from any of the known catechol, indole, or imidazole alkylamines (*13*). Of known nonprotein toxins, only batrachotoxin (*2*), tetrodotoxin (*14*), and saxitoxin (*15*) are more toxic than the concentrate from *A. zeteki* which has been prepared in our laboratory (*7*). Atelopidtoxin can be differentiated chemically from batrachotoxin, which is a steroidal alkaloid readily soluble in chloroform and other organic solvents (*2*). The insolubility of atelopidtoxin in chloroform is indicated by our procedure for extraction. Furthermore, in separate experiments we have been unable to extract atelopidtoxin with chloroform from aqueous solutions at *p*H 2, 8, or 11. Thus, the markedly different solubilities of atelopidtoxin and batrachotoxin demonstrate that they are not identical. Atelopidtoxin differs in pharmacological action from both tetrodotoxin and saxitoxin, which block axonal conduction without significant cardiotoxic effect. In addition, atelopidtoxin has been differentiated from tetrodotoxin and saxitoxin by direct comparison using thin-layer chromatography (*7*).

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References and Notes

1. L. Lewin, *Die Pfeilgifte* (Barth, Leipzig, 1923).
2. F. Märki and B. Witkop, *Experientia* **19**, 329 (1963); J. W. Daly, B. Witkop, P. Bommer, K. Biemann, *J. Amer. Chem. Soc.* **87**, 124 (1965).
3. J. W. Daly and B. Witkop, *Mem. Inst. Butantan* **33**, 425 (1966); T. Tokuyama, J. Daly, B. Witkop, I. L. Karle, J. Karle, *J. Amer. Chem. Soc.* **90**, 1917 (1968).
4. J. W. Daly and C. W. Myers, *Science* **156**, 970, 1967.
5. E. R. Dunn, *Occas. Pap. Boston Soc. Natur. Hist.* **8**, 65 (1933).
6. Another atelopid, *Atelopus* (*Dendrophryniscus*) *stelzneri*, found in Paraguay and Argentina, is described by D. Cochran [*Living Amphibians of the World* (Doubleday, Garden City, N.Y., 1969)] as having poisonous skin. J. S. Budgett [*Quart. J. Microscop. Sci.* **42**, 305 (1899)] relates that this brightly colored frog (designated *Phryniscus nigricans* by him) was shunned by a grass snake (*Paludicola signifera*) that readily devoured an agile olive-green frog in the same cage. According to H. B. Cott [*Adaptive Coloration in Animals* (Oxford Univ. Press, New York, 1940)], Professor Graham Kerr found that his pet bird, a crested screamer (*Cariama cristata*), while out for a walk, enjoyed eating many amphibians but always found *Atelopus stelzneri* to be highly unpalatable. We know of no experiments on the nature of the poison in this atelopid.
7. J. Schindelman, M.S. thesis, Stanford University (1969); ———, F. A. Fuhrman, H. S. Mosher, *Toxicon*, in press.
8. B. K. Ranney, G. J. Fuhrman, F. A. Fuhrman, in preparation.
9. D. A. Rytand, *Ann. Int. Med.* **65**, 125 (1966).
10. F. A. Fuhrman and G. J. Fuhrman, in preparation.
11. H. Michl and E. Kaiser, *Toxicon* **1**, 175 (1963).
12. G. Habermehl, *Z. Naturforsch.* **20**, 1129 (1965).
13. V. Erspamer, T. Vital, M. Roseghini, J. M. Ceig, *Arch. Biochem. Biophys.* **105**, 620 (1964).
14. H. S. Mosher, F. A. Fuhrman, H. D. Buchwald, H. G. Fischer, *Science* **144**, 1100 (1964).
15. E. J. Schantz, J. M. Lynch, G. Vayvada, K. Matsumoto, H. Rapoport, *Biochemistry* **5**, 1191 (1966).
16. Supported by grants HD-00218 and GM-16031 from NIH.

26 May 1969; revised 4 August 1969 ■

Visual Stimuli and Evoked Responses in the Rat

Abstract. *Rats were presented with a series of visual stimuli, each described by shape, size, and position. Cortical recordings were made in medial and lateral positions of rat visual cortex. Average responses were calculated and correlated. This analysis revealed that information regarding size was contained in electrical activity from medial areas, and informtion regarding shape in electrical activity from lateral areas.*

Changes in visual stimuli to the rat usually result in perturbations of the electrical activity of certain areas of the cortex. The perturbation appears to end a short time after the change, and the section of the electroencephalograph record beginning with the stimulus and ending with the disappearance of the perturbation will be known as an individual evoked response (IER). If a particular change in a visual stimulus is repeated, and the resulting IER's are averaged, one may calculate an averaged evoked response (AER).

