failure of mantle rocks at the site where slab pull (as indicated by focal mechanism analysis[27]) results in fracturing at the stress maximum near the expected oceanic brittle–ductile transition (500–700 °C).

Our seismic images of the central Andes show striking similarities with reflection data obtained by the LITHOPROBE group[6,7] at the plate boundary between the oceanic Juan de Fuca plate and the North American continent, although our study penetrates to larger depths. Deep reflections from the Vancouver island experiment at 30–40 km depth were initially interpreted as the image of the shear zone at the top of the relatively young oceanic plate beneath the North American continent[6]. Subsequent discussion that integrated newly acquired offshore reflection data and magnetotelluric data has equally included the possibility of fluids trapped at the base of accreted terranes[28] or within the subduction boundary itself[29].

The Nazca reflector and the active seismicity image different parts of the northern Chilean subduction zone. They are transient features that are probably due to the fluid-associated petrological processes that are driven by continuing subduction and the related thermal regime. □

Received 16 January; accepted 4 November 1998.

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Acknowledgements. We thank the Chilean Navy for providing a vessel for firing the marine shots. We also thank the following for logistical support: the mining companies Compañia Minera Punta de Lobos SA, Compañia Minera Doña Inés de Collahuasi and Compañia Quebrada Blanca, and the local authorities Carabineros de Chile, Zona de Tarapacá y Antofagasta (Retenes de Ollagüe, Ujina), Instituto Geográfico Militar La Paz and Ministerio de Hacienda y Desarrollo Económico, Secretaia Nacional de Minera, Bolivia. A great deal of the success of this project is due to the more than 100 helpers and field operators from all three countries. This Letter benefited substantially from constructive criticism by R. Clowes. The ANCORP '96 project was funded by the Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie (BMBF, Bonn) within the DEKORP programme (Deutsches Kontinentales Reflexionsseismisches Program) and by the Deutsche Forschungsgemeinschaft (DFG, Bonn) within the Collaborative Research Center 267 (SFB 267—Deformationsprozesse in den Anden).

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High genomic deleterious mutation rates in hominids

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It has been suggested that humans may suffer a high genomic deleterious mutation rate[1,2]. Here we test this hypothesis by applying a variant of a molecular approach[3] to estimate the deleterious mutation rate in hominids from the level of selective constraint in DNA sequences. Under conservative assumptions, we estimate that an average of 4.2 amino-acid-altering mutations per diploid per generation have occurred in the human lineage since humans separated from chimpanzees. Of these mutations, we estimate that at least 38% have been eliminated by natural selection, indicating that there have been more than 1.6 new deleterious mutations per diploid genome per generation. Thus, the deleterious mutation rate specific to protein-coding sequences alone is close to the upper limit tolerable by a species such as humans that has a low reproductive rate[4], indicating that the effects of deleterious mutations may have combined synergistically. Furthermore, the level of selective constraint in hominid protein-coding sequences is atypically low. A large number of slightly deleterious mutations may therefore have become fixed in hominid lineages.

It has been estimated that there are as many as 100 new mutations in the genome of each individual human[1]. If even a small fraction of these mutations are deleterious and removed by selection, it is difficult to explain how human populations could have survived. If the effects of mutations act in a multiplicative manner, the proportion of individuals that become selectively eliminated from the population (proportion of 'genetic deaths'[5]) is $1 - e^{-U}$ (ref. 4), where U is the deleterious mutation rate per diploid, so a high rate of deleterious mutation ($U \gg 1$) is paradoxical in a species with a low reproductive rate. Furthermore, if a significant fraction of new mutations is mildly deleterious, these may accumulate in populations with small effective sizes, or in populations in which selection has been relaxed, leading to a gradual decline in fitness[2,6]. It has been argued that an accumulation of mildly deleterious mutant alleles could have long-term consequences for human health[2]. For inbreeding plants, indirect estimates for U approach 1 (ref. 7), but these estimates assume that variation is maintained solely by a balance between mutation and selection. Results of studies of mutation accumulation in *Drosophila* also suggest values for U approaching or exceeding 1 (ref. 8), but mutations with small effects, perhaps crucial for evolution, cannot be detected on the basis of measured phenotypic differences, and the validity of the experimental controls has been challenged[9]. There are no direct estimates of U for mammals or other vertebrates.

