The relation between BR and NPP (Fig. 1*b*) was constructed with data extracted from 17 published articles, and reports of several measurements for the same site were averaged. A total of 81 individual data points collected from the literature resulted in 47 average values, 18 for open ocean and coastal marine sites, 7 for estuaries and 22 for lakes worldwide. Volumetric rates of net primary production per unit time were converted to $\mu g\,C\,litre^{-1}\,d^{-1}$, assuming 12 times the hourly rate. The complete data sets are available from the authors or from the Repository of Unpublished Data, CISTI, National Research Council of Canada, Ottawa, Ontario KIA 0S2.

Statistical analysis. All the data were analysed using ordinary least squares (o.l.s.) regression, for comparison with previously published empirical models. The o.l.s. slope will systematically underestimate the true slope, however, owing to error in both the dependent and the independent variables, so we also calculated the reduced major axis (r.m.a.) slopes for the structural relationship between BR–BA and BR–NPP[30]. the complete o.l.s. and r.m.a. regression parameters are shown in Table 1.

Calculation of BGE. For Fig. 2, bacterial growth efficiencies were calculated as BGE = BP/(BP + BR), where BR are bacterial respiration data collected from the literature and used in Fig. 1*a*, *b*. For each BR point, bacterial production (BP, in $\mu g\,C\,l^{-1}\,d^{-1}$) was calculated from either the accompanying net primary production data in Fig. 1*b* using the equation of Cole *et al.*[1], or from the accompanying bacterial abundance data in Fig. 1*a* using the equation of White *et al.*[7]. Data are presented as box-and-whisker plots showing the median and range of BGE for each system for the two methods for estimating bacterial production.

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Episodic adaptive evolution of primate lysozymes

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ALTHOUGH the darwinian concept of adaptation was established nearly a century ago, it has been difficult to demonstrate rigorously that the amino-acid differences between homologous proteins from different species have adaptive significance. There are currently two major types of sequence tests for positive darwinian selection on proteins from different species: sequence convergence, and neutral rate violation (reviewed in ref. 1). Lysozymes from the stomachs of cows and langur monkeys, two mammalian species displaying fermentation in the foregut, are an example[2,3] of amino-acid sequence convergence among homologous proteins[4–6]. Here we combine tests of neutral rate violation with reconstruction of ancestral sequences to document an episode of positive selection on the lineage leading to the common ancestor of the foregut-fermenting colobine monkeys. This analysis also detected a previously unsuspected adaptive episode on the lineage leading to the common ancestor of the modern hominoid lysozymes. Both adaptive episodes were followed by episodes of negative selection. Thus this approach can detect adaptive and purifying episodes, and localize them to specific lineages during protein evolution.

The colobine Old World monkeys are unique among the primates in having a complex foregut in which bacteria ferment leafy plant materials[7], followed by a true stomach that expresses high levels of the bacteriolytic enzyme, lysozyme[4,6,8]. Other primates, including the cercopithecine Old World monkeys, have simple stomachs[7] with lysozyme expressed only in the pyloric region[6]. The cercopithecines, therefore, can serve as closely related 'negative controls' for the comparative study of the evolution of lysozyme in the colobines. Using the phylogeny of the primates (Fig. 1) as a guide, we sequenced the protein-coding region of the lysozyme gene from species representing each of the major lineages of catarrhines (colobines, cercopithecines and hominoids), as well as from some New World monkeys for primate outgroup comparisons (see Supplementary Information). Visual analysis of these sequences shows that there are many non-synonymous nucleotide differences (that is, differences that would cause amino-acid replacements) compared with the number of synonymous nucleotide differences (those that would not cause amino-acid replacements); this suggests that the proteins might have evolved under positive darwinian selection.

