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.

Received 26 November 2002; accepted 24 April 2003; doi:10.1038/nature01765.

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).

Supplementary Information accompanies the paper on **www.nature.com/nature**.

Acknowledgements We thank B. Baker, D. Hartl, J. Hermisson, D. Kennedy, J. Masel, C. Meiklejohn, D. Petrov, G. Wagner, C. Yanofsky and E. Zuckerkandl for helpful discussions. This study was supported by the Center for Computational Genetics and Biological Modeling, Stanford University. M.L.S. was supported by a National Institutes of Health National Research Service Award Individual Postdoctoral Fellowship, and thanks B. Baker for his support. A.B. thanks the Paul G. Allen Charitable Foundation for its continual support.

Competing interests statement The authors declare that they have no competing financial interests.

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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

cells comprises a cell assembly. The latter feature should be reflected by a statistical preference in the probability with which a neuron joins its various peers in synchronous firing.

The activity of simultaneously monitored pyramidal cells, with neurons arranged according to their physical position within the CA1 region, showed no apparent spatio-temporal patterns[18,19] (Fig. 1a, b). When the units were rearranged so that synchronously firing cells were displayed near each other, however, the patterns were often suggestive of a cell assembly organization, with different sets of cells repeatedly showing synchronous activity at different times (Fig. 1c).

To verify that the visualized assembly organization was not simply due to chance coincidences of spikes, we used a 'peer prediction' method (Fig. 2). Because hippocampal pyramidal cells have place correlates[20], their instantaneous firing probabilities can be predicted from the spatial position of the rat. Under the assumption of a 'firing rate code' for position[16,21,22], this is the best prediction that can be made for a cell's activity. By contrast, the cell assembly hypothesis predicts that cells should show synchronous activation beyond that predicted by their common modulation by sensory input. Thus, it should be possible to predict a cell's exact spike times better from the activity of simultaneously recorded assembly members than from spatial position alone.

