and B), which suggests that NMDA alone is not sufficient to open the channel. Indeed, at early times in culture, NMDA elicited very little current unless glycine was added (Fig. 4A). By 6 to 10 days in vitro, however, the response to rapid perfusion of NMDA in solutions lacking glycine was often a substantial fraction of the maximal current obtained with glycine added (Fig. 4A). This change over time in culture probably reflects the continual release of glycine by glial cells (*6*) that proliferate to confluence during the first week in vitro. Johnson and Ascher (*6*) have shown that medium conditioned by cultures contains significant levels of glycine; rapid perfusion apparently can reduce but not eliminate this contamination.

These experiments demonstrate that I2CA selectively and competitively inhibits the potentiating action of glycine on current elicited by NMDA. Together with recent results on another antagonist, 7-chlorokynurenic acid (*9*), they strengthen the proposal (*7*) that glycine is essential for channel activation (although it cannot be ruled out that I2CA may act as an inverse agonist at the glycine site). A number of studies have implicated NMDA receptors in the control of synaptic plasticity (*2*) and in excitotoxic cell death (*18*). Investigations of antagonists of the glycine potentiation site, such as I2CA, should lead to a better understanding of glycine's role in these processes; these antagonists may have therapeutic value for the treatment of stroke, epilepsy, and other neurodegenerative disorders (*18*).

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12. Neurons were bathed in a continuous stream of control or agonist-containing external solution flowing from one of a linear array of microcapillary tubes. A complete change of solution could be achieved within a few hundred milliseconds by moving the array of tubes relative to the cell. The external solution contained 160 m*M* NaCl, 10 m*M* Hepes (*p*H 7.4), 1 m*M* EDTA, 3 m*M* $CaCl_2$, and 300 n*M* tetrodotoxin. Pipettes were filled with 120 m*M* cesium methanesulfonate, 5 m*M* CsCl, 10 m*M* Cs_2EGTA, 10 m*M* Hepes (*p*H adjusted to 7.4 with CsOH), 5 m*M* MgATP, and 1 m*M* Na_2GTP (ATP, adenosine triphosphate; GTP, guanosine triphosphate).
13. Dose-response relations were fitted with the Langmuir equation [H. P. Rang, *Nature* **231**, 91 (1971)]:

    $$I/I_{\max} = 1/(1 + EC_{50}/[\text{glycine}])$$

    A competitive inhibitor shifts the EC_{50} for agonist by a factor of

    $$(1 + [\text{inhibitor}]/K_i)$$
14. I2CA and its derivatives were obtained from Aldrich.
15. The differences in potency among the six compounds may be due to direct interaction of the substituent group with the receptor or could result from the tendency of the substituent to release or withdraw electrons, which would affect the stability of structures in which nitrogen forms four bonds and bears a positive charge. Further experiments with other derivatives of I2CA should resolve these two possibilities.
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20. I am grateful to B. Bean for advice, encouragement, and support and to L. Regan and D. Sah for helpful comments on the manuscript. Supported by the Harvard University Society of Fellows and the Esther A. and Joseph Klingenstein Fund and by grants to B. P. Bean from the National Institutes of Health (HL-35034) and the Rita Allen Foundation.

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Technical Comments

Asymmetries of Clade Shape and the Direction of Evolutionary Time

We argued recently (*1*) that an "arrow of time" could be demonstrated by characteristic differences between early and late arising clades in the fossil record. Early arising clades tend to be more bottom heavy than late arising clades, in the sense that they tend to diversify more rapidly after their origin. Jennifer A. Kitchell and Norman MacLeod (*2*) criticize our thesis that asymmetries of clade shape are useful for inferring temporal directionality. Their challenge is based principally on their statement that "neither the early arising (Cambro-Ordovician) nor the later arising clades have an asymmetry that is distinguishable from clades produced by a random branching process." Their objection is invalid for several reasons.