The changes in visual stimuli to the subject may be described by a number of observables. In these experiments black and white visual stimuli were used, so they can be completely defined by edge positions, lengths, and orientations. We may specify brightness and shape, with position and orientation, without significant loss of information.

Each visual stimulus is therefore a point in "stimulus space" whose location is specified by the values of the observables. Similarly, each IER is a point in function space. Since the IER's are measured with equipment of limited frequency response, the sampling theorem predicts that the functions can be represented by a finite number of samples of the finite record. The subject serves as a transform from stimulus space to "response space" within the limits of their definition. As our chosen observables will probably not completely define the stimulus, apparently equivalent stimuli need not map to the same point in response space, and never do. Rather, the points form a cluster whose "center" is approximated by the AER.

Several investigators have considered the information loss through this transformation. The AER or IER resulting from a given visual stimulus may be said to contain information regarding a particular observable if its position in response space changes when the value of that observable in stimulus space is changed. This may be difficult to determine because of uncontrolled variables producing unpredictable changes with repeated trials.

The AER to visual stimuli in monkeys (*1*) and in man (*2*) apparently contain more information about the shape parameter of the stimulus than about size or brightness parameters. The present study considers the relation between AER's and cortical recording sites in the rat.

Long-Evans hooded rats (male, 350 to 450 g) were used. Prior to the presentation of the stimuli they were curarized and respirated by the method of Miller and DiCara (*3*) and then placed in a stereotaxic instrument; Xylocaine was applied to the ears to minimize pain. While curare may have distorted the visual evoked responses, it seems unlikely that it could have been responsible for the effects reported. The rats were allowed to dark-adapt for 15 minutes before the presentations. While visual stimuli were being shown, the eye was observed with cross hairs and a dissecting microscope. No eye or pupil movements could be seen in the curarized rat, nor could any obvious deterioration of the eye be detected during the experiments.

The visual stimuli consisted of white shapes projected onto a dark background by a modified Sawyer projector. The projection bulb was replaced by the bulb of a Grass photostimulator, which allowed accurate control of the triggering of stimuli. The use of opaque slides showed that there were no appreciable electrical or acoustical arti-

facts. To produce maximum response, the screen was positioned 45 deg above the horizontal plane, 45 deg from midline, and 8 inches (20 cm) from the eye. With this arrangement, shapes covered between 5 and 30 deg of the visual field. No shape was sufficiently large to be confused with a uniformly lit screen.

Each visual stimulus was presented by flashing the Grass lamp at a rate of 90 per second for 0.75 second. This rate was above the flicker-fusion frequency of the rat. Lowering the frequency to 70 per second produced no substantial change in the AER, and power spectrum analysis (*5*) of the AER's and IER's disclosed no components that varied with the frequency of the lamp flashes. Therefore, the burst of flashes was roughly equivalent to constant illumination for 0.75 second.

Four intensity levels could be selected with the Grass photostimulator. When a uniformly lit visual field was used as a stimulus, increasing intensities produced monotonically increasing maximum amplitudes of AER, with a medial recording position. Therefore, use of the third brightness level produced IER's that were below a possible saturation level.

In order to test the effects of various interstimulus intervals, a particular stimulus, such as a circle or square, was used to produce a response with 4-second and 10-second intervals. No appreciable difference could be noted in the AER. In order to reduce the effects of noise with the same periodicity as the stimulus, the interstimulus interval was varied randomly between 10 and 15 seconds.

After local application of Xylocaine, the rat's skull was exposed, and a small hole was drilled by hand. Insect pins insulated with Formvar served as monopolar electrodes, which were placed just below the dura. Electrical activity was amplified by a Bioelectric amplifier and a Tektronix 502a oscilloscope. The system had low pass qualities with an upper cutoff frequency of approximately 300 hz. All recordings were made 1 to 3 mm anterior to the lambda. Medial recordings were 1 to 2.5 mm lateral to midline, and lateral recordings 2.5 to 5 mm lateral to midline.

Stimulus display was controlled by a Linc-8 computer. The Linc-8 also gathered the data through its analog-to-digital converters, sampling at a rate of 512 points per 0.75 second. This rate was sufficiently high to avoid aliasing errors. On-line averages were computed with the Linc-8 or a Fabritek signal averager. For computations of power spectra, autocorrelations, and cross correlations, data were transferred to a 160-G computer (Control Data Corp.).

Table 1. Averaged cross correlations.

