mal circumstances using the technique of transneuronal transport.

19. The presence in some instances of ocular dominance patches in the cortical plate lateral to the cannula site in area 18 as in, for example, Fig. 1C, might be a result of variable or asymmetric diffusion of the neurotrophin or an inability of neurotrophin infusion at this age to affect the formation of ocular dominance patches in area 18, which is known to occur earlier than in area 17.
20. Dark-field images were directly imported from microscope slides by means of a video camera with the use of Metamorph (Universal Imaging). For each slide, layer 4 was subdivided into a series of overlapping linear segments. A window was drawn around each segment and a grain density profile was generated with Scion 1.55 (National Institutes of Health). Grain density profile data were spliced to create the continuous linear profiles shown (Fig. 2).
21. Measurements of grain density in the BDNF- or NT-4/5–treated brains indicate that the average diameter of the region over which grain density fluctuation was clearly diminished was 3.4 ± 0.4 mm for BDNF (n = 4 animals) and 5 ± 0.8 mm for NT-4/5 (n = 4 animals).
22. The specificity of these antibodies has been demonstrated biochemically, on protein immunoblots, and immunohistochemically, through the visualization of neurotrophins injected into rat brain and after preabsorption with antigen (J. Dugich-Djordjevic *et al.*, unpublished results). After perfusion, brains were dissected, postfixed in 4% paraformaldehyde in 0.1 M phosphate buffer, cryoprotected in 25% sucrose in 0.1 M phosphate buffer, frozen, and stored at −80°C. Sections were cut on a freezing microtome, washed in PBS three times, and incubated with 3% normal serum in PBS containing 0.5% bovine serum albumin and 0.1% Tween-20 (blocking buffer) for 2 hours at room temperature. Sections were then incubated with primary antibodies overnight at 4°C, washed three times for 10 min with blocking buffer, incubated with secondary antibodies for 2 hours at room temperature, washed, and visualized with the ABC kit (Vector) using diaminobenzidine (0.05%) with hydrogen peroxide (0.006%) and nickel ammonium sulfate (0.05%) as chromogen.
23. Although it is not possible to relate directly the immunohistohemical staining of neurotrophins to their free effective concentrations in the tissue (the micrographs shown in Fig. 3 probably represent a minimum estimate of the diffusion distance of the infused neurotrophins), it is likely that an excess of all four neurotrophins over the amount necessary to saturate their receptors has been provided. First, the rate of infusion of neurotrophins in this study (2.4 μg/day) is identical to the rate of infusion of NGF used by Gu *et al.* (*25*) to induce monocular deprivation plasticity in the adult cat. It is also well within the range of rates of ventricular NGF infusion that prevent the physiological shift in responsiveness after monocular deprivation (*26*). Second, assuming that the daily dosage of neurotrophin was able to diffuse throughout the affected area or, in the case of NT-3, the area in which it is detectable by immunohistochemistry, approximately 24 μg of neurotrophin would diffuse into 1 ml of cortex each day. Given that half-maximal neurotrophin responses typically occur at concentrations less than 1 ng/ml, we almost certainly provided a significant excess, even if concentrations within the cortical plate were smaller than those in the white matter and with some metabolism of the infused neurotrophins. Finally, the neurotrophins maintain at least 80% of their activity upon storage for 2 weeks at 37°C [L. R. Williams, *Neurobiol. Aging* **2**, 39 (1991); F. Hefti, personal communication]. Thus, it is unlikely that differences in the effective concentrations, diffusion properties, or stability of the different neurotrophins could account for the lack of effect of NGF and NT-3.
24. In several animals, the infusion itself created a large hole in the white matter. However, in control animals this did not have any effect on cortical plate morphology or on the formation of ocular dominance columns, even in close proximity to the infusion site. Furthermore, the presence of a hole at the site of infusion did not affect the diffusion of neurotrophins into the surrounding white matter and cortex, as determined immunohistochemically. Cortical plate morphology was indistinguishable from that of controls in two of the four BDNF-infused brains and in two of the four NT-4/5–infused brains (as well as in those brains in which NGF or NT-3 had been infused). In the remaining brains (two with BDNF; two with NT-4/5), some disorganization and decrease in cell number was observed in cortical layers 4 and 5, but only in very close proximity to the site of infusion and over a much smaller area than that in which ocular dominance patches were absent.
25. Q. Gu, Y. L. Liu, M. S. Cynader, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 8408 (1994).
26. L. Domenici *et al.*, *ibid.* **88**, 8811 (1991); L. Maffei *et al.*, *J. Neurosci.* **12**, 4651 (1992); G. Carmignoto *et al.*, *J. Physiol. (London)* **464**, 343 (1993).
27. The cholinergic inputs from the basal forebrain to the cortex express TrkA [M. E. Vazquez and T. Ebendal, *NeuroReport* **2**, 593 (1991); D. M. Holtman *et al.*, *Neuron* **9**, 465 (1992)] and are thought to allow ocular dominance plasticity, as assessed physiologically [M. F. Bear and W. Singer, *Nature* **320**, 172 (1986)]. Thus, it is possible that the NGF infusions could have acted to alter these inputs.
28. As an alternative, it is possible that the effects of the infused NT-4/5 and BDNF are mediated indirectly, through interactions with TrkB-expressing cortical neurons. For example, it is known that many pyramidal neurons in cortical layers 3 and 5 are also immunoreactive for TrkB (*16*). However, given the absence of direct connections between LGN axons and layer 3 or layer 5 neurons, we consider it unlikely that the responses of the neurons to neurotrophins would have a direct effect on the behavior of LGN axons.
29. Subplate neurons have been shown to be required for the formation of ocular dominance columns [A. Ghosh and C. J. Shatz, *J. Neurosci.* **14**, 3862 (1994)]. However, it is unlikely that the results presented here reflect a modulation of subplate neuron survival, which might in turn have altered column formation. The major phase of subplate cell death is already over by P28 [J. J. M. Chun and C. J. Shatz, *J. Comp. Neurol.* **282**, 555 (1989)], the time at which these infusions were begun, and preliminary analysis does not indicate significant changes in subplate neuron survival that are a result of neurotrophin infusion at this particular age.
30. We wish to thank F. Hefti, G. Burton, C. Schmelzer, and G. Bennett at Genentech for their generous gifts of neurotrophins and antibodies to NT-3 and NT-4/5. We also acknowledge the able technical assistance of S. Tavazoie. Supported by NIH grants EY02858 and MH 48108 (C.J.S.), NIH postdoctoral fellowship EY06327 (R.J.C.), and the Swiss National Foundation (A.H.). C.J.S. is an investigator of the Howard Hughes Medical Institute.

