

## Time to taste

### Circadian clock function in the *Drosophila* gustatory system

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**C**ircadian clocks keep time in the digestive, circulatory, reproductive, excretory and nervous systems even in absence of external cues. Central oscillators in the brain control locomotor activity of organisms ranging from fruit flies to man, but the functions of the clocks in peripheral nervous system are not well understood. The presence of autonomous peripheral oscillators in the major taste organ of *Drosophila*, the proboscis, prompted us to test whether gustatory responses are under control of the circadian clock. We find that synchronous rhythms in physiological and behavioral responses to attractive and aversive tastants are driven by oscillators in gustatory receptor neurons (GRNs); primary sensory neurons that carry taste information from the proboscis to the brain. During the middle of the night, high levels of G protein-coupled receptor kinase 2 (GPRK2) in the GRNs suppresses tastant-evoked responses. Flies with disrupted gustatory clocks are hyperphagic and hyperactive, recapitulating behaviors typically seen under the stress of starvation. Temporal plasticity in innate behaviors should offer adaptive advantages to flies. In this Extra View article we discuss how oscillators inside GRNs regulate responsiveness to tastants and influence feeding, metabolism and general activity.

Daily changes in the environment are perceived via multiple sensory systems. To anticipate and respond to these diurnal changes, eukaryotes evolved time-keeping devices made up of autoregulatory interlocked transcriptional feedback

loops.<sup>1</sup> In fruit flies, photoreceptors and thermoreceptors provide input to the oscillator,<sup>2</sup> and the clock in turn exerts control on various sensory modalities, courtship and mating, locomotion, feeding, metabolism, sleep, memory formation and immunity among others.<sup>3</sup> The functional ramifications of having clocks in different tissues is a fundamental question in chronobiology that has not been adequately addressed. Cellular and molecular mechanisms of clock output are understudied despite being the machinery that connects the molecular feedback loop oscillator with behavioral, physiological and metabolic function of the organism.

#### Taste Tested Over Time

We showed that in *Drosophila*, physiological responses of the GRNs to tastants vary in a circadian manner. Single-unit responses, which are extracellular recordings of action potentials in the form of voltage spikes, were recorded from GRNs in taste sensilla. GRN spikes displayed circadian modulations in amplitude, frequency and duration across multiple functional classes of GRNs from different types of taste-sensilla that express different repertoires of GRs. The amplitude, frequency and duration of GRN spikes in response to the same dose of tastant solution were highest around subjective early morning and lowest during mid-evening (Fig. 1A). As the concentration of tastant increases, firing frequency, spike amplitude and spike duration also increase.<sup>4</sup> Accordingly, gustatory physiology in the fly appears to be tuned to a higher gain