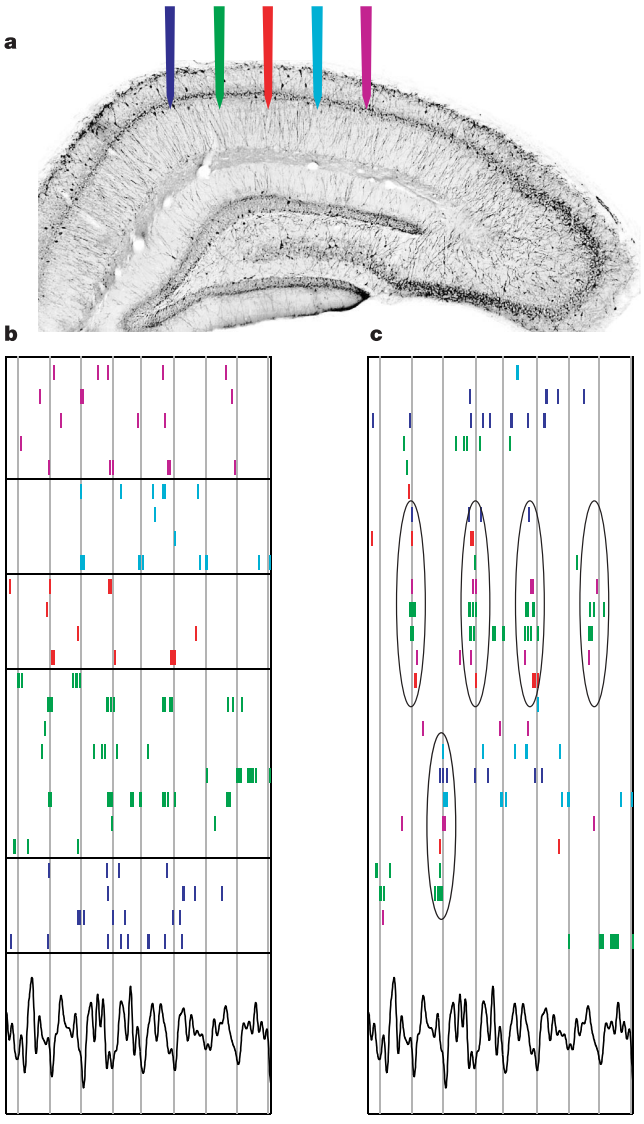

Figure 1 Cell assembly activity in a population. **a**, Location of the recording electrodes. **b**, **c**, Raster plots of 25 pyramidal cells that were active during a 1-s period of spatial exploration out of 68 simultaneously recorded neurons. **b**, Neurons are arranged in order of physical position in the CA1 pyramidal layer (colour-code refers to locations in **a**). Vertical lines indicate troughs of theta waves (bottom trace). Location-specific synchrony is not apparent in the population activity. **c**, The same spike rasters shown in **b**, reordered by stochastic search over all possible orderings to highlight synchrony between anatomically distributed populations. 'Cell assembly' organization is now visible, with repeatedly synchronous firing of some subpopulations (circled).

To predict the spike train of a target cell, we estimated the discharge probability of the target cell at every instant of time (the 'instantaneous intensity function'[23]). To predict intensity from position alone, the mean firing rate for the animal's location was determined from the place field (Fig. 2b). To predict from peer activity (Fig. 2a), each peer cell was assigned a 'weight', which was either positive or negative (Methods and Supplementary Information). The activity of positively or negatively weighted cells increased or decreased the predicted intensity of the target cell within a specific time window (the 'peer prediction timescale').

To determine the weights and to quantify the success of prediction, we used a cross-validation method: weights were chosen to maximize the success of prediction on one part of the data (the training set), and the success of prediction was quantified on the remaining part (the test set). The cross-validation method is essential to ensure that the predictions are not due to statistical fluctuations (Supplementary Information). The prediction method is shown in Fig. 2c, d. Typically, positively and negatively weighted cells showed peaks and dips in their cross-correlograms (CCGs) with the target cell (Fig. 2e).

Of the 200 pyramidal cells that met our unit isolation criteria (Methods and Supplementary Information), the spike trains of 189 (95%) were predictable from space, which were therefore classified as 'place cells'. For most place cells ($n = 185$, 98%), adding population activity to the place information further improved spike timing predictability (Fig. 3a). Additional peer predictability increased with the number of simultaneously monitored neurons ($r = 0.55$, $P < 10^{-14}$; Supplementary Information).

By varying the peer prediction timescale, the temporal window within which spike times were best predictable from the population could be determined. For short temporal windows (~1 ms) prediction was poor, indicating that spike timing was not coordinated to this accuracy in the population. Prediction improved with longer peer prediction timescales, reaching a maximum predictability of 4.8 bits s^{-1} at 25 ms for a representative neuron (Fig. 3b), as compared with 3.2 bits s^{-1} for prediction from space alone. The distribution of the optimal time windows across the population showed a peak between 10 and 30 ms (Fig. 3c; 95% confidence interval for median, 21–26 ms). These observations suggest that pyramidal neurons are organized into assemblies whose activity synchronizes transiently with a temporal resolution of ~25 ms. Positively weighted cells are those with which the target cell frequently joins in an assembly, and negatively weighted cells are those with which the target cell rarely joins in an assembly.

A mechanism that can affect neuronal firing times is the hippocampal theta oscillation[24]. The timing of spikes within the theta cycle, in addition to the cell's firing rate, is correlated with the rat's position in space[5–8,25]. It may be argued, therefore, that correlated assembly firing arises from the simultaneous phase modulation of cell populations. We considered two schemes. In the first, neuronal synchronization is due to the dependence of the mean theta phase of each cell on position, but fluctuations in timing about this mean are random and independent between cells. In the second, both mean phase and correlated phase fluctuations contribute to synchronization, in which case the phase–space correlation is only one manifestation of a more general mechanism determining exact spike times.