Medial recording site	Lateral recording site
Shape change	
0.77 (S.D. = 0.02)	0.16 (S.D. = 0.01)
Size change	
.28 (S.D. = .02)	.81 (S.D. = .02)

Each visual stimulus was determined by shape, position, and size parameters. There were two possible shapes for each stimulus (horizontal or vertical stripes), three possible positions (linear shift of the lines perpendicular to their direction by ½- and 1-line width), and two possible sizes (wide, 1-inch stripes; narrow, ½-inch stripes). Each of the 12 possible visual stimuli were presented 24 times. With each presentation, a recording was made from a medial and lateral recording position over rat visual cortex. The first and last 12 IER's from each series were examined separately to check for trend. Therefore, each visual stimulus produced 48 IER's and 4 AER's (medial or lateral times the first 12 or the last 12). As there were 12 visual stimuli, each rat produced 48 AER's and 576 IER's. Stimuli were presented in random order, except for the following constraints: (i) No more than two visual stimuli of the same type were presented in succession, and (ii) the density of a particular stimulus within the total series was approximately uniform. In practice, the size of the sample was large enough so that these constraints were seldom imposed, and the series was usually well balanced.

Four rats were used in the experiment described. Nine others were used in simplified experiments in which one of the parameters was not varied. These experiments used crosses versus circles, triangles versus circles, and horizontal versus vertical stripes. Shapes were varied over a range of sizes. The details of these experiments will not be presented separately, for their results were entirely consistent with those of the more comprehensive experiment. The IER from a typical experiment showed a great deal of variability. No information regarding the parameters of visual stimuli could be reliably detected without averaging.

Experiments involving interpretation of the AER are subject to artifacts resulting from the misuse of the averaging technique. The statistics of averaging are reviewed by Parsons (*4*). It is particularly important that the variance of the latency of the signal be small and that the signals that are averaged come from a homogeneous population. Averaging of signals with a large variance of latency produces distortion of the signal configuration. The distorted AER is the convolution of the latency probability density and the signal configuration. In this experiment, consideration of the distributions of first maxima and first minima of each IER showed a variance of latency that was of considerably shorter time course than the main features of the typical AER. However, a detailed structural study of the individual AER would have been meaningless.

To examine the homogeneity of the population of IER, the autocorrelation of each IER was calculated. The population of IER from a particular visual stimulus was never unimodal, and usually trimodal. Therefore, averaging results in a loss of significant information about the IER population. Fortunately, one "class" of IER usually predominated, so that the configuration of the AER was not seriously disturbed; but again, an analysis of the details would be misleading. These errors are discussed at greater length elsewhere (see *6*).

Inspection of the 48 AER's from each experiment showed: (i) The configuration of the AER from the first 12 IER's in a sequence was very similar to that of the second 12. Therefore, no trend could be detected. (ii) Position in the visual field did not significantly affect the configuration of the AER. Consequently, position information was not included in the AER from either medial or lateral recording positions. (iii) In medial recording positions, the form of the AER was markedly changed by varying the size parameter of the visual stimulus and was not changed by varying the shape parameter. The AER from medial recording positions contain information about the size, but not the shape of the visual stimulus. (iv) In lateral recording positions, the shape of the AER was markedly changed by varying the shape parameter of the stimulus but was not changed by varying the size parameter. The AER from lateral recording positions contain information about the shape, but not the size, of the visual stimulus.

In order to obtain a more objective analysis, each of the 48 AER's were correlated with each other (at zero time lag) to produce a 48 × 48 correlation matrix. From such a matrix one can determine the effect of changing one of the parameters that determines the visual stimulus. For example, in a typical experiment (i) the AER from the first 12 IER's correlated with the AER from the last with an average correlation of 0.89 (standard deviation, 0.014); (ii) the correlations of an AER from a particular visual stimulus and a similar visual stimulus, differing only in the position variable, gave an average value of 0.89 (standard deviation, 0.06); and (iii) the effect of changing other parameters can be similarly computed, as in Table 1. Highly similar patterns of correlations were secured for the other experiments. The correlations were greatly reduced by a change of size, with a medial recording position, or by a change in shape, with a lateral recording position. Information regarding size appears to be contained in the configuration of the AER from medial recording positions, and that regarding shape is contained in the configuration of the AER from lateral recording positions.

Spinelli (*1*) working with monkeys, reported that 8 out of 84 medial positions could produce AER's that contained information differentiating one shape of visual stimuli from another. No points of this type could be found with medial recording positions in the rat, although this may be a sampling error.

On rare occasions a medial position could be found which served as a "nonspecific shape detector." That is, all shapes presented, regardless of size or position, produced a remarkably uniform AER, which differed from the AER from a uniformly lit field, despite appropriate controls of the total energy transmitted.

