Inhibitory Interplay between Orexin Neurons and Eating

Highlights

- Brain orexin cell activity is rapidly inactivated upon the act of eating.
- This occurs with different foods, including calorie-free and liquid food.
- Complete orexin cell inactivation in adult brain can promote overeating.
- Overweight resulting from orexin cell loss is reversible by mild dieting.

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In Brief

Brain orexin cells control arousal, but pathological deregulation of their activity can produce sleepiness, obesity, and anxiety. González et al. show that the voluntary act of eating can rapidly downregulate orexin cell activity irrespective of taste or calories, whereas obesity resulting from orexin underactivity can be reversed by a mild diet.
Inhibitory Interplay between Orexin Neurons and Eating

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SUMMARY

In humans and rodents, loss of brain orexin/hypocretin (OH) neurons causes pathological sleepiness [1–4], whereas OH hyperactivity is associated with stress and anxiety [5–10]. OH cell control is thus of considerable interest. OH cells are activated by fasting [11, 12] and proposed to stimulate voluntary action. [13]. However, OH cells are also activated by diverse feeding-unrelated stressors [14–17] and stimulate locomotion and “fight-or-flight” responses [18–20]. Such OH-mediated behaviors presumably preclude concurrent eating, and loss of OH cells produces obesity, suggesting that OH cells facilitate net energy expenditure rather than energy intake [2, 21–23]. The relationship between OH cells and eating, therefore, remains unclear. Here we investigated this issue at the level of natural physiological activity of OH cells. First, we monitored eating-associated dynamics of OH cells using fiber photometry in free-feeding mice. OH cell activity decreased within milliseconds after eating onset, and remained in a down state during eating. This OH inactivation occurred with foods of diverse tastes and textures, as well as with calorie-free “food,” in both fed and fasted mice, suggesting that it is driven by the act of eating itself. Second, we probed the implications of natural OH cell signals for eating and weight in a new conditional OH cell-knockout model. Complete OH cell inactivation in adult brain induced a hitherto unrecognized over-eating phenotype and caused overweight that was preventable by mild dieting. These results support an inhibitory interplay between OH signals and eating, and demonstrate that OH cell activity is rapidly controllable, across nutritional states, by voluntary action.
that OH cells are rapidly inactivated by the act of eating, irrespective of food properties or body energy state.

**Natural Impact of OH Neurons on Eating**

The above correlative data have two possible causal interpretations: (1) OH cells oppose eating, and are disabled to enable eating, or (2) OH cells drive eating, and so eating stops shortly after OH cells are silenced. To distinguish between these possibilities, we investigated causality between natural OH activity and eating by inactivating OH cells in adult mice through a toxin receptor-mediated cell-knockout strategy [27, 28].

We generated new transgenic mice in which the expression of the human diphtheria toxin receptor (DTR) is driven by the OH promoter (see the Supplemental Experimental Procedures). In OH-DTR mice, but not in control WT mice, the injection of diphtheria toxin ablated all OH cells, but not the neighboring melanin-concentrating hormone-containing cells, within a couple of days (Figures 2A–2D). This complete inactivation of OH cells, which is not as readily achievable through alternative silencing methods such as opto- and chemo-genetics, may be critical for elucidation of their full impact, because key deficiency phenotypes are not apparent upon partial inactivation [13].

See also Figures S1–S3 and Movie S1.
DT injection led to greater weight gain in DTR+ mice than in their DTR− littermates (Figure 2E), confirming that OH cells oppose overweight. Next, we probed food intake patterns at an hourly temporal resolution, using a food hopper specifically designed to re-capture any food spillage and a food-weighing system whose errors were sufficiently low to report changes greater than 0.01 g (Figures S4A–S4D). In DTR− mice injected with DT (control mice) this revealed a robust daily eating rhythm, where food intake was largely restricted to the lights-off phase (“night”) but had a pronounced “dip” late at night (Figures 2F and 2G). However, in DTR+ mice injected with DT, this dip in eating was significantly reduced, causing them to consume significantly more food during the late night (Figures 2F–2H; note that the magnitude of this overeating is well...
above the sensitivity limit of the food-weight detector; Figures S4A–S4D). Interestingly, this overeating did not cause compensatory undereating at other times of day (Figures 2F–2H).