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TECHNICAL COMMENTS

The Body Temperature of *Tyrannosaurus rex*

R. E. Barrick and W. J. Showers (*1*) purport to show that the oxygen isotope signature of *Tyrannosaurus rex* bone phosphate indicates that this species was homeothermic. A statistical analysis of their results based on our knowledge of bone remodelling processes shows that the data is consistent with a widely varying body temperature for *T. rex*.

Living compact bone is remodeled by the construction of individual Haversian systems, so that the shortest event that could possibly be investigated for palaeothermophysiology is the time taken for this process. The same applies to other techniques including palaeodietary reconstruction (*2*). In adult animals this process does not take place in adjacent Haversians in sequence, but essentially randomly throughout the bone. Samples taken across a bone do not, therefore, "record physiological conditions over time," as Barrick and Showers state, but events randomly scattered through an individual creature's life. Moreover, samples which include mineral from more than one Haversian system will produce results averaging different times in the individual's life. The method used by Barrick and Showers (*3*) uses a sample of some 30 mg of phosphate, which is therefore a volume of at least 1 mm^3 of bone (at an overestimated density for bone of 3 g cm^{-3}). Haversian systems are typically cylinders of diameter 200 μm, which if a 1 mm length of a system is taken has a volume of 0.031 mm^3. I therefore estimate that each sample of bone taken represented a minimum of 32 Haversian systems, and therefore that each of the measurements made was the average of the values for 32 Haversian systems.

Taking a series of measurements, each of which is the average of 32 individual events, will lead to the same mean value, but the variance of the measurements will be 32 times smaller than the true variance for those events, and thus the spread of the data will be $\sqrt{32} = 5.7$ times smaller than the true spread. Using this factor to multiply up the ranges observed for single skeletal elements of 1.7° to 3.8°C gives ranges of 9.7° to 21.7°C. These ranges must be regarded as minimum estimates, because of the small value taken for the sample volume.

Such large temperature variations preclude the classification of *T. rex* as a homeotherm, but they are concordant with Barrick and Showers' estimate of 20°C as the annual variation in the core body temperature of a 5000-kg bradymetabolic hadrosaur. It must be concluded that *T. rex* was an ectotherm, not a homeotherm.

