17. Rosenberg, N. D. *et al.* Estimation of heat and chemical fluxes from a seafloor hydrothermal vent field using radon measurements. *Nature* **334,** 604–607 (1988).
18. Thomson, R. E., Delaney, J. R., McDuff, R. E., Janecky, D. R. & McClain, J. S. Physical characteristics of the Endeavour Ridge hydrothermal plume during July 1988. *Earth Planet. Sci. Let.* **111,** 141–154 (1992).
19. Lupton, J. E., Delaney, J. R., Johnson, H. P. & Tivey, M. K. Entrainment and vertical transport of deep-ocean water by buoyant hydrothermal plumes. *Nature* **316,** 621–623 (1985).

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Evolutionary capacitance as a general feature of complex gene networks

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An evolutionary capacitor buffers genotypic variation under normal conditions, thereby promoting the accumulation of hidden polymorphism. But it occasionally fails, thereby revealing this variation phenotypically[1]. The principal example of an evolutionary capacitor is Hsp90, a molecular chaperone that targets an important set of signal transduction proteins. Experiments in *Drosophila* and *Arabidopsis* have demonstrated three key properties of Hsp90: (1) it suppresses phenotypic variation under normal conditions and releases this variation when functionally compromised; (2) its function is overwhelmed by environmental stress; and (3) it exerts pleiotropic effects on key developmental processes[1,2]. But whether these properties necessarily make Hsp90 a significant and unique facilitator of adaptation[1–10] is unclear. Here we use numerical simulations of complex gene networks, as well as genome-scale expression data from yeast single-gene deletion strains, to present a mechanism that extends the scope of evolutionary capacitance beyond the action of Hsp90 alone. We illustrate that most, and perhaps all, genes reveal phenotypic variation when functionally compromised, and that the availability of loss-of-function mutations accelerates adaptation to a new optimum phenotype. However, this effect does not require the mutations to be conditional on the environment. Thus, there might exist a large class of evolutionary capacitors whose effects on phenotypic variation complement the systemic, environment-induced effects of Hsp90.

Several studies on other genes than that encoding Hsp90 have also reported increased phenotypic variance in mutant strains[11,12]. These results have been taken as evidence that wild-type organisms are buffered, or 'canalized', against environmental and genetic variation[13]. We previously demonstrated that canalization does not require a dedicated mechanism (such as that provided by chaperone proteins), but instead arises as an emergent property of complex developmental–genetic networks that reach a steady state

of gene expression[14]. We therefore proposed that a loss of buffering might be induced by compromising the function of an evolved network, namely by removing the function of an arbitrary gene in the network. To test this hypothesis, we use simulations of gene networks and ask how an arbitrary null ('knockout') mutation affects the expression of other genes. Does a network with a single gene knocked out exhibit a greater range of phenotypes, across environmental conditions or genetic backgrounds, than does the wild-type network from which it was derived? If so, the knocked-out gene has the potential to be an evolutionary capacitor.

First we consider the effect of knockouts in different genetic backgrounds. We use a representation of a gene network in which each of N genes produces a factor that is capable of influencing the expression of each other gene as well as itself[14,15] (see Fig. 1 and Supplementary Information; $N = 10$ for all simulations presented here). An $N \times N$ matrix encapsulating the regulatory relationships is considered the 'genotype' of an individual. Unlike standard quantitative-genetic and population-genetic frameworks, this representation lends itself to the study of developmental buffering, because development is explicitly modelled as the progression from an initial gene-expression state to a state of gene-expression equilibrium. The equilibrium gene-expression profile is considered to be the 'phenotype', and those genotypes that do not produce an equilibrium state are considered lethal (see refs 14 and 15 for a justification of this definition of lethality). A knockout of a gene is represented by zeroing the corresponding row and column of the regulatory matrix.

