4. Roberts, D.N., Wilson, B., Huff, J.T., Stewart, A.J., and Cairns, B.R. (2006). Dephosphorylation and genome-wide association of Maf1 with polymerase III-transcribed genes during repression. Mol. Cell *22*, 633–644.
5. Moir, R.D., Lee, J., Haeusler, R.A., Desai, N., Engelke, D.R., and Willis, I.M. (2006). Protein kinase A regulates RNA polymerase III transcription through the nuclear localization of Maf1. Proc. Natl. Acad. Sci. USA, in press.
6. Boguta, M., Czerska, K., and Zoladek, T. (1997). Mutation in a new gene *MAF1* affects tRNA suppressor efficiency in *Saccharomyces cerevisiae*. Gene *185*, 291–296.
7. Pluta, K., Lefebvre, O., Martin, N.C., Smagowicz, W.J., Stanford, D.R., Ellis, S.R., Hopper, A.K., Sentenac, A., and Boguta, M. (2001). Maf1p, a negative effector of RNA polymerase III in *Saccharomyces cerevisiae*. Mol. Cell Biol. *21*, 5031–5040.
8. Adelman, K., Marr, M.T., Werner, J., Saunders, A., Ni, Z., Andrulis, E.D., and Lis, J.T. (2005). Efficient release from promoter-proximal stall sites requires transcript cleavage factor TFIIS. Mol. Cell *17*, 103–112.
9. Rasmussen, E.B., and Lis, J.T. (1993). In vivo transcriptional pausing and cap formation on three *Drosophila* heat shock genes. Proc. Natl. Acad. Sci. USA *90*, 7923–7927.
10. Desai, N., Lee, J., Upadhya, R., Chu, Y., Moir, R.D., and Willis, I.M. (2005). Two steps in Maf1-dependent repression of transcription by RNA polymerase III. J. Biol. Chem. *280*, 6455–6462.
11. White, R.J. (2004). RNA polymerase III transcription and cancer. Oncogene *23*, 3208–3216.

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Animal Cognition: An Insect's Sense of Time?

For Immanuel Kant, time was the very form of the inner sense, the bedrock of our consciousness and also the origin of arithmetic ability. New research on bumblebees has shown that even an invertebrate with a brain the size of a pinhead can actively sense the passage of elapsed time, allowing it to predict when certain salient events will occur in the future.

Peter Skorupski and Lars Chittka

It has long been known that bees have circadian rhythms that allow them to estimate the time of day [1,2]. This helps them to use a sun compass to determine correctly the direction of home or a feeder [2]; they can also learn to schedule their visits to food sources to certain times of day [3]. But can bees also measure shorter, flexible intervals that are not directly driven by an endogenous biological oscillator such as their circadian clock (Figure 1) [4]? The assumption that insects can measure time — or its reciprocal, rate — is implicit in the literature on foraging, where there is evidence that bees might measure flower profitability by assessing nectar gained per unit time [5], and cost in terms of floral handling time [6]. An ability to measure time is implied in the literature on insect flight speed and distance measurement [7,8]. And to understand their dance language, honeybees need to be able to attend to the times of the various moves [2]. The measurement of time or rate is implicit in all of these studies.

Nevertheless, a basic question about the neural representation of time arises: is it emergent in the activity of any neural circuit that subserves processing with a temporal dimension, or is it necessary to posit a special cognitive representation of time [9]? The ability to attend to the passage of time is termed interval timing, which has been demonstrated in a range of vertebrate species [10,11]. This shows that time can be represented explicitly in non-human animals — in estimating, and then waiting for, a fixed time interval, an animal is, in effect, attending to the future, and at the same time, referring to a memory from the past. But despite the many studies predicated on the assumption that insects can measure time or some correlate of time, empirical evidence that time itself can be measured by insects was, until now, lacking.

