axonal structure and function. Further, the ability of ceramide to inhibit the growth of T9 cells, which only express $p75^{NTR}$, suggests that the SM cycle may be a prominent signaling pathway in cells primarily expressing $p75^{NTR}$, such as glioma, schwannoma (*17*), and melanoma cells (*25*) or cells overexpressing $p75^{NTR}$ in response to axotomy or senile dementia (*26*). The antiproliferative and differentiative effects of ceramide may occur by modulation of cellular protein phosphorylation and the down-regulation of the c-myc proto-oncogene (*7*). Both a ceramide-activated kinase (*7*, *8*) and phosphatase (*27*) have been identified that may provide direct molecular targets for further downstream effects of ceramide (*10*).

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20. Rat T9 cells were transfected by the calcium phosphate precipitation method with 1 μg of pCMVEN10 complementary DNA (cDNA) (*19*) and 0.1 μg of *pSV2neo* (neomycin resistance gene). Transfection with *pSV2neo* only served as the control. Twenty-four hours after transfection, cells were placed in Dulbecco's modified Eagle's medium (DMEM) containing 10% bovine calf serum (BCS) and G418 (0.6 mg/ml). After 2 to 3 weeks, neomycin-resistant colonies were pooled and subcultured in DMEM containing 10% BCS and G418 (0.25 mg/ml) for use in all subsequent experiments. Cells were designated T9EN10 and T9SV2neo. To examine integration of chimeric cDNA, genomic DNA was isolated and Southern blot analysis was performed. The pooling of stable colonies avoids the bias associated with the analysis of single colonies and, therefore, is representative of clones expressing various amounts of receptor.
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29. Rat T9 cellls were grown for 72 hours in the presence of [^{3}H]choline (0.5 μCi/ml, 86 Ci/mmol) in DMEM containing 10% BCS. The medium was removed, and the cells were washed with phosphate-buffered saline (PBS) and incubated in serum-free DMEM containing 25 mM Hepes (pH 7.4) for 4 to 6 hours before treatment. Cells were treated with either PBS or mouse 2.5S NGF in PBS (Harlan Bioproducts for Science) at 37°C for the indicated times, the medium was aspirated, and the cells were fixed with 2 ml of methanol. Lipids were extracted and the amount of SM was quantitated by resuspending 10 to 15 nmol of total lipid phosphorous in 50 μl of 200 mM tris-HCl (pH 7.4), 10 mM $MgCl_2$, and 1% Triton X-100. Reactions were initiated by the addition of 50 μl of 10 mM tris-HCl (pH 7.4) containing 100 mU of bacterial sphingomyelinase (*Streptomyces* sp., Sigma), and the mixture was incubated for 2 hours at 37°C. The reaction was terminated by the addition of 1.5 ml of chloroform:methanol (2:1, v/v). Hydrolyzed [^{3}H]phosphocholine was recovered in the aqueous phase after the addition of 0.2 ml of water. The amount of [^{3}H]phosphocholine released was normalized to total phospholipid mass [S. Jayadev, C. M. Linardic, Y. A. Hannun, *J. Biol. Chem.* **269**, 5757 (1994)]. Ceramide mass was quantitated as described [P. P. VanVeldhoven, D. P. Matthews, D. P. Bolognesi, R. M. Bell, *Biochem. Biophys. Res. Commun.* **187**, 209 (1992)].
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TECHNICAL COMMENTS

Entropic Elasticity of λ-Phage DNA

DNA is unique among polymers both for its size, and for its long persistence length, $A \approx 50$ nm (*1*). Since A encompasses many base pairs, and thus to a large degree is averaged over sequence, a continuum elastic description of DNA bending is plausible. Recently, S. B. Smith *et al.* made a direct mechanical measurement of the force versus extension $F(x)$ for a 97-kb λ-DNA dimer (*2*). Here we show that these experimental data may be precisely fit by the result of an appropriate elastic theory and thereby provide a quantitative baseline, departures from which will signal effects of more biological interest.

If the force is used as a Lagrange multiplier to fix the extension, the free energy of a stretched worm-like polymer corresponds to the quantum-mechanical ground state energy of a dipolar rotator with moment of inertia A, subject to an electric field F (*3*). Although the quality of the experimental data required us to supply a complete numerical solution, both the large- and small-force limits admit analytical asymptotic solutions that are summarized by the following interpolation formula:

$$FA/kT = \frac{1}{4}(1 - x/L)^{-2} - \frac{1}{4} + x/L$$

where k is Boltzmann's constant, T is temperature, and L is the molecular contour length. For large $F \gg kT/A$, the accessible conformations reduce to quadratic fluctuations around a straight line, while for $F \ll kT/A$ the polymer conformation becomes a directed random walk. The force needed to extend a freely jointed chain model diverges less strongly as $x \to L$ [$F \propto (1 - x/L)^{-1}$] as fluctuations inside each segment are suppressed.

