no LTP in any of 13 animals[3]. These results weaken the evidence for our earlier conclusion that LTP in the dentate gyrus may not be necessary for spatial learning. The reduction in LTP in the awake *Thy1*$^{-/-}$ animal may simply be insufficient to produce a spatial learning impairment.

Neither the *in vitro* preparation nor the anaesthetized mouse necessarily provides an accurate prediction of the potential for LTP in the awake animal. The wide variability in the degree of LTP exhibited by animals in the same alert, awake state (a variability which may reflect individual differences in the level of inhibitory tone) prompts the cautionary observation that attempts to correlate LTP with behaviour are unlikely to be interpretable unless both are assessed in the same animal.

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Rhodopsin evolution in the dark

The gene for the visual pigment rhodopsin has been extensively studied from biochemical, molecular and evolutionary perspectives. This makes it ideal for investigating the relationship between protein structure and function[1,2], and the effects of loss of functional constraint on the evolution of a gene. We specifically addressed the question of what happens when there is no light available by studying rhodopsin genes from three pairs of cave-dwelling and surface-dwelling freshwater crayfish species. Contrary to predictions, we found no differences in the rate of evolution between the cave and surface species or between the conserved and variable structural motifs of the rhodopsin protein. This suggests that rhodopsin might have a previously unknown function in the absence of light.

We sequenced a 900-base-pair segment of the rhodopsin gene from three species pairs of crayfish, each from a different genus (*Cambarus, Orconectes* and *Procambarus*), as well as the gene from an outgroup species, *Cambarellus schufeldtii*. Each species pair consisted of a cave-dwelling species and its closest surface-dwelling relative. Using phylogenetic information about the species relationships[3–5], it is possible to reconstruct nucleotide substitutions and amino-acid replacements along the two independent lineages of each pair, and test the effects of loss of functional constraint on the rhodopsin gene (Fig. 1).

Table 1 Synonymous and non-synonymous nucleotide substitutions

	Synonymous		Non-synonymous		*P* value
C. hubrichti C	13	(7.30–22.3)	6	(2.61–13.3)	0.206
C. maculatus	7	(3.29–14.3)	6	(2.61–13.3)	
O. australis C	3	(0.82–8.81)	10	(5.32–18.3)	0.127
O. virilis	8	(3.77–15.8)	9	(4.46–17.3)	
P. orcinus C	2	(0.36–7.29)	2	(0.36–7.29)	0.255
P. seminolae	6	(2.61–13.3)	1	(0.052–5.76)	
Total Cave	18	(11.2–28.3)	18	(11.2–28.3)	0.157
Total Surface	21	(13.3–32.3)	16	(9.60–25.9)	

A comparison between the number of synonymous and non-synonymous nucleotide substitutions in the cave (C) and surface lineages. Numbers in parentheses are the 95% confidence limits based on sampling from a Poisson distribution[12]. *P* values were determined using Fisher's Exact Test for independence.

Our expectation was that, with a loss of functional constraint, there would be a significant difference in nucleotide substitutions between cave and surface lineages, with the cave lineages having a higher rate of evolution, similar to that in pseudogenes[6]. In addition, we expected that the amino-acid replacements would be distributed randomly over the structure of the protein, irrespective of known functional constraints.

Ancestral states for both nucleotide and amino-acid changes were reconstructed using the maximum-likelihood procedure[7] of PAML[8] software. Although there was a large number of changes along the phylogeny, the numbers of nucleotide substitutions along the cave and surface lineages were nearly identical (Fig. 1). Using a sign test, we could not reject the null hypothesis that there was no difference in the number of substitutions in all three lineages ($P = 0.452$). Amino-acid replacements were also nearly equal in number (Fig. 1; $P = 0.367$).

