ably already contains heme iron in a lipophilic environment (*14*), these facts suggest to us that a lipophilic environment is necessary for heme–group IVB complex formation. This suggestion is strengthened by the observation that sucrose is required in some instances for a spectrum to be obtained. Since sucrose is freely water-soluble but not lipophilic, its presence would tend to divert the solvating power of water and to force tin(II) species, probably charged by coordinated phosphate in the aqueous phase, into the lipophilic phase as SnX_2 or $LSnX_2$ species.

The group IVB dihalides, having the ns^2 outer electron configuration and available orbitals, are electronically similar to carbenes, CO, and ethyl isocyanide. Ethyl isocyanide and CO complex with cytochrome P-450 to give spectra having large extinction coefficients and absorption maxima at 455 or 450 nm (*15*). Isocyanide and CO are classified as π-acceptor ligands since each has a free pair of electrons used in dative σ-bond formation and each also has available π-orbitals which are involved in π-bonding by accepting electrons from a filled metal *d*-orbital. We suggest that the similarity in electronic structure between the group IVB dihalides on the one hand and CO and isocyanide on the other leads to similar binding in their heme iron complexes, and consequently similar electronic spectra.

We have investigated the interactions of the group IVB dihalides with artificial systems that behave spectrally like cytochrome P-450 (*16*). We have also examined molecules that appear to produce carbene–cytochrome P-450 complexes after metabolism. These molecules include the methylene dioxyphenyl compounds, which are well-known drug synergists, and their aliphatic analogs the dioxolanes (*17*). No toxicological implications for the use of SnF_2 are immediately apparent from our findings. Further investigations are merited, however, particularly with regard to the possible synergism of toxic materials.

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References and Notes

1. M. Klingenberg, *Arch. Biochem. Biophys.* **75**, 376 (1958); D. Garfinkel, *ibid.* **72**, 493 (1958).
2. T. J. Marks and A. R. Newman, *J. Am. Chem. Soc.* **95**, 769 (1971) and references therein.
3. J. D. Donaldson, *A Review of the Chemistry of Tin(II) Compounds* (Tin Research Institute, Greenford, Middlesex, England, 1964), pp. 28–29.
4. R. B. Mailman, A. P. Kulkarni, R. C. Baker, E. Hodgson, *Drug Metab. Dispos.* **2**, 301 (1974); A. P. Kulkarni, R. B. Mailman, R. C. Baker, E. Hodgson, *ibid.*, p. 309.
5. T. Omura and R. Sato, *J. Biol. Chem.* **239**, 2370 (1964).
6. Cytochrome P-448, specific activity 7 nmole per milligram of protein and used as a solution of 0.6 nmole/ml, was obtained from R. M. Philpot of the National Institute of Environmental Health Sciences, Research Triangle Park, N.C. [R. M. Philpot, *Chem. Biol. Interact.* **9**, 169 (1974)].
7. A. Y. H. Lu and W. Levin, *Biochim. Biophys. Acta* **344**, 205 (1974).
8. Lecithin was used at 0.05 to 1 percent and sucrose at 3 to 20 percent (weight/volume).
9. Dipalmitoyl DL-α-phosphatidylcholine, approximately 99 percent pure, Sigma Chemical Co.
10. B. K. Tam and D. B. McCoy, *J. Biol. Chem.* **245**, 2295 (1970).
11. P. G. Stecher, Ed., *The Merck Index* (Merck & Co., Inc., Rahway, N.J., ed. 8, 1968), p. 521.
12. G. B. Ludwig, W. S. Blakemore, D. L. Drabkin, *Biochem. J.* **66**, 38P (1957); R. Coburn, *N. Engl. J. Med.* **282**, 207 (1972).
13. E. Hodgson and M. Juchau, *J. Steroid Biochem.* **8**, 669 (1977).
14. Y. Imai and R. Sato, *J. Biol. Chem.* **62**, 464 (1967).
15. ———, *Biochem. Biophys. Res. Commun.* **23**, 5 (1966); J. Peisach and G. J. Mannering, *Mol. Pharmacol.* **11**, 818 (1975).
16. R. Chiang, R. Makine, W. C. Spomer, L. P. Hager, *Biochemistry* **14**, 4166 (1975); C. K. Cheng and D. Dolphin, *J. Am. Chem. Soc.* **97**, 5948 (1975); J. P. Collman and T. N. Sorrell, *ibid.*, p. 4133.
17. E. Hodgson and R. M. Philpot, *Drug. Metab. Rev.* **3**, 231 (1974).
18. Supported in part by NIH grants ES-00044 and ES-00083.