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References and Notes

1. D. N. Spinelli, *Brain Res.* **5**, 511 (1967).
2. E. R. John, R. N. Herrington, S. Sutton, *Science* **155**, 1439 (1967).
3. N. E. Miller and L. V. DiCara, *J. Comp. Physiol. Psychol.* **63**, 12 (1967).
4. Power spectra were calculated by means of the Welch method [J. W. Cooley, P. A. W. Lewis, P. D. Welch, *IBM Res. Rep. No. RC1743* (1967)].
5. D. Parsons, thesis, Washington University (1963).
6. C. Fields, in preparation.
7. I thank Dr. Neal Miller for his advice and assistance, and Dr. Floyd Ratliff for his critical examination of the experiment. Supported in part by PHS grant MH-13189 to Dr. Neal Miller.

13 June 1969

Growth Pattern of Leishmania in Phlebotomine Sandflies

Anderson and Ayala (*1*) report a trypanosome of the toad *Bufo boreas halophilus* transmitted by *Phlebotomus vexator occidentis*, a sandfly that feeds on reptiles and anurans (*2*). A remarkable feature of these infections is that the flagellates in the sandfly gut and in culture occur only in the leptomonad (promastigote) form, with no indication of their trypanosomal nature until put into the toad.

For many years the leptomonad stages of *Leishmania* in the sandfly gut (infection rate, growth pattern, and so forth) have been used as indices of potential vector species of the various leishmaniases. In the case of Old World species of *Leishmania* affecting man and other mammals, growth of the flagellates is typically at the anterior part of of the midgut (anterior station) with, at times, growth forward into the foregut in the head and mouthparts, whereas the leptomonads of reptilian leishmaniae are typically at the posterior station in the hindgut of the sandfly (*3*).

As Anderson and Ayala point out, the exclusively leptomonad morphology of this toad trypanosome in the sandfly, and its position in the posterior part of the hindgut, will need to be considered in interpreting flagellate infections of wild-caught sandflies in studies of leishmaniasis. With regard to hindgut infections, however, they overlook the fact that the view that the human strains could be expected to adopt only the anterior position is no longer valid for the cutaneous leishmaniases of the New World. They refer specifically to our work (*4*), in which a large number of wild-caught sandflies were found naturally infected with leptomonad flagellates, often limited to the hindgut. They make the sweeping statement that "all evidence so far indicates that posterior station infections in sandflies are not associated with *Leishmania* of man." They ignore the fact that we repeatedly produced similar hindgut infections with various human strains of *Leishmania braziliensis* (s. lat.) by two different methods. (i) Over 700 laboratory-reared phlebotomine sandflies of several species were fed artificially by the micropipette technique on cultures of several different Panamanian strains (*5*). The infection rate was about 80 percent; hindgut infections, together with growth at the anterior station, were characteristic. (ii) Sandflies were fed on hamsters infected with several human strains from Panama and a Peruvian strain from a case of mucocutaneous leishmaniasis (*4*, *6*). The resulting infections, often limited to the hindgut, were quite similar to the natural infections in sandflies.

In the Panamanian infections the flagellates in the hindgut tended to be limited to the short, thin-walled section (the hind triangle) just posterior to the junction with the midgut, with no concentration in or near the rectal ampulla, as with some of the nonmammalian flagellates.

The following are additional data bearing on the relation of sandfly flagellates and human *Leishmania*.

1) Pure cultures were obtained over 100 times from the naturally infected sandflies. Nineteen of these strains were tested in hamsters; five produced lesions indistinguishable from those produced by human strains (*4*, *7*, *8*).

2) Sandflies fed on hamsters infected with wild-sandfly strains acquired the same type of hindgut infection as those fed on human-strain lesions (*4*).

3) Agar gel diffusion tests were made with Panamanian human and sandfly strains (*8*). Two different immunological groups of the human leishmaniae were recognized; five sandfly strains, two of which had failed to infect hamsters, were divided between the two groups; three other sandfly strains were unclassified.

4) Seven of these eight sandfly strains were compared with one of the human strains by electron microscopy (*9*). The ultrastructure of the kinetoplast of the human strain and of six sandfly strains was leishmanial. The other sandfly strain (unique among the 500-odd natural infections and recognized at the original dissection as different morphologically) was probably *Crithidia* sp.

5) Coelho *et al.* (*10*) infected two Brazilian species of sandflies, *P. longipalpis* and *P. renei*, by feeding them on hamster lesions produced by four species of *Leishmania*—*L. braziliensis* (from Brazil), *L. tropica* (Israel), *L. mexicana* (British Honduras), and *L. donovani* (Brazil). The overall infection rate was 57 percent (825 out of 1451). Infections of the anterior portion of the gut predominated, but there were also hindgut infections in 118 (14 percent) of the 825 infections, varying from 8 of 147 (5 percent, *L. tropica*) to 79 of 422 (19 percent, *L. braziliensis*), with an individual high of 43 of 61 (70 percent)