6. Johnston, R.J., and Hobert, O. (2003). A microRNA controlling left/right neuronal asymmetry in *Caenorhabditis elegans*. Nature *426*, 845–849.
7. Stark, A., Brennecke, J., Russell, R.B., and Cohen, S.M. (2003). Identification of Drosphila MicroRNA Targets. PLoS Biol. *1*, 1–13.
8. Lai, E.C. (2004). Predicting and validating microRNA targets. Genome Biol. *5*, 115.
9. Bartel, D.P. and Chen, C.Z. (2004). Micromanagers of gene expression: the potentially widespread influence of metazoan miRNAs. Nat. Rev. Genet. *5*, 396–400.
10. Hobert, O. (2004). Common logic of transcription factor and miRNA action. Trends Biochem. Sci. *29*, 462–468.
11. Giraldez, A.J., Cinalli, R.M., Glasner, M.E., Enright, A.J., Thomson, M.J., Baskerville, S., Hammond, S.M., Bartel, D.P., and Schier, A.F. (2005). MicroRNAs regulate brain morphogenesis in zebrafish. Science, epub ahead of print [DOI: 10:1126/Science. 1109020].
12. Murchison, E.P., and Hannon, G.J. (2004). miRNAs on the move: miRNA biogenesis and the RNAi machinery. Curr. Opin. Cell Biol. *16*, 223–229.
13. Wienholds, E., Koudijs, M.J., van Eeden, F.J., Cuppen, E., and Plasterk, R.H. (2003). The microRNA-producing enzyme Dicer1 is essential for zebrafish development. Nat. Genet. *35*, 217–218.
14. Ciruna, B., Weidinger, G., Knaut, H., Thisse, B., Thisse, C., Raz, E., and Schier, A.F. (2002). Production of maternal-zygotic mutant zebrafish by germ-line replacement. Proc. Natl. Acad. Sci. USA *99*, 14919–14924.
15. Schier, A.F. (2001). Axis formation and patterning in zebrafish. Curr. Opin. Genet. Dev. *11*, 393–404.
16. Kanellopoulou, C., Muljo, S.A., Kung, A.L., Ganesan, S., Drapkin, R., Jenuwein, T., Livingston, D.M., and Rajewsky, K. (2005). Dicer-deficient mouse embryonic stem cells are defective in differentiation and centromeric silencing. Genes Dev. *19*, 489–501.
17. Chang, S., Johnston, R.J., Frokjaer-Jensen, C., Lockery, S., and Hobert, O. (2004). MicroRNAs act sequentially and asymmetrically to control chemosensory laterality in the nematode. Nature *430*, 785–789.
18. Chen, C.Z., Li, L., Lodish, H.F., and Bartel, D.P. (2004). MicroRNAs modulate hematopoietic lineage differentiation. Science *303*, 83–86.
19. Bernstein, E., Kim, S.Y., Carmell, M.A., Murchison, E.P., Alcorn, H., Li, M.Z., Mills, A.A., Elledge, S.J., Anderson, K.V., and Hannon, G.J. (2003). Dicer is essential for mouse development. Nat. Genet. *35*, 215–217.

Department of Biochemistry and Molecular Biophysics, Center for Neurobiology and Behavior, Columbia University, College of Physicians and Surgeons, New York, New York 10032, USA. E-mail: or38@columbia.edu

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Time Perception: Components of the Brain's Clock

We know the human brain contains some kind of clock, but determining its neural underpinnings and teasing apart its components have proven difficult. New work on the parietal cortex illustrates how single unit recording may be able to help.

Penelope A. Lewis[1] and Vincent Walsh[2]

Our brains measure time continuously. We are aware of how long we have been doing a particular thing, how long it has been since we last slept, and how long it will be until lunch or dinner. We are ready, at any moment, to make complex movements requiring muscle coordination with microsecond accuracy, or to decode temporally complex auditory signals in the form of speech or music. Our timing abilities are impressive, diverse and worthy of investigation. But they are not very well understood.

Many models of time perception have been put forward (for example, see [1–3]), collectively postulating a wide variety of different mechanisms. Regardless of their diversity, the models all agree that temporal information is processed in many ways: it is remembered, compared to other temporal information, combined with sensory information, and used in the production of motor outputs.