As reported recently in *Current Biology*, Boisvert and Sherry [12] used a standard fixed interval procedure from the vertebrate literature to probe the interval timing capacity of bumblebees. The behaviour was first shaped by training a bee to obtain a sugar reward by inserting its proboscis through a small hole in the wall of an experimental chamber. Proboscis extension interrupted a fine infra-red beam, which triggered delivery of sucrose reward. For the experimental sessions, the apparatus was programmed so that the reward would only be delivered after a fixed time interval had elapsed (Figure 2). The onset of this interval was cued by illumination of the experimental chamber. A response by the bee — proboscis

Figure 1. Can bumblebees sense the passage of time?
It has been long known that bees can correctly estimate the time of day by relying on their circadian clocks. As discussed in the text, a new article by Boisvert and Sherry [12] shows that bumblebees can also measure the duration of short intervals, potentially allowing them to predict the refill schedules of nectar-yielding flowers.

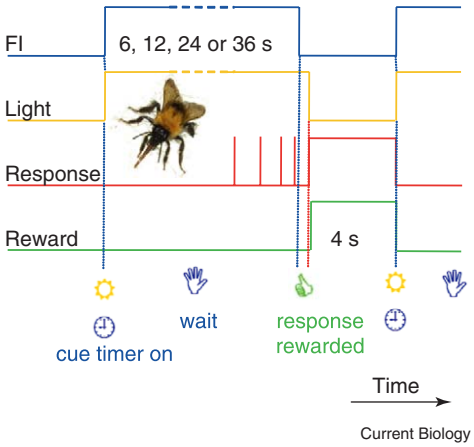

Figure 2. Diagram of the fixed-interval procedure used by Boisvert and Sherry [12].

Once a bee was in the experimental chamber and had consumed an initial reward by extending its proboscis through a hole in the wall, the interval timer was switched on (FI, upward deflection) and illumination of the chamber (light) served to indicate that timing was to begin. Any proboscis extensions (response) made by the bee during the timed interval had no effect, but the first response following the end of the interval triggered delivery of a sucrose reward and also switched off the light. After a delay of 4 seconds to allow reward consumption, the next trial in the session was initiated by switching the light and interval timer on again. A session consisted of 20 trials. Different experimental groups received either long and short intervals in separate sessions, or long and short intervals (for example 12 and 36 seconds) randomly intermixed , to test the bees' concurrent timing ability.

extension — during this fixed interval has no effect, but the first response after the time interval has elapsed triggers sucrose reward (Figure 2). When rats and pigeons are trained with similar procedures, the animal soon learns about the time delay involved, and then typically withholds its response for the first part of the fixed interval. Boisvert and Sherry [12] found that bumblebees behave in a similar manner. When trained on fixed intervals of either 12 or 24 seconds, responses were delayed appropriately. The delay from the beginning of the timed interval to the first response — the wait time — was significantly longer for the 24 second than for the 12 second intervals. In both cases, however, mean wait times accounted for about a third of the interval duration, and the maximal rate of response occurred at or near the end of the time interval. The bees, therefore, can predict the anticipated end of the interval and delay their responses accordingly.

In a second experiment, Boisvert and Sherry [12] probed the bees' ability to time different intervals concurrently, by mixing fixed intervals of either short or long duration. What would happen when long and short intervals were randomly mixed, so that the bees could not anticipate whether the current interval was to be long or short? Would the bees learn and attempt to track the durations of both intervals? One would expect an initial response in anticipation of the end of the short interval, followed by a later response in anticipation of the end of the long interval (on those trials in which the short interval expired with no reward). The bees did indeed behave as if they were initially timing the shorter interval, although the overall rate of response did not show two clearly distinguishable peaks. Nevertheless, detailed examination of within-trial response patterns were suggestive of concurrent timing: during long interval trials, responses tended to occur in bursts, with an early burst centred near the end of the short interval, followed significantly later by a second burst anticipating the end of the long interval.

