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# Nutrient homeostasis — translating internal states to behavior

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Behavioral neuroscience aims to describe a causal relationship between neuronal processes and behavior. Animals' ever-changing physiological needs alter their internal states. Internal states then alter neuronal processes to adapt the behavior of the animal enabling it to meet its needs. Here, we describe nutrient-specific appetites as an attractive framework to study how internal states shape complex neuronal processes and resulting behavioral outcomes. Understanding how neurons detect nutrient states and how these are integrated at the level of neuronal circuits will provide a multilevel description of the mechanisms underlying complex feeding and foraging decisions.

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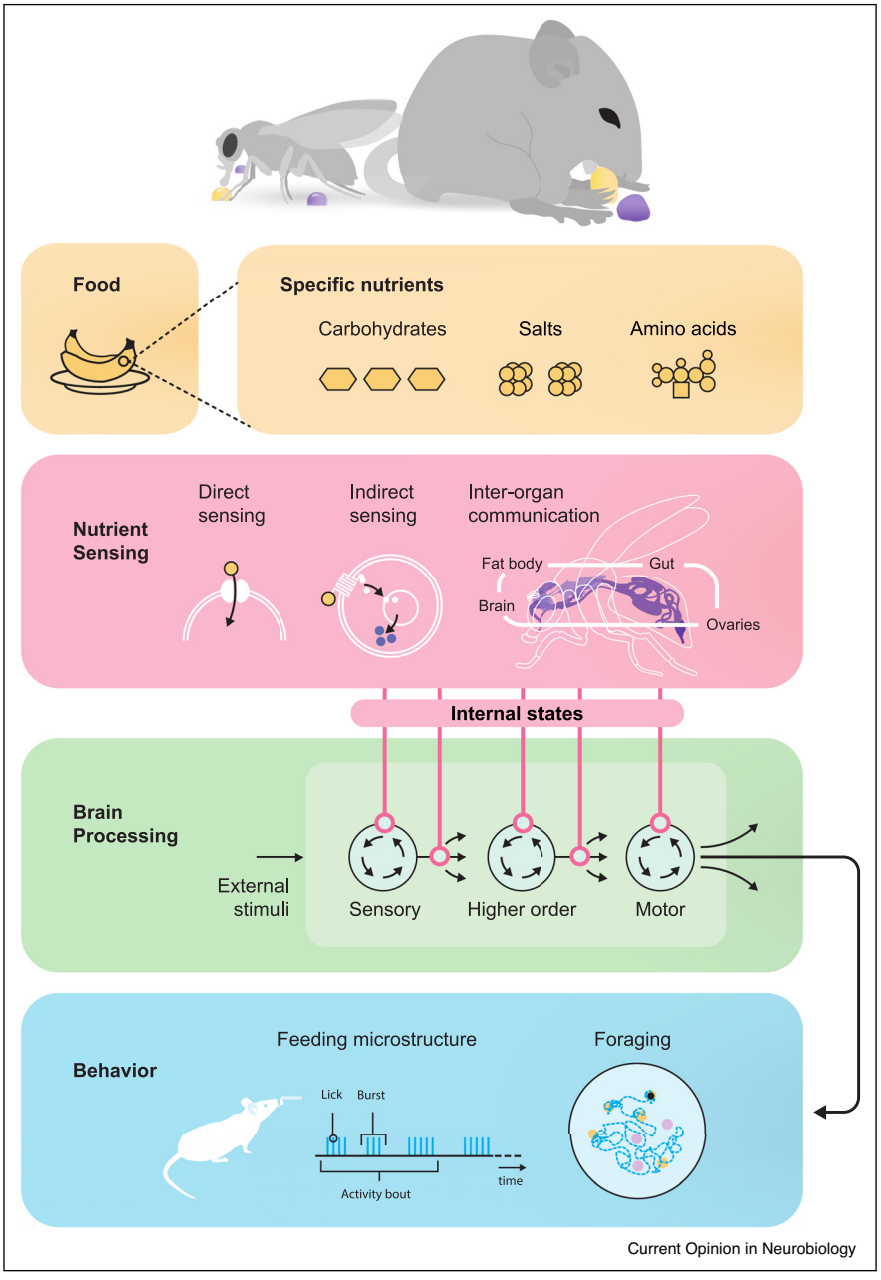
## Introduction

