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Authors' Response

Sir,

We thank Dr. Mash for her interest in our findings regarding cocaine abuse and heat shock protein 70 (HSP70) gene expression, which were recently published in this journal (1). In her lengthy Commentary, Dr. Mash reviewed the literature on excited delirium (ED) syndrome, cocaine effects on cerebral vasculature, terminal hypoxia, data normalization and outlier determination, and the intricacies of quantitative PCR. We limit our response to a few issues meriting comment.

To place our findings in context, Mash et al. previously reported (2) that HSP70 gene expression was elevated in the postmortem brains of cocaine abusers who had exhibited ED prior to death, relative to other (non-ED) cocaine fatalities and control subjects. In the course of our recent postmortem studies to determine the global profile of gene expression associated with human cocaine abuse, we found that the abundance of two HSP70-related gene transcripts was, in fact, significantly increased in our cocaine abuse cohort *as a group* compared with well-matched drug-free control subjects (1); thus, our data are not in agreement with this earlier publication. We subsequently sought to identify variables that might account for this unexpected discrepancy, as well as the variance in HSP70 transcript abundance observed within our cocaine abuse cohort. We determined that elevated HSP70 expression was not correlated with cocaine or metabolite levels, but *was* associated with a documented period of survival and contact with medical personnel and/or police between last cocaine use and death. In fact, HSP70 transcript abundance was statistically predictive as a diagnostic test for such a survival period (1). Behavioral evidence of ED syndrome was clearly observed in two of our cocaine fatalities, but these cases did *not* exhibit significantly increased HSP70 expression (conversely, the subjects with the highest HSP70 expression were *not* reported to exhibit ED prior to death).

In her Commentary, Dr. Mash questioned the determination of ED syndrome in two of our cocaine subjects. As discussed in our study (1), the final classification of cocaine-related fatalities was made after review of case files (police reports, medical files, toxicology reports, and autopsy results) by a trained forensic pathologist working in a jurisdiction with a large number of drug-related deaths and well versed in the nuances associated making the determination of cocaine intoxication/abuse and ED. Some additional details regarding these ED cases were provided during the peer-review process, but excluded from the final manuscript precisely so as to avoid undue emphasis on ED—as clearly discussed (1), our conclusions about ED are tempered by our very limited sample. Like the majority of ED cases previously studied by Mash et al. (2), our two cases did not have ED listed as the cause of death.

Other criticisms raised regarding subject postdrug survival time and possible cocaine-induced seizures are surprising, given that the Mash et al. publication featured ED subjects with survival times of up to 2 days (with over one half of ED subjects surviving at least 1 h postintervention) and a reported 13% incidence of seizures prior to death (2). In our study, measures of tissue sample quality and RNA quality for the high HSP70 subjects (pH 6.5 ± 0.2 and RNA integrity number 6.1 ± 0.4, respectively) did not differ from the other subjects with lower HSP70 values (1). Furthermore, unbiased profile-wide hierarchical clustering of microarray data did not identify the cases with high HSP70 levels as outliers (data not shown).

Putting aside these distractors, the fundamental point of our study (1) is the finding that HSP70 gene expression was increased in an important subset of cocaine-related fatalities *independent* of ED, in contrast to the conclusion of Mash et al. that increased HSP70 expression is seen only in ED subjects but not other cocaine fatalities (2). Those authors concluded that HSP70 "affords an objective measure to assess ED at autopsy" and (along with antemortem behavior and another protein marker) "has validity for use in assigning ED as a cause of death" (2, p. e18). The proposed use of HSP70 as a forensic tool should be considered in light of the well-established principles of validation of a diagnostic test: 1. Sensitivity—that is, the ability to obtain positive results (in this case, high HSP70) in essentially all ED cases. 2. Specificity—that is, the ability to obtain uniformly negative results (low HSP70) in non-ED cocaine fatalities and control subjects. 3. Selection of assay threshold—for a low prevalence syndrome such as ED, even a low rate of false positives could result in detection of more false positive cases than true ED cases. 4. Reproducibility across laboratories—to our knowledge, there is no independent replication of an ED-specific elevation in brain HSP70 expression. The discrepancies between our data (1) and the previous report (2) indicate that, at least at the present time, HSP70 determinations fall well short of validation as a diagnostic tool for ED.

In her Commentary, Dr. Mash calls for methodologically sound studies and a specimen repository to support research into ED. In fact, Dr. Mash currently serves as the Director of such a biorepository, which measures purported ED biomarkers such as HSP70 on a fee-for-service basis. Our group supports the continued search for, and appropriate validation of, diagnostic tests for ED and other drug-related fatalities.

References

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2. Mash DC, Duque L, Pablo J, Qin Y, Adi N, Hearn WL, et al. Brain biomarkers for identifying excited delirium as a cause of sudden death. Forensic Sci Int 2009;190(1–3):e13–9.

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