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Molecular Graphics: Application to the Structure Determination of a Snake Venom Neurotoxin

Abstract. *Atomic coordinates have been determined for a snake venom α-neurotoxic protein by fitting a molecular model to a crystallographically derived 2.2-angstrom electron density map. The fitting was carried out entirely on a computer-operated molecular graphics system without going through any mechanical model stage.*

The fitting of a molecular model to a calculated electron density map is an important step in the determination of the structure of a protein. This fitting is commonly done with a device called a Richards box (*1*). Contour lines drawn on a series of transparent sheets represent the map; a molecular model is built in front of the map, but a half-silvered mirror creates the illusion that the model is being built "inside" the map. Since even a small protein has at least 500 atoms, the precise fitting of model to map and subsequent measurement of the coordinates of each atom are tedious and error-prone steps. In this report we describe how the entire process of fitting a model of a protein to its electron density map and then measuring the coordinates was done in a short time by means of a special-purpose interactive computer graphics system, a molecular graphics system, without ever constructing a physical model.

A number of molecular graphics systems have been built, which are each unique in some ways and which together represent a wide variety of design choices (*2*). For the work reported here we used the GRIP (Graphics Interaction with Proteins) molecular graphics system at the University of North Carolina (*3*). GRIP makes available to the user most of the facilities of other systems plus a few unique features. We briefly describe the GRIP system as it appears to the user, stressing features that we found to be effective.

GRIP is operated with explicit commands and with hand controls. The former are given by entering any needed numerical values from a keyboard and then selecting a displayed key word with the light pen or pressing a button on a small console. (The light pen may also be used to select an atom or a contour.) The system's response to these commands is usually not instantaneous. The hand controls are analog devices which specify viewpoint and conformation. The display's response to the use of the hand controls is essentially immediate.

The "seeing" controls of the system include window selection, scaling, detail suppression, suppression of selected contours, viewpoint selection, and creation of a three-dimensional illusion. Execution of several commands specifies the contents of the viewing volume. Once this is done, a hand-controlled lever can be used to change the viewpoint by rotating the contents of the viewed space, which aids the user in comprehending the view and provides a strong depth cue. This is but one of the various monocular and binocular depth cues included in the GRIP system. Of the former, we use intensity depth cueing and continuous changes of viewpoint. (Intensity variation may also be used to distinguish the molecule and the electron density map.)

GRIP uses three analog devices to control smooth changes of the conformation of the displayed molecule. One device, a three-dimensional positional lever, has a knob that may be moved anywhere within a certain vol-

ume; a selected residue moves correspondingly in the screen space. The second is a three-dimensional isometric lever specifying angular velocities. The residue appears to rotate according to the way the handgrip is stressed. The third device is a set of eight one-turn potentiometers, each of which can be made to control the internal rotation about a chemical bond. The combination of analog devices and GRIP software gives a molecular modeling system in which the map and model can be manipulated almost by hand.

The user originally provides values proportional to the electron density at the points of a rectangular lattice. During the sessions he invokes an on-line contouring program to convert these data into a series of contours at one or more selected levels. The contours may be calculated for three sets of perpendicular planes, and the user may select for display combinations of the calculated contour maps. Individual contours may be edited out after selection with the light pen.

After the molecular model has been manipulated, and hence distorted, the user may invoke an on-line geometric refinement program which causes the molecular geometry to approach that of an idealized molecule while keeping the changes in the coordinates to a minimum. Several methods have been developed for this (*4*); the method used in GRIP is that of Hermans and McQueen (*5*, *6*), which seems best suited to an interactive environment.

On-line contouring and on-line geometric idealization seem to add substantially to the power furnished the user. These calculations are made more acceptable in the interactive environment by the system's time-shared use of a high-speed host computer (*3*).