Unequivocal evidence that bees can concurrently time multiple intervals would require demonstrating that response onset and offset bracket the anticipated end times of both the short and the long intervals. This could be done by omitting the sugar reward, but will require many more experiments, as it means interspersing occasional unreinforced trials among many more reinforced ones. Nevertheless, it seems clear that Boisvert and Sherry's [12] bees are predicting the future, in so far as their behaviour suggests an expectation tuned to the lapse of different time intervals; timing, by definition, is attending to the future.

Time, in vertebrates, can partly be described as a mental magnitude that obeys Weber's law [13] — that is, like a perception mediated by the conventional senses. According to Weber's law the precision of a sensory quality scales with the magnitude: for example, you could distinguish a 10 gram weight from one of 20 grams, or 1000 grams from 2000 grams, but probably not 1000 grams from 1020 grams. Similarly, if an organism can time (say) a 6 second interval with ±2 seconds accuracy, then its accuracy on a 60 second interval will likely be ±20 seconds [13]. Further work is required before this can be confirmed for insects. Interestingly — though perhaps not surprisingly, considering the reciprocal of time is rate, or events over time — number (strictly speaking, countable quantity) also appears to be represented as a scalar variable according to Weber's law in vertebrates [14]. Rate is quantity over time, and quantity may be countable or non-countable. Given this, and given also the demonstrated cognitive capacities of bees [15], one might ask if they also have the cognitive ability to attend to countable quantity in time, as well as non-countable scalar duration.

In fact there is already some evidence that estimation of distance travelled by bees and ants can be modulated in a predictable way by countable quantity [16,17]. If it is implicit, say from considering optimal foraging, that an organism can represent time, then it seems to be equally so that it can represent quantity, and specifically, countable quantity or numerosity. Stomach distension in bees, for example, is a non-countable quantity, but the quantity of flowers visited is countable. The two together provide useful information concerning flower profitability. Velocity, again, is distance over time, and distance is formally non-countable. But if you are gazing out of the passenger window, your sensation of speed is not based on distance in this formal sense, it is based on

something countable, such as trees flashing past. Even if you are not actually counting, you are still having a sensation of countable quantity. Perhaps this is how numerosity is measured in animals. In a range of vertebrate species, the representation of time and numerosity seem to share common principles [14,18], as Kant posited; Boisvert and Sherry's [12] elegant demonstration of a timing sense in bees opens the way for further investigation of these fundamental questions in invertebrates.

References

1. Chittka, L., Williams, N.M., Rasmussen, H., and Thomson, J.D. (1999). Navigation without vision: bumblebee orientation in complete darkness. Proc. R. Soc. Lond. B *266*, 45–50.
2. Frisch, K.v. (1967). The dance language and orientation of bees (Cambridge: Harvard Univ. Press).
3. Menzel, R., Geiger, K., Joerges, J., Müller, U., and Chittka, L. (1998). Bees travel novel homeward routes by integrating separately acquired vector memories. Anim. Behav. *55*, 139–152.
4. Stanewsky, R. (2003). Genetic analysis of the circadian system in *Drosophila melanogaster* and mammals. J. Neurobiol. *54*, 111–147.
5. Chittka, L., Ings, T.C., and Raine, N.E. (2004). Chance and adaptation in the evolution of island bumblebee behaviour. Pop. Ecol. *46*, 243–251.
6. Saleh, N., Ohashi, K., Thomson, J.D., and Chittka, L. (2006). Facultative use of repellent scent marks in foraging bumblebees: complex versus simple flowers. Anim. Behav. *71*, 847–854.
7. Srinivasan, M.V., Zhang, S., Altwein, M., and Tautz, J. (2000). Honeybee navigation: nature and calibration of the 'odometer'. Science *287*, 851–853.
8. Chittka, L., and Tautz, J. (2003). The spectral input to honeybee visual odometry. J. Exp. Biol. *206*, 2393–2397.
9. Ivry, R.B., and Richardson, T.C. (2002). Temporal control and coordination: The multiple timer model. Brain Cognit. *48*, 117–132.
10. Gallistel, C.R., and Gibbon, J. (2000). Time, rate, and conditioning. Psychol. Rev. *107*, 289–344.
11. Bateson, M. (2003). Interval timing and optimal foraging. In Functional and Neural Mechanisms of Interval Timing, W.H. Meck, ed. (CRC Press), pp. 113–141.
12. Boisvert, M.J., and Sherry, D.F. (2006). Interval timing by an invertebrate, the bumble bee Bombus impatiens. Curr. Biol. *16*, 1636–1640.
13. Gibbon, J. (1977). Scalar expectancy theory and Weber's law in animal timing. Psychol. Rev. *84*, 279–325.
14. Gallistel, C.R., and Gelman, R. (2000). Non-verbal numerical cognition: from reals to integers. Trends Cogn. Sci. *4*, 59–65.
15. Menzel, R., and Giurfa, M. (2001). Cognitive architecture of a mini-brain: the honeybee. Trends Cogn. Sci. *5*, 62–71.
16. Chittka, L., and Geiger, K. (1995). Can Honey-bees count landmarks. Anim. Behav. *49*, 159–164.
17. Wittlinger, M., Wehner, R., and Wolf, H. (2006). The ant odometer: Stepping on stilts and stumps. Science *312*, 1965–1967.
18. Roberts, W.A., and Boisvert, M.J. (1998). Using the peak procedure to measure timing and counting processes in pigeons. J. Exp. Psychol. Anim. Behav. Process. *24*, 416–430.