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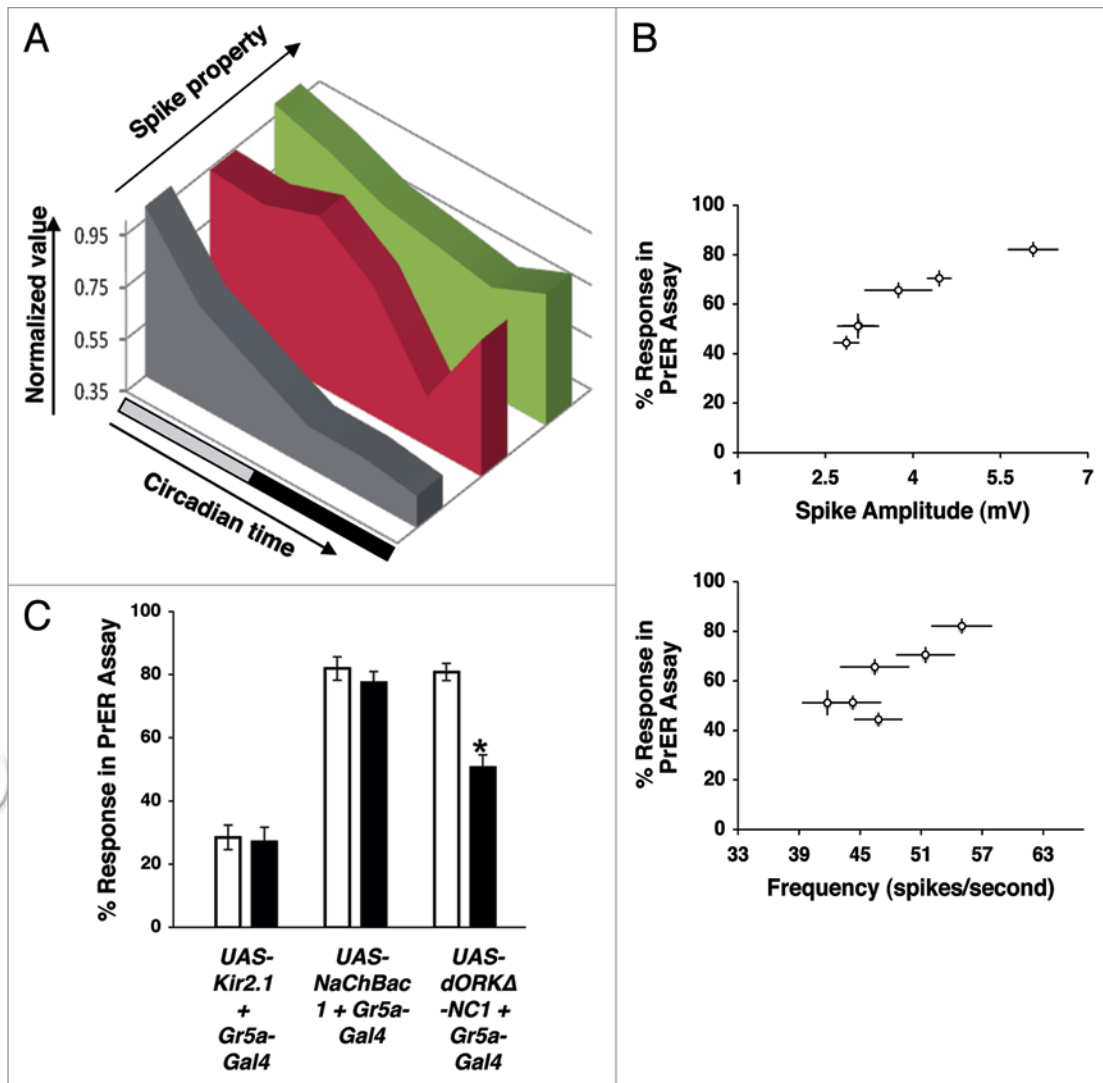
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**Figure 1.** Circadian regulation of gustatory physiology and behavior. (A) The amplitude (gray), duration (red) and frequency (green) of S spikes in response to 100 mM sucrose exhibit synchronous circadian rhythms under constant darkness (DD). The overall effects of time of day on all the three spike parameters are significant by one-way ANOVA ( $p < 0.001$  for amplitude,  $p < 0.02$  for frequency,  $p < 0.01$  for duration). Single-sensillum recordings were carried out on separate populations of age and sex-matched flies every four hours starting from CT1. (B) PrER response levels are positively correlated with the amplitude (upper part) and frequency (lower part) of GRN spikes. PrER responses were plotted as a function of the amplitude and frequency of S spikes recorded from I-type sensilla from WT flies stimulated with 100 mM sucrose during DD. Spike amplitude and PrER response levels are strongly correlated ( $r^2 = 0.92$ ) and highly significant ( $p < 0.003$ ). S spike firing rate and PrER response levels show a significant ( $p < 0.036$ ) correlation ( $r^2 = 0.71$ ). (C) Disruption of membrane excitability of GRNs abolishes PrER rhythms. PrER responses of *Gr5a-Gal4* flies carrying *UAS-Kir2.1*, *UAS-NaChBac1* or *UAS-dORK-ΔNC1* at ZT1 and ZT17. There are no significant ( $p > 0.40$ ) differences in PrER responses at ZT17 compared with ZT1 in *Gr5a-Gal4* flies carrying either *UAS-Kir2.1* or *UAS-NaChBac1*. The differences in mean PrER responses at ZT1 and ZT17 are significant ( $p < 0.001$ ) in *Gr5a-Gal4* flies carrying *UAS-dORK-ΔNC1*. White columns indicate mean PrER responses to 100 mM sucrose at ZT1; black columns represent mean PrER responses to the same stimulus at ZT17. Asterisk denotes a significant ( $p < 0.05$ ) decrease in PrER responses at ZT17 compared with ZT1. Error bars indicate the S.E.M.