Here we use a variation of a molecular method[3] to estimate U in hominids. By using related species, whose time of divergence from a common ancestor is known, one can, in theory, estimate U by

measuring the level of divergence between DNA sequences of related species for a random sample of DNA sequences, and comparing this with divergence of non-functional sequences. In non-functional regions of the genome, the rate of nucleotide substitution is equal to the mutation rate[10]. Random samples of the genome will include functionally important regions under selective constraint (for example, protein-coding sequences), and will show slower evolution, as a proportion of mutations in these regions will be rejected by selection. U is estimated from the difference between the divergences in a non-functional region and a random DNA sample. For example, zero divergence for the random DNA sample would imply complete constraint, and a deleterious mutation rate equal to the neutral mutation rate. The method therefore has its roots in the neutral theory of molecular evolution[10]. A difficulty with this approach is that mutation rates may differ across the genome[11]. However, we can apply a modified approach to estimate the deleterious mutation rate in protein-coding sequences alone. We assume, conservatively, that synonymous mutations are neutral in hominids; the synonymous substitution rate can therefore be used as an estimate of the mutation rate for each gene.

We estimated rates of synonymous substitution per nucleotide, and rates of substitution that result in changes in amino acids per codon, along the human lineage after the human–chimpanzee split for 46 protein-coding sequences from humans and chimpanzees, using another primate species as an outgroup (Table 1). We estimated the rates of transition (K_{ts}) and transversion (K_{tv}) mutations at synonymous sites separately, because the former occur more frequently. We also calculated the proportion of sites at which transition (N_{ts}) and transversion (N_{tv}) mutations would change the amino-acid sequence of the protein. The total number of non-synonymous mutations expected in a gene of length L is therefore $L(K_{ts}N_{ts} + K_{tv}N_{tv})$. To remove sequencing errors, we constructed consensus sequences for most of the human genes (see Methods). Randomly distributed errors in the chimpanzee and outgroup sequences will inflate the mutation-rate estimates for these lineages, but not for the human lineage.

If all non-synonymous mutations were neutral, we estimate that in human lineage there would have been 231 new non-synonymous substitutions in our entire sample of 46 genes, which together contain 41,471 nucleotides, resulting in an average of 0.0056 non-synonymous substitutions per nucleotide. However, the estimated actual number of non-synonymous substitutions is 143, an average of 0.0034 per nucleotide. Mammals are estimated to have a minimum of 60,000 genes[12], and the average length of human protein-coding sequences is $1,523 \pm 19$ base pairs (mean $\pm$ s.e.m.)[13]. Humans and chimpanzees diverged $\sim 6 \times 10^6$ years ago[14,15], so, if we assume that the average generation time in the human lineage has been 25 years since the split with chimpanzees, the estimated total rate of amino-acid-altering mutations, M, is 4.2 ($\pm$0.5) mutations per diploid genome per generation, and the deleterious mutation rate, U, is 1.6 ($\pm$0.8). Estimates of genomic mutation rate

Table 1 Estimates of synonymous substitution rates and amino-acid-substitution rates