To study a population of individuals that differ in genotype but, for the most part, have the same phenotype, we perform 100 evolutionary simulations in which a randomly generated, non-lethal individual founds a population of size 500 that is allowed to evolve, with mutation of the elements of the regulatory matrix, for 400 generations. During evolution there is strong selection both against lethal disruptions in gene-expression equilibrium and for the phenotype of the founder individual, as described previously[14]. Setting the founder's phenotype as optimal in this way ensures that the range of phenotypes in the final population of 500 individuals at generation 400 is very narrow, thus permitting comparison with the range of phenotypes seen in the knockout derivatives of each of the 500 individuals. Out of 1,000 such comparisons (100 simulations × 10 gene knockouts), only 4 showed higher phenotypic variation for the wild-type population than for its knockout derivatives ($P \approx 0$, sign test; for a detailed analysis see Supplementary Information). Results of a typical simulation are shown in
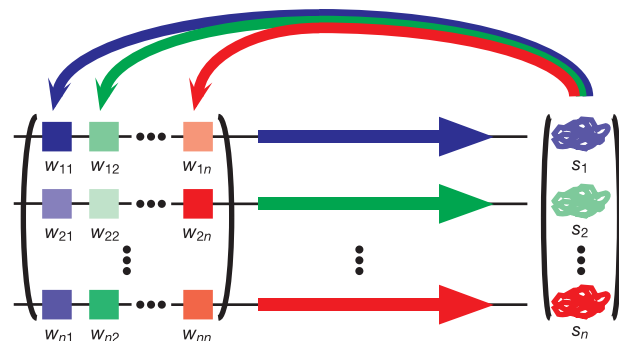

Figure 1 Representation of a gene network. Each gene (horizontal arrow) is regulated by the products of the other genes by means of upstream enhancer elements (boxes). The strength and direction of regulation (depicted as different colour saturation levels) are a function of both the regulatory element and the abundance of its corresponding gene product. Genotype is represented as the matrix, W, of regulatory interactions, and phenotype is the vector, $\hat{S}$, of gene-product levels at equilibrium.

Fig. 2. The range of phenotypes in the final population is indeed quite narrow, with nearly all individuals having the optimum phenotype. In striking contrast, the range of phenotypes seen when each individual has a given gene knocked out is quite broad. As shown in Fig. 2, the exact proportion of genetic backgrounds on which a knockout is lethal, and also the distribution of non-lethal phenotypes, depend on which gene is knocked out, but the evolved population clearly harbours genetic variation that is not expressed phenotypically unless a gene is knocked out. Indeed, each knockout reveals phenotypic variation that is not seen in the wild-type population. Interestingly, the most frequent phenotypic class is generally that corresponding to the optimum phenotype, indicating that the evolved networks have some robustness even to these major-effect mutations. Also noteworthy is that the property of buffering genetic variation seems to be relatively independent of the exact network topology, because we observed it in all simulations performed as described here and also in similar simulations in which the network of the population founder was generated not from a Poisson distribution of connections but rather from a power-law distribution of outgoing connections, creating a so-called 'scale-free' topology, which has been inferred for the gene-regulation network of yeast[16] and other biological networks[17]. We therefore conclude that arbitrary genes in complex gene networks have the property of buffering genetic variation and therefore have the potential to act as evolutionary capacitors.

As Hsp90's effects are dependent on the environment, and as the relationship between genetic and environmental variation features prominently in theoretical considerations of canalization[10,18], we also investigated the effect of knockouts on individuals subject to environmental 'noise'. Noise is introduced into the model used above by the random perturbation of an individual's initial gene-expression state. The variance in phenotypes induced by variation in initial conditions is then measured for an individual and for each single-gene knockout derivative of that individual. In addition to this comparison between wild type and knockout, we also compare individuals 'before' and 'after' evolution, to assess the effect of genetic canalization on the response to environmental perturbation.

The evolutionary simulations are performed as above (but without selection for an optimum phenotype), and from each the population founder is assayed as a before-evolution individual, and the most canalized (that is, the least sensitive to mutations) descendant present in the final generation is assayed as an after-evolution individual. Thus, each simulation contributes to four categories of individuals tested for their sensitivity to initial conditions: wild type before evolution, knockout before evolution, wild type after evolution, and knockout after evolution.