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REFERENCES

1. R. E. Barrick and W. J. Showers, *Science* **265**, 222 (1994).
2. B. Herrman, in *Prehistoric Human Bone: Archaeology at the Molecular Level*, J. B. Lambert and G. Grupe, Eds. (Springer-Verlag, Berlin, 1993), pp. 203–215.
3. R. C. Crowson, W. J. Showers, E. K. Wright, T. C. Hoering, *Anal. Chem.* **63**, 2397 (1991).

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Response: Millard concludes that *T. rex* is an ectotherm from an analysis of the data in our (R.E.B. and W.J.S.) report (*1*). We disagree with his conclusion because his application of a statistical model to the isotopic variations found within the bones of *T. rex* is inappropriate. Millard's argument depends on the statistical and physiological independence of each Haversian system within the body of *T. rex*. It is unlikely this assumption holds, as all Haversian systems are part of the same vascular system of a single animal. Millard cannot "multiply up the ranges" of the isotopic values within single bones without an assumption about the statistical distribution of the putative population of measurements theoretically available from each Haversian system within the 30-mg samples analyzed in the report (*1*). Millard implicitly assumed normality in his statistical argument, but such an assumption is unjustified in the absence of data that would help constrain the form of the underlying distribution. Common fluid flow within adjacent systems would result in physiological dependence, and therefore statistical dependence of isotopic variations.

Millard suggests that the true spread of the isotopic data within single samples of bone is inversely proportional to the number of Haversian systems, and therefore the size of the sample taken will control the isotopic heterogeneity. The average 30-mg sample size analyzed by the silver phosphate technique cited by Crowson *et al.* (*2*) was of NBS 120 phosphorite rock which is 33% PO_4 (about 10 mg of phosphate). The *T. rex* samples were found to contain 2.25 to 23% PO_4, so between 3 to 13 mg of PO_4 (about 6 to 25 mg of apatite) were analyzed in each *T. rex* sample. No significant correlation is found ($r = 0.19$, $P(r) = 0.29$) when the isotopic range for each bone sample is plotted against the average number of Haversian systems contained within each sample estimated from Millard's calculations (data not shown). This suggests that Millard's Haversian system independence assumption is invalid.

Furthermore, body temperatures in endotherms are controlled within narrow limits (±2°C), resulting in a restricted range of bone isotopic values. Measurements of intrabone isotopic heterogeneity ranged from 0.4 to 0.8 per mil, respectively, from a modern cow and deer (*3*), using sample sizes similar to those taken from *T. rex*. Millard's statistical reasoning would suggest that these endotherms underwent body temperature variations of 10° to 20°C. With these modern animals we know that this is not the case, which also suggests that the Haversian system independence assumption is invalid. We agree that the spread of the isotopic data may be a minimum estimate of variation.

Statistical tests of means and variances from data in the report (*1*) may be used to evaluate a hypothesis of endothermy in *T. rex*. These tests were completed independently (by those of us at the University of Alabama, Birmingham) and the results suggest the following. (i) If *T. rex* were an endotherm, isotopic variance of extremity bones (tibia, metatarsal, phalange, and mid and distal caudal vertebrae) ought not to differ significantly from isotopic variance of core bones (rib, gastralia, and dorsal vertebrae). A two-tailed *F* test yields a test statistic of 1.010, the probability of which is 0.49; thus the null hypothesis of endothermy cannot be rejected. (ii) If *T. rex* were an endotherm, the mean isotopic value of the bone recording the coldest temperature (distal caudal vertebra) ought to differ by less than 4°C from the mean isotopic value of the bone recording the warmest temperature (dorsal vertebra). A one-tailed, two-sample *t* test on these two bones yields a test statistic of 0.659, the probability of which is 0.26; thus once again providing no evidence to reject the null hypothesis of endothermy. These additional statistical analyses are consistent with the hypothesis of endothermy in *T. rex*. Our point still stands that the lack of significant intrabone or interbone δ_p variation in *T. rex* suggests it was homeothermic.

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REFERENCES

1. R. E. Barrick and W. J. Showers, *Science* **265**, 222 (1994).
2. R. C. Crowson *et al.*, *Anal. Chem.* **63**, 2397 (1991).
3. R. E. Barrick and W. J. Showers, unpublished data.

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