Gene	Outgroup	Length (base pairs)	K_{ts}	K_{tv}	K_n	N_{ts}	N_{tv}
α-A-crystallin	M	123	0.03027	0.00000	0.00000	0.634	0.780
α-Fetoprotein precursor	G	1,830	–0.00002	0.00455	0.00163	0.643	0.861
α-Globin	O	429	0.00000	0.00000	0.00000	0.636	0.804
β_2-Microglobulin	G	360	0.00000	0.00000	0.00000	0.650	0.842
β-Globin	G	363	0.00000	0.00000	0.00000	0.631	0.818
Blue opsin	G	1,044	0.00335	0.00599	0.00000	0.663	0.824
BRCA-1	G	3,423	0.00193	0.00000	0.01509	0.645	0.872
C5a receptor	G	1,020	0.00345	0.00000	–0.00004	0.629	0.801
CC-chemokine receptor-5	G	1,059	0.00335	0.00000	0.00570	0.649	0.839
CD4	M	1,377	0.00533	0.00521	0.00071	0.641	0.832
Chemokine receptor-4	M	1,059	0.00345	–0.00014	0.00000	0.647	0.836
Complement receptor CR1	B	5,733	0.00591	0.00262	0.00571	0.656	0.839
Cytochrome *c* oxidase subunit IV	G	435	–0.00004	0.01961	0.00000	0.678	0.876
c-Myc	Gi	1,320	0.00250	0.00000	0.00228	0.637	0.841
DAZL1	M	291	0.00000	0.00000	0.00000	0.653	0.844
Decay-accelerating factor	O	165	0.00000	0.00000	0.00000	0.655	0.770
Eosinophil cationic protein	G	483	–0.00005	0.00000	0.00623	0.634	0.833
Eosinophil-derived neurotoxin	G	486	0.01427	–0.00022	–0.00002	0.654	0.847
EPI-1	M	456	0.00766	0.00000	0.00000	0.662	0.839
Factor IX	M	1,383	0.00252	0.00556	0.00217	0.654	0.855
Fut2–α(1,2)fucosyltransferase	G	1,032	0.00710	0.00000	0.00292	0.657	0.820
γG globin	G	444	0.00000	0.00000	0.00000	0.646	0.830
Glycophyorin A	G	366	0.00000	0.00000	0.08952	0.653	0.794
Haptoglobin	M	438	–0.00024	0.01589	0.00682	0.648	0.825
Interleukin-8 receptor	G	1,059	0.00669	0.00000	0.00284	0.626	0.820
Interleukin-16	M	1,893	0.00975	0.00658	0.00974	0.638	0.811
Interleukin-3	M	432	–0.00041	0.00000	0.01533	0.630	0.837
Involucrin	G	1,635	0.01636	0.00000	0.04863	0.631	0.880
Kruppel-like protein–ZNF75	G	633	0.00000	0.00000	0.00476	0.651	0.885
L-selectin	O	1,119	0.00989	0.00000	0.00270	0.667	0.869
Leptin	G	441	0.01689	0.00000	0.00685	0.637	0.824
Low-affinity *N*-formyl-peptide receptor	G	1,044	–0.00007	–0.00003	0.00286	0.646	0.810
Lysozyme C	G	447	0.01539	0.00000	0.00000	0.644	0.849
Melanin-concentrating hormone	Gi	186	0.06279	0.00000	0.08480	0.634	0.847
N-formyl-peptide receptor-2	G	1,047	0.00359	0.00000	0.00870	0.654	0.827
N-formyl-peptide receptor	G	1,038	0.01065	0.00000	0.00287	0.651	0.805
Preproinsulin	M	333	–0.00070	0.01819	0.00448	0.625	0.805
Prion protein	G	762	0.00474	0.00000	0.00395	0.685	0.831
Protamine P1	G	150	0.00000	0.00000	0.12090	0.553	0.913
Protamine P2	G	309	0.02300	0.00000	0.05058	0.576	0.885
Rhesus-like protein	G	1,254	0.01605	0.00000	0.07110	0.643	0.811
Ribonuclease K6	G	453	0.01520	0.00000	–0.00002	0.653	0.858
SRY	G	615	0.00000	0.00000	0.00985	0.647	0.873
Triosephosphate isomerase	M	750	0.01524	0.00870	0.00000	0.657	0.819
ZNF80	G	822	0.00647	0.00000	0.00898	0.654	0.870
ZNF91	O	180	0.00000	0.00000	0.00000	0.628	0.917

K_{ts} and K_{tn}, estimates of synonymous transition and transversion substitution rates, respectively, per nucleotide; K_n, estimates of amino-acid-substitution rates per codon; calculated as described in the Methods for 46 hominid genes. Negative estimates arise because of sampling errors. N_{ts} (N_{tv}), proportion of transition (transversion) mutations that change an amino acid. Sequences for all genes were obtained for humans and chimpanzees, plus a primate outgroup species: G, gorilla; O, orang-utan; Gi, gibbon; M, macaque; B, baboon.

for our closest hominid relatives are similar to those that we have obtained for humans (Table 2). These are conservative estimates, as data on exon abundance[12] and the frequency of CpG islands[16] indicate that there may be as many as 70,000–80,000 genes in the human genome. Furthermore, 25 years may be an underestimate of the generation time. From studies of modern hunter-gatherer human societies, three estimates for the mean age at reproduction for mothers are 27 years (±0.3)[17], 28 years (±0.5)[18] and 28 years (±0.5)[19]; for fathers, three estimates are 32 years (±0.4)[17], 35 years (±0.5)[18] and 34 years (±0.5)[19]. In wild common chimpanzees, the average age of mothers giving birth to offspring that survive infancy is estimated to be 23 years (±1.1) (data in ref. 20), whereas males have more extended reproductive profiles than females[20].