Rigorous demonstration that a protein has evolved more rapidly than the neutral substitution rate requires more than simply counting non-synonymous and synonymous differences between sequences. Proper consideration of the genetic code requires a comparison of the number of non-synonymous substitutions per non-synonymous site (termed K_A in the method[9,10] used here) with the number of synonymous substitutions per synonymous site (termed K_S) between pairs of homologous protein-coding genes, corrected for multiple hits[9–11]. Such comparisons are conventionally presented as ratios between the K_A and K_S values, although statistical significance for such comparisons must be calculated using the differences between the values, a convention we follow here. K_A/K_S ratios less than 1.0 are generally taken as evidence that the proteins have evolved under negative or purifying selection; most pairwise comparisons between extant genes show this pattern[11–13]. Conversely, K_A/K_S ratios significantly greater than 1.0 are considered to be strong evidence of positive selection for amino-acid replacements, because they imply that the rate of non-synonymous substitution exceeds that which can be explained solely by the neutral substitution rate at the locus under study. Significantly elevated K_A/K_S ratios have been documented for very few whole proteins[13–15], although domains of certain proteins display this phenomenon[1,12,16].

K_A and K_S calculations were made for all possible pairwise comparisons of the primate lysozyme DNA sequences (see Supplementary Information). As predicted by previous studies on lysozyme in the Hanuman langur[4–6], nearly all pairwise comparisons between the sequences from the colobines and the cercopithecines showed K_A/K_S ratios greater than 1.0 (range 0.77–3.68). Many of these comparisons are statistically significant, and are

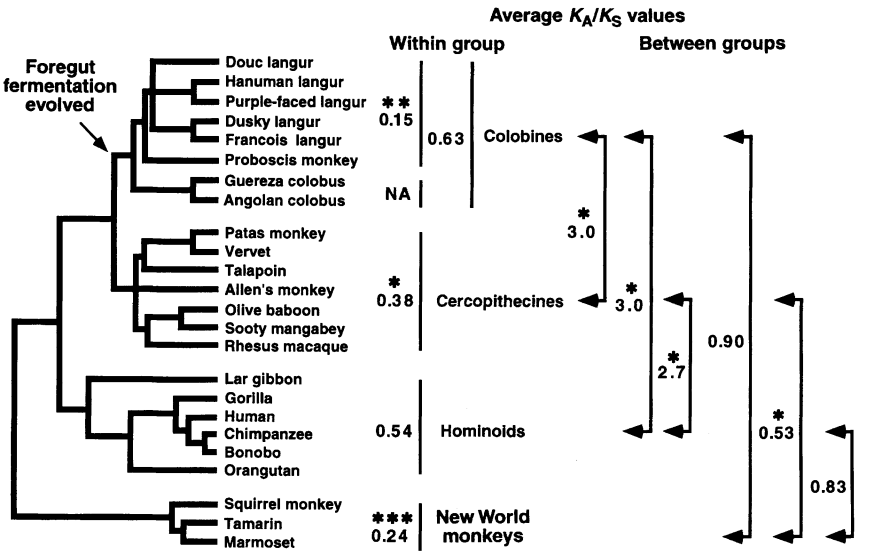

FIG. 1 Phylogeny of the primate species used in this study, showing average K_A/K_S ratios within and between groups. K_A and K_S group averages were calculated as described[17] using the *SEND* computer program. The within-group average from the two African colobus species could not be calculated because the two sequences are identical. Statistical significance was calculated by *t*-tests, with *P* values as follows: *, <0.05; **, <0.01; ***, <0.005. The branching order of the major groups of anthropoid primates (New World monkeys, hominoids and Old World monkeys) has been established by numerous studies. The well-supported mitochondrial DNA phylogeny of the hominoids is presented. The cercopithecine phylogeny represents a combination of molecular and morphological studies. The overall colobine phylogeny is consistent with both our mtDNA phylogeny of this group (R.V. Collura and C.-B.S., manuscript in preparation) and phylogenetic analysis of the complete coding region of the lysozyme sequences (data not shown). Those branching orders shown as multifurcations were not resolved by available sequence data; however, changing the branching order of these lineages did not change the results of our analyses in any meaningful manner, because the DNA sequences from the species involved are so similar.