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G-Protein Signaling: A New Branch in an Old Pathway

A recent study provides evidence for a new branch of the yeast mating pathway in which a G-protein alpha subunit directly activates phosphatidylinositol 3-kinase at endosomes.

Lee Bardwell

The signal transduction pathway by which yeast cells respond to peptide mating pheromone secreted by nearby cells is arguably one of the most well-understood signaling pathways in eukaryotes [1]. Nevertheless, workers in the field confidently expect the pathway to provide important insights into fundamental signaling mechanisms for decades to come. Few anticipated, however, that a completely new branch of the pathway had remained hidden from thousands of person-years of genetic and biochemical assault, waiting to be revealed by the right approach. Now Slessareva *et al.* [2], by cannily combining functional genomics with a revealing mutant allele, appear to have found such a branch.

G-protein-coupled receptor pathways respond to hormones, neurochemicals, light, odors, and tastes, and constitute a plurality of known drug targets [3]. As with other G-protein-coupled receptor pathways, when the yeast pheromone receptor binds ligand, it stimulates the alpha subunit of an associated heterotrimeric G protein to bind GTP. GTP-bound Gα then detaches from the receptor and releases the G$\beta\gamma$ subunits. One or both members of the newly liberated pair (Gα and G$\beta\gamma$) then go on to bind to downstream effectors and thus propagate the signal. Gα is also a GTPase; after hydrolyzing GTP to GDP, it rebinds G$\beta\gamma$, thus terminating signaling. In the yeast pheromone response pathway (Figure 1), G$\beta\gamma$ transmits the mating signal to a scaffolded mitogen-activated protein (MAP) kinase cascade [4]. The two MAP kinases in this pathway, Kss1 and Fus3, then phosphorylate transcription factors, cell-cycle regulators, and other targets that coordinate mating.

Gα proteins were first discovered in mammalian cells as 'transducers' that propagated signals from hormone receptors to second-messenger producers like adenylate cyclase [5]. For many years it was thought that G$\beta\gamma$ did nothing but bury the business end of Gα so that Gα could not signal until it scored some GTP and disengaged. Studies of the yeast mating pathway helped turn that dogma on its head, however, when genetic and (eventually) molecular studies showed that G$\beta\gamma$ transmitted the mating signal to downstream effectors like the Ste5 scaffold protein and the Ste20 protein kinase. As the evidence favoring a signaling role for yeast G$\beta\gamma$ mounted, most workers presumed that Gα did nothing more to transmit the signal than release G$\beta\gamma$ (and perhaps activate a desensitization pathway [6]). Now the dogma is chasing its tail, as the new work indicates a positive signaling role for yeast Gα as well.