tion had been through 270° clockwise (*17*).

Finally, these results were obtained from one animal: because cognitive problems could be solved in different ways by different subjects, it is important that techniques for reading out brain operations be sensitive enough to be applied to single subjects. Indeed, the findings of our study indicate that the population vector is a sensitive tool by which an insight can be gained into the brain processes underlying cognitive operations in space.

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10. The apparatus was as described in A. P. Georgopoulos and J. T. Massey [*Exp. Brain Res.* **65**, 361 (1987)]. Briefly, it consisted of a 25 cm by 25 cm planar working surface made of frosted plexiglass onto which a He-Ne laser beam was back-projected with a system of mirrors and two galvanometers. The monkey (5 kg) sat comfortably on a primate chair and grasped a freely movable, articulated handle at its distal end, next to a 10-mm diameter transparent plexiglass circle within which the animal captured the center light.
11. The eight positions were equally spaced on the circle, that is, at angular intervals of 45°, and were the same throughout the experiment. The brightness condition (dim or bright) and the position of the light were mixed. The resulting 16 brightness-position combinations were randomized. Eight repetitions of these 16 combinations were presented in a randomized block design.
12. The term "counterclockwise" is simply descriptive; no counterclockwise or clockwise directions were indicated to the animal. The direction in which the animal was required to move can be described equivalently as either 90° counterclockwise or 270° clockwise. The animal received a liquid reward when its movement exceeded 3 cm and stayed within ±25° of the direction required. The average direction of the actual movement trajectories was within ±5° of the direction required. Performance was over 70% correct trials.
13. The square root transformation was used as a variance-stabilizing transformation for counts [G. W. Snedecor and W. G. Cochran, *Statistical Methods* (Iowa State Univ. Press, Ames, Iowa, ed. 7, 1980), pp. 288–290.] Although the results obtained without this transformation were similar, the transformation is more appropriate because of the small size of the time bins (10 ms), and, therefore, the small number of counts.
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18. We thank D. Brandt and N. Porter for help during some of the experiments. Supported by USPHS grants NS17413 and NS20868.

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Technical Comments

Analysis of Ligand Binding Specificity of Receptor Chimeras

The elegant studies of Kobilka *et al.* (*1*) define the effects of exchange of individual transmembrane segments of α_2- and β_2-adrenergic receptors on the binding of the α_2-specific agonist *p*-aminoclonidine (PAC) and the β_2-specific agonist isoproterenol (ISO). Their results reveal a dominant role for transmembrane segment 7 in determining the specificity of binding of α_2-specific agonists versus that of β_2-specific agonists (*1*). Here I offer a quantitative analysis based on a calculation of the relative free energy of binding that further strengthens their conclusion.

The K_d value for a ligand defines its free energy of binding according to the relation $\Delta G = -RT\ln(1/K_d)$. Binding specificity of each receptor species for α_2-specific agonists versus that for β_2-specific agonists depends on the difference in the free energy of binding of the two ligands $\Delta(\Delta G) = RT\ln[K_d(\text{PAC})/K_d(\text{ISO})]$. A plot of $\Delta(\Delta G)$ for binding of PAC versus that of ISO by each receptor chimera as a function of the number α_2 transmembrane segments in the chimera reveals progressive changes in the binding energy preference for these two ligands (Fig. 1). For the β_2-receptor and three chimeras having transmembrane segment 7 of the β_2-receptor (□), replacement of transmembrane segments 1 to 5 causes a reduction in the binding energy preference for ISO of approximately 0.8 kcal/mol for each segment replaced, as indicated by the linear relation of these points. Similarly, for the α_2-receptor and three chimeras having transmembrane segment 7 of the α_2-receptor (■), replacement of transmembrane segments 1 to 5 causes an increase in the binding energy preference for ISO of ap-

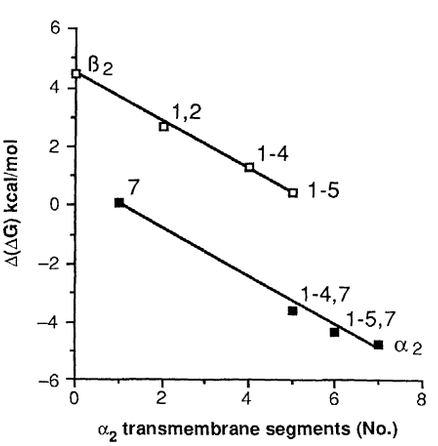

Fig. 1. Binding energy difference for α_2/β_2 receptor chimeras. The difference in Gibb's free energy of binding [$\Delta(\Delta G)$] of PAC and ISO was calculated from the agonist binding data of (*1*) with the use of the equation described in the text. $\Delta(\Delta G)$ values for the β_2-receptor and chimeras containing transmembrane segment 7 of the β_2-receptor are plotted versus the total number of α_2 transmembrane segments in the molecule (□). Similarly, $\Delta(\Delta G)$ values for the α_2-receptor and the chimeras containing transmembrane segment 7 of the α_2-receptor are also plotted versus the total number of α_2 transmembrane segments in the molecule (■). The individual α_2-receptor transmembrane segments in each molecule are indicated by the numerals with each data point. Note that substitution of transmembrane segment 7 displaces the linear relation by approximately 3.7 kcal/mol.

proximately 0.8 kcal/mol for each segment replaced, as indicated by the parallel line drawn through these points. These two parallel relations show that transmembrane segments 1 to 5 of these receptors each contribute approximately 0.8 kcal/mol to the difference in binding energy between PAC and ISO.