among the highest known for any whole protein[1,12,13]. The large range in these ratios reflects the stochastic nature of the synonymous nucleotide-substitution process[17], as K_S was small and varied more widely than K_A in these pairwise comparisons. Because all colobines are equally related to all cercopithecines, having diverged from a common ancestor about 15 Myr ago[18], comparisons between group averages would be statistically more meaningful than individual comparisons of K_A and K_S[16]. We therefore calculated average K_A and K_S values both within and between phylogenetic groups of these primate lysozymes; K_A/K_S ratios for these group averages are summarized in an evolutionary context in Fig. 1. The between-group average K_A/K_S ratio for all colobine lysozymes versus all cercopithecine lysozymes is statistically significant ($K_A/K_S = 3.0$; $P < 0.05$). Similarly, all comparisons between the colobine and the hominoid lysozymes gave K_A/K_S ratios greater than 1.0 (range 1.33–3.49) and the average is statistically significant ($K_A/K_S = 3.0$; $P > 0.05$). We had expected that comparisons between lysozymes from primates with simple stomachs would yield K_A/K_S ratios less than 1.0, as there was no known change in role for these enzymes, as there had been in the colobine case[4,6,8]. Contrary to this expectation, all comparisons between the cercopithecine and hominoid sequences yielded elevated K_A/K_S ratios (range 1.02–3.28), with a statistically significant average ($K_A/K_S = 2.7$; $P < 0.05$). These significantly high K_A/K_S values strongly suggest that positive selection for amino-acid replacements occurred during the evolution of catarrhine lysozymes, but cannot identify the lineage.

In contrast to the between-group comparisons, all within-group averages of catarrhine lysozymes show K_A/K_S ratios less than 1.0 (Fig. 1). These within-group comparisons involve sequences that are so closely related that these low ratios suggest that purifying selection has been active during the recent evolution of primate lysozymes. The striking contrast of the purifying selection within groups and the positive selection between groups (Fig. 1) suggests that the adaptive evolution of lysozyme occurred early in catarrhine evolution. If this is the case, then K_A/K_S ratios between the lysozymes from the ancestors of the major lineages should be elevated.

To test this hypothesis, we reconstructed ancestral DNA sequences representing all internal nodes on the tree shown in Fig. 2, using both newly developed maximum-likelihood methods[19–21] and traditional maximum-parsimony methods[20,22]. Because the divergence of these primate species was relatively recent, and there are a large number of lysozyme sequences of appropriate phylogenetic spread, these ancestral sequences were reconstructed with relatively few equally parsimonious alternatives by the maximum-parsimony method, and with generally high probabilities by the maximum-likelihood method. Although maximum-likelihood methods can theoretically reconstruct a different ancestral sequence from that found by maximum-parsimony methods[21], in all cases examined the maximum-likelihood ancestral lysozyme sequence was identical to one of the maximum-parsimony sequences. The reconstructed DNA sequences representing key primate ancestors (labelled internal nodes in Fig. 2) were then used in pairwise K_A/K_S calculations.

This ancestral analysis of K_A and K_S pinpointed an episode of adaptive lysozyme sequence evolution ($K_A/K_S = 4.7$; $P < 0.05$) on the ancestral colobine lineage (that is, on the colobine lineage after its divergence from the cercopithecine lineage, but before the speciation event that gave rise to the African colobus and the Asian langur clades; between nodes OW and Co in Fig. 2). This period corresponds to the time in evolutionary history when lysozyme was most likely to have been recruited to digest foregut bacteria in the true stomachs of colobine monkeys. In comparison, on the comparable ancestral lineage leading to the cercopithecine monkeys (between nodes OW and Ce in Fig. 2), the K_A/K_S ratio is not significantly different from neutral expectation. This analysis also identified an episode of positive selection ($K_A/K_S = 5.1$; $P < 0.05$) on the ancestral hominoid lineage (between nodes Ca and Ho in Fig. 2) that was not anticipated by previous analyses[4–6]. We do not have an adaptive explanation for this episode of positive selection, although it might be related to increased neutrophil expression of lysozyme in hominoids as compared with other catarrhines[23]. That these episodes of positive selection were followed by periods of purifying selection is supported by the average K_A/K_S ratios of less than 1.0 for all within-group comparisons (Fig. 1). Therefore, combining ancestral sequence reconstruction with pairwise K_A and K_S comparisons allows the detection of specific episodes of positive and negative selection, and localization of these episodes to distinct branches on an evolutionary tree.