Mechanistic behavioral neuroscience aims at identifying the molecular, cellular and computational processes that act within neuronal circuits to generate and control behavior. This endeavor requires mapping these different processes with high temporal and spatial resolution and measuring the effect of perturbing these processes on specific cellular and behavioral outcomes. Using this strategy our understanding of how sensory information is processed by neuronal circuits to control behavior has made tremendous advances over the last decade. Animals, however, do not always produce the same behavior when exposed to the same sensory stimuli. Some of this behavioral variability can be attributed to variations in the physiological condition of the animal, which are not static but highly dynamic. These physiological conditions are thought to be represented by the state of a set of molecular and cellular processes within the organism. These states, which are commonly referred to as 'internal states', in turn, influence a wide variety of physiological parameters, including brain activity. Within the brain, internal states influence neural processing in order to adapt animals' behavior to their current needs. We propose that internal states, especially nutrient states, provide a unique framework to understand the molecular, cellular, circuit, and behavioral implementation of neuronal computations that direct animals' behavior toward achieving specific homeostatic goals.

Nutrient homeostasis is critical for health and well-being in all organisms. The balanced intake of specific nutrients (e.g. amino acids, carbohydrates) is essential to optimize lifespan and reproductive success across animal phyla [1,2]. Any deviation from 'nutrient homeostasis' (lack, imbalance or overconsumption) can have a negative impact on fitness. Thus, the tight regulation of nutrient intake is pivotal for the fitness of all animals. Most work in the field of the neuroscience of homeostasis has focused on what might be called 'indiscriminate hunger' and 'satiety' without considering the specific impact that individual nutrients have on the organism. These studies have made seminal contributions to our understanding of how feeding behavior is organized and its underlying neural mechanisms [3]. Yet, it is clear that these studies do not tackle a key question underlying nutrition: how does the animal balance the intake of specific nutrients to optimize its fitness? Classic behavioral studies [4–8] clearly demonstrate that animals control the intake of specific macronutrients and micronutrients to compensate for their lack, or in anticipation of different physiological needs (Figure 1). For example, the high demand for sodium during pregnancy drives rats to increase their sodium intake, while calcium appetite increases only later during lactation to support milk production [4]. These shifts in food intake are thought to be driven by specific appetites for the nutrients in demand. While the behavioral evidence for such nutrient-specific appetites is strong, the underlying mechanisms remain mostly obscure [9••,10]. This is partially due to the fact that designing experiments to explore such appetites is not trivial (also discussed in Refs. [9••,10]). A powerful approach is to separate the experiments into two phases: One in which the diet is changed to induce a nutrient-specific deficit in the animal. And a second one, in which different diets are presented to the animal to reveal possible homeostatic alterations in feeding preference.

**Figure 1**




How internal states control behavior to achieve nutritional homeostasis. The levels of specific nutrients are detected by different molecular sensing mechanisms, ultimately informing the nervous system of the nutritional needs of the animal (pink box). This internal state information is integrated at different levels of neuronal information processing, modulating brain function to change sensory-motor transformations (green box) and generate appropriate behavioral strategies to achieve nutrient homeostasis (blue box).

These alterations can reveal if the animal displays nutrient-specific appetites. Within these two phases three specific aspects need to be given special consideration: 1) Given the complexity of natural foods, it is not trivial to deprive animals of a specific nutrient without affecting other nutritional variables. Proteins, for example, are not only sources for amino acids but also provide nitrogen and calories. This problem can be largely circumvented nowadays by the use of diets that are holidic, meaning fully chemically defined, and when necessary, ensuring the success of the manipulation by measuring the level of the nutrient of interest in the animal [11,12]. 2) Diets with different nutritional content can also differ in their sensory properties. It is therefore important to validate that changes in dietary preference are not due to neophobia or other non-specific effects of exposing animals to foods

with different sensory properties. Different experimental designs can be used to show that either the different diets are perceived as sensory equivalent or by showing that the changes in preference do not correlate with the sensory properties of the different diets. 3) Both learned and innate mechanisms are likely to contribute to forming appetites. It is therefore important to dissociate between changes in diet preference which require the animal to associate the effect of eating from a specific food with possible ameliorations in their nutritional state from mechanisms that are independent of the formation of such associations. In this review we will focus mainly on the innate mechanisms as these are less well understood and should by definition also be required for forming nutrient-specific learned associations. While sounding exceedingly complex, these special precautions are not more complicated than the ones that have to be used when studying specific behaviors. The difference in this case being that they need to be focused on ensuring that the changes in behavior are indeed linked to changes in the internal nutrient state of the animal [13••,14,15].