The holy grail of timing research is to understand the 'time-dependent process': a mechanism equivalent to a piezoelectric crystal in a man-made clock or the movement of a shadow on a sundial. This has proven an elusive goal, to the extent that ideas about how this mechanism might work remain near the level of conjecture. Researchers have had great difficulty in pinning timing-related activity in the brain to any specific type of function. This is largely because most time measurement tasks draw upon more than one process, making it difficult to tease the various components apart. In their recent study, Janssen and Shadlen [4] have shown how single unit recording can be used to partially bypass this issue.

Janssen and Shadlen [4] recorded time-sensitive responses in the lateral inferior parietal (LIP) cortex of the macaque. They trained two monkeys to perform a visual delay task: the monkeys first fixated a light, then, in response to a 'go' signal, moved their eyes to a peripheral visual target as quickly as possible (Figure 1). The delay between target onset and 'go' signal varied according to two schedules: a bimodal schedule in which the 'go' cue could come early or late, but not between 0.75 and 1.75 seconds, and a unimodal schedule in which it came between 0.5 and 2 seconds. The schedules were presented in alternating blocks. The observed neural spike frequency in LIP correlated with the expectancy — 'hazard function' — of the 'go' cue

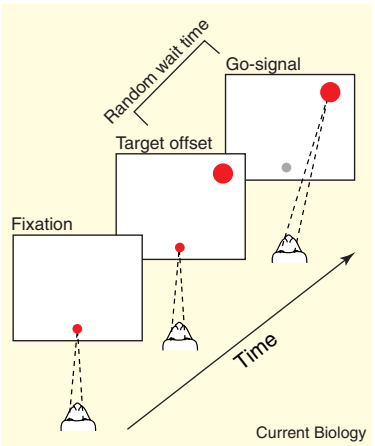

Figure 1. The task used by Janssen and Shadlen [4].

The monkey made eye movements to the red target as soon as the fixation point dimmed. Only trials in which the target appeared in the response field of the LIP neuron were reported. A bracket demarcates the random waiting time between target onset and 'go' signal. (Reproduced with permission [4].)

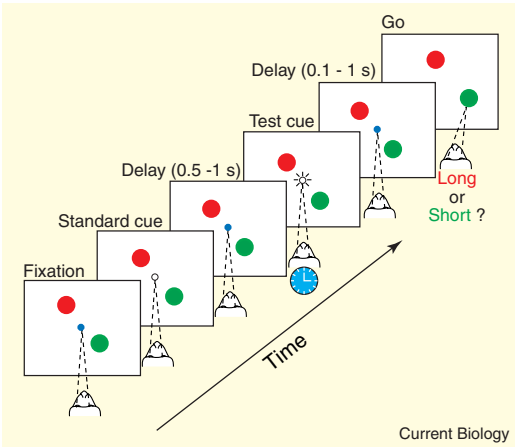

Figure 2. The time-discrimination task used by Leon and Shadlen [5].

A central, blue fixation point turned white for either 316 or 800 ms (the standard cue). After delay of between 500 and 1000 ms, the cue again turned white for a variable period (the test cue). Following a second variable delay between 100 and 1000 ms the blue fixation point disappeared and the monkey made a saccade to one of the two peripheral targets, indicating whether the monkey had judged the test cue to be longer (red) or shorter (green) than the standard cue.

for each of the two delay schedules.

These results build upon the findings of an earlier paper from the same group [5] in which LIP neurons were recorded during a temporal discrimination task. Monkeys fixated during presentation of a standard time interval, as defined by a light, followed by a variable probe interval defined in the same manner. They then indicated that the probe was *longer* than the standard by saccading to a peripheral green target, or *shorter* by saccading to a peripheral red target (Figure 2). Recordings from LIP neurons showed that those with the short (green) light in their receptive field responded at a high frequency until the duration of the standard had elapsed, at which time their response gradually decreased. Neurons with the long (red) light in their receptive field gradually increased responding, such that their response rates eventually 'crossed over' and exceeded those of the green light neurons.