Both for before-evolution and for after-evolution individuals, the average variance in gene expression across all genes tends to be lower for a wild-type individual subjected to environmental noise than for its knockout derivatives under the same conditions. The amplitude of noise required to induce non-zero variance differs from individual to individual, so for each comparison between wild type and knockout we assayed a range of noise levels and used the lowest level at which the wild-type and knockout individuals had different variance. By this measure, of 500 wild-type before-evolution individuals, 434 displayed lower gene-expression variance than in the corresponding knockouts, and 66 displayed higher variance than in the corresponding knockouts. The results are similar for after-evolution individuals, with 479 lower in the wild type and 21 higher in the wild type. Notice, however, that for the after-evolution individuals there is a greater proportion of wild-type individuals with lower variance than the corresponding knockouts (G-test with Williams correction, $P = 2.6 \times 10^{-7}$). This could be a result of either a lower sensitivity to initial conditions for the after-evolution wild-type individuals or a greater sensitivity to initial conditions for the knockout derivatives of the after-evolution individuals. The former seems to be true. Of 500 pairs of before-evolution and after-evolution individuals, 390 displayed higher gene-expression variance in the before-evolution individual than in the after-evolution individual; for the corresponding knockouts, 364 displayed higher gene-expression variance before evolution than after evolution. Thus, not only are evolved individuals less sensitive to initial conditions, but their knockout derivatives are also less sensitive (no significant difference between wild type and
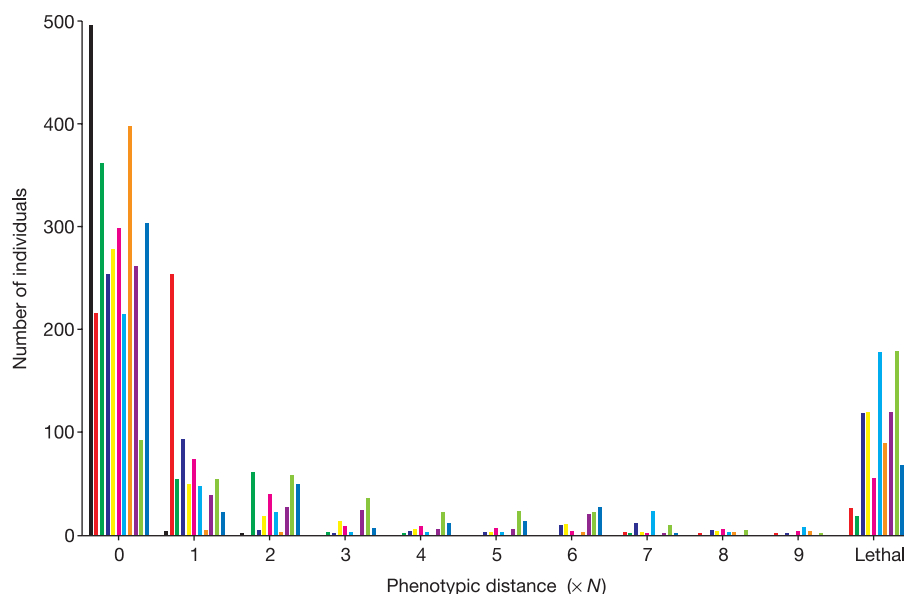

Figure 2 Greater phenotypic variation in single-gene knockouts than in the wild-type networks from which they derive. The histogram shows results of a typical simulation, in which a population of 500 individuals evolved for 400 generations under strong stabilizing selection. Black bars show the distribution of phenotypes in the final population, as the phenotypic distance (defined previously[14]) to the selected optimum (phenotypic distances are multiplied by N, the number of genes in the network (10 for wild type, 9 for knockouts)). Each set of bars of the same colour shows the distribution of phenotypes resulting from introducing a given single-gene knockout into each of the 500 members of the final population. The rightmost group of bars shows the number of lethal genotypes for each knockout.

knockouts in this respect, $P = 0.056$). We conclude that the evolved individuals, although not selected directly to become less sensitive to variation in initial conditions, have achieved this property. It is also clear that knockout mutations significantly increase the sensitivity to initial conditions of both before-evolution and after-evolution individuals. These results were not noticeably changed when the evolved individuals were also subject to selection for a phenotypic optimum (not shown), similar to what was seen previously for the evolution of canalization itself[14].