A nonlinear least-squares fit of the exact $F(x)$ to experimental data (Fig. 1) gives $L = 32.80 \pm 0.10$ μm and $A = 53.4 \pm 2.3$ nm (90% confidence level errors; $\chi^2/n = 1.04$ for $n = 303$ data points). This L is close to the crystallographic value of 32.7 μm, while A is in good agreement with the results of cyclization studies (*1*). Refinements of the present technique may well become the most accu-

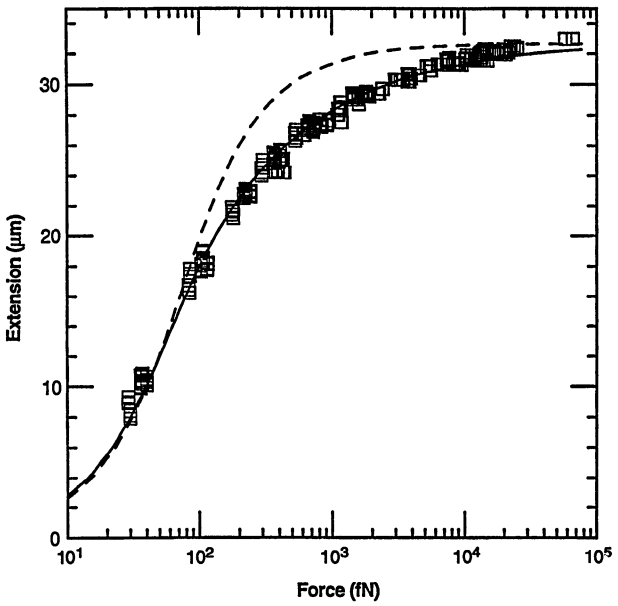

Fig. 1. Squares are experimental force versus extension data for 97 kb λ-DNA dimers from figure 3 of (*2*); solid line is a fit of the entropic force required to extend a worm-like polymer. The fit parameters are the DNA length (L = 32.80 ± 0.10 μm) and the persistence length (A = 53.4 ± 2.3 nm). Shown for comparison (dashed curve) is the freely jointed chain model (*2*) with L = 32.7 μm and a segment length b = 100 nm chosen to fit the small-x data.

rate method for determination of L and A for DNA in solution, in part because the interpretive theory is simple. For example, excluded volume effects are minimal for unstretched DNA with $L \leq 100$ kb, and are further reduced by extension. The systematic underestimation of F for $x > 31$ μm may signal the breakdown of the conventional bending elasticity, because beyond that point the correlation length $(kTA/F)^{1/2}$ becomes less than the double helix period. Further mechanical studies of DNAs that are supercoiled, single-stranded, intrinsically bent, or in contact with proteins should prove even more interesting.

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Explicit and Implicit Learning and Maps of Cortical Motor Output

Alvaro Pascual-Leone *et al.* (*1*) report that cortical motor output maps change systematically as subjects practice a reaction time task when a sequence of stimuli is patterned, but not when the sequence is random. Implicit learning, measured by comparing improvement in reaction time in patterned conditions with that in random conditions, was correlated with growth in the maps. Pascual-Leone *et al.* assessed explicit learning every 120 trials by asking subjects to try to describe the pattern; by their definition, explicit learning had occurred only when the subject could describe the pattern with complete accuracy. The maps returned to baseline conditions about the time explicit learning occurred. Pascual-Leone *et al.* suggest that the growth in the maps reflected implicit learning and that the return to baseline reflected some kind of transfer from implicit to explicit learning. This conclusion is post hoc and is inconsistent with other research on implicit learning.

Implicit and explicit learning can occur independently (*2*, *3*), whereas Pascual-Leone *et al.* seem to regard the former as a precursor of the latter. In their experiment (*1*), subjects were asked to recall the pattern after every block of 120 trials, a procedure that is likely to have induced an explicit learning strategy. Assuming that implicit learning is automatic, both forms of learning probably occurred simultaneously under these conditions. If so, the changes observed in the maps could reflect a number of stages in implicit learning or explicit learning, or both, and not necessarily a shift from one to the other.

But the maps may not reflect implicit learning at all. Pascual-Leone *et al.* apparently assumed that implicit learning in the reaction time task is a motor process, but that assumption is suspect. Implicit learning is evident in this task even after the mapping of effectors to responses is changed (*4*) and when subjects are first exposed to the repeating pattern only by watching it without making a response (*5*). Without a specific rationale for relating implicit learning to the cortical motor output maps, it is not clear that the growth in the maps is related to implicit learning.

It seems more likely that explicit learning caused the growth in the maps. Mean reaction time was about 200 ms five blocks before explicit learning supposedly occurred and was under 100 ms two blocks before. Such fast reaction times suggest that subjects knew in advance what stimulus to expect, which suggests explicit learning had occurred. Reaction times faster than 100 ms have previously been regarded as anticipations, and such responses are strongly correlated with, although perhaps not completely diagnostic of, explicit knowledge (*3*). The subjects in the study by Pascual-Leone *et al.* had apparently acquired explicit knowledge well before they were so classified, perhaps because the procedure induced an explicit learning strategy. Thus, the greatest growth in the maps was strongly related to explicit learning. Moreover, implicit learning has been shown to begin early in practice, in the first 100 trials (*3*, *6*). At that stage of the experiment of Pascual-Leone *et al.*, there was little, if any, change in the maps. Both the growth in the maps and the return to baseline were most likely caused by explicit learning. Perhaps the growth is caused by increments in explicit knowledge and the return to baseline by overlearning or automatization.

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