First, as Kitchell and MacLeod acknowledge, our criterion for establishing directionality hinges on the following question: "If you were handed a chart of clade diversity diagrams with unlabeled axes, would you know whether you were holding the chart upside down or right side up?" (*1*, *2*). We held that an affirmative answer would be a sufficient demonstration of temporal directionality. As our question was clearly meant to be answered using real *empirical* data, it is inappropriate (and also wrong in this case) to criticize our thesis by comparing our results to those of a *theoretical* model. The relevant comparisons are between early and late arising fossil clades. The regression analyses we presented, which were not mentioned by Kitchell and MacLeod, clearly show that early arising clades are significantly more bottom heavy than those arising later (*3*). New comparisons of mean centers of gravity (*CG*) of Cambro-Ordovician and later arising clades in which standard statistical tests were used also show early arising clades to be significantly more bottom heavy ($P = 0.013$, *t* test; $P = 0.017$, Mann-Whitney *U* test) (*4*).

However, even if a comparison of our empirical data to the results of their theoretical model were relevant, Kitchell and MacLeod's own analysis would have proved their contention wrong if they had performed the correct statistical test. They found that the mean *CG* determined using their model was 0.503 and that the 95%

confidence limits about the mean ranged from 0.431 to 0.577. According to Kitchell and MacLeod, these "results indicate statistically significant ($P < 0.05$) bottom-heavy asymmetry only for clades of $CG < 0.431$ and top-heavy asymmetry only for clades of $CG > 0.577$." Noting that our value of 0.482 for the mean CG of Cambro-Ordovician clades falls within their 95% range, they infer that 0.482 "is not significantly different from the expected shape of randomly branching (symmetrical) clades."

But it is incorrect to compare our *mean* value of 0.482 to the 95% confidence intervals for *individual* clades. To compare our mean to the expectation of their simulation model (which is also a mean) requires a test for comparing *means*, such as the standard t test. Correctly comparing our mean CG of 0.482 to Kitchell and MacLeod's simulated mean of 0.503 using a t test, we find that our mean for Cambro-Ordovician taxa *is* statistically different from the mean for their randomly branching clades ($P < 0.001$) (*5*).

We agree that identification of evolutionary patterns rests on methods of statistical inference. When the relevant statistical tests are made, time's arrow emerges as a fact of life (*6*).

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3. $P = 0.0038$, $P = 0.0161$, and $P = 0.0124$ for regression analyses based on three different measures of bottom-heaviness. Details of the analyses are presented in (*1*).
4. Data on families within orders are from J. J. Sepkoski, Jr., *Milwaukee Pub. Mus. Cont. Biol. Geol.* **51** (1982). For Cambro-Ordovician clades, $n = 47$, mean $CG = 0.484$ and SD of $CGs = 0.083$. For post-Ordovician clades, $n = 11$, mean $CG = 0.560$, and SD of $CGs = 0.109$.
5. P value is from a t test comparing the mean CG of Cambro-Ordovician genera within families discussed in (*1*), to the mean CG of simulated clades discussed in (*2*). For Cambro-Ordovician genera within families, $n = 353$, mean $CG = 0.482$, and SD of $CGs = 0.079$. For simulated clades, $n = 1000$, mean $CG = 0.503$, and SD of $CGs = 0.033$; data from table 1 in (*2*). We also used a t test to compare the mean CG of 47 Cambro-Ordovician families within orders [data source cited in (*4*)] to the mean CG of 1000 simulated clades discussed in (*2*) and discovered that here, too, $P < 0.001$, despite the smaller sample size. For Cambro-Ordovician families within orders, $n = 47$, mean $CG = 0.484$, and SD of $CGs = 0.083$. For simulated clades, $n = 1000$, mean $CG = 0.503$, and SD of $CGs = 0.033$; data from table 1 in (*2*).
6. David Backus assisted with the tabulation of the data.