Snake venom α-neurotoxins are small basic proteins of 60 to 70 amino acids with four or five disulfide bridges, which are found in the venom of elapid land snakes and sea snakes. They act by specifically blocking the membrane-bound acetylcholine receptor of the muscle motor end plate (*7*, *8*). The available sequence information has been summarized by Ryden *et al.* (*8*), who emphasize the importance of the disulfide bridges and certain amino acids around residue 33 for the maintence of features of structure and function which are presumably common to all neurotoxins. Because of their specific, tight binding to the acetylcholine receptor, snake venom α-neurotoxins are much used in isolating and assaying the receptor (*9*).

The crystal structure of one such toxin, the 62-residue neurotoxin b from a sea snake from the Philippine Sea, was solved by Tsernoglou and Petsko (*10*) at a resolution of 2.2 Å. A small-scale electron density map (0.5 cm/Å) was plotted which revealed the general folding of the polypeptide chain and the positions of the four disulfide bridges. Dots were placed on the map at approximate α-carbon positions (determined by looking for side-chain density branching out from the main chain), and crude α-carbon coordinates were obtained by overlaying a 1-Å grid [figure 2 in (*10*)]. We did not build a molecular model at this stage, nor did we ever use a Richards box in interpreting the map. We proceeded immediately to the molecular graphics system.

As a preliminary step, a set of coordinates for all atoms in the molecule (excluding hydrogens) was calculated on the basis of the α-carbon coordinates and the stereochemistry required by the amino acid sequence of a homologous neurotoxin (*11*, *12*). This coordinate set was used as a starting approximation in which each residue was not too far from where it belonged.

To fit the model to the map the following procedure was used. A portion of the model, usually some six consecutive amino acid residues, was chosen for display. With a single command, the scale was adjusted so that these residues would just fit inside the viewed volume. Within this volume, the electron density contours were then calculated at some

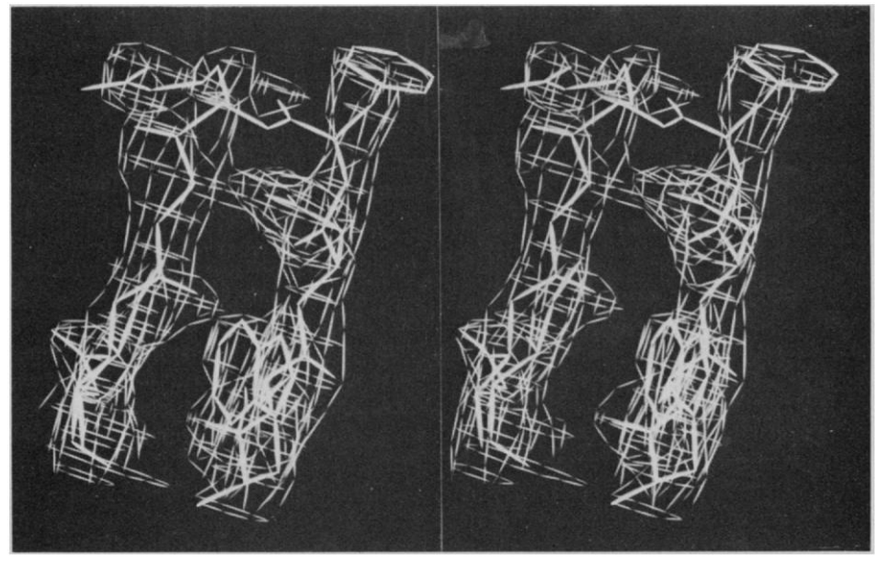

Fig. 1. View of the toxic loop (residues 29 to 35). This and Figs. 2 and 3 are reproductions of Polaroid photographs taken directly of the graphics screen. A gap in the density can be seen where the main chain goes across to make the bend. This gap caused some difficulty in fitting. (Stereo pairs; model bright, map dim.)