Nutrient-specific appetites are generally thought to emerge from homeostatic feedback, in which the deficiency of a certain nutrient triggers compensatory consumption of that nutrient [5,6,8,14–17]. However, an increasing number of nutrient-specific appetites are found to emerge from feedforward mechanisms driven by the anticipation of a future need, leading the animal to consume nutrients before they become rate-limiting, and thereby avoiding nutritional imbalances [18••,19–21]. We suggest that dissecting the mechanisms controlling nutrient-specific appetites beyond generic hunger and satiety states is crucial for two reasons: (1) it will allow us to reach a holistic picture of the processes underlying nutrition; (2) it will also provide a powerful framework to dissect and understand how complex brain functions emerge from the integrated action of multiple processes acting at different scales within the brain, ranging from the molecular to the circuit and the behavioral level.

## Reading out nutritional needs

In order to generate compensatory behaviors to overcome nutritional imbalances, an organism needs distinct mechanisms to be able to sense the need for a variety of specific macronutrients and micronutrients. While our knowledge of the molecular basis of nutrient sensing in cells in culture is advancing at a breathtaking pace (i.e. TOR signaling [22]), we know surprisingly little about nutrient sensing mechanisms *in vivo*, in the context of a whole organism. In particular, relatively little is known about mechanisms of neuronal nutrient sensing and how it relates to behavior. Conceptually, these 'nutrient state'-sensing mechanisms can be divided into three broad categories: (1) direct, and (2) indirect sensing within the nervous system; and (3) inter-organ communication (endocrine or paracrine signaling) (Figure 1).

Direct sensing in the nervous system (1) includes dedicated receptors on or in specific neurons that directly respond to nutrients, leading to the alteration of intracellular signaling cascades. An interesting example is Gr43a, a taste receptor that is also expressed in a small subset of neurons in the *Drosophila* brain, in which it serves to detect fructose [23]. Upon activation, it is thought to promote feeding in starved flies, whereas in satiated flies it suppresses feeding. In vertebrates, both nutrient receptors and transporters expressed in feeding-relevant brain regions such as glucose transporter 2 and short transient receptor potential channel have been proposed to act as glucose sensors to alter the feeding behavior of mice [24,25].

In contrast, indirect sensing mechanisms (2) also act in neurons but are slower to respond, as they rely on the metabolic consequences of nutrient availability. GCN2, a serine/threonine-protein kinase is one of the classic examples of such a nutrient sensing pathway [26]. In nutrient balanced conditions, transfer RNAs (tRNAs) are bound to amino acids; when tRNA is not bound to amino acids, it instead binds to GCN2. Accumulation of GCN2-bound tRNA activates the GCN2 signaling cascade. In *Drosophila* larvae, GCN2 is thought to act in a subset of dopaminergic neurons to eliciting the rejection of amino acid-imbalanced diets [27]. While classic work on the neuronal functions of GCN2 was performed in mice [28,29], the involvement of neuronal GCN2 in coordinating amino acid feeding in rodents has been recently challenged [13••]. This highlights how little we still know about amino acid sensing in neurons, and the challenges in identifying the relevant molecular basis of neuronal nutrient sensing and how it is linked to behavior.

Nutrient sensing via inter-organ communication (3) relies on nutrient sensing in a particular organ, which sends a signal conveying the nutritional information over short or long ranges to an effector organ, like the brain. This is probably the most studied nutrient sensing strategy, especially in the context of metabolic disorders (for an extensive review see Ref. [3]). The levels of many hormones, such as cholecystokinin (CCK), insulin, leptin, and ghrelin are clearly modulated by nutrient availability [3,30–32]. Yet, while some, like leptin and ghrelin, are certainly involved in altering feeding, the behavioral impact of many of these factors remains unclear. It is possible that some hormonal signals are relevant only for controlling metabolism while having no direct effect on behavior. It is especially unclear if these nutrient state signals regulate nutrient-specific appetites. Interestingly though, some hormonal signals have been shown to respond to the levels of specific nutrients,

as in the case of fibroblast growth factor 21 (FGF21) [33•]. FGF21 is a member of the endocrine FGF family and is produced primarily by the liver and signals to various tissues by endocrine signaling which has significant impact on metabolism. Importantly, FGF21 expression is responsive to dietary conditions, and when injected intracerebrally it specifically increases protein preference in mice. This hormone thus represents an example of a signal mediating inter-organ communication involved in regulating nutrient-specific preferences linked with internal metabolic state. If we are to have an understanding of how nutrition is regulated by the brain it will be important to systematically test the different hormones which have been proposed to react to the nutrient state of the animal or control feeding, using nutrient-specific behavioral paradigms aiming at uncovering possible functions in regulating specific appetites.