Glucose tolerance in OH cell-deficient mice was normal (Figure S4E), as previously observed for OH-deficient humans [29], suggesting that OH cell loss does not prevent glucose uptake from blood into tissues. Overall, these data show that the natural OH cell activity prevents overeating and suppresses weight gain.

**Temporal Dissociation of Fasting-Dependent and OH-Dependent Eating**

The above findings demonstrate that the natural activity of OH cells opposes eating, and therefore challenge current models postulating that OH cells stimulate eating. However, because OH cells are activated by fasting, it is still possible that they become critical for compensatory eating after fast-
Conclusions

Our findings reveal population activity dynamics of OH cells during eating, and suggest that eating is less likely to occur when OH cells are naturally active. OH cells stimulate “non-eating” behaviors such as locomotion [18]. Although these behaviors expend energy, it is difficult to eat while performing them. Thus, a possible evolutionary rationale for our findings is that suppression of OH-dependent non-eating behaviors—by silencing OH cells upon food contact—would facilitate eating (e.g., by making the mouse less likely to move away from the food). It is tempting to speculate that increased eating after experimental stimulation of the OH system [11, 13] may be a secondary response to OH-driven energy expenditure [33], rather than a primary function of OH cells.

Our findings identify interesting directions for future work. We found that the weight gain in OH-cell-deficient mice could be prevented by mild caloric restriction. This suggests that development of non-pharmacological interventions may be useful in managing excessive weight gain in neuropsychiatric conditions associated with reduced OH signaling [3, 21, 30, 34]. Furthermore, our results suggest that OH cells—whose hyperactivity has been implicated in pathological states such as panic anxiety [5]—could be inhibited by simple voluntary actions such as eating, irrespective of the nature of the food. Further research into neuroscience-based lifestyle interventions for anxiety and obesity might lead to treatments that are easier to implement and have fewer side effects.

EXPERIMENTAL PROCEDURES

OH cell activity was recorded in vivo using fiber photometry of the GCaMP6s activity indicator targeted to OH cells either using previously characterized orexin-Cre mice and Cre-inducible GCaMP6s viral vectors [35, 36] (Figure 1; Figure S1B) or using a newly generated orexin promoter-dependent adenovirus (AAV)-GCaMP6s vector (characterized in Figures S2C–S2F and described in the Supplemental Experimental Procedures). OH cells were specifically and completely inactivated using a diphtheria toxin receptor-mediated cell-ablation strategy in newly generated OH-DTR transgenic mice described in the Supplemental Experimental Procedures. Food intake was monitored using a TSE PhenoMaster system, whose sensitivity and accuracy were directly determined in our laboratory (Figures S4A–S4D). Immunohistochemistry and glucose tolerance tests were performed using standard techniques (see the Supplemental Experimental Procedures).

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, and one movie and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2016.07.013.

AUTHOR CONTRIBUTIONS

J.A.G. conducted most of the experiments; L.T.J. created and characterized the DTR transgenic mice; P.I. conducted the experiments in Figures S2C–S2F; M.S. contributed to the experiments in Figure S2C; D.B. and L.F. designed the study and obtained funding; and D.B., L.F., and L.T.J. wrote the paper.

ACKNOWLEDGMENTS

We thank Drs. Cornelia Schöne, Rui Costa, and Guohong Cui for their assistance with fiber photometry. This work was funded by The Francis Crick Institute, which receives its core funding from Cancer Research UK, the UK Medical Research Council, and the Wellcome Trust. L.F. is supported by the MRC and the Wellcome Trust.

Received: June 15, 2016
Revised: July 5, 2016
Accepted: July 8, 2016
Published: August 18, 2016
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