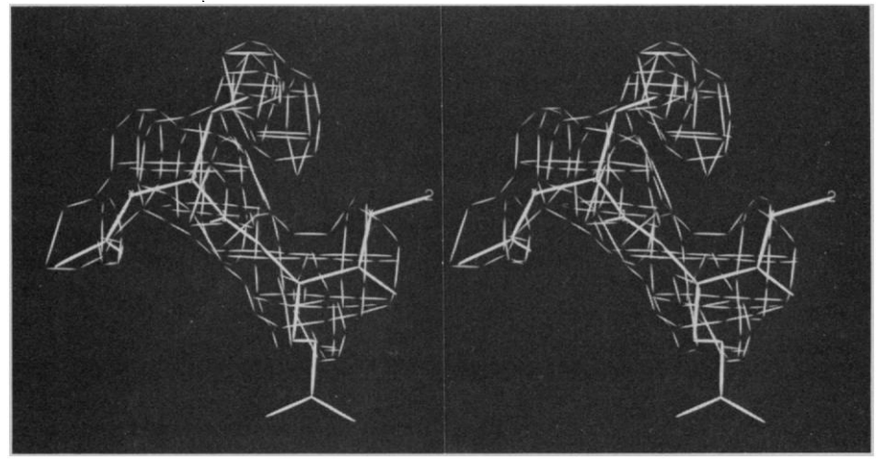

Fig. 2. Sequence reversal: residues 21 and 22 in electron density. The extra density at the end of the short side chain and the lack of density at the end of the long side chain can be clearly seen.

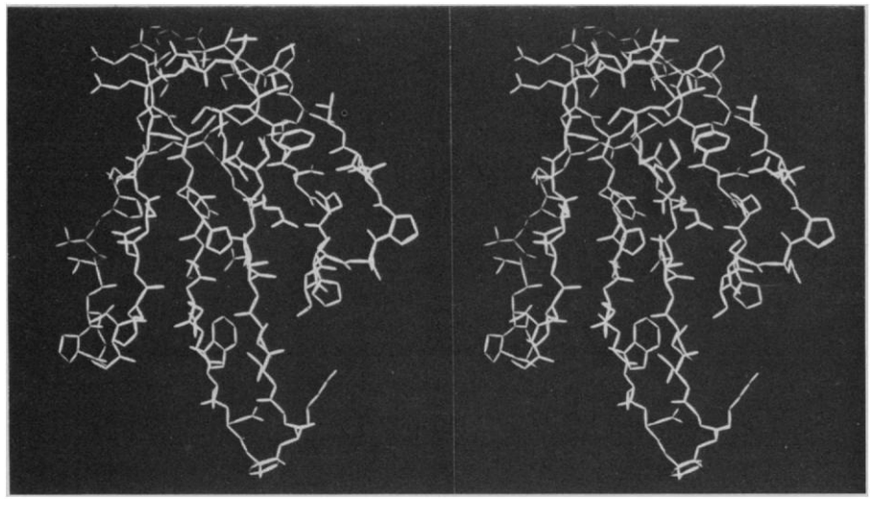

Fig. 3. View of the whole molecule with intensity depth cueing. The β-bend region (called the toxic loop in Fig. 1) is at the bottom center.

specified level. We found the "chicken wire" display of contours at one level in three perpendicular sets of planes to be the most useful (Figs. 1 and 2). Usually the display contained electron density outside the area of direct interest. These contours were selected with the light pen and removed from the display with a command.

The remaining contours and model were rotated by use of one of the levers to obtain the best view for model building. It is not possible to change significantly the viewing direction in a Richards box, and the ability to do this with the graphics system is a great advantage. Model and map were displayed at two different levels of intensity. A monocular view of the model space was used, and depth was perceived by viewing the model while frequently adjusting the viewing angle with the lever (*13*).

The fitting was done residue by residue. Each residue was in turn selected to be moved into the appropriate region of high electron density. If necessary, the movements were alternated with changes in the residue conformation by bond twisting. After this had been done for several residues, stereochemical errors existed in the connections of each fitted residue to adjacent residues. These errors were largely corrected by means of on-line refinement. The refinement was restricted to several consecutive residues and improved both the stereochemistry within this zone and the connections to other residues already fitted to the map. We frequently defined target positions for selected atoms, most often main-chain nitrogens, carbonyl oxygens, and β-carbon atoms. Refinement with specification of target positions (*6*) was found to be a means of obtaining a more precise positioning of the residues in the density than had been achieved during the fitting step.