level during daytime. The phase relationship between rhythms in spike frequency, amplitude and duration during constant darkness (DD) implies that common molecular factors and/or cellular processes are involved in their regulation (Fig. 1A). It would be interesting to determine whether spontaneous impulse activity is rhythmic in GRNs since such rhythms would demonstrate that the circadian

oscillator does not act only on the tastant-evoked signaling cascade.

When a fly's taste hairs are stimulated with a solution containing phagostimulants, the fly reflexively extends its proboscis to attempt feeding. Consequently, this proboscis extension reflex (PrER) is used to measure a fly's motivation to eat.<sup>5</sup> We used the probability of PrER behavior to quantify circadian variation

in gustatory responses. The probability of PrER responses changed as a function of time of day in parallel to the phase of neurophysiological rhythms of GRNs, particularly the amplitude and frequency of GRN impulses (Fig. 1B). This result suggests that amplitude, in addition to frequency of spikes, may code information translatable into behavior. Circadian rhythms in spike amplitude have been

recently reported also in the olfactory system of flies,<sup>6</sup> where the phases of electrophysiological rhythms, likewise, mirror the rhythms in odor-dependent chemotactic behavior.<sup>7</sup> Together these data demonstrate that chemosensory ability, which offers survival advantages,<sup>8</sup> shows plasticity depending on the organism's internal temporal environment.

A wide variety of sensory inputs are known to undergo processing by the clock. It is clear that the quantitative relationship between sensory stimulus and its corresponding perceptual output is dependent on stimulus intensity and also on the circadian time when the signal is registered. Time of day seems to be a widespread psychophysical parameter for vision,<sup>9</sup> audition,<sup>10</sup> olfaction,<sup>11</sup> electroreception,<sup>12</sup> and taste.<sup>13</sup> Daily variation in the responsiveness of larval GRNs was previously suggested to occur in the moth *Spodoptera littoralis*.<sup>14</sup> Gustation in *Drosophila* has long been used as a model for sensory transduction, information coding and processing, cue-driven behavior and associative learning.<sup>15</sup> In light of our finding that physiological and behavioral responses to tastants are under circadian control, it will be important to consider time of day in the design and interpretation of experiments regarding taste.

### GRN Clocks Reach Beyond Taste

Our results indicate that a fly is most sensitive to tastants in the morning. This may allow the organism to temporally couple increased morning activity with enhanced food-detection ability, thereby leading to increased feeding. Taste sensitivity peaks around the same time of day as food consumption.<sup>16</sup> Rhythmic GRN activity also seemed to correlate with food intake in moths.<sup>14</sup> Circadian rhythms in feeding-motivation provide a unique strategy to optimize food consumption. Thus, plasticity in gustatory physiology and behavior possibly provide the organism advantages in food acquisition at particular time of the day. This strategy can, on the other hand, minimize energy expenditure by shutting down the frequency of innate taste-evoked PrER behavior when flies are resting.