To compare these possibilities, we refined the prediction of spike

trains from position by incorporating a position-dependent theta modulation, determined from the neuron's theta phase field[7]. In the first scheme, this is the best prediction that can be made for a cell's spike times. In the second scheme, this prediction should be further improved by peer activity. Figure 4a, b shows an example where the rat traversed the periphery of the neuron's place field, producing a phase pattern considerably more variable than the stereotyped forward phase advancement seen on linear tracks[5]. This irregular spike train was better predicted by peer activity than by the refined spatial prediction (Fig. 4a).

At the group level, to ensure that phase prediction was not compromised by poor theta detection, only cases with high theta signal-to-noise ratio were used (122 cells, 11 recording sessions; Methods). Incorporation of theta phase improved over prediction from the place field alone, as expected ($n = 89$, 73% of cells); however, peer prediction further improved on the refined spatial prediction in 89% of these cells ($n = 79$, Fig. 4c), indicating that neurons show coordinated activity beyond that predicted by their simultaneous phase precession.

Hippocampal population firing patterns are therefore different from those predicted by independent coding of spatial position, even when position-dependent phase modulation is taken into account. This interdependence suggests that the temporal structure of neuronal activity can be understood only at a population level[26]. Here, a picture emerges of neurons organized into temporary coalitions, whose activity lasts for roughly 25 ms, and whose timing and membership are determined only partially by the animal's location in space. Although a given neuron may be part of several assemblies, there are statistical preferences in partner selection, as reflected by the positively and negatively weighted cells. Could the observed assembly activity result from the precisely timed activation of hippocampal cells by sensory, but non-spatial, stimuli[27]? For the timing of non-spatial sensory events to be the sole cause of the observed synchronization, the external events would themselves need to show exquisite temporal coordination, on the scale of tens of milliseconds, with respect to the theta rhythm. Rather, we propose that the assembly organization arises from the internal dynamics of neuronal circuits and reflects the operation of non-sensory cognitive phenomena.

