7905.
4. Cox RW, Chen G, Glen DR, Reynolds RC, Taylor PA (2017): fMRI clustering and false-positive rates. Proc Natl Acad Sci U S A 114:E3370–E3371.
5. Pulini AA, Kerr WT, Loo SK, Lenartowicz A (2019): Classification accuracy of neuroimaging biomarkers in attention-deficit/hyperactivity disorder: Effects of sample size and circular analysis. Biol Psychiatry Cogn Neurosci Neuroimaging 4:108–120.
6. Cardoso de Almeida JR, Phillips ML (2013): Distinguishing between unipolar depression and bipolar depression: Current and future clinical and neuroimaging perspectives. Biol Psychiatry 73:111–118.