

Reply to Gregory Francis

Brian G. Dias^{1,2} and Kerry J. Ressler^{1,2,3}

¹Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, Atlanta, GA 30329

²Center for Behavioral Neuroscience, Yerkes National Primate Research Center, Atlanta, GA 30329

³Howard Hughes Medical Institute, Chevy Chase, MD 20815

WE thank Gregory Francis for his careful reading of our article and associated commentary. We too remain amazed at this phenomenon, and we agree that much remains to be done to understand the underlying mechanisms. However, we have now replicated these effects multiple times within our laboratory with multiple colleagues as blinded scorers, and we fully stand by our initial observations. Here we focus on addressing some of the broader implications of his letter.

1. The principal assertion made by Dr. Francis for his analyses are that “the reported experiments were uniformly successful” in our reported studies. This is a false statement. While we wish that all our behavioral, neuroanatomical, and epigenetic data were successful and statistically significant, one only need look at the Supporting Information in the article to see that data generated for all four figures in the Supporting Information did not yield significant results. We do not believe that these nonsignificant data support our theoretical claims as is suggested. If that were the case, there ought to be have been correspondence between our DNA methylation data in the sperm (Figure 6), and Main Olfactory Epithelium (Figure S6). In addition, we do not observe differences among groups in levels of histone modifications around the M71 gene in sperm (Figure S7). These multiple cases of nonsignificant data were clearly reported by us within the primary paper and the supporting information. Therefore, we strongly reject the assertion that we only presented data that confirmed the hypotheses. We are actively searching for mechanisms that support these robust findings.
2. We opine that the real story and mechanism is likely to be found probing concepts like penetrance. For it is most likely that epigenetic mechanisms might not affect all the

germ cells, and understanding the spread of data will give us a more nuanced view of the mechanism.

3. We wholeheartedly disagree with the shadow that Francis and the accompanying commentary casts on our experimental design and data analysis. All experiments conducted were reported in the article, which means that no experimental data were excluded. When one conducts transgenerational studies that are dependent on the vagaries of breeding and husbandry, one uses all that are given and is never wasteful. All experiments were run blind by the experimenter, and data were analyzed in a double-blind fashion as we have emphasized in the article. The one point of agreement between Francis and us is the need for higher sample sizes, and this is something that we would like to address in subsequent work. This said, it must be emphasized that our sample sizes are consistent with what animal behaviorists use.
4. It is also asserted by Dr. Francis that perhaps the manuscript did not undergo rigorous peer review. The manuscript in fact went through numerous rounds of rigorous peer-review at *Nature Neuroscience*, with at least 3 anonymous and critical external reviewing scientists, along with substantial editorial review. The review process resulted in multiple additional experiments and analyses, some positive and some negative, which we believe improved the manuscript.

In summary, while we appreciate some of Francis’ concerns, we stand by our results as robust, reproducible, and verified by blinded assessment. We believe our findings withstand the test of ‘extraordinary evidence’. Science is built on the synergy between findings from independent research groups. We wholeheartedly welcome this process, and are excited about the direction of this research.