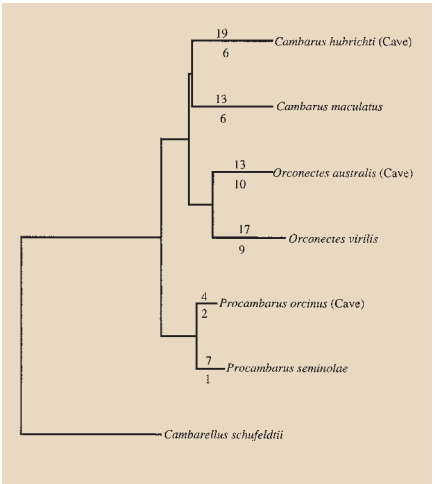

Figure 1 Maximum-likelihood reconstruction of nucleotide substitutions and amino-acid replacements for the crayfish phylogeny. Phylogenetic relationships were reconstructed with morphological[3,4] and nucleotide sequence data[5], independent of rhodopsin data. The number of nucleotide substitutions (above the branch) and amino-acid replacements (below the branch) was estimated using the programs BASEML and CODEML in the PAML package[8]. Branch lengths are proportional to the amount of change. Similar results were obtained using MacClade[13].

Next we tested to see if habitat (cave or surface) was independent of substitution pattern. We determined whether nucleotide substitutions were synonymous (substitutions that do not result in amino-acid replacement) or non-synonymous (substitutions that do result in amino-acid replacement) using the maximum-likelihood reconstructions. We then compared these values to habitat type, and again failed to reject the null hypothesis of independence (Table 1). This indicates that substitution pattern was not affected by surface/subterranean environmental differences. Indeed, in comparing nucleotide substitution matrices we found no significant difference between the cave lineages and the surface lineages ($P = 0.2558$, Kolmogorov–Smirnov test). So there is no detectable difference in selection pressures on the patterns of nucleotide substitutions.

Finally, we examined amino-acid replacements related to different structural motifs of the rhodopsin protein. We partitioned the protein into structural units and identified the number of amino-acid replacements in these units. Again, we found no significant differences between the cave and surface lineages in the distribution of amino-acid replacements with respect to the secondary protein structure ($P = 0.1549$, Kolmogorov–Smirnov test).

These lines of evidence indicate that there has been no loss of functional constraint for rhodopsin in the cave lineages of crayfishes, which in turn suggests that the protein is still functional, contrary to previous studies of cave-adapted organisms[6,9]. Rhodopsin cannot have its conventional function, as there is no light available to initiate a biochemical cascade. We conclude

that rhodopsin has an additional, previously unrecognized function, perhaps a role in circadian rhythms[10,11], unrelated to light absorption.

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Chirality errors in nucleic acid structures

The prevalence of errors as opposed to true anomalies in protein structures was discussed in Correspondence last year[1]. We have analysed the nucleic acid structures in the Brookhaven Protein Data Bank using a simple automatic procedure (program available at http://www.mbi.ucla.edu/people/peter/chiral.html) to determine the configurations at tetrahedral chiral centres. It appears that a number of structures containing significant "chirality errors that no-one would argue with"[2] have made their way into the data bank.

Of 562 data bank files containing nucleic acid coordinates, we found that 34 contained reversed configurations (Table 1) of at least one chiral centre (Fig. 1). Incorrect configurations at C1′, C3′ and C4′ are detectable in both NMR and crystal structures. C2′ represents a special case for DNA structures where H2′ and H2″ are included. Errors in the naming of H2′/H2″ atoms would be irrelevant in X-ray structures but are significant in NMR structures of DNA because H2′ and H2″ are almost always stereospecifically assigned. In the case of RNA, the correct chirality of C2′ would be equally significant in both X-ray and NMR structures.

In 25 further coordinate files, not listed in Table 1, the naming of H2′/H2″ was reversed throughout compared with the IUPAC-IUB convention[3,4]. Whether these cases are merely in discrepancy with the convention in a particular file or inconsistent with the underlying experimental data would depend on whether the same nomenclature was used for the determination of the stereospecific resonance assignment in each case.

