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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, consumption of food lacking a single EAA leads to 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 three hours of feeding (Figure 1); that GCN2 was not activated in APC by EAA-deficient food (Figure 2); and that GCN2 knockout mice were not impaired in any aspect of feeding behavior (Figures 1-4). We then went on to develop new feeding paradigms in which we could detect rapid sensing of dietary EAAs (Figures 3 and 4). 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 protocol. They focus on two differences between our study design and theirs: (1) the length of food deprivation prior to feeding experiments, and (2) the timepoints 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, Gietzen et al claim that their studies used a longer period of fasting prior to feeding than we did (16–21 hours vs 3 hours). This longer fast could potentially lead to more rapid food ingestion, 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 (Figure 1). In addition,

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Gietzen et al's argument is directly contradicted by several of the papers that they cite, one of which reported rapid dietary EAA sensing following only a 3-hour fast (Koehnle et al., 2003) and another of which 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 claim that we measured food intake too long after providing the mice with food (3 hours). They argue that the GCN2-dependent effect is transient, appearing at 20–40 minutes 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 hours after food presentation (Maurin et al., 2005). (2) We chose a later timepoint 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 vs. 0.19±0.03 g threonine- and leucine-deficient food consumed after 1 hour, mean ± 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 attempt to find any evidence to support their previously reported model. (3) If the behavioral response is as ephemeral as Gietzen et al 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, more robust, and we believe represent the major behavioral response.

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