Circadian clocks can be reset by social interaction amongst flies.<sup>17</sup> Social influence is communicated by chemosensory cues such as pheromones, some of which are synthesized in a circadian manner.<sup>18</sup> A number of gustatory receptors are thought to be involved in pheromone sensing.<sup>19</sup> The gustatory clock may impose temporal gating on chemosensory input that may be transmitted to protocerebral clock cells controlling locomotor activity of flies. Circadian gating of pheromonal input that is relayed to the protocerebrum may also modulate the likelihood of mating.

Clocks in *takeout*-expressing metabolic tissues were shown to restrict feeding and neuronal clocks were found to promote feeding.<sup>16</sup> In addition to fat bodies, antennal olfactory receptor neurons (ORNs) and labellar GRNs also express *takeout*.<sup>20,21</sup> We disrupted the molecular clockwork within sugar-responsive GRNs and found those flies to be hyperphagic. Therefore, in contrast to other neuronal clocks, the gustatory clock helps the animal to restrict daily food consumption. We did not observe any uniform correlation between PrER behavior and food intake, suggesting that the oscillators inside GRNs influence food consumption possibly by altering the feedback of GRNs on tissues controlling the metabolic status of the fly. Flies with a compromised gustatory clock also showed gross metabolic defects in the form of significantly increased levels of stored fat and carbohydrate. GRNs are predicted to convey output to mushroom bodies (MB) and the corpora cardiaca (CC) via interneurons in the suboesophageal ganglion (SOG).<sup>22</sup> Alteration of clock function in the olfactory receptor neurons, which ultimately send information to the MB and lateral horn of protocerebrum, also influences feeding in flies.<sup>16</sup> It is possible that clock-mediated changes in outputs from gustatory interneurons control the levels of food-intake regulating factors such as short neuropeptide F in MB,<sup>23</sup> and/or secretion of endocrine factors such as adipokinetic hormone from CC.<sup>24</sup> The fact that a small number of peripheral neurons known to be concerned with only taste reception may also control locomotor activity, feeding and metabolism of an organism indicate that homeostatic

regulation of the 'milieu intérieur' is multilayered and complex.

### Mechanism of Gustatory Rhythms

We previously demonstrated that clocks within the GRNs are both necessary and sufficient for PrER rhythms.<sup>13</sup> Silencing GRNs via *Gr5a-Gal4*-driven *UAS-Kir2.1*,<sup>25,26</sup> and hyperexciting GRNs via *Gr5a-Gal4*-driven *UAS-NachBac1*,<sup>26,27</sup> gave rise to arrhythmic phenotypes with constant low and constant high levels of PrER responses, respectively, whereas control *Gr5a-Gal4*-driven *UAS-dORK-ΔNCL* flies<sup>26,28</sup> remained rhythmic (Fig. 1C). This reinforces the idea that the GRN clock changes taste-driven behavior by altering membrane excitability on a daily basis. The flow of rhythm-information from primary sensory neurons to downstream cells is required to generate rhythmic behavior, although clocks within these relay neurons, if present, are not necessary for PrER rhythms. It is also possible that the circuit for generating PrER behavior and the circuit for executing PrER rhythms are not completely identical. Both these circuits do share the GRNs, but neuroanatomical structures that are not required for PrER behavior per se, such as MB neurons,<sup>29</sup> may form a functional part of the neural circuit for PrER rhythms.

Colocalization of a circadian oscillator and a rhythmic output in GRNs suggests a number of potential targets for clock regulation. Gustatory signal transduction in *Drosophila* probably begins with the capture of tastants by odorant binding proteins (OBP) present in the sensillar lymph and CheB family of proteins present in the inner lumen of sensillae.<sup>30,31</sup> Some *obp* transcripts are, in fact, known to oscillate in *Drosophila* heads.<sup>32</sup> A single GRN generally expresses multiple GRs,<sup>33</sup> which ultimately recognize tastants either directly or in conjunction with OBPs and/or CheB proteins. A number of G proteins are known to be expressed in the labellum and disruption of their function affects sugar reception.<sup>34-36</sup> The second messenger-generating enzyme associated with G protein-mediated signaling, PLCβ, encoded by *norpA* gene, is also present in the gustatory organ.<sup>37</sup> Our work demonstrated that rhythms in PrER behavior