Although elevated K_A/K_S ratios are usually taken to suggest that amino-acid replacements are being fixed faster than the neutral substitution rate, it is possible that they might result from a decrease in the fixation of synonymous mutations, rather than an adaptive increase in the fixation of non-synonymous mutations[1]. To test for this possibility, we sequenced intron 3 of the lysozyme gene from phylogenetically diverse catarrhines. The

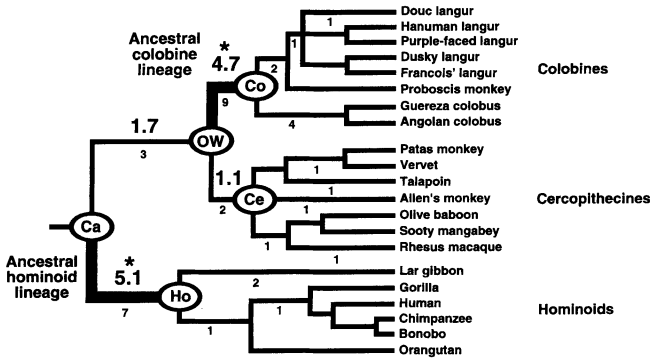

FIG. 2 K_A/K_S ratios between ancestral lysozyme DNA sequences detect early episodes of adaptive molecular evolution. Ancestral sequences (available from C.-B.S. on request) were reconstructed on the full primate phylogeny shown in Fig. 1. K_A/K_S values between ancestral sequences reconstructed by maximum likelihood are shown in a larger typeface above the internal lineages; those ratios with (K_A/K_S) differences that are significant at the 0.05 level are indicated by an asterisk. The minimum number of amino-acid replacements required to explain the sequence differences between these maximum-likelihood reconstructed ancestral sequences are given below the lineages in a smaller typeface. Nodes representing common ancestors of the extant primate groups of interest are labelled as follows: Co, colobine; Ce, cercopithecine; Ho, hominoid; OW, Old World monkey; Ca, catarrhine. Maximum-likelihood ancestral sequences were reconstructed by the marginal and joint methods in *PAUP* 4.0* (ref. 20) and by the marginal method in *PHYLIP 4.0* (ref. 19), with identical results; these sequences were used for the analyses shown. Ancestral sequences were also inferred by maximum parsimony using the computer programs *PAUP* 4.0* (ref. 20) and *MacClade 3.0* (ref. 22). For each labelled node there was more than one equally parsimonious ancestral sequence, with the number of ambiguities increasing at earlier nodes; for example, using the primate DNA sequences, there were 2 equally parsimonious ancestral sequences for node Ho, 4 for node Ce, 6 for node Co, 16 for node OW, and 16 for node Ca. The ranges of K_A/K_S values for all equally parsimonious sequences are as follows: Ca to Ho, 3.0–5.9*; Ca to OW, 0.85–3.8; OW to Co, 1.5–5.3*; OW to Ce, 0.56–2.3. Use of a larger data set containing 50 DNA sequences, including non-primate vertebrate lysozymes (both of the conventional chicken and calcium-binding types), gave similar overall results to those from the primate data set, except that the K_A/K_S ratio for the ancestral hominoid lineage was less (maximum-likelihood $K_A/K_S = 2.5$).

neutral rate of nucleotide substitution calculated for this intron is almost identical to the synonymous substitution rate at this locus. Furthermore, the neutral substitution rate in the lysozyme gene is similar to that of the well-studied η- and δ-globin pseudogenes (W.M. *et al.*, manuscript in preparation). Thus the high K_A/K_S ratios for the catarrhine lysozymes cannot be explained by significant suppression of synonymous substitutions in this gene.