The neurotoxin has four disulfide bridges and the tertiary structure consists of three long and two short loops (*10*). The fitting was begun near the disulfide bridges. First, the bridges were found as peanut-shaped regions of density in a map contoured at a high level (that is, close to the maximum density). We then recontoured the map at a lower level and could work out from the bridges, fitting the chains until two ends met. By using an even lower contour level, we could frequently locate hydrogen bonds. These were a decisive factor in determining the orientations of many peptide groups.

Some sections of the map were interpreted and fitted with surprising ease; in others we had some difficulties. The tip of the "toxic loop" (Fig. 1, residues 29 to 35) was hard to interpret and we obtained two alternative conformations. One of these was subsequently discarded as it contained several consecutive residues with a high-energy conformation (*14*). The other conformation is that shown in Figs. 1 and 3. Difficulties were also encountered in fitting residues 21 and 22. The fit obtained in this region (Fig. 2) indicates that the assumed chemical sequence is incorrect and contains an inversion (*15*).

A permanent record of the work was obtained in various forms. As the fitting progressed, the permanent file containing the molecular description was frequently updated. This file was copied to other media (printer, cards, and tape) at the end of the fitting. The machine-readable output has been used as input for subsequent refinement calculations. Polaroid photographs of the screen yielded both conventional and stereographic photographs, which have proved useful in studying the structure when away from the graphics system.

It took two of us, neither of whom had ever built a protein model before, about 60 hours to produce coordinates for all the atoms in the protein (Fig. 3) (*16*). The model had a crystallographic reliability index (R) of 0.47 at 2.2-Å resolution, which is comparable to R values for other proteins for which the model-building was done conventionally. The model was of sufficiently good quality to be used for constrained crystallographic least-squares refinement (*17*).

On the basis of our experience, we conclude that molecular graphics systems provide a fast, accurate, and efficient means of displaying and manipulating protein electron density maps and models, a means that is superior to conventional methods of model building. Using the GRIP molecular graphics system, we were able to reduce a tedious and slow step in protein crystallography to one that is fast and enjoyable.

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References and Notes

1. F. M. Richards, *J. Mol. Biol.* **37**, 225 (1968).
2. C. Levinthal, *Sci. Am.* **214** (No. 6), 42 (1966); ———, C. D. Barry, S. A. Ward, M. Zwick, in *Emerging Concepts in Computer Graphics*, D. Secrest and J. Nievergelt, Eds. (Benjamin, New York, 1968), pp. 231–253; C. D. Barry, R. A. Ellis, S. M. Graesser, G. R. Marshall, in *Proceedings of the IFIP Congress 1971*, C. V. Freiman, Ed. (North-Holland, Amsterdam, 1971), pp. 1552–1557; C. D. Barry and A. C. T. North, *Cold Spring Harbor Symp. Quant. Biol.* **36**, 577 (1972); L. Katz and C. Levinthal, *Annu. Rev. Biophys. Bioeng.* **1**, 465 (1972); W. V. Wright, thesis, University of North Carolina, Chapel Hill (1972); C. M. Collins, F. A. Cotton, E. F. Hazen, Jr., E. F. Meyer, Jr., C. N. Morimoto, *Science* **190**, 1047 (1974). Other systems have been implemented by R. Diamond at Cambridge University; R. Langridge at the University of California, San Francisco; and J. Cornelius at the University of California, San Diego.
3. The hardware of this system consists of a Vector General graphics unit model 3 and a PDP-11/45 processor with 45,056 16-bit words of memory. The user communicates with this system through a variety of devices: light pen, control levers, potentiometers, and keyboards. Many of the user's commands are executed by a host computer, an IBM S/360 model 75, which communicates with the 11/45 processor through a selector channel.
4. R. Diamond, *Acta Crystallogr.* **21**, 253 (1966); P. K. Warme, N. Go, H. A. Scheraga, *J. Comput. Phys.* **9**, 303 (1972).
5. J. Hermans and J. E. McQueen, Jr., *Acta Crystallogr. Sect. A* **30**, 730 (1974).
6. Each atom is moved, in turn, to reduce the discrepancies between current bond lengths, bond angles, and fixed dihedral angles and their corresponding ideal values. When every atom has

been shifted, one cycle of refinement is complete; the refinement consists of several such cycles. Selected atoms may be kept flexibly tethered to target positions determined by fitting the model to the density manually. This prevents well-fit atoms from wandering too far, and also provides points of stability which constrain the entire idealized structure to lie within the electron density. It is also possible to encourage atoms to move in a particular direction by proper choice of target positions.