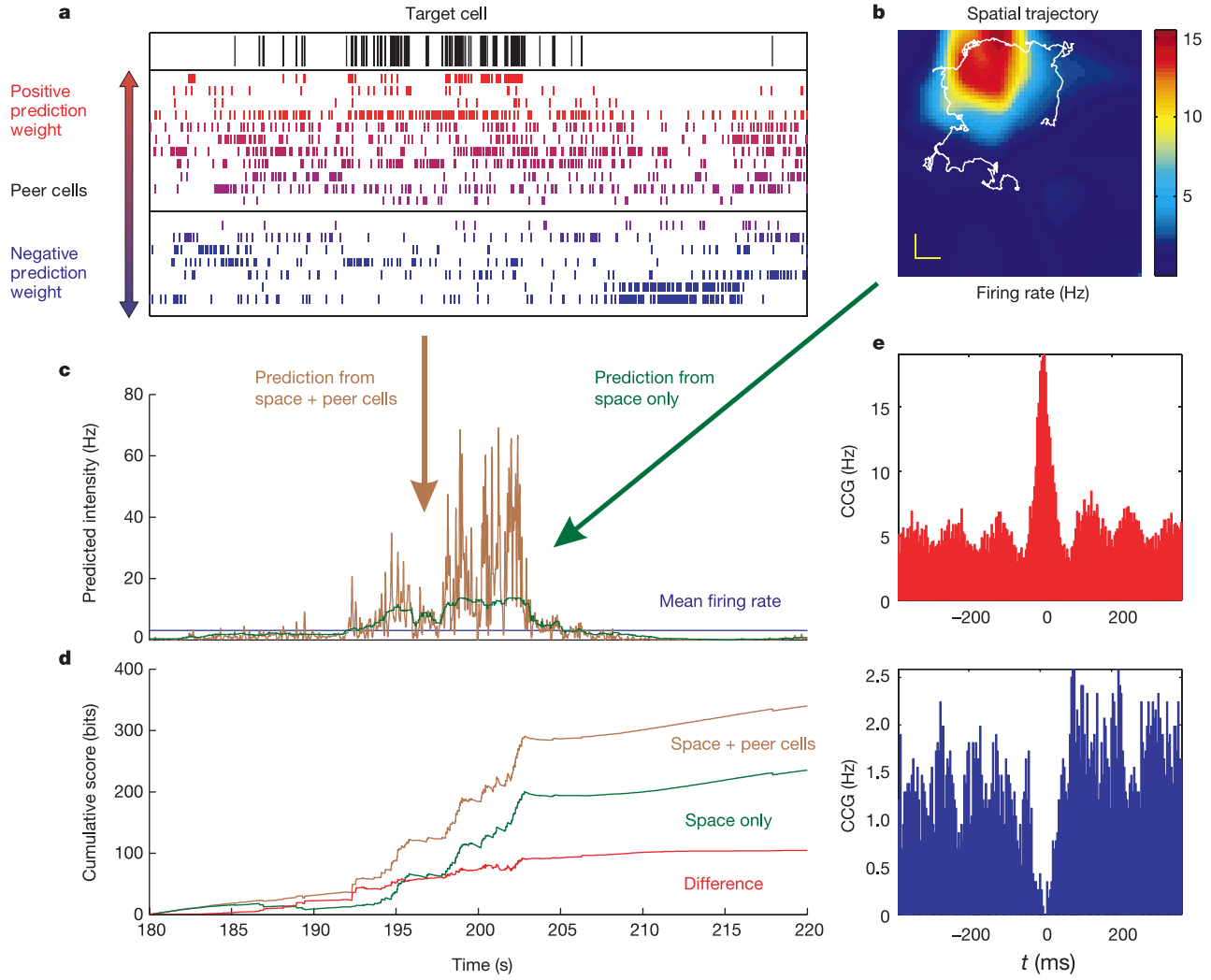

Figure 2 Spike train prediction method. **a**, Activity of the target cell (top) and a group of simultaneously recorded cells (bottom). Each peer cell is assigned a prediction weight by the cross-validation procedure (Methods). The activity of positively or negatively weighted cells predicts increased or decreased the probability of synchronous spikes in the target cell. **b**, The target cell's place field and the animal's trajectory (white trace). Scale bar, 10 cm. **c**, Instantaneous firing intensity of the target cell predicted from position (green), or position and peer activity (brown). **d**, Prediction quality is quantified by assessing the fit of the actually observed spike train against the prediction. **e**, CCG of the target cell with a positively (top) and a negatively (bottom) predicting cell, showing a peak or trough in the CCG, respectively.