Taken together, the above analyses strongly suggest that there have been two major episodes of positive darwinian selection during the evolution of catarrhine lysozymes. To enumerate and identify the amino-acid replacements that probably occurred during these adaptive episodes, we used maximum-likelihood and maximum-parsimony methods to assign events to specific lineages (Fig. 2). Strikingly, maximum-likelihood analysis suggests that there were nine amino-acid replacements during the brief adaptive episode on the ancestral colobine lineage (Fig. 2), five of which are among those originally identified to have occurred in parallel or convergently along the lineage leading to cow[4] and other ruminant[24] stomach lysozymes. During a similar period along the ancestral cercopithecine lineage, only two amino-acid replacements are reconstructed. Similarly, maximum-likelihood analysis suggests that seven amino-acid replacements occurred during the adaptive episode on the ancestral hominoid lysozyme lineage (Fig. 2). Thus, as suggested by the ancestral K_A/K_S calculations, numerous amino-acid replacements were fixed during the brief adaptive episodes on the ancestral colobine and hominoid lysozyme lineages. A more complete discussion of these matters will be presented elsewhere.

The evidence for these two adaptive episodes becomes masked when K_A/K_S comparisons are made between lysozymes from more distantly related species. For example, most comparisons of the catarrhine lysozyme sequences with those from the New World monkeys show K_A/K_S ratios less than 1.0 (range 0.38–1.03), and all between-group averages for these comparisons are less than 1.0 (Fig. 1). Similarly, pairwise comparisons between the extant rodent, artiodactyl and primate lysozyme genes produce K_A/K_S ratios significantly less than 1.0 (see Supplementary Information), even though published analyses of artiodactyl lysozymes[24–26] and our analyses of rodent lysozymes (data not shown) suggest that episodes of positive selection have occurred within both of these lineages. However, these mammalian lysozymes seem to have been mainly under negative selection, so the adaptive episodes are obscured in comparisons involving distantly related sequences. This demonstrates the importance of comparing sequences that diverged within appropriate time frames, if positive darwinian selection is to be detected.

For example, a recent survey of the genetic databases, in which predominantly distantly related sequences were compared, led to the conclusion that positive selection in protein-coding genes is very rare[12]. However, this survey failed to identify either mammalian lysozymes or abalone fertilization proteins[14,15], although both of these gene families show significantly elevated K_A/K_S ratios when pairwise comparisons are made in a proper phylogenetic context. Thus the survey probably missed many other cases of positive selection, particularly if most proteins evolve in an episodic manner, as has been proposed[3]. The phylogenetic approach we used in comparing K_A and K_S values is capable of teasing apart adaptive and purifying episodes, and so might be able to determine whether darwinian selection or neutral drift is primarily responsible for the differences between homologous proteins. □