7. For reviews of the protein chemistry and pharmacology of snake venom neurotoxins see A. T. Tu [*Annu. Rev. Biochem.* **42**, 235 (1973)] ➡ C. Y. Lee [*Annu. Rev. Pharmacol.* **12**, 265 (1972)]. A discussion of possible structure-function features in neurotoxins has been given by Ryden *et al.* (*8*).
8. L. Ryden, D. Gabel, D. Eaker, *Int. J. Pept. Protein Res.* **5**, 261 (1973).
9. For example, see J. P. Changeux, M. Kasai, C. Y. Lee, *Proc. Natl. Acad. Sci. U.S.A.* **67**, 1241 (1970).
10. D. Tsernoglou and G. A. Petsko, *FEBS Lett.* **68**, 1 (1976).
11. The Philippine sea snake toxin has not been sequenced. However, a sequence was available for neurotoxin b from Japanese sea snake venom [S. Sato and N. Tamiya, *Biochem. J.* **122**, 453 (1971)] and this was used. Although one might expect the two proteins to have very similar or identical sequences, chemical work had indicated considerable differences [A. T. Tu, B. Hong, T. N. Solie, *Biochemistry* **10**, 1295 (1971)]. Our fitting and subsequent refinement of the Philippine sea snake neurotoxin indicates that the two toxins are either identical or only slightly different. The sequence question is discussed in detail elsewhere (*12*). A complete description of the structure will be published separately (D. Tsernoglou and G. A. Petsko, in preparation).
12. D. Tsernoglou, G. A. Petsko, A. T. Tu, *Biochim. Biophys. Acta* **491**, 605 (1977).
13. Rotating the model to obtain depth perception during the fitting comes closest to the natural process of making small head movements, a technique for depth perception that is used to some extent by everyone. Two-image depth illusion techniques are available on the system, but we did not use them in fitting because one of us does not have stereoscopic vision and therefore cannot benefit from these techniques.
14. Another feature of GRIP that we found to be useful is its ability to produce dihedral angles on command. One can immediately inspect them, and if they fall in high-energy regions in Ramachandran-type diagrams other conformations can be considered. Such a process would be very difficult and time-consuming with conventional model-building.
15. It is possible that this is a sequence difference between our toxin and the Japanese sea snake toxin, but we regard this as unlikely (*12*).
16. A report has appeared concerning the backbone structure of another, almost identical, neurotoxin from a sea snake from the Sea of Japan [B. W. Low, H. S. Preston, A. Sato, L. S. Rosen, J. E. Searl, A. D. Rudko, J. S. Richardson, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2991 (1976)]. The backbone structure appears identical to that of our toxin. No information on the structure of this homologous toxin was available to us during the work described in this report.
17. Constrained refinement is being carried out by the method of J. H. Konnert [*Acta Crystallogr. Sect. A* **32**, 614 (1976)]. The current R factor is 0.27 based on data at 5.0- to 2.0-Å resolution.
18. We thank F. P. Brooks, Jr., and W. V. Wright for help and advice at various stages of this work and M. E. Pique and J. S. Lipscomb for assistance. Supported by research grants from the National Institutes of Health (RR-00898 and HL-15958) and from the National Science Foundation (BMS74-21633). The GRIP system was built by the Department of Computer Science, University of North Carolina, following specifications laid out and regularly revised in conjunction with the system's early users: biochemists at UNC and crystallographers at Duke University (the latter included D. Richardson, J. Richardson, S. Kim, and J. Sussman). Major contributions were made by E. G. Britton, M. E. Pique, and J. S. Lipscomb under the direction of W. V. Wright and by J. E. McQueen, Jr., under the direction of J. Hermans. F. P. Brooks, Jr., is the principal investigator. Development of the GRIP system has been supported by AEC contract AT(40-1)-3817, NSF grant GJ-34697, NIH Biotechnology Research Resource grant RR-00898, and the IBM Corporation.