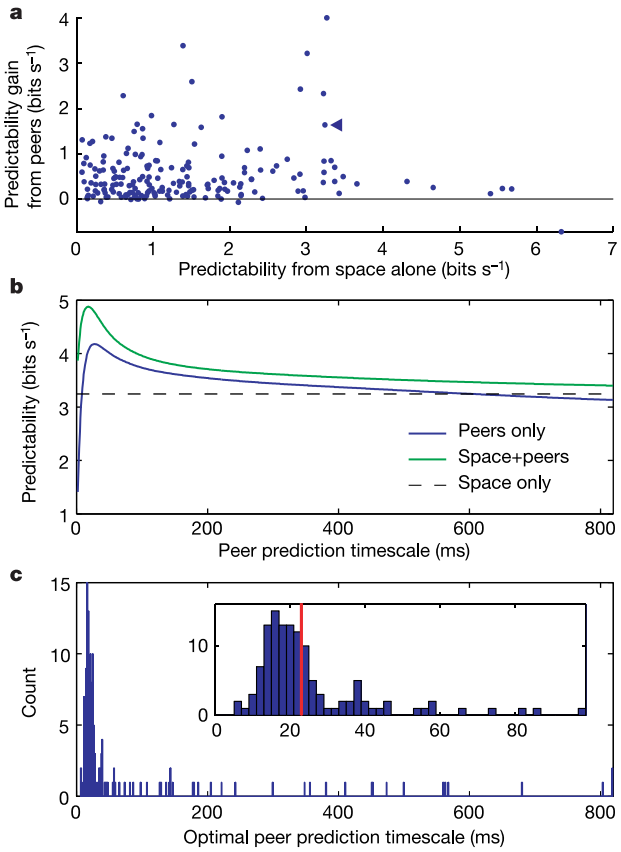

Figure 3 Spike timing is predictable from peer activity. **a**, Gain in predictability of spike trains from peer activity in excess of predictability from location, for all pyramidal cells. Peer activity improved spike train prediction for 98% of cells. **b**, Predictability versus peer prediction timescale for a representative cell (indicated by arrowhead in **a**). Predictability peaks at ~25 ms, indicating that this is the timescale of synchronization for the assemblies in which this cell participates. **c**, Distribution of timescales at which peer activity best improved spike time prediction, for all cells (expanded in inset). The median optimal timescale is 23 ms (red line).

The observed 25-ms timescale of synchrony may be physiologically significant. Because it closely matches the membrane time constant of pyramidal neurons in the hippocampal region[9], activity synchronized with this timescale may be optimal for inducing spiking in downstream neurons. In addition, this timescale matches the period of the hippocampal gamma oscillation[10] and the effective window for synaptic plasticity[11]. We therefore suggest that assembly activity may be optimal for propagating and storing information in neuronal circuits. □

Methods

Animals and recordings

Male rats (Sprague Dawley or Long Evans) were implanted with either a 64-site silicon probe or several movable tetrodes. All experimental procedures were in accordance with Rutgers University guidelines. Up to 68 cells were recorded simultaneously in 30 recording sessions from six rats, which were either collecting food pellets in an open environment or walking on an elevated square track for food reward. An LED was attached to the head stage to track the position of the animal. Extracellular spikes were extracted from the traces as described[24,28]. To avoid spurious synchrony, unit activity was not predicted from cells on the same tetrode[29]. For silicon probes, units were not predicted from peer cells for which the two channels of largest amplitude were the same as for the target cell. We predicted spike trains only for pyramidal cells that had sufficient isolation quality (isolation distance ≥ 20)[30] and at least five simultaneously recorded peers. Theta phase was determined by a Hilbert transform[7] for epochs where the theta–delta power ratio exceeded 6 (ref. 24). Theta was filtered at 8–20 Hz.

Analyses

Analyses were done with custom-written MATLAB and C^{++} software. Prediction quality was evaluated by tenfold cross-validation. We computed firing rate maps $f(x)$ (place fields) and maps of preferred theta phase and modulation depth (phase fields) from the training set by a local smoothing method[7,30]. Instantaneous firing intensity was predicted in time bins of size 3.2 ms as the product of a spatial term $f(x_t)$ and a peer prediction term:

$$g\left(\sum_{\alpha} s_{t\alpha} w_{\alpha}\right)$$

where $s_{t\alpha}$ is the temporally smoothed spike count of cell α in time bin t, w_{α} is the weight of cell α, and $g(\eta)$ is $\exp(\eta)$ for $\eta < 0$ and $1 + \eta$ for $\eta \geq 0$. Weights were fit by maximum likelihood on the training set, penalized by the term:

$$-\sum_{\alpha} w_{\alpha}^2/4$$

Prediction quality was quantified by the ratio of log-likelihoods:

