

Rapid Sensing of Dietary Amino Acid Deficiency Does Not Require GCN2

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Animals are unable to synthesize the nine essential amino acids (EAAs) and consequently must obtain them from their food. In 2005, two papers proposed an extraordinary mechanism for dietary amino-acid sensing (Hao et al., 2005; Maurin et al., 2005). According to these reports, the consumption of food lacking a single EAA leads to the development of an amino-acid imbalance in the anterior piriform cortex (APC) within minutes. This amino acid imbalance was proposed to be sensed in the APC by activation of the protein kinase GCN2, enabling animals to reject the EAA-deficient food within the first hour of feeding.

The idea that cortical neurons functioned as nutrient sensors to control feeding behavior was unprecedented, which prompted us to reinvestigate this phenomenon. However, we were unable to replicate any aspect of the proposed model (Leib and Knight, 2015). We found that mice were unable to sense deficiency of the EAAs threonine and leucine within the first 3 hr of feeding, that GCN2 was not activated in APC by EAA deficiency, and that GCN2 knockout mice were not impaired in any aspect of feeding behavior. We then went on to develop new feeding paradigms in which we could detect rapid sensing of dietary EAAs. Our results from these new assays reveal that the development of need states for specific EAAs plays an important role in dietary EAA sensing. However the mechanism for this need-dependent dietary EAA sensing remains unclear and does not require GCN2.

The authors of the original reports now reply (Gietzen et al., 2016) by contending that our inability to replicate their findings reflects differences in experimental protocols. They focus on two differences between our study design and

theirs: (1) the length of food deprivation prior to feeding experiments, and (2) the time points at which food intake was measured. However, as we explain below, neither of these explanations can account for the differences between our results.

Regarding the first difference, Gietzen et al. (2016) claim that their studies used a longer period of fasting prior to feeding than we did (16–21 hr versus 3 hr). This longer fast could potentially lead to more rapid food ingestion and greater EAA imbalance in the blood and thereby enhanced dietary EAA detection. In fact, we explicitly tested overnight fasting as a parameter in our feeding experiments for this reason (Leib and Knight, 2015), and we found that it did not enable rapid sensing of dietary EAAs or reveal any role for GCN2. In addition, Gietzen et al. (2016)'s argument is directly contradicted by several of the papers they cite, one of which reported rapid dietary EAA sensing following only a 3 hr fast (Koehnle et al., 2003), and another that makes no reference to fasting at all (Hao et al., 2005). We could not find any statement in these earlier reports indicating that fasting was required for this phenomenon. On the contrary, the authors previously argued the opposite: "We observed slight increases in the time it took to recognize amino acid-deficient diets when rats were deprived of food for long periods before testing" (Koehnle et al., 2004).

Second, Gietzen et al. (2016) claim that we measured food intake too long after providing the mice with food (3 hr). They argue that the GCN2-dependent effect is transient, appearing at 20–40 min and then disappearing shortly thereafter. In response to this, we make three points. (1) This claim directly contradicts their

own published observations of purported EAA sensing between 1 and 4 hr after food presentation (Maurin et al., 2005). (2) We chose a later time point in an effort to *enhance* our ability to detect dietary EAA sensing after we failed in pilot experiments to replicate their finding of an effect at earlier times (e.g., 0.13 ± 0.07 g control versus 0.19 ± 0.03 g threonine- and leucine-deficient food consumed after 1 hr, mean \pm SEM, $n = 9$). Indeed, the primary focus of our paper is a description of how we systematically and extensively varied the parameters of our feeding experiments in an attempt to find any evidence to support their previously reported model. (3) If the behavioral response is as ephemeral as Gietzen et al. (2016) suggest, then that itself raises the question of what physiologic significance this phenomenon has. As we state in the conclusion to our paper: "Whereas we cannot exclude the possibility that experimental conditions exist in which normal mice can rapidly identify and reject these diets, our data clearly show that this phenomenon is not nearly as robust or universal as is implied by the existing literature." In contrast, the GCN2-independent effects we reported are larger and more robust, and we believe they represent the major behavioral response.

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REFERENCES

- Gietzen, D.W., Anthony, T.G., Fafournoux, P., Maurin, A.M., Koehnle, T.J., and Hao, S. (2016). Measuring the ability of mice to sense dietary essential amino acid deficiency: the importance of amino acid status and timing. *Cell Rep.* *16*, this issue, 2049–2050.
- Hao, S., Sharp, J.W., Ross-Inta, C.M., McDaniel, B.J., Anthony, T.G., Wek, R.C., Cavener, D.R., McGrath, B.C., Rudell, J.B., Koehnle, T.J., and Gietzen, D.W. (2005). Uncharged tRNA and sensing of amino acid deficiency in mammalian piriform cortex. *Science* *307*, 1776–1778.
- Koehnle, T.J., Russell, M.C., and Gietzen, D.W. (2003). Rats rapidly reject diets deficient in essential amino acids. *J. Nutr.* *133*, 2331–2335.
- Koehnle, T.J., Russell, M.C., Morin, A.S., Erecius, L.F., and Gietzen, D.W. (2004). Diets deficient in indispensable amino acids rapidly decrease the concentration of the limiting amino acid in the anterior piriform cortex of rats. *J. Nutr.* *134*, 2365–2371.
- Leib, D.E., and Knight, Z.A. (2015). Re-examination of dietary amino acid sensing reveals a GCN2-independent mechanism. *Cell Rep.* *13*, 1081–1089.
- Maurin, A.C., Jousse, C., Averous, J., Parry, L., Bruhat, A., Cherasse, Y., Zeng, H., Zhang, Y., Harding, H.P., Ron, D., and Fafournoux, P. (2005). The GCN2 kinase biases feeding behavior to maintain amino acid homeostasis in omnivores. *Cell Metab.* *1*, 273–277.