At this point one might ask whether there is any corroborating experimental evidence that knockout mutations tend to increase variation in the expression of other genes. Indeed there is: a systematic project has been undertaken to delete in turn each gene in the yeast *Saccharomyces cerevisiae*; each knockout strain is then assayed for the expression levels of all remaining genes as well as for its growth rate relative to the other strains[19–21]. We have analysed the available data from this project for evidence that the buffering of other genes' expression levels is disrupted when one gene is knocked out. The yeast strains are isogenic, so it is buffering against environmental, not genetic, variation that is being probed. However, this distinction is not likely to be important because it has been argued that environmental and genetic buffering are likely to be achieved by the same mechanisms[10,22], and because our simulations above indicate that the evolution of genetic canalization makes individuals less sensitive to environmental variation as well. In an initial analysis of the yeast data[20] a measure called a 'scale factor' was introduced, which was calculated for each gene using data from 63 wild-type control experiments and captures the degree of biological variability in expression for that gene. When we do the same calculation using instead the expression data from 53 knockouts, the resulting value for each gene tends to be higher than its scale factor; this is true even for those genes that do not show any evidence of being a regulatory target of any one of the knockouts ($P < 0.0001$, Friedman's test). Thus, deleting a gene tends to increase the variation in expression of other genes. Moreover, the increase in gene-expression variation is linked to phenotypic variation, in that the magnitude of revealed variation is negatively correlated with fitness in the standard growth condition: knockout strains with lower fitness tend to be those with greater variation in the expression levels of the remaining non-target genes and vice versa (product-moment correlation $r = -0.1973$, significant at $P = 0.0278$ by non-parametric permutation test).

So far we have shown that removing the function of an arbitrary gene tends to increase the variance in expression of other genes in its network, ultimately increasing the phenotypic variance. The key question now is whether this effect has important evolutionary consequences. Because one of the special features of Hsp90 is the environmental sensitivity of its function, a related question is whether only genes with conditional alleles can serve as evolutionary capacitors. To answer these questions we perform evolutionary simulations as before, but with an additional mutational process by which a gene's activity can be knocked out and, at a lower rate, restored to function. We allow populations to evolve for 400 generations under strong selection for an optimum phenotype, then shift to a new optimum at which the equilibrium expression states of three of the 10 network genes flip from on to off or vice versa. We simulated 200 founder populations with their corresponding initial and shifted optima, and allowed each population to evolve under two models: with and without the knockout mutation process. Populations evolving with the occurrence of knockout mutations reached the new optimum an average of 77 generations faster than those with no knockouts (314 generations with knockouts compared with 391 generations without; $P = 0.0030$, Friedman's test), indicating both that evolutionary capacitance does

indeed speed adaptation to a new phenotypic optimum and that mutations that disrupt buffering need not be conditional on the environment that enforces the new optimum. Interestingly, if the population is shifted to its new optimum immediately after its founding (instead of after 400 generations), the average number of generations to reach the new optimum is not significantly dependent on the presence or absence of knockout mutations (379 generations with knockout compared with 461 generations without; $P = 0.066$). This indicates that the accumulation of allelic variation during the evolution of a canalized genetic system might be a prerequisite for the adaptive advantage of loss-of-function mutations. The interplay between accumulated, but hidden, variation and its knockout-induced revelation could provide a mechanistic link to a theoretical model that predicts that selection towards a new optimum promotes decanalization[23].

We showed previously that genetic canalization, or phenotypic insensitivity to mutation, is an emergent property of complex gene networks, in that such networks evolve greater insensitivity to mutation without direct selection for this property and even without selection for an optimum phenotype[14]. Here we have extended this result by showing that both environmental canalization and genetic canalization break down when an arbitrary gene is deleted and that the availability of knockout mutations increases the rate of adaptation to a new phenotypic optimum. These results indicate that evolutionary capacitance is not limited to dedicated buffering mechanisms such as Hsp90 but might also be exhibited by many other genes, by virtue of their being part of a complex genetic network. This finding is consistent with the previously mentioned studies[11,12] demonstrating increased phenotypic variation in mutants compared with the wild type. It is also consistent with theoretical models that predict an increased phenotypic effect of new mutations after mutating a gene with epistatic relationships to other genes[24,25], although such models do not predict canalization to exist unless selectively favoured.