$$L_f = -\int f(t)dt + \sum_{s} \log f(t_s)$$

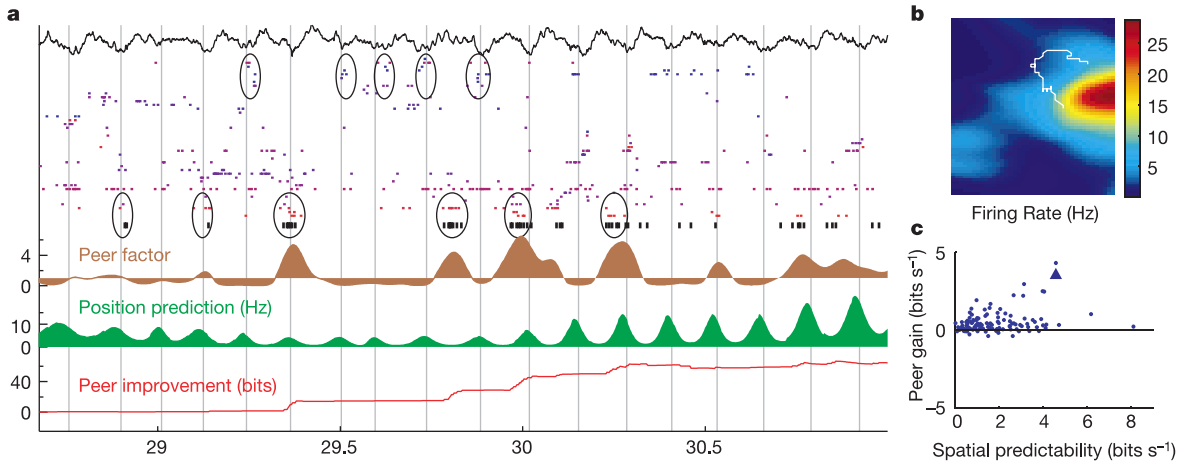

Figure 4 Assembly structure is not fully accounted for by spatially dependent phase modulation. **a**, Top, theta oscillation and spike rasters for a 2-s data segment. Neurons are colour-coded by prediction weight onto the target cell (bottom, black raster; indicated by arrowhead in **c**). Brown indicates peer prediction factor (fill level 1, neutral prediction). Green indicates refined spatial prediction, taking into account theta-phase modulation (fill level 0). Red indicates cumulative gain in predictability of target cell spike train using peer activity. **b**, Animal's trajectory for this data segment, superimposed on target cell place field. **c**, Gain in predictability of spike trains by using peer activity for the cell population, plotted against predictability from the refined (phase-modulated) spatial prediction. Use of peer activity further improved the refined spatial prediction.

of the test set spike train under the predicted firing probability relative to a constant probability given by the mean firing rate on the training set. Computed to base 2, this ratio estimates the number of bits a communicator of the test set spike train can save by knowing the predictor variables (position and/or peer cell activity), as compared with knowing only mean rate. Spike rasters were re-ordered for display by stochastic search over all possible orderings, to maximize the fraction of spike pairs between neighbouring rasters that lay within 25 ms (Figs 1b and 4a).

See Supplementary Information for a full description of methods.

Received 14 April; accepted 9 June 2003; doi:10.1038/nature01834.