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Response: Norman L. Gilinsky *et al.* are making an argument where there is none. We did not question the *statistical* results of the t tests as reported in their 1987 article (*1*), nor would we question the *statistical* results of the t tests presented in this comment. The t test is a standard statistical test for assessing differences between means. However, whether or not this statistical difference is meaningful depends on the question posed. With the use of the t test, it has been shown that the widths of hairs from the left side of a sheep are statistically different from the widths of hairs from the right side of the same sheep. Similarly, although it can be shown that the mean height of class A basketball teams is greater than the mean height of class B basketball teams, it would be erroneous to conclude that the shorter team was short. In this case, "short" is a nominal category whose definition derives from some other expectation.

The purpose of our work on clade shape (*2*) was to provide *expectations of nominal categories*. Three categories of clade shapes had been previously recognized (*1, 3*): the asymmetrical categories of "bottom heavy" and "top heavy" and the intermediate "symmetrical" category. Idealized diagrams of each clade shape category were given in figure 1 of Gould *et al.* (*1*). These three patterns are the outcome of distinct processes. In the symmetrical case, the probabilities of speciation and extinction are equiprobable over time, whereas, in the asymmetrical cases, the probabilities of speciation and extinction are inequiprobable over the clade's duration.

A common error is to assume that the distribution of a novel statistic is known. In the case of the center of gravity (CG) statistic, however, the relevant distributions were unknown. Figure 1 of Gould *et al.* (*1*) limits the symmetrical clade shape category derived from an equiprobable process to $CG = 0.5$, defining clades of $CG < 0.5$ to the category of bottom heavy and clades of $CG > 0.5$ to the category of top heavy. What was needed, and what our work provides, are distributions for the CG statistic of individual clade shape. Our work generates probability distributions of the CG statistic for individual clades in which the generative process is equiprobable with respect to speciation and extinction (the symmetrical assumption). Using these distributions, we addressed the following question: Is the Cambro-Ordovician group, defined on the basis of time of origin, a "bottom-heavy" group (meaning a group whose individual members are characteristically bottom heavy) and the post-Ordovician group, similarly defined on the basis of time, a "symmetrical" group (whose individual member clades are characteristically symmetrical)? The question follows from the assertion in Gould *et al.* (*1*) that the "Cambro-Ordovician clades show a significant tendency to bottom heaviness . . . whereas clades arising later are temporally symmetrical."

Our original contention stands. The probability distributions we generated show that the equiprobable process gives rise [even for the most conservative cases; see (*4*)] to CG statistics for individual clades whose 95% confidence intervals include values ranging from 0.388 to 0.571. One can now determine the nominal category of individual clades. On the basis of the reported mean CG and standard deviation, approximately 74% of the individual clades placed by Gould *et al.* within the Cambro-Ordovician group are not bottom heavy (*5*). Instead, like the post-Ordovician group, these clades predominantly belong to the symmetrical (or top heavy) nominal category. Because the symmetrical nominal category dominates both the early and later arising groups, it is not easy to determine whether one is holding a chart of clade diversity diagrams "upside down or right side up." A test more appropriate to the question would involve assigning individual clades to nominal categories and testing for serial randomness of these nominal categories.

[We are perplexed by the statement by Gilinsky *et al.* that one should not compare empirical data, such as that from the fossil record, to expectations from theoretical models. The comparison of sets of numbers with theoretical models is one of the principal activities of statistics. Also, we did not mention the results of the regression analysis in Gould *et al.* (*1*, figure 3) because we determined that the statistical significance of this result was not robust to the removal of a single (extreme) datum. When we excluded this single observation (highest CG), analysis of variance accepted the null hypothesis of zero slope, that is, no linear trend.]

Our contribution is a positive one: we have provided the probability distributions from the relevant equiprobable generative process, thereby enabling individual clade shapes to be placed into nominal (process) categories. Because the two groups selected by Gould *et al.* were each dominated by individual clades belonging to the *same* nominal categories and not to different nominal categories, as stated, we rejected their conclusion. Our work, however, provides a