Three further factors may indicate that we have underestimated M and U. First, insertions and deletions also contribute to the genomic mutation rate. In human pseudogenes, estimated rates of deletions and insertions are only 2.5% and 1%, respectively, of the point mutation rate[21], but insertions and deletions in coding sequences are likely to be unconditionally deleterious, indicating that they might increase our estimate of U for hominids by about 10%.

Second, we have estimated the rate of deleterious mutation only within protein-coding sequences, but there are elements important for gene expression that are found upstream and downstream of genes and within introns. Rates of substitution in 3′ and 5′ untranslated regions are lower than those for synonymous sites, suggesting moderate selective constraint[22]. Our data do not allow reliable estimation of the level of constraint for hominids in transcribed untranslated regions, but data in ref. 22 indicate that mutations in these regions might contribute an extra ~10% to our estimate of U. However, sequences controlling gene expression that can be some distance from coding sequences may be a more important source of selective constraint.

Third, our sample of genes may be unrepresentative. The average level of constraint in our gene sample is remarkably low. Constraint (C) as determined by U/M is 0.38, 0.53 and 0.38 in humans, chimpanzees and gorillas, respectively, and a joint estimate for constraint in hominids is 0.45 (±0.09). However, C values, measured as $1 - K_a/K_s$, where K_a and K_s are non-synonymous and synonymous substitute rates, respectively, typically exceed 0.7. For example, in primate, artiodactyl and rodent lineages, C has been reported to be 0.73, 0.74 and 0.83, respectively[23]. To determine whether the low level of constraint is specific to our sample, we compiled the 34 available pairs of mouse and rat gene sequences that are homologous to genes present in humans and chimpanzees. The constraint in the subset of hominid genes for which we have homologous rodent sequences is 0.44 (±0.16), whereas it is 0.68 (±0.04) for the rodent genes; these values are nearly significantly different ($P < 0.06$). However, the average constraint for these 34 rodent genes is significantly lower than the average from 363 rodent genes (0.82 ± 0.01)[24], so our constraint level for hominid genes may be underestimated. A corrected constraint level is 0.46 (that is, $(0.38 \times 0.82)/0.68$). The low level of constraint is partly attributable, therefore, to an effect specific to the hominid clade (this effect contributes about two-thirds of the low level of constraint), whereas the remaining third is due to the nature of the genes in our sample. We may therefore have underestimated the deleterious mutation rate in protein-coding sequences by an extra ~20%.

An independent estimate of the non-synonymous mutation rate can be obtained from the frequency at which new electrophoretic alleles appear in human pedigrees. The analysis of 30 loci in children of the Hiroshima and Nagasaki atomic-bomb survivors, and in a control cohort whose parents were not close to the bombings, is summarized in ref. 25. In the control cohort, three band-morph mutations were detected in $\sim 4.7 \times 10^5$ allele tests, giving an estimate for the band-morph-mutation rate of 6.4×10^{-6} (95% confidence interval 1.3×10^{-6} to 19×10^{-6}). The band-morph-mutation rate in the exposed cohort was similar. About one-third of amino-acid-altering mutations lead to a change in electrophoretic mobility[25,26], giving a genomic non-synonymous mutation rate of 2.3, an estimate that is about one-half of that obtained here from DNA-sequence data.

Although calculations of mutation rates in humans have been made previously[3,27], our study is, to our knowledge, the first detailed analysis of molecular constraint in hominid lineages. With conservative assumptions, we estimate that there have been about 1.5 new deleterious mutations per generation in hominid protein-coding sequences. Assuming less conservative values for gene number (80,000), generation interval (30 years), and constraint (0.46), estimates of M and U for humans become 6.7 and 3.1, respectively. However, these remarkably high rates cannot be generalized because U appears to vary widely across taxa. For example, similar calculations for rodent lineages give estimates of U specific to protein-coding sequences that are about one order of magnitude lower than our estimates of U for humans (our unpublished results).