Conventionally, molecular nutrient sensing is incorporated in models which have feedback mechanisms at their center. However, internal states can also induce specific compensatory behaviors in a feedforward manner. In *Drosophila*, for example, mating is a well-established feedforward signal driving salt and protein appetites [16,18••]. Various sensory inputs, such as olfaction and gustation, may also be seen as feedforward, predictive nutrient sensing mechanisms driving the decision to feed or to avoid food, based on the expectation that the food will contain specific nutrients [34,35]. Embracing more sophisticated control theory models for nutrient regulation should open the doors to a better understanding of how nutrient sensing signals control behavior [19].

Understanding neuronal nutrient sensing remains an exciting field with many additional important open questions. While traditionally nutrient-specific appetites have focused on salts, amino acids, and carbohydrate sensing, mechanisms for detecting the availability of other circulating nutrients remain unknown in most organisms. It is, however, known that *Caenorhabditis elegans*, for example, senses vitamins [36], which raises the question of how many specific nutrients can be sensed in different organisms. Moreover, it is largely unclear how molecular nutrient state information is transformed into a change in the activity of specific neurons, which then alter circuit function. Neuromodulators are good candidates for mediating these transformations and their individual contributions have recently been reviewed in detail [17,37–39] but neuronal nutrient signaling could also lead to cell autonomous changes at the level of connectivity and synaptic physiology which would directly lead to alterations in behavior [40]. Furthermore, it is also not clear if internal sensing of different nutrients converges on and activates a common feeding circuit or whether there are dedicated nutrient-specific circuits acting in parallel.

## How nutrient states alter neuronal circuit functions to modify behavior

To induce adaptive changes in behavior, particularly feeding, neuronal circuit computations must be altered in response to the detection of changes in nutrient state (Figure 1). Here we will discuss two circuit processes which implement these adaptations: those affecting overall 'feeding motivation', and those that specifically affect the processing of chemosensory information. The first can be thought of as increasing the effort put into initiating and sustaining feeding-related behaviors. The latter tunes sensory systems to enable a more efficient detection of required nutrients provided by different food sources and/or modulates their saliency.

As previously discussed, in mammals, a large body of work shows that homeostatic feedback signals from peripheral organs modulate hypothalamic circuits to alter feeding behavior. The activity of two small populations of interconnected neurons in the arcuate nucleus expressing either POMC or AgRP is modulated by satiety and hunger signals such as leptin and ghrelin [3,32,41]. *Ex vivo* recordings and optogenetic and chemogenetic experiments led to the classic view that activity in AgRP neurons promotes food intake, while activity in POMC neurons decreases it. However, recent recordings of these neurons in freely behaving animals have challenged this view. While the basal activity in AgRP and POMC neurons is modulated by hunger or satiety signals, preingestive sensory inputs from food sources lead to rapid modulations of activity in these neurons [42••,43••,44••]. Importantly, *in vivo*, increased AgRP and decreased POMC activity correlate with the detection of food and not with feeding initiation, challenging the idea that they drive ingestion directly. This suggests that this microcircuit is rather involved in coordinating foraging behavior and that it not only integrates feedback information about internal states but also sensory information in a feedforward fashion. It is currently unclear if these neurons are also involved in mediating nutrient-specific appetites, making this an important avenue of future research. Surprisingly, given the available tools, a similar microcircuit integrating hunger and satiety signals to drive foraging and feeding has not yet been identified in *Drosophila*. The current view is that neurons in the pars intercerebralis (PI) such as Taotie neurons could be part of such a microcircuit [45]. However, more work is clearly required before the PI can be labeled the 'invertebrate hypothalamus'.

Apart from acting on central circuits altering feeding motivation, internal states also modulate sensory processing. This has been clearly shown in *Drosophila*, where starvation induces bi-directional modulation of olfactory and gustatory sensory neurons [38]. At the neuronal activity level, hunger lowers the detection thresholds for attractive, food-related, olfactory and gustatory cues

such as sugar, while at the same time it increases the detection threshold of aversive stimuli, such as bitter tastants [46•,47•,48•,49•,50]. Related observations have been made in mice, where the satiety signal leptin seems to act on chemosensory processing [51,52]. Since tastants generally have an innate behavioral saliency, one can think of changing their sensory perception as also affecting the motivational behavior of the animal.