Methods

The protein-coding region of the primate lysozyme gene was sequenced as follows. Total cellular RNA was extracted by the single-step method[27] from EDTA-anticoagulated blood or frozen tissue. Most lysozymes were sequenced directly from cDNA (prepared with the GeneAmp RNA PCR kit, Perkin Elmer Cetus); this was possible because primates have one chicken-type lysozyme gene that is expressed in both the stomach and white blood cells[5]. When an RNA source was not available, the protein-coding regions were sequenced from 'long PCR' products (GeneAmp XL PCR kit, Perkin Elmer Cetus) using genomic DNA as template. DNAs were extracted from blood or frozen tissue[28]; purified DNAs from Allen's monkey, patas, talapoin and douc langur were obtained from O. Ryder. Amplification primers were as in ref. 5, and sequencing primers were designed as needed. Sequencing was accomplished with the Taq DyeDeoxy Terminator Cycle sequencing kit and analysed on a 373A DNA sequencer (Applied BioSystems). Both strands were sequenced several times to verify the sequences carefully. If there was evidence of an allelic difference within an individual, the PCR products were cloned (TA cloning kit, Invitrogen) when necessary to obtain unambiguous sequences. The species included in this study are listed by common name, scientific name, GenBank accession number, sources (see below) and number of individuals sampled (in parentheses): human, *Homo sapiens*, J03801; chimpanzee, *Pan troglodytes*, U76912, Yerkes (1); bonobo, *Pan paniscus*, U76930-933, Yerkes (1); gorilla, *Gorilla gorilla*, U76913, Yerkes (2); orangutan, *Pongo pygmaeus*, U76914, Yerkes (1); gibbon, *Hylobates lar*, U76915, Yerkes (1); guereza colobus, *Colobus guereza*, U76916, HZG (2) and SL (1); Angolan colobus, *Colobus angolensis*, U76934-37, MMZ (2); Hanuman langur, *Semnopithecus entellus*, X60235, UCB (3); purple-faced langur, *Semnopithecus vetulus*, U76938-40, BR (1); dusky langur, *Trachypithecus obscurus*, U76917, HZG (3); Francois' langur, *Trachypithecus francoisi*, U76918, HZG (3); douc langur, *Pygathrix nemaeus*, U76941-44, SD (1); proboscis monkey, *Nasalis larvatus*, U76945-48, NY/SD (1); olive baboon, *Papio cynocephalus*, U76919, SFBR (1); sooty mangabey, *Cercocebus atys*, U76920, Yerkes (1); rhesus macaque, *Macaca mulatta*, X60236, NE (1); Allen's monkey, *Allenopithecus nigroviridis*, U76949-51, SD (1); talapoin, *Miopithecus talapoin*, U76952-55, SD (1); patas, *Erythrocebus patas*, U76956-59, SD (1); vervet, *Cercopithecus aethiops*, X60237, NE (1); squirrel monkey, *Saimiri sciureus*, U76921, NE (1); cotton-top tamarin, *Saguinus oedipus*, U76922, NE (1); common marmoset, *Callithrix jacchus*, U76923, NE (1). Intron 3 of the lysozyme gene was amplified and sequenced (primer sequences are available from C.-B.S. on request) from orangutan (U76924),

Allen's monkey (U76951), mangabey (U76928), guereza colobus (U76925), and from Hanuman (U76929), dusky (U76926), Francois' (U76927) and purple-faced (U76940) langurs. The new lysozyme sequences have been deposited into GenBank under the accession numbers listed above. Institutional abbreviations are as follows, with contact persons in parentheses: NE, New England Regional Primate Research Center (D. Lee-Parritz); Yerkes, Yerkes Regional Primate Research Center (H. McClure); SD, The Zoological Society of San Diego (O. Ryder); HZG, Houston Zoological Gardens (B. Lester and J. Flanagan); UCB, University of California at Berkeley (P. Dolhinow); SL, St. Louis Zoological Park (I. Porton); SFBR, Southwest Foundation for Biomedical Research (K. Rice); MMZ, Miami MetroZoo (W. Zeigler); BR, Greater Baton Rouge Zoo (C. Lehn); NY, New York Zoological Society (G. Amato). The previously published[5] non-human primate sequences were verified in the present study; as suggested[5], the baboon lysozyme sequence differs slightly from the published protein sequence used in earlier analyses[4,6]. Only in gorilla and orangutan did we find allelic differences in the coding region (G. Maston and C.-B.S., manuscript in preparation); the less-derived alleles are considered here. For each species, we sequenced the entire coding region; only the mature region is used in the present analyses. K_A and K_S values for all pairwise comparisons were calculated with the computer program *Li93* (ref. 10). Similar overall results were found using the method of Nei and Gojobori[29,30]; the results from the Li method[9,10] are presented because the primate lysozyme sequences best meet its assumptions. Statistical significances for the K_A and K_S values were calculated by *t*-tests[16,30]. For a matrix of K_A-to-K_S values, see Supplementary Information. Rate comparisons between the introns and synonymous sites were done using *MEGA*[30], and included the published human sequence.