We also observed inconsistencies in the naming of the amino protons, which are distinguishable even when they are not involved in hydrogen bonds simply by their *cis/trans* positions with respect to other base atoms. We disregarded inconsistencies in the naming of the diastereotopic atom pairs H5′/H5″ and O1P/O2P as they are generally not relevant to the experimental results in either NMR or X-ray structures. However, these atoms should be consistently named for accurate structure comparisons by root mean square deviations of atomic positions.

The overall topology of most affected structures would probably not change substantially on correction of their chirality problems and re-refinement. Nevertheless, these errors are significant both because the structures are stereochemically wrong and because of the importance of ion and hydrogen bonding interactions with the phosphodiester backbone which stabilize the structures of nucleic acids.

We note that current refinement programs and their parametrization for nucleic acids do not always prevent this type of avoidable error, especially when high-temperature simulated annealing protocols are used. The higher number of errors found in the NMR structures compared with the X-ray structures does not reflect a weakness of NMR experiments, but rather depends on problems in the refinement protocols.

It might be reasonable to test all structures for rigorous adherence to the existing conventions of stereochemical nomenclature[3,4] at the time of deposition into the data bank.

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Table 1 Chirality problems in nucleic acid coordinate sets from the Brookhaven Protein Data Bank

PDB entry code	Total number of nucleosides	Chirality errors 1′, 3′, 4′	Chirality errors 2′	Method	PDB entry code	Total number of nucleosides	Chirality errors 1′, 3′, 4′	Chirality errors 2′	Method
106D	96	0	8	NMR	1DSD	16	0	1	NMR
108D	640	0	20*	NMR	1HRY	16	2	1	NMR
143D	132	21	20	NMR	1HRZ	560	70	35	NMR
149D	294	0	147	NMR	1LBG	84	7	0	X-ray
170D	22	0	10	NMR	1QDF	15	7	15	NMR
171D	24	0	12	NMR	1QDG	15	7	0	Theory
173D	16	1	0	X-ray	1QDH	15	7	0	NMR
186D	168	9	5	NMR	1RCS	600	36	0	NMR
1ARA	136	34	87†	NMR	1URN	55	0	2†	X-ray
1BUF	12	2	0	NMR	203D	640	0	60*	NMR
1D31	26	1	0	X-ray	204D	640	20	40*	NMR
1D42	80	1	0	NMR	228D	88	9	73	NMR
1D70	64	1	0	NMR	229D	14	2	7	NMR
1D98	24	1	0	X-ray	28DN	8	1	0	X-ray
1D99	24	1	0	X-ray	2DA8	96	0	2	NMR
1DDP	200	0	20*	NMR	5BNA	24	1	0	X-ray
1DSC	16	0	1	NMR	5ZNA	32	0	16	Theory

A chiral centre was designated wrong if the dihedral angle defined by its four ligands – or three ligands and the central atom in cases where no hydrogen coordinates are given – was found to have the opposite sign of the correct configuration. A complete, detailed table of chirality problems is available at http://www.mbi.ucla.edu/people/peter/chiral.html.
*In these cases the majority of H2′/H2″ configurations are inconsistent with the convention as given in ref. 3. The number given represents configurations opposite to the nomenclature used within these entries. Coordinate files where all H2′/H2″ configurations are reversed throughout are not listed.
†RNA, number of flipped O2′ atoms.

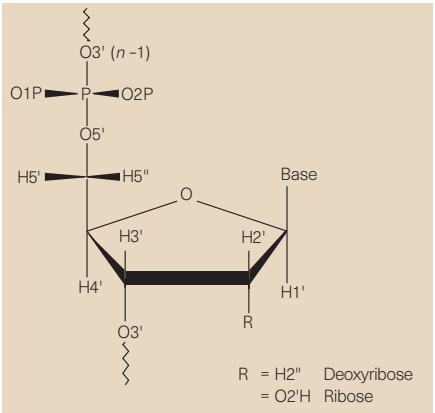

Figure 1 Stereochemical nomenclature of a nucleotide. Carbon atoms are numbered from that bound to the base.