Importantly, changes in sensory processing can be nutrient-specific. Animals specifically deprived of dietary amino acids for example only increase the gustatory detection of and feeding on proteinaceous food while sweet sensory neurons remain unaffected [53]. Interestingly, other internal states, such as reproductive state, also induce nutrient-specific appetites, for example, for protein or salt, acting synergistically with nutrient state signals [4,16,18••,53]. This is an important reminder that while in our laboratories we think of individual internal states as altering behavior, in reality animals are constantly in a multitude of internal states which need to be compared and traded-off. How multiple states are integrated into circuit computations to elicit appropriate behavioral outputs is a key question for future research. Given that in flies we know the circuits signaling the mating state of the female and modifying their nutrient preferences [18••,54,55], *Drosophila* food choice provides an attractive platform for studying this important question.

Overall a picture emerges, in which internal states affect general feeding motivation to increase the likelihood to find, initiate and sustain the intake of specific foods. At the same time changes in sensory processing allow for the differential detection of foods containing specific nutrients. In vertebrates, it is clear that sensory information is also integrated into circuits controlling motivational drives in a feedforward manner, shaping expectations of food availability. In the future, it will be interesting to investigate if such a feedforward integration also exists in insects and how it shapes behavior.

## Nutrient states and behavior: feeding microstructure and foraging

The previously described molecular and circuit elements ultimately exist to control behavior. Here we argue that understanding how these mechanisms control nutrient homeostasis requires high-resolution quantitative analysis of behavior, ideally in naturalistic conditions.

### Feeding microstructure

In almost all animals, including rodents and flies, feeding can be described as hierarchically organized sequences of behavioral submodules [56,57,58•,59]. These modules range from the exploration of possible food sources to the organization of consummatory behavior, or 'feeding microstructure' (Figure 1). At the finest motor level, rodents use rhythmic licking behavior with their tongues to ingest liquid food, while flies perform corresponding rhythmic extension-retraction cycles of the proboscis named 'sips'. At a higher organizational level, these licks or sips can be further grouped into bursts, which are organized into activity bouts at a higher level (Figure 1). The duration and frequency of behavior at these different hierarchical levels are tightly regulated by the integrated action of sensory cues, nutrient value of the food, and the internal states of the animal. For example, rats modulate both the number and length of feeding bursts and bouts depending on the sucrose concentration in the food [56]. Starvation also differentially modulates the number and length of the bursts and bouts depending on the sucrose concentration [60,61]. These findings suggest that both the quality of the food and internal states are important determinants of feeding microstructure in mice. Frequency and number of feeding bursts and bouts in *Drosophila* are also tightly regulated by sensory cues and nutrient states [53,58•,62]. These different aspects of the feeding microstructure are likely to be regulated independently, as different durations of starvation differentially affect the length and frequency of feeding bursts [58•]. This idea is supported by the discovery that it is possible to functionally separate these regulatory variables at the circuit level [53]. Furthermore, at least in flies, the animal is able to differentially regulate different aspects of the feeding microstructure in a food source-specific way depending on their nutrient content.

Overall, total intake of a specific food is controlled by varying these submodules composing the feeding microstructure (i.e. licks or sips, bursts, and bouts) in a complex yet hierarchical fashion [58•]. This is likely to happen by altering distinct neuromodulatory pathways which integrate nutrient state and sensory experience to modulate different aspects of the feeding microstructure [18••,50,63••]. Successful mapping of neural circuits controlling nutrient-specific appetites will therefore have to take into account the differential regulation of feeding microstructure, rather than using the total food intake as the only readout to assess the impact of experimental manipulations.

### Foraging behavior

Animals need to optimize the intake of various nutrients while minimizing their net energy use, avoiding risks (e.g. predators, thermal fluctuations), and competing for resources with other organisms [64]. Indeed, in ecological settings starved mice are more willing to search beyond their safe zone compared to satiated conspecifics [65]. Therefore, in addition to tightly regulating the motor structure of consummatory behaviors, nutrient states also shape food-seeking behavior to maintain an optimal balance between exploration and exploitation [63••,65,66]. To achieve this, animals must integrate

external sensory stimuli, internal states as well as idiothetic cues to compute strategies that optimize the balance between exploration and exploitation. Given their complexity, these computations are likely to represent an evolutionary ancient form of proto-cognitive processing.