The deleterious mutation rate appears to be so high in humans and our close relatives that it is doubtful that such species, which have low reproductive rates, could survive if mutational effects on fitness were to combine in a multiplicative way. Our results instead indicate that synergistic epistasis may occur between deleterious mutations, in hominids at least. However, the level of constraint in hominid protein-coding sequences is very low; roughly half of all new non-synonymous mutations appear to have been accepted. Low constraint could result from the fixation of slightly deleterious mutations in species with small long-term effective population sizes (N_e), from the relaxation of selection, or from a high rate of adaptive substitution. The first of these explanations seems the most plausible, because N_e in hominids is expected to be atypically low. If deleterious new mutations are accumulating at present, this could have damaging consequences for human health[3], but this would depend critically on the frequency distribution of fitness effects of mutant alleles, about which we know little. □

Table 2 Estimates of genomic mutation rates

Species	u ($\times 10^{-9}$) (s.e.m.)	M (s.e.m.)	U (s.e.m.)	Constraint (s.e.m.)
Human	1.33 (0.18)	4.2 (0.5)	1.6 (0.8)	0.38 (0.17)
Chimpanzee	1.22 (0.31)	3.2 (0.8)	1.7 (0.8)	0.53 (0.16)
Gorilla	1.23 (0.19)	3.1 (0.5)	1.2 (0.6)	0.38 (0.17)

Estimates of per nucleotide mutation rates, u (per site per year; the sum of estimates for rates of transition and transversion mutations averaged over genes, weighted by gene length), genomic rates of mutation in amino-acid-coding sequences, M (per diploid), genomic deleterious mutation rates, U (per diploid), and levels of constraint in protein-coding sequences (see Methods). We assumed that humans have a generation time of 25 years, that chimpanzees and gorillas have generation times of 20 years, and that humans diverged 6 million and 7 million years ago from chimpanzees and gorillas, respectively[14,15]. The calculations are based on a weighting by sequence length (Table 1), although similar estimates are obtained if the mutation rates are calculated with unweighted averages; for example, $M = 4.7(\pm 0.8)$ and $U = 1.4(\pm 1.1)$ in humans. Estimates of u are consistent with previous estimates of divergence between humans and chimpanzees[30].

Methods

Nucleotide sequences of genes from humans, chimpanzees and the closest available primate species were extracted from GenBank. The third primate was chosen on the basis of its proximity to humans and chimpanzees, for example gorilla sequences were used in preference to orang-utan sequences. All genes were checked for paralogy using HOVERGEN[13] and by BLAST searches. Major histocompatibility complex genes were excluded because many of the polymorphisms segregating in humans pre-date the human/chimpanzee split[22]. Multiple sequences were obtained for 40 of the 46 human genes (90% of the total sequence length) and were used to construct consensus sequences, thereby reducing the impact of sequencing errors. Where only two human sequences were available, the chimpanzee sequence was used to resolve differences. Sequences were aligned using ClustalX[28] and corrected by hand. Synonymous and non-synonymous substitution rates were calculated by two different methods. In both methods, the calculation of the synonymous substitution

rate was restricted to codons that code for the same amino acid in all three taxa.

Method (1). For each pair of sequences (i and j), the rates of synonymous transition ($K_{ts4(i,j)}$) and transversion ($K_{tv(i,j)}$) mutations were estimated separately at fourfold degenerate sites using the equations of Ina[29] for Kimura's two-parameter method[22]. The rate of transition substitution at twofold degenerate sites ($K_{ts2(i,j)}$) was estimated as $K_{ts2(i,j)} = -\frac{1}{2}\ln(1-2P_{s(i,j)})$, where $P_{s(i,j)}$ is the proportion of sites that show a difference between sites. The overall synonymous transition rate (K_{ts}) was calculated as a weighted (by number of sites) average of the twofold and fourfold rates. The non-synonymous substitution rate per codon was calculated as $K_{n(i,j)} = -\ln(1-P_{n(i,j)})$, where $P_{n(i,j)}$ is the proportion of amino acids that differed between sequences. The distances along each branch were calculated using Fitch and Margoliash's method; for example, $K_{ts(i)} = (K_{ts(i,j)} + K_{ts(i,k)} - K_{ts(j,k)})/2$.