Hsp90 has three features that make it appealing as a candidate evolutionary capacitor: its effect of revealing phenotypic variation when its function is impaired or overwhelmed, its pleiotropic effects on several key developmental processes, and the environmental sensitivity of its buffering capacity. We have presented an alternative mechanism with partly overlapping features. The revelation of phenotypic variation is central to the proposed mechanism, as it is to Hsp90, and we have shown that this property is expected to be shared by many genes. These genes will differ in their degree of pleiotropy, but it is likely that collectively they affect a range of phenotypes as broad as, or broader than, that affected by Hsp90; even individually some might approach the level of pleiotropy of Hsp90, because key genetic pathways tend to be reused during development. Sensitivity to environmental conditions is also not unique to Hsp90, because conditional (for example, temperature-sensitive) alleles are fairly commonly recovered in genetic screens. Conditionality might also be achieved by other mechanisms; for example, a gene's function might be eliminated by the insertion of a transposable element and then later restored by excision of the element[26]. Beyond this, our results suggest that conditionality is not an absolute requirement of an evolutionary capacitor, because in our simulations the existence of non-conditional knockouts accelerated adaptation to a new phenotypic optimum. Why are such knockouts tolerated, let alone favoured during adaptation to a new optimum? The answer might lie in robustness itself. That is, some genes might exist solely to stabilize the function of a network; these genes would seem expendable in the laboratory, yet would be selectively maintained in nature until their loss facilitated adaptation. The existence in model organisms of many gene knockouts without apparent phenotypic effect[27] lends plausibility to this idea. It remains to be seen what types of mutations other than knockouts can serve to decanalize a genetic system. In particular, it will be important to explore the effects of partial loss-of-function

mutations, perhaps in the context of a diploid model analogous to the haploid one presented here.

Hsp90 might be a member of a fairly large class of genes with the ability to serve as evolutionary capacitors, most of which might have more subtle effects than Hsp90 has. The existence of this complementary set of capacitors means that adaptation by means of the release of canalized variation might be possible even under conditions that do not involve chronic environmental stress, such as sexual selection and predator–prey 'arms races'. The proposed capacitance mechanism could be tested by the analysis of genotypic and phenotypic data from populations of a laboratory microorganism, such as *S. cerevisiae* or *Escherichia coli*, at successive stages in adaptation to an artificial selection regime. As for natural adaptations, establishing the role of Hsp90 or another potential capacitor in facilitating the evolution of any particular trait will require intensive investigation of the relevant ecological, population-biological and evolutionary-genetic history.   □

Methods

Gene networks and their evolution were modelled as described previously[14]. The original model, and extensions of it used here, are described fully in Supplementary Information. The yeast gene-expression data are from ref. 20. The statistical tests presented use the same stringent criterion for declaring a gene a regulatory target of the knocked out gene as that used by those authors. To ensure the robustness of our results we repeated the tests with a range of more lenient criteria; in all cases the test results remained significant. Details of our analysis, including a list of microarray experiments used, are also available in Supplementary Information.