are dependent on GRs and G-protein Receptor Kinase 2 (GPRK2).<sup>13</sup>

GPRKs promote termination of GPCR signaling in mammals.<sup>38</sup> Two *Gprk* genes are present in *Drosophila*: *Gprk1* and *Gprk2*. Disruption of *Gprk1* function in photoreceptors leads to increased electroretinogram (ERG) amplitude,<sup>39</sup> consistent with a role in terminating GPCR signaling. Likewise, we found that *Gprk2* mutants have enhanced gustatory responses. Rhythms in GPRK2 expression were recently found to underlie olfaction rhythms in *Drosophila*.<sup>6,40</sup> GPRK2 is also expressed in sensory neurons of gustatory sensilla and its protein level cycles in abundance.<sup>13</sup> GPRK2 levels peak around the middle of the night, when PrER responses are lowest, and GPRK2 levels are at their lowest in the early morning, when PrER responses reach their peak.<sup>13</sup> By altering GPRK2 levels in *Gprk2* mutant and overexpression flies, we demonstrated that oscillating levels of GPRK2 protein drive PrER rhythms.<sup>13</sup> Given that GPRKs typically target GPCRs, it is possible that GPRK2 alters taste sensitivity by modulating the activity of GRs. Although a hypomorphic *Gprk2* mutant enhances low nighttime PrER responses to morning-like high levels,<sup>13</sup> the same mutant reduces physiological responses to odorants,<sup>40</sup> indicating that *Gprk2* affects olfactory and gustatory responses in opposite ways. The predominant isoform of GPRK2 protein present in antennal extracts is absent in the proboscis.<sup>13</sup> In the olfactory system, GPRK2 has been shown to promote the dendritic trafficking of olfactory receptors (ORs),<sup>40</sup> but it is not known whether GPRK2 changes the subcellular localization/stability/activity of GRs or how GPRK2 brings about its effects at biochemical level in the olfactory or the gustatory system.

We cannot exclude other possible mechanisms for clock-dependent taste modulation; the clock may in parallel control rhythmic oscillation of other molecules in the GRNs, such as *takeout*, a clock-controlled molecule expressed in the proboscis known to modulate GRN firing rate.<sup>21,41</sup> GPRK2 may be controlling the activity of other components of gustatory signal transduction machinery, e.g., ion channels.<sup>42</sup> Alternatively, non-neuronal

accessory cells in gustatory sensilla may undergo GRN-clock driven oscillations to bring about perireceptor changes in the taste sensilla. However, the simplest model would posit that circadian changes in the levels of GPRK2 from GRNs drive electrophysiological and behavioral rhythms in taste, though less direct and more complex regulatory mechanisms may operate simultaneously.

### Concluding Remarks

We examined gustatory physiology, tastant-evoked appetitive behavior, food ingestion and storage in *Drosophila* to understand the breadth and depth of clock-dependent regulation of the gustatory system. Our studies showed that circadian clocks in GRNs control neuronal output and drive behavioral rhythms in taste responses in a GPRK2-dependent manner. We also revealed a connection between peripheral GRN clocks and feeding regulation. Our results may be applicable well beyond fly sensory biology because circadian clock components, gustatory system organization and function, feeding behaviors and circadian control of lipid and carbohydrate metabolism are remarkably similar in flies and mammals.<sup>16,43-47</sup> For instance, the acuity of the gustatory sensitivity in humans was recently shown to change in a diurnal manner, with the highest sensitivity to sweet compounds occurring in the morning—when the fly also elicits its strongest taste response.<sup>48</sup> Determining how the *Drosophila* clock controls rhythms in gustatory physiology, appetitive behavior and feeding will provide a basis for understanding the eco-ethological significance of rhythms in taste in *Drosophila* and perhaps humans.

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