Taken together, these datasets demonstrate that neurons in macaque LIP can respond to temporal information. The origin of that information and the purpose of such responses remain open for debate. If you are searching for the holy grail and you come across a shiny golden cup, it is natural to speculate that this is your object. Shadlen and colleagues have done so by suggesting that the neurons they observed measure time: ".. the monkey could base its judgement of time on the discharge of neurons with properties like the ones we observe" [5]. One of their arguments in favour of this interpretation is that the gradual change from high to low firing rates precludes input from an outside timer because the smooth shift in responding is inconsistent with information from a discreet decision. This pattern does not, however, exclude the involvement of input from a graded external timing signal [6].

In the more recent paper, Janssen and Shadlen [4] admit that they "cannot determine whether the timing-related anticipatory activity arises in area LIP or is simply passed to LIP from other structures that have been implicated in interval timing". Their results show that LIP responds along an expectation function or 'hazard rate' predicting the time of eye movements. It is unlikely that a central clock, providing time signals to a variety of brain regions for a variety of purposes, would compute such a function. These data therefore suggest either a localized parietal timer for eye movements or at least a localized calculation of the hazard rate based upon external timing signals. From the perspective of those not involved in finding the golden cup, the latter possibility appears just as likely as the former. It therefore seems imprudent to assume that these neurons actually measure time until more evidence is forthcoming. Furthermore, the pattern of response in these cells is not consistent with activity patterns predicted by network models of timing, for which one might expect either a periodic signal similar to the ticking of a clock [1,3] or a gradual and predictable ramping up or down of activity [2]. The authors have proposed no mechanism for such a function, and no mechanism is obvious from the literature at either the cellular or network level. Because LIP is widely connected, temporal information could easily be passed to it from other parts of the brain, a scenario which would be in better keeping with the large existing literature implicating structures such as cerebellum [7], basal ganglia [7,8], SMA [9] and dorsolateral prefrontal cortex [10,11] as the seat of time measurement. Thus, there is little evidence to suggest that this is the true grail, and quite a bit to suggest that it is not.

If LIP neurons do not measure time, what is the function of their temporally sensitive response? The most obvious possibility is a role in preparation for eye movements. This is certainly worth considering given that the neurons in question were selected because they responded during preparation for such movements. Leon and Shadlen [5] argue against this explanation, pointing out, amongst other things, that they did not find a correlation between the response functions of these neurons and eye movements. The subsequent demonstration by Janssen and Shadlen [4] of a correspondence between activity in these cells and the expectation that a movement will be cued undermines this argument, as does the observation that neural response frequency or 'expectation' is reflected in movement response times.

Taken together with the clear adaptive advantage conveyed by a tendency to prepare movement only when a cue to move is *expected,* these data provide

substantial support for the possibility that the LIP activity in question is associated with preparation for eye movement. The alternative interpretation offered by Janssen and Shadlen [4], "the intention-related signals seen in our experiments could underlie a shift in spatial attention that is not in competition with an eye movement plan" [4], is not obviously compelling. However, a related scenario in which these cells code for intentionality regarding a response, without being involved in a specific motor plan or tied to a specific motor affector, should also be considered.

The likelihood that LIP neurons do not actually measure time, and that their temporally sensitive responding codes instead for eye movement, do not render these findings uninteresting to the field of time measurement. The parietal cortex is frequently activated in neuroimaging studies of timing — for example, see [8,12], but see also review [11] — and damage to this region [13], as well as temporary disruption via transcranial magnetic stimulation, have both been shown to cause temporal deficits. A number of authors have speculated about the role of parietal cortex in temporal processing, with some papers [8,14] suggesting an attentional function, while another[15] suggests a system for calculating magnitude. Until now, little has been known about how these involvements could be manifest at the neural level.

