



February 13, 1986

Neil M. Nathanson, Ph.D.
Associate Editor
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Dear Dr. Nathanson,

We appreciate your review of our manuscript entitled "The Binding of L-[³H] Nicotine to a Single Class of High Affinity Sites in Rat Brain Membranes" (No. 4982). The reviewer's comments have been carefully considered, and we feel that the revised manuscript speaks adequately to the concerns that were expressed. Our responses to the individual issues are detailed below, and specific changes to the text have been appropriately noted.

Reviewer I

General Comments:

1. (a,b) - Although negative cooperativity effects cannot be entirely ruled out, we agree that, at this point, an equally likely explanation for the data (based on increased Bmax values) is binding to additional low affinity sites that result from proteolysis. This is stated in the revised text. We do not feel that the evidence is conclusive enough to make a clear-cut choice between the two.

(c) - The data were also fit to a three-site model, using "LIGAND", and there was no significant improvement. This is also stated in the revised text under "Results".

2. We feel that the inclusion of data from additional studies using muscarinic agonists/antagonists would not add to the completeness of the manuscript. However, we have included some additional data from an inhibition binding experiment with acetylcholine that is relevant. This has been added to Table II and comments have been included in the revised text under "Results" (Inhibition Binding), and in the "Discussion".

Comparison of receptor numbers determined in our studies and those of Schwartz et al. (Ref. 15) and Marks and Collins (Ref. 13) have been added under "Discussion".

3. The experiment suggested would probably not tell us anything about conformational changes since the shift will occur anyway (to the extent that nicotine can bind in the presence of a given concentration of a competitor) during the establishment of new equilibrium conditions. However, we are presently completing detailed kinetic experiments that speak more directly to the issue.
4. These experiments have been done but would be more appropriately detailed in a subsequent report.

Specific Comments: The legend to Figure 2 has been enlarged as requested.

Reviewer II

Specific Comments:

1. We agree that this is certainly an experiment worth doing. However, we feel that the observation of a decreased B_{max} at 37°C is not the main focus of the present publication and could be better pursued in more detail elsewhere.
2. The question of cooperativity has been dealt with in the revised text. Alternative explanations for the curvature of Scatchard plots have been suggested as equally plausible (e.g. additional low-affinity binding sites). Even in the original text, negative cooperativity effects were meant to be suggested as one possible explanation, but certainly not the only one.
3. We agree! We have in fact done these studies and are preparing them for publication.
4. This experiment has been done and the result is stated in the revised text under "Results" (Binding Kinetics).

Reviewer III

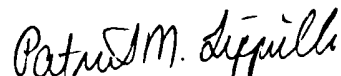
1. See Reviewer I, Part 2.
2. The Hill slopes were in fact close to 1 and this has been stated in Table 2 of the revised manuscript.
3. Yes, it would, in the intermediate concentration range. In the extremes of high and low concentration, the slope would of course approach unity. If a wide concentration range is used (e.g., 0.1 nM to 500 nM) as we did here, the slope would be biased toward lower values because of the lower slopes of intermediate points. However, even if one invokes a model based on a 100-fold difference in K_d between two classes of sites, the slope predicted by linear regression analysis would be on the order of 0.7. In the present studies, the maximum difference in K_d between the high and low affinity sites was actually found to be in around 100-fold. Therefore,

Hill slopes on the order of 0.5 or 0.6 may be indicative of some additional cooperativity effects. Since we feel it would not significantly improve the text to add such a lengthy discussion, we have simply offered alternative explanations for curved Scatchard plots as equally plausible.

4. This correction has been made in the "Discussion" of the revised text.
5. The suggested syntax changes have been made.

Thank you for your kind attention. It is hoped that the revised manuscript, together with our comments to the reviewer, will help expedite publication.

Sincerely,



Patrick M. Lippiello
Senior R&D Biochemist

PML/rb