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SUPPLEMENTARY INFORMATION is available on *Nature*'s World-Wide Web site (http://w.w.w.nature.com) or as paper copy from Mary Sheehan at the London editorial office of *Nature*.

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CORRESPONDENCE and requests for materials should be addressed to C.-B.S. (e-mail: c.stewart@albany.edu). Primer sequences of intron 3 of the lysozyme gene are available from C.-B.S., and the GenBank accession numbers for the new lysozyme sequences are listed in the Methods section.

Abnormal temporal dynamics of visual attention in spatial neglect patients

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When we identify a visual object such as a word or letter, our ability to detect a second object is impaired if it appears within 400 ms of the first[1–5]. This phenomenon has been termed the attentional blink or dwell time and is a measure of our ability to allocate attention over time (temporal attention). Patients with unilateral visual neglect are unaware of people or objects contralateral to their lesion[6,7]. They are considered to have a disorder of attending to a particular location in space (spatial attention)[6–11]. Here we examined the non-spatial temporal dynamics of attention in patients, using a protocol for assessing the attentional blink. Neglect patients with right parietal, frontal or basal ganglia strokes had an abnormally severe and protracted attentional blink. When they identified a letter, their awareness of a subsequent letter was significantly diminished for a length of time that was three times as long as for individuals without neglect. Our results demonstrate for the first time that visual neglect is a disorder of directing attention in time, as well as space.

Visual neglect is a common disorder following stroke. It is most severe after right hemisphere lesions, affecting over 70% of such patients[12]. These individuals are unaware of people or objects to their left and have a poor prognosis for recovery of independent function[12,13]. Despite the intense interest in neglect, the mechanisms underlying this disorder remain obscure[6,7]. One prominent theory considers left-sided neglect to be the result of a bias to attend to the right[8]. An alternative hypothesis is that neglect patients have a direction-specific impairment of disengaging attention from a stimulus on the right when they are required to shift attention to the left[9–11]. Both theories make the same fundamental assumption that neglect is an impairment of spatial attention. We investigated whether there is a non-spatial component of attention in neglect by measuring the temporal dynamics of attention at one location. The protocol we used allowed us to measure directly the time required to discriminate an object and release processing capacity for another, when no directional shift of attention is required.

Individuals without any neurological abnormality experience a significant 'loss' of attention for ~400 ms after engaging a target for purposes of identification[1–5]: this has been referred to as the attentional blink or dwell time. A standard procedure for determining this loss of temporal attention requires individuals to view a rapid serial visual presentation (RSVP) sequence of letters presented successively at the same location (see Fig. 1 and Methods for further details). In each RSVP sequence, all the letters are black except one, which is white. This is the first target (T1) the subject is asked to identify. In half the trials, T1 is followed at some point in the sequence by a black 'X'. This is the second target (T2). Individuals' ability to detect T2 correctly after successfully identifying T1 in this dual-target task is then plotted over time (interval between T1 and T2).

The performance of ten right-handed volunteers who had not suffered a stroke (mean age, 73 years) is shown in Fig. 2. When asked to identify T1 and also say whether T2 was present (dual-target task), their ability to detect T2 varied according to its temporal position in the RSVP sequence: if it occurred within 360 ms of T1, detection was significantly impaired. This is the standard attentional blink outcome[1–5]. These subjects were also tested on a control task in which they viewed similar RSVP