Several studies have corroborated starvation-induced shifts in food-seeking behavior in laboratory conditions. It has been shown that starvation leads to increased foraging-related behaviors (i.e. walking and digging) in mice [67,68]. Fasting also overrules the learned avoidance of locations that have been paired with electric shocks or the innate avoidance of exposed areas and leads to decreased aggression [69]. The decrease in learned avoidance depends on the presence of food, suggesting that the hunger state might not be sufficient to induce risk-taking behavior in the absence of anticipated gains (i.e. desired nutrients or calories). This fits well with the emerging feedforward perspective of how hunger circuits regulate behavior. In addition to food consumption and foraging, AgRP neurons also modulate learned avoidance, anxiety, and aggression [68,70–72]. Chemogenetic and optogenetic activation of distinct subpopulations of AgRP neurons has demonstrated that foraging and aggression behaviors can be segregated at the level of AgRP subpopulations, their projection areas, and the neuromodulatory mechanisms [68,71]. However, whether subpopulations of AgRP neurons also contribute to nutrient-specific appetites remains to be determined.

*Drosophila* foraging behavior is also tightly regulated by nutrient states. Flies implement multiple changes in their exploratory and food consumption strategies upon amino acid deprivation, leading to increased local exploration of protein-rich food sources and reduced global exploration [63••]. Starved flies exhibit local search behavior upon finding a nutrient-rich spot by repeatedly looping toward and away from the spot they are eating from [7,63••,73••,74••]. This is likely to help the fly search for more nutritious food spots even if it has encountered a satisfactory one. Sweet sensation by pharyngeal gustatory sensory neurons is important for this local search behavior, although experiments with optogenetic gustatory stimulation have also proposed that other gustatory neurons are involved [73••,74••,75••,76••]. Strikingly, flies maintain this local search pattern even if visual and olfactory cues are removed, suggesting that they could perform path integration [74••]. Given that foraging computations are complex and require the integration of sensory information, internal states and idiothetic cues, identifying the circuits integrating this information in the fly brain and the computations used to control the trade-off between food exploration and consumption will serve as an attractive and tractable opportunity to mechanistically dissect these proto-cognitive brain processes.

## Conclusion

How animals select food and balance the intake of different nutrients is an important problem with deep implications in many fields outside of classical neuroscience such as metabolism, physiology, immunology, reproductive biology, microbiome research, and cellular signaling. It also has important practical consequences for the understanding, prevention, and treatment of many pathologies which go beyond metabolic diseases, such as neuropsychiatric diseases and cancer, for which nutritional interventions have been proposed and are being tested in the clinic. Understanding the mechanisms underlying the neuroscience of nutrition should be an integrative endeavor, bringing together classical neuroscience approaches as well as insights from molecular, cellular, and metabolic disciplines. Explanatory frameworks will have to go beyond understanding pure neuronal processes and take a whole-animal systems view. As such, the neuroscience of nutrition is by definition a prime example of integrative neuroscience.

Unraveling the mechanisms of how nutrient-specific appetites are generated by the brain is a relatively new venture. We hope to have highlighted that we are just at the beginning of understanding how the brain detects nutritional needs and changes sensory-motor transformations to adapt behavior. While being a complex problem, it is also one of the most ancient problems neuronal systems must have evolved to tackle. As such, the mechanisms directing nutrient-specific appetites are very likely to be conserved. Furthermore, they are likely to be the ancient substrate on which complex cognitive economic trade-off computations have evolved. Because this system reads out molecular entities (nutrients) to define the internal state of the animal rather than abstract features of the environment or the past history of the animal, it is likely to be based on dedicated molecular and cellular machineries acting in specific circuits. This makes this problem experimentally tractable using molecular and genetic manipulations.

The emerging picture is that to understand how the animal maintains nutrient homeostasis it is necessary to combine precise internal state and sensory perturbations with quantitative behavioral readouts. This in conjunction with advanced *in-vivo* imaging and electrophysiological approaches should make it possible to map specific molecular and cellular processes to neuronal and behavioral effects. As such, nutrient-specific appetites and their related behaviors open an experimentally tractable window into how the brain generates and implements complex computations. This approach will be best tackled in versatile animal models that exhibit strong and specific behavioral changes upon nutrient manipulations. These organisms also need to allow researchers to precisely control their internal states, while allowing them to perturb and read out behavioral, cellular and physiological

parameters with high spatial and temporal resolution in the freely foraging animal. By integrating data from different model organisms fulfilling these requirements we should be able to gain exciting and generalizable mechanistic insights into how internal states act on the brain to control behavior.

## Conflict of interest statement

Nothing declared.

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