1. Rutherford, S. L. & Lindquist, S. Hsp90 as a capacitor for morphological evolution. *Nature* **396**, 336–342 (1998).
2. Queitsch, C., Sangster, T. A. & Lindquist, S. Hsp90 as a capacitor of phenotypic variation. *Nature* **417**, 618–624 (2002).
3. Cossins, A. Cryptic clues revealed. *Nature* **396**, 309–310 (1998).
4. Dickinson, W. J. & Seger, J. Cause and effect in evolution. *Nature* **399**, 30 (1999).
5. McLaren, A. Too late for the midwife toad: Stress, variability and Hsp90. *Trends Genet.* **15**, 169–171 (1999).
6. Wagner, G. P., Chiu, C.-H. & Hansen, T. F. Is Hsp90 a regulator of evolvability? *J. Exp. Zool.* **285**, 116–118 (1999).
7. Rutherford, S. L. From genotype to phenotype: buffering mechanisms and the storage of genetic information. *BioEssays* **22**, 1095–1105 (2000).
8. Pigliucci, M. Developmental genetics: buffer zone. *Nature* **417**, 598–599 (2002).
9. Mitchell-Olds, T. & Knight, C. A. Chaperones as buffering agents? *Science* **296**, 2348–2349 (2002).
10. Meiklejohn, C. D. & Hartl, D. L. A single mode of canalization. *Trends Ecol. Evol.* **17**, 468–473 (2002).
11. Scharloo, W. Canalization: Genetic and developmental aspects. *Annu. Rev. Ecol. Syst.* **22**, 65–93 (1991).
12. Gibson, G. & Wagner, G. Canalization in evolutionary genetics: A stabilizing theory? *BioEssays* **22**, 372–380 (2000).
13. Waddington, C. H. Canalization of development and the inheritance of acquired characters. *Nature* **150**, 563–565 (1942).
14. Siegal, M. L. & Bergman, A. Waddington's canalization revisited: Developmental stability and evolution. *Proc. Natl Acad. Sci. USA* **99**, 10528–10532 (2002).
15. Wagner, A. Does evolutionary plasticity evolve? *Evolution* **50**, 1008–1023 (1996).
16. Featherstone, D. E. & Broadie, K. Wrestling with pleiotropy: Genomic and topological analysis of the yeast gene expression network. *BioEssays* **24**, 267–274 (2002).
17. Ravasz, E., Somera, A. L., Mongru, D. A., Oltvai, Z. N. & Barabási, A.-L. Hierarchical organization of modularity in metabolic networks. *Science* **297**, 1551–1555 (2002).
18. Wagner, G. P., Booth, G. & Bagheri-Chaichian, H. A population genetic theory of canalization. *Evolution* **51**, 329–347 (1997).
19. Winzeler, E. A. et al. Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. *Science* **285**, 901–906 (1999).
20. Hughes, T. R. et al. Functional discovery via a compendium of expression profiles. *Cell* **102**, 109–126 (2000).
21. Giaever, G. et al. Functional profiling of the *Saccharomyces cerevisiae* genome. *Nature* **418**, 387–391 (2002).
22. Stearns, S. C., Kaiser, M. & Kawecki, T. J. The differential genetic and environmental canalization of fitness components in *Drosophila melanogaster*. *J. Evol. Biol.* **8**, 539–557 (1995).
23. Rice, S. H. The evolution of canalization and the breaking of von Baer's laws: Modeling the evolution of development with canalization. *Evolution* **52**, 647–656 (1998).
24. Wagner, G. P. & Mezey, J. Modeling the evolution of genetic architecture: A continuum of alleles model with pairwise A × A epistasis. *J. Theor. Biol.* **203**, 163–175 (2000).
25. Hansen, T. F. & Wagner, G. P. Modeling genetic architecture: A multilinear theory of gene interaction. *Theor. Popul. Biol.* **59**, 61–86 (2001).
26. Edwards, R. J. & Brookfield, J. F. Y. Transiently beneficial insertions could maintain mobile DNA sequences in variable environments. *Mol. Biol. Evol.* **20**, 30–37 (2003).
27. Tautz, D. A genetic uncertainty problem. *Trends Genet.* **16**, 475–477 (2000).

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Organization of cell assemblies in the hippocampus

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Neurons can produce action potentials with high temporal precision[1]. A fundamental issue is whether, and how, this capability is used in information processing. According to the 'cell assembly' hypothesis, transient synchrony of anatomically distributed groups of neurons underlies processing of both external sensory input and internal cognitive mechanisms[2–4]. Accordingly, neuron populations should be arranged into groups whose synchrony exceeds that predicted by common modulation by sensory input. Here we find that the spike times of hippocampal pyramidal cells can be predicted more accurately by using the spike times of simultaneously recorded neurons in addition to the animals location in space. This improvement remained when the spatial prediction was refined with a spatially dependent theta phase modulation[5–8]. The time window in which spike times are best predicted from simultaneous peer activity is 10–30 ms, suggesting that cell assemblies are synchronized at this timescale. Because this temporal window matches the membrane time constant of pyramidal neurons[9], the period of the hippocampal gamma oscillation[10] and the time window for synaptic plasticity[11], we propose that cooperative activity at this timescale is optimal for information transmission and storage in cortical circuits.

In sensory brain regions, the temporal pattern of spikes can correlate precisely with the time course of an external stimulus[12–14]. In high-level structures, however, neural responses are often more variable than is expected from sensory control[15]. Is this variability simply noise[16], or does it reflect the operation of internal, non-sensory processes? In the hippocampus, the timing of pyramidal cell spikes with respect to a clock ('theta') rhythm is correlated with the animal's location in space[5,6]. This timing does not reflect the occurrence of external sensory events precisely timed with respect to the theta rhythm, but rather must arise because of dynamics internal to the brain, a conclusion that is reinforced by the existence of similar phenomena during non-spatial behaviours[7].

We have investigated the hypothesis that hippocampal neurons are organized in time into 'cell assemblies' whose activity can reflect both external sensory input and internal cognitive processes[2,17]. A signature of assembly organization is the existence of anatomically distributed groups of neurons whose activity is synchronized more than is predicted by common sensory modulation. A second postulated signature is that, although individual neurons may participate in many assemblies, not every possible combination of