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Visual Input to the Visuomotor Mechanisms of the Monkey's Parietal Lobe

Abstract. *A newly identified class of neurons of the parietal cortex, studied in waking monkeys* (Macaca mulatta), *is activated by visual stimuli, perhaps via the retino-collicular visual pathway. This afferent input is thought to provide the visual cues activating the visuomotor mechanisms of the parietal lobe for the direction of visual attention.*

When a stimulus is presented in the peripheral visual field of a monkey, the monkey may, after a latency of 150 to 200 msec, make a saccadic eye movement to bring the image of the target onto its fovea. The neural mechanisms that mediate this eye movement are of great interest, and considerable progress has been made in elucidating the neural circuitry of the brainstem that controls eye movements (*1*). Less is known about the cortical mechanisms thought to link the central neural representation of such targets of interest with those brainstem circuits. It was thought that neurons of area 8 of the frontal lobe, in the so-called frontal eye fields, form the cerebral efferent pathway for voluntary or "spontaneous" eye movements, and that visually evoked eye movements are mediated via the visual cortical areas of the occipital lobe. However, electrophysiological studies of the frontal eye fields and of the geniculo-striate visual system in waking animals have not revealed neurons that discharge before eye movements occur (*2*). In recent studies of the cerebral cortex of alert, behaving monkeys we found several classes of neurons of the inferior parietal lobule that have visuomotor functions. They appear to provide a neural mechanism for the direction of visual attention; that is, for the fixation of gaze upon objects of interest, for maintaining fixation of the object when it moves slowly, and for loosening fixation and initiating saccadic eye movements toward new objects that appear in the visual field (*3*).

Here we describe the results of another series of experiments in which we sought to define the visual input to this area. A class of cells previously called visual space neurons (*3*) has been found to be sensitive to light and to subtend large, peripherally placed receptive fields. We suggest that these cells and the pathways that project upon them provide one afferent input to the visuomotor mechanisms of area 7 that are active before visually evoked eye movements.

The electrical signs of the activity of single cells of area 7 were recorded by way of platinum-iridium microelectrodes inserted transdurally into the cortex of waking rhesus monkeys trained on the visual fixation task described by Wurtz (*4*). The behavioral training procedures and electrophysiological methods were described perviously (*3*). Four male monkeys (*Macaca mulatta*) were used. Each was trained to fixate a small gallium-arsenide (λ = 660 nm) light-emitting diode (LED) positioned on a tangent screen in the animal's primary line of gaze, 34 to 57 cm in front of him. The screen contained an array of 16 other LED's; several different spatial arrays of the target lights were available. The animal was required to depress the key after the central light came on, and to hold it down until he detected a dimming cue in order to receive a drop of liquid reward. He also learned, if the center LED disappeared and another appeared simultaneously, to saccade to the second light and fixate it until it dimmed (after a preset delay). If the center light remained on, the monkey maintained fixation of it until it dimmed, and made no eye movements toward any other light that came on during the fixation period. The head was held stationary and eye movements were monitored by recording the horizontal and vertical electroculograms (EOG's) with silver–silver chloride electrodes implanted in the orbital bone. A PDP 11/20 computer was used to control light intensity, deliver rewards, and monitor EOG signals, behavioral events, and cell discharges. Small electrolytic lesions were made at the end of each penetration by passing current through the tip of the microelectrode (4 μa for 4 seconds); they were used to identify the location of neurons after histological preparation of the brains. A total of 350 neurons was studied in this series of experiments, in six hemispheres. All cells were located in area 7.

The light-sensitive cells of area 7 (98 cells out of 350) are activated when visual stimuli are presented at or near the far peripheral edges of the visual fields, whether or not a saccade is made in that direction. Figure 1 illustrates the results of the study of one such cell, located in the right hemisphere of a monkey. When the animal was fixating the central point, *FP*, and a test light positioned 30° to the left came on midway through the fixation