The demonstration by Janssen and Shadlen [4] that individual LIP neurons can respond to visuo-temporal information confirms that temporal information is available to the parietal cortex. These findings also provide novel and welcome insight into what this area may be doing in time measurement tasks, suggesting that the role of parietal cortex in multisensory integration and the planning of action extends to the modality of time. Thus, by exploring temporal processing in the parietal cortex with single unit recording, Shadlen's group has taken a useful step towards describing the type of temporal processes performed in that region. Their work is also novel because it illustrates the potential of single unit recording as a tool for discrimination between the different forms of temporal processing: perception, memory, preparation for movement, and so on. Similar work in other structures associated with timing may lead to even greater insights. Thus, Shadlen and colleagues may not have found the holy grail of timing research, but they have certainly discovered a treasure trove of information which will undoubtedly lead to a better understanding of this system. And really, its about *time.*

References

1. Gibbon, J. (1977). Scalar expectancy theory and Weber's law in animal timing. Psychol. Rev. *84*, 279–325.
2. Staddon, J.E., and Higa, J.J. (1999). Time and memory: towards a pacemaker-free theory of interval timing. J. Exp. Anal. Behav. *71*, 215–251.
3. Grossberg, S., and Schmajuk, N.A. (1989). Neural dynamics of adaptive timing and temporal discrimination during associative learning. Neural Netw. *2*, 79–102.
4. Janssen, P., and Shadlen, M.N. (2005). A representation of the hazard rate of elapsed time in macaque area LIP. Nat. Neurosci. *8*, 234–241.
5. Leon, M.I., and Shadlen, M.N. (2003). Representation of time by neurons in the posterior parietal cortex of the macaque. Neuron *38*, 317–327.
6. Walsh, V. (2003). Time: the back-door of perception. Trends Cogn. Sci. *7*, 335–338.
7. Ivry, R.B., and Spencer, R.M. (2004). The neural representation of time. Curr. Opin. Neurobiol. *14*, 225–232.
8. Rao, S.M., Mayer, A.R., and Harrington, D.L. (2001). The evolution of brain activation during temporal processing. Nat. Neurosci. *4*, 317–323.
9. Macar, F., Vidal, F., and Casini, L. (1999). The supplementary motor area in motor and sensory timing: evidence from slow brain potential changes. Exp. Brain Res. *125*, 271–280.
10. Onoe, H., Komori, M., Onoe, K., Takechi, H., Tsukada, H., and Watanabe, Y. (2001). Networks recruited for time perception: a monkey positron emission tomography (PET) study. Neuroimage *13*, 37–45.
11. Lewis, P.A., and Miall, R.C. (2003). Distinct systems for automatic and cognitively controlled time measurement: evidence from neuroimaging. Curr. Opin. Neurobiol. *13*, 250–255.
12. Coull, J.T., Vidal, F., Nazarian, B., and Macar, F. (2004). Functional anatomy of the attentional modulation of time estimation. Science *303*, 1506–1508.
13. Harrington, D.L., Haaland, K.Y., and Knight, R.T. (1998). Cortical networks underlying mechanisms of time perception. J. Neurosci. *18*, 1085–1095.
14. Macar, F., Lejeune, H., Bonnet, M., Ferrara, A., Pouthas, V., Vidal, F., and Maquet, P. (2002). Activation of the supplementary motor area and of attentional networks during temporal processing. Exp. Brain Res. *142*, 475–485.
15. Walsh, V. (2003). A theory of magnitude: common cortical metrics of time, space and quantity. Trends Cogn. Sci. *7*, 483–488.

[1]Institute of Science and Culture, 187 The Terrace, Wellington, New Zealand. E-mail: plewis@fil.ion.ucl.ac.uk
[2]Institute of Cognitive Neuroscience & Department of Psychology, University College London, 17 Queen Square, London WC1N 3AR, UK.

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Axis Formation: Redundancy Rules

The role of BMP antagonists in the Spemann-Mangold organizer of vertebrate embryos is a controversial issue. A study using combined knock down of multiple antagonists finally reveals dramatic effects.

Christof Niehrs

The Spemann-Mangold organizer of vertebrate embryos plays a paramount role during embryogenesis by releasing a cocktail of molecules that induce the embryonic axes and various cell fates. Bone morphogenetic protein (BMP) antagonists are an important class of such inducers, as was discovered in *Xenopus*, where their over-expression has dramatic effects, such as inducing a secondary embryonic axis. By contrast, studies in higher vertebrates — such as chicken and mouse — have yielded less impressive results and have led to a controversy over how important BMP antagonists really are and what their precise role is. In a bold approach, Khoka *et al*. [1] have now knocked down in parallel three BMP antagonists in *Xenopus* embryos and observe dramatic effects on embryonic axis formation.