Method (2). Primate sequences are sufficiently similar that parsimony can also be used to estimate substitution rates. We estimated the numbers of synonymous transition and transversion and amino-acid substitutions for the human and chimpanzee lineages. Dividing these numbers by the relevant number of sites gave the substitution rate per site (as above). By reconstructing the sequence ancestral to the human/chimpanzee divide, we could also separately estimate the transition rate at CpG dinucleotides and incorporate this rate into the calculation of mutation rates. All methods gave quantitatively similar results.

Estimates of non-synonymous and deleterious mutation rates and constraint. Rates of non-synonymous (M) and deleterious (U) mutation were estimated using weighted (by length) and unweighted substitution-rate estimates. Weighted method: $M = Z\Sigma(L(K_{ts}N_{ts} + K_{tv}N_{tv}))/\Sigma L$, $U = M - Z\Sigma(LK_n/3)/\Sigma L$; unweighted: $M = Z(\overline{K_{ts}N_{ts}} + \overline{K_{tv}N_{tv}})$, $U = M - Z\overline{K_n}/3$; where Z is a constant that incorporates the number and length of genes and the generation time; for example, for humans $Z = 2(\text{genomes}) \times 60,000(\text{genes}) \times 1,523(\text{base pairs}) \times 25(\text{years})/6 \times 10^6$ (years), and all summations were across genes.

As the estimate of constraint is subject to large sampling error, we produced a joint estimate of the level of constraint in hominid protein-coding genes in the following manner. Data sets for all genes with homologues in humans and chimpanzees ($n = 53$), humans and gorillas (30), chimpanzees and gorillas (28), humans and orang-utans (25) and chimpanzees and orang-utans (25) were compiled and rates of substitution were measured as in method (1). We then estimated the number of non-synonymous substitutions predicted to occur in all sequences should all non-synonymous mutations be neutral from:

$$X = \sum_{\text{data sets}} \sum_{\text{genes}} L(K_{ts}N_{ts} + K_{tv}N_{tv})$$

and the number of non-synonymous substitutions that have occurred from:

$$Y = \sum_{\text{data sets}} \sum_{\text{genes}} LK_n/3.$$

Constraint was then estimated as $C = 1 - Y/X$.

All estimates of standard error were obtained by bootstrapping the data, by gene, 1,000 times.

Received 16 October; accepted 1 December 1998.

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Acknowledgements. We thank B. Charlesworth, J. F. Crow, E. K. Davies, W. G. Hill, T. Johnson, A. S. Kondrashov, G. McVean, J. R. Peck, A. D. Peters, M. W. Simmen, D. B. Smith and H. B. Trotter for comments and helpful discussions; K. H. Wolfe for a database of rodent gene sequences; and the Royal Society for support.

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Monocular deprivation induces homosynaptic long-term depression in visual cortex

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Brief monocular deprivation during early postnatal development can lead to a depression of synaptic transmission that renders visual cortical neurons unresponsive to subsequent visual stimulation through the deprived eye. The Bienenstock–Cooper–Munro (BCM) theory[1] proposes that homosynaptic mechanisms of long-term depression (LTD) account for the deprivation effects[2,3]. Homosynaptic depression, by definition, occurs only at active synapses. Thus, in contrast to the commonly held view that the synaptic depression caused by monocular deprivation is simply a result of retinal inactivity, this theoretical framework indicates that the synaptic depression may actually be driven by the residual activity in the visually deprived retina[4]. Here we examine the validity of this idea by comparing the consequences of brief monocular deprivation by lid suture with those of monocular inactivation by intra-ocular treatment with tetrodotoxin. Lid suture leaves the retina spontaneously active, whereas tetrodotoxin eliminates all activity. In agreement with the BCM theory, our results show that monocular lid suture causes a significantly greater depression of deprived-eye responses in kitten visual cortex than does treatment with tetrodotoxin. These findings have important implications for mechanisms of experience-dependent plasticity in the neocortex.

Previous work has shown that monocular inactivation with tetrodotoxin (TTX), like monocular lid suture, shifts the ocular dominance of cortical neurons strongly towards the non-deprived eye[5,6]. However, those studies used prolonged TTX treatment