Reply to Deighton et al.: Neuronal activity regulates distinct antioxidant pathways in neurons and astrocytes

We thank Deighton et al. (1) for highlighting the importance of antioxidant pathway regulation in the brain, the topic of our recent study (2). In their letter, Deighton et al. show that expression of antioxidant genes Srxn1 and xCT [which can be regulated by nuclear factor erythroid 2-related factor 2 (Nrf2)] is increased by neuronal activity in cortical cultures that lack both Nrf2 and astrocytes (1). Although this finding is in agreement with their previous work, which showed that activity regulates neuronal Srxn1 expression through transcription factors ATF4 and AP-1, the authors raised a possibility that activity-induced increase in Gclc and Nqo1 expression in mixed hippocampal cultures (2) was also Nrf2- and astrocyte-independent. However, in contrast to Srxn1, xCT, and other genes that are regulated by multiple transcription factors, Nqo1 gene expression is controlled exclusively through Nrf2-antioxidant response element (ARE) interaction (3) [a possible regulation by AP-2 was reported in 1996 (4), but has not been confirmed since]. Consistent with this single mode of transcriptional regulation, both basal and tert-butylhydroquinone (tBHQ)-induced Nqo1 enzyme activities were absent in Nrf2−/− astrocyte cultures (5). Thus, activity-induced increase in the Nqo1 mRNA level observed in hippocampal cultures (2) can be considered reliable evidence of Nrf2 involvement. Moreover, this activity-induced and Nrf2-mediated increase in antioxidant gene expression is restricted to astrocytes: in brain slices from ARE-human placental alkaline phosphatase (hPAP) transgenic mice [in which the hPAP reporter gene is under control of the Nqo1-derived ARE promoter (6)], neuronal activity led to increase in the number of hPAP+ astrocytes, but not hPAP+ neurons (2).

In contrast to us (2), Deighton et al. did not observe a statistically significant increase in Nrf2 protein level following bicuculline/4-aminopyridine (Bic/4-AP) and high K+ treatments of mixed cortical cultures (1). We do not think that this discrepancy is a result of different antibodies used by the two groups: although the antibody we used does recognize several nonspecific bands, only the 84-kDa band (which was specifically attenuated by anti-Nrf2 siRNA transfection) demonstrated increase in response to Gab/4-AP and high K+ treatments of mixed cultures, as shown in ref. 2 and in Fig. 1. Similarly, it is unlikely that the discrepancy is a result of animal model differences: in our hands, activity-dependent up-regulation of astrocytic Nrf2 signaling was detectable both in rat hippocampal cultures and mouse cortical slices (2). Instead, the discrepant findings are most likely a result of a methodological difference: although we observed a significant increase in Nrf2 protein level only in nuclear fractions (2), Deighton et al. used whole-cell lysates (in which nuclear proteins are only a small part of the total) for their experiments (1). (Other methodological differences may have contributed, but are difficult to evaluate given the lack of experimental detail in the letter.)

Taken together, the findings suggest the existence of two distinct activity-mediated antioxidant pathways: in neurons, expression of one group of antioxidant genes is induced through activation of ATF4 and AP-1 (1), whereas in astrocytes, expression of another group of antioxidant genes is induced through activation of Nrf2 (2). As suggested by Deighton et al. (1), potential cooperation between these two pathways will be an important subject of future investigation.

ACKNOWLEDGMENTS. We thank Ms. Christine Lin for aid with the figure preparation. This work was supported by a University of California, San Francisco (UCSF) Program for Breakthrough Scientific Research start-up award, a UCSF Academic Senate start-up award, and National Institutes of Health Grants NS054113 and NS073765 (all to M.M.).

Agata Habas, Junghyun Hahn, Xianhong Wang, and Marta Margeta1
Department of Pathology, University of California, San Francisco, CA 94143

Author contributions: A.H., J.H., and M.M. designed research; A.H., J.H., and M.M. performed research; A.H., J.H., X.W., and M.M. analyzed data; and A.H., J.H., X.W., and M.M. wrote the paper.

The authors declare no conflict of interest.

1To whom correspondence should be addressed. E-mail: Marta.Margeta.ucsf.edu.

1Deighton RF, et al. (2014) Nrf2 target genes can be controlled by neuronal activity in the absence of Nrf2 and astrocytes. Proc Natl Acad Sci USA 111:E1818–E1820.

www.pnas.org/cgi/doi/10.1073/pnas.1403291111
Fig. 1. Summary graphs for nuclear fraction immunoblot densitometry from multiple repeat experiments are shown, with Nrf2 84-kDa band density normalized to lamin B band density for each sample; for drug dosage, treatment duration, and other experimental details, please see the original publication (2). Because of the narrow dynamic range of the chemiluminescence-based immunoblotting technique, the data were not normally distributed and were therefore analyzed with Kruskal–Wallis one-way ANOVA on ranks, followed by Dunn’s posttest to compare each treatment to vehicle control. Results are shown as box charts; the horizontal lines in the box denote 25th, 50th, and 75th percentile values, and error bars denote 10th and 90th percentile values ($n = 7–17; *P<0.05, **P<0.001; ns, not significant). Please note that the apparently larger effect of sulforaphane (SLF) treatment in the context of high K+ experiments (C and D) is primarily a reflection of differences in the vehicle Nrf2 level (used for normalization), which was lower in artificial cerebrospinal fluid than in culture medium used for Gab/4-AP experiments (A and B).