1. Mainen, Z. F. & Sejnowski, T. J. Reliability of spike timing in neocortical neurons. *Science* **268,** 1503–1506 (1995).
2. Hebb, D. O. *The Organization of Behavior* (Wiley, New York, 1949).
3. Freiwald, W. A., Kreiter, A. K. & Singer, W. Synchronization and assembly formation in the visual cortex. *Prog. Brain Res.* **130,** 111–140 (2001).
4. Engel, A. K., Fries, P. & Singer, W. Dynamic predictions: oscillations and synchrony in top-down processing. *Nature Rev. Neurosci.* **2,** 704–716 (2001).
5. O'Keefe, J. & Recce, M. L. Phase relationship between hippocampal place units and the EEG theta rhythm. *Hippocampus* **3,** 317–330 (1993).
6. Skaggs, W. E., McNaughton, B. L., Wilson, M. A. & Barnes, C. A. Theta phase precession in hippocampal neuronal populations and the compression of temporal sequences. *Hippocampus* **6,** 149–172 (1996).
7. Harris, K. D. *et al.* Spike train dynamics predicts theta-related phase precession in hippocampal pyramidal cells. *Nature* **417,** 738–741 (2002).
8. Mehta, M. R., Lee, A. K. & Wilson, M. A. Role of experience and oscillations in transforming a rate code into a temporal code. *Nature* **417,** 741–746 (2002).
9. Spruston, N. & Johnston, D. Perforated patch-clamp analysis of the passive membrane properties of three classes of hippocampal neurons. *J. Neurophysiol.* **67,** 508–529 (1992).
10. Csicsvari, J., Jamieson, B., Wise, K. D. & Buzsáki, G. Mechanisms of gamma oscillations in the hippocampus of the behaving rat. *Neuron* **37,** 311–322 (2003).
11. Magee, J. C. & Johnston, D. A synaptically controlled, associative signal for Hebbian plasticity in hippocampal neurons. *Science* **275,** 209–213 (1997).
12. Reinagel, P. & Reid, R. C. Precise firing events are conserved across neurons. *J. Neurosci.* **22,** 6837–6841 (2002).
13. Kara, P., Reinagel, P. & Reid, R. C. Low response variability in simultaneously recorded retinal, thalamic, and cortical neurons. *Neuron* **27,** 635–646 (2000).
14. Buracas, G. T., Zador, A. M., DeWeese, M. R. & Albright, T. D. Efficient discrimination of temporal patterns by motion-sensitive neurons in primate visual cortex. *Neuron* **20,** 959–969 (1998).
15. Fenton, A. A. & Muller, R. U. Place cell discharge is extremely variable during individual passes of the rat through the firing field. *Proc. Natl Acad. Sci. USA* **95,** 3182–3187 (1998).
16. Shadlen, M. N. & Newsome, W. T. Noise, neural codes and cortical organization. *Curr. Opin. Neurobiol.* **4,** 569–579 (1994).
17. Lisman, J. E. & Idiart, M. A. Storage of 7 ± 2 short-term memories in oscillatory subcycles. *Science* **267,** 1512–1515 (1995).
18. Redish, A. D. *et al.* Independence of firing correlates of anatomically proximate hippocampal pyramidal cells. *J. Neurosci.* **21,** RC134 [online] ⟨http://www.jneurosci.org/cgi/content/full/21/10/RC134⟩ (2001).
19. Hirase, H., Leinekugel, X., Csicsvari, J., Czurko, A. & Buzsáki, G. Behavior-dependent states of the hippocampal network affect functional clustering of neurons. *J. Neurosci.* **21,** RC145 [online] ⟨http://www.jneurosci.org/cgi/content/full/21/10/RC145⟩ (2001).
20. O'Keefe, J. & Nadel, L. *The Hippocampus as a Cognitive Map* (Clarendon, Oxford, 1978).
21. Wilson, M. A. & McNaughton, B. L. Dynamics of the hippocampal ensemble code for space. *Science* **261,** 1055–1058 (1993).
22. Brown, E. N., Frank, L. M., Tang, D., Quirk, M. C. & Wilson, M. A. A statistical paradigm for neural spike train decoding applied to position prediction from ensemble firing patterns of rat hippocampal place cells. *J. Neurosci.* **18,** 7411–7425 (1998).
23. Cox, D. R. & Isham, V. *Point Processes* (Chapman and Hall, London, 1980).
24. Csicsvari, J., Hirase, H., Czurko, A., Mamiya, A. & Buzsáki, G. Oscillatory coupling of hippocampal pyramidal cells and interneurons in the behaving rat. *J. Neurosci.* **19,** 274–287 (1999).
25. Jensen, O. & Lisman, J. E. Position reconstruction from an ensemble of hippocampal place cells: contribution of theta phase coding. *J. Neurophysiol.* **83,** 2602–2609 (2000).
26. deCharms, R. C. & Zador, A. Neural representation and the cortical code. *Annu. Rev. Neurosci.* **23,** 613–647 (2000).
27. Wood, E. R., Dudchenko, P. A. & Eichenbaum, H. The global record of memory in hippocampal neuronal activity. *Nature* **397,** 613–616 (1999).
28. Harris, K. D., Henze, D. A., Csicsvari, J., Hirase, H. & Buzsáki, G. Accuracy of tetrode spike separation as determined by simultaneous intracellular and extracellular measurements. *J. Neurophysiol.* **84,** 401–414 (2000).
29. Quirk, M. C. & Wilson, M. A. Interaction between spike waveform classification and temporal sequence detection. *J. Neurosci. Methods* **94,** 41–52 (1999).
30. Harris, K. D., Hirase, H., Leinekugel, X., Henze, D. A. & Buzsáki, G. Temporal interaction between single spikes and complex spike bursts in hippocampal pyramidal cells. *Neuron* **32,** 141–149 (2001).