In contrast to the progressive change in binding energy preference observed when segments 1 to 5 are exchanged, substitution of transmembrane segment 7 of the α_2-receptor for the corresponding segment of the β_2 receptor causes a dramatic change of 3.7 kcal/mol for a single segment (compare □ and ■ in Fig. 1). The size of this change is independent of the source of transmembrane segments 1 to 5 as illustrated by the parallel lines in Fig. 1. Evidently segment 7 has unique determinants of agonist binding specificity, as concluded by Kobilka *et al.* (*1*).

This quantitative analysis of agonist binding specificity emphasizes the additive contributions of individual transmembrane segments in determining binding energy preference. The use of binding free energy as the measured parameter makes these additive relationships clearer than simple inspection of binding curves and relative K_d values. This approach may prove valuable in similar analyses of chimeras of other members of the family of G-protein–coupled receptors or of other proteins with multiple membrane-spanning segments.

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Response: We are pleased that Catterall's quantitative analysis of our data strengthens the conclusions that we drew about the importance of various transmembrane domains in determining the α- versus β-adrenergic binding specificity of these receptors. Combination of the experimental approaches used in our studies with analytic approaches such as that suggested by Catterall should provide a powerful means of analyzing the structural basis of the function of receptors coupled to guanine nucleotide regulatory proteins.

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Predation on Ocean Krill

In developing the hypothesis that "high-density demersal layers" of krill (*Meganyctiphanes norvegica*) at the bottom of submarine canyons are a major prey of fishes on Georges Bank, Greene *et al.* (*1*) may be missing a major facet of the trophic interactions among these organisms. According to their hypothesis, the fishes make descents into deep water next to the Bank, where, it is suggested, there is advantage in feeding on these vertical migrators when they are in their normal daytime aggregations. But this is not how the interactions proceed in what probably are similar situations elsewhere.

It has been widely reported (*2–4*) that fishes which inhabit relatively shallow banks or shelves feed heavily by day on organisms that, like *M. norvegica*, make extensive diel vertical migrations in adjacent deep water. The reports have come from the continental shelves of North America (*2*) and Australia (*3*), as well as from a central Pacific atoll (*4*); and in addition to various species of krill, the vertically migrating prey have included copepods and myctophid fishes. In the reported cases, however, the predatory fishes do not descend from the shelf or bank into the adjacent depths to take prey from the concentrations that form there by day. Rather, they feed on individuals that, after having been carried by currents (or swimming) over the shelf-bank while in the surface waters at night, are trapped by the relatively shallow shelf-bank when in the morning they descend toward their normal daytime depths. Apparently these organisms are especially vulnerable to predators in this setting, which is very different from their normal daytime habitat.

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Response: In our paper (*1*), we hypothesized that squid and demersal fish production attributed to Georges Bank might be subsidized by the exploitation of krill from the submarine canyons and other deep waters surrounding the Bank. At present, the evidence for such a subsidy is circumstantial; krill are an important but variable dietary component of the Bank's commercially important squid and demersal fish stocks, and many of these stocks seasonally move off the Bank (as defined by the 200-meter isobath) into the surrounding deep waters where the high-density krill demersal layers are found. Unfortunately, little is known about the behavior and diets of these species when they move into deeper water. As we stated, closer examination of the spatial and temporal coupling between predator and prey populations will be essential to determine the validity of our hypothesis.

Hobson (*2*) raises a valid point with regard to the spatio-temporal coupling between predator and prey populations. If krill are the missing link in the Georges Bank food chain, then they must move onto the Bank either through vertical migration and advection by currents (or active swimming), as Hobson suggests, or the squid and fish stocks must descend into deeper water and feed, as we implied. Initially, we favored the mechanism hypothesized by Hobson, since there is ample evidence for such events occurring on other banks (*3*) and seamounts (*4*) around the world. However, extensive zooplankton and micronekton surveys on Georges Bank (*5*) indicate that krill rarely intrude on the shallower portions of the Bank, and thus the circumstantial evidence for Hobson's hypothesis does not appear to exist. On the other hand, fishery surveys on and around Georges Bank (*6*) indicate that many squid and demersal fish stocks move off the Bank seasonally into the deeper waters, where high-density krill demersal layers have been observed. Therefore, we chose to emphasize the latter hypothesized mechanism for the trophic linkage rather than the one Hobson suggests. So little is