TECHNICAL COMMENT

# Comment on "Food wanting is mediated by transient activation of dopaminergic signaling in the honey bee brain"

Andrew Barron[1], Susan E. Fahrbach[2], Alison R. Mercer[3], Karen A. Mesce[4], David J. Schulz[5], Brian H. Smith[6]*, Eirik Søvik[7]

Huang *et al*. (*1*) make an exciting claim about a human-like dopamine-regulated neuromodulatory mechanism underlying food-seeking behavior in honey bees. Their claim is based on experiments designed to measure brain biogenic amine levels and manipulate receptor activity. We have concerns that need to be addressed before broad acceptance of their results and the interpretation provided.

Biogenic amines drive the motivation to find food—"wanting" behavior—in mammals (*2*). It is important to establish whether these mechanisms are phylogenetically conserved in insects or whether they have had different origins and endpoints. Huang *et al*. (*1*) use HPLC to measure dopamine levels in individual brains of foragers at different phases of food seeking. Then, using manipulations of dopamine receptors, they claim that blockade or activation of dopamine receptors affects food wanting. Given the implication of this claim, that there is a phylogenetically ancient mechanism for meditating food wanting, we feel compelled to engage in a constructive conversation about this important work. Our primary concerns center around two questions.

### How can the use of HPLC on whole brain extracts resolve the reported changes in biogenic amine levels across such short time scales?

Biogenic amines can be measured in several ways. Changes in biogenic amine levels in small areas of the brain (*3*) can be detected using microdialysis or in vivo voltammetry, both of which measure changes in extracellular levels that reflect the acute release of amines and the efficiency of their reuptake. In what might be characterized as a relatively fast, zero-sum game, biogenic amines cycle from being vesicle-bound inside the cell, to released and acutely active, and back (*4*).

In contrast, HPLC of biogenic amine levels extracted from whole brains does not specifically measure active, acutely released biogenic amines. Rather, whole brain sampling provides the total of the intracellular reserves and extracellular pools of biogenic amines: a total that at any point in time is assumed to reflect primarily biogenic amines stored in vesicles. Therefore, changes in biogenic amine levels measured in this way reflect a slower, changed-sum process. To date, global changes in biogenic amine levels in bees have reflected differences in behavioral state that take place over, and persist for, days. This is a time frame over which changes in synthesis and degradation could leave their mark in brain-wide, HPLC-based measurements of biogenic amines.

As changes in acute release and reuptake are not expected to appear as dramatic changes in total brain biogenic amine levels, this brings into question the results in figure 1 (*1*). The authors report changes in brain-wide dopamine levels on time scales that do not seem physiological. For example, the authors report dopamine levels going from 200% or 300% of baseline and back within one or a few minutes, which reflects physiological events not yet documented in any animal. We also find it unlikely that the reported rapid changes in brain dopamine reflect release into the hemolymph (blood). For all insects examined to date, dopaminergic neurons do not project to the periphery, unlike serotonergic and octopaminergic neurons (*5*). Dopamine is predominantly confined to brain compartme[illegible] There is no quick way to release dopam[illegible] into the hemolymph after its action on the brain.

Two additional concerns are important in this context. First, Huang *et al*. utilized between-animal sampling at different time points—prior to or after dancing or feeding, for example. This method of sampling contrasts with the within-animal method typically used to study mammalian brains, wherein biogenic amine levels before and after an event are sampled in the same individual. The assumption here is that an animal that is sampled before an event accurately reflects the initial state of a different animal sampled after an event. If that assumption does not hold, between-animal sampling can give rise to errors. There is not enough information in the methods to indicate what precautions were taken. Second, all other studies using HPLC to measure biogenic amine levels in honey bee brains have quantified octopamine (*6*, *7*). As octopamine has been consistently implicated as a mediator of reward in honey bees (*8*, *9*), a potential role for octopamine in driving food motivation must be considered. Huang *et al*. report that octopamine was not consistently observed in their samples. This omission raises the concern that dopamine and octopamine may have been co-detected as a single peak, particularly because of the pH of 4 reported for the mobile phase, which is lower than the pH used in separation of dopamine and octopamine in other studies (*10*). The authors also report a temperature of their mobile phase of 40°C, which is potentially damaging to heat labile amines.

### Conclusions

The claims made by Huang *et al*. about a dopamine-based drive for food wanting, and its phylogenetic homology with mammals, are exciting. However, for reasons described here, we feel that such claims are not supported by the data.


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[1]Department of Biological Sciences, Macquarie University, Sydney, NSW, Australia. [2]Department of Biology, Wake Forest University, Winston-Salem, NC 27109, USA. [3]Department of Zoology, University of Otago, Dunedin, New Zealand. [4]Departments of Entomology and Neuroscience, University of Minnesota, St. Paul, MN 55108, USA. [5]Division of Biological Sciences, University of Missouri-Columbia, Columbia, MO, 65211, USA. [6]School of Life Sciences, Arizona State University, Tempe, AZ 85287, USA. [7]Department of Science and Mathematics, Volda University College, 6100 Volda, Norway.
*Corresponding author. Email: brian.h.smith@asu.edu
†These authors contributed equally to this work.

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