Supplementary Information accompanies the paper on **www.nature.com/nature**.

Acknowledgements We thank J. E. Lisman, D. L. Buhl, S. M. Montgomery, P. E. Bartho and I. Creese for comments on the manuscript. This work was supported by grants from the National Institutes of Health

Competing interests statement The authors declare that they have no competing financial interests.

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Role of the prolyl isomerase Pin1 in protecting against age-dependent neurodegeneration

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The neuropathological hallmarks of Alzheimer's disease and other tauopathies include senile plaques and/or neurofibrillary tangles[1–4]. Although mouse models have been created by over-expressing specific proteins including β-amyloid precursor protein, presenilin and tau[1–10], no model has been generated by gene knockout. Phosphorylation of tau and other proteins on serine or threonine residues preceding proline seems to precede tangle formation and neurodegeneration in Alzheimer's disease[11–14]. Notably, these phospho(Ser/Thr)-Pro motifs exist in two distinct conformations, whose conversion in some proteins is catalysed by the Pin1 prolyl isomerase[15–17]. Pin1 activity can directly restore the conformation and function of phosphorylated tau or it can do so indirectly by promoting its dephosphorylation, which suggests that Pin1 is involved in neurodegeneration[14,18,19]; however, genetic evidence is lacking. Here we show that Pin1 expression is inversely correlated with predicted neuronal vulnerability and actual neurofibrillary degeneration in Alzheimer's disease. *Pin1* knockout in mice causes progressive age-dependent neuropathy characterized by motor and behavioural deficits, tau hyperphosphorylation, tau filament formation and neuronal degeneration. Thus, Pin1 is pivotal in protecting against age-dependent neurodegeneration, providing insight into the pathogenesis and treatment of Alzheimer's disease and other tauopathies.

Pin1-catalysed prolyl isomerization can regulate the function and/or dephosphorylation of some phosphoproteins, many of which are also recognized by the mitosis- and phosphorylation-specific monoclonal antibody MPM-2. Notably, induction of MPM-2 epitopes is a prominent common feature of Alzheimer's disease (AD), frontotemporal dementia with Parkinsonism linked to chromosome 17, Down's syndrome, corticobasal degeneration, progressive supranuclear palsy and Pick's disease[13,14]. In fact, the pattern of tau phosphorylation in AD is similar to that in mitotic cells[12,14]. Taking these observations together with the reduced amount of soluble Pin1 in brains at a late stage of AD[18], we previously proposed that Pin1 might protect against neurodegeneration[14,18]. However, it has been reported that in AD hippocampal expression of Pin1 occurs primarily in a few tangle-free degenerative neurons in CA1 and CA2, and not in CA3 and CA4 non-degenerative neurons, and it has been proposed that Pin1 promotes neurodegeneration[20]. Thus, the neuronal function of Pin1 remains elusive.

Neurons in different subregions of the hippocampus and neo-

