small ganglion cells of the area centralis (*10*), and I have experienced the same difficulty. If the tungsten electrodes preferentially recorded from larger ganglion cells and if most of the suppressed-by-contrast cells are small, it is possible that they are more abundant than the present sample of receptive field types would indicate.

Quantitative considerations aside, the suppressed-by-contrast type of receptive field is yet another to be explained in terms of retinal organization. This type of unit appears unique in sensory neurophysiology in that stimuli may suppress the firing but not increase it.

R. W. RODIECK

Brain Research Unit, Department
of Physiology, University of Sydney,
Sydney, N.S.W., Australia

References and Notes

1. S. W. Kuffler, *J. Neurophysiol.* **16**, 37 (1953).
2. D. H. Hubel and T. N. Wiesel, *J. Physiol. London* **160**, 106 (1962); W. Kozak, R. W. Rodieck, P. O. Bishop, *J. Neurophysiol.* **28**, 19 (1965).
3. For rabbit: H. B. Barlow and R. M. Hill, *Science* **139**, 412 (1963); frog: H. R. Maturana, J. Y. Lettvin, W. S. McCulloch, W. H. Pitts, *J Gen. Physiol.* **43**(6), suppl., 129 (1960); pigeon: H. R. Maturana and S. Frenk, *Science* **142**, 977 (1963); and squirrel: C. R. Michael, *ibid.* **152**, 1092 (1966).
4. J. Stone and M. Fabian, *Science* **152**, 1277 (1966).
5. D. N. Spinelli, *ibid.*, p. 1768.
6. H. B. Barlow, W. R. Levick, G. Westheimer, D. N. Spinelli, *ibid.* **154**, 920 (1966).
7. R. W. Rodieck, *J. Neurophysiol.*, in press.
8. W. Kozak, R. W. Rodieck, C. J. Mears, *Vision Res.* **3**, 389 (1963); R. W. Rodieck and J. Stone, *J. Neurophysiol.* **28**, 819 (1965).
9. A. Donovan, *J. Anat.* **101**, 1 (1967).
10. J. Stone, *J. Comp. Neurol.* **124**, 337 (1965).
11. Aided by grants from the National Health and Medical Research Council of Australia and the Postgraduate Medical Foundation of the University of Sydney.

27 March 1967

The (±) Reference: Accuracy of Estimated Mean Components in Average Response Studies

Abstract. *The (±) reference is defined as the result of alternate addition and subtraction and division by* N *(the number of sample functions). Under suitable conditions both the (±) reference and the variable component (noise) of the usual average tend to be derived from the same Gaussian process, and the former can be used as a measure of the latter. This property is most easily applied when the noise is derived from a stationary process. Application of the (±) reference and the average of the square of the voltage in studies of evoked response is discussed.*

In recent years, biologists have begun to average random functions, particularly electrophysiological potentials, where the underlying mathematics is a combination of applied statistics and the theory of stochastic processes (random functions). Investigators using this technique have obtained new and important results but they generally have not applied measures of precision to their results. This could be due to the difficulty of the task. In this situation many investigators tend to distrust results obtained by averaging. For example, Perry (*1*) concluded a discussion of this question as follows: "Thus, disregard of noise in summation techniques weakens an otherwise impressive research tool."

It is the purpose of this report to clarify some of the problems attendant upon estimating the so-called "noise". A special kind of average is defined, called the (±) reference, which is computed by alternate addition and subtraction instead of by the usual addition. It is especially useful because its mean component is zero, while its statistical structure tends to be otherwise similar to that of the regular average. The computation of the (±) reference, whether for playback or on line, may be instrumented easily and inexpensively for a special-purpose averaging computer, either by manually or automatically alternating the "add" and "subtract" modes, or by alternating the polarity of the input.

The (±) reference, either alone or in conjunction with the average of squares, can be used in several ways (*2*): (i) for guiding the investigator in detecting and measuring mean evoked components; (ii) for indicating the nature of the variability of the evoked activity; (iii) for determining the size and structure of averaged ongoing background activity, including its frequency structure; and (iv) for revealing interaction between the stimulus and ongoing background activity.

Let v be the random function of time which is being studied. It will be assumed that v can be decomposed into the sum of two hypothetical components, u and x, where x is an oscillatory background component which is approximately stationary in the random process sense and is unaffected by the delivery of a stimulus, and u is taken to represent the activity evoked by the stimulus and is a nonstationary process. Neither u nor x nor their averages can be measured directly, but the decomposition hypothesis leads to interesting analytical results which can be tested empirically.

The following notation is used:

$\bar{v}$, $\bar{u}$, $\bar{x}$—the random functions obtained by averaging v, u, x, respectively, at each time point for N sample functions.

$\overline{\pm v}$, $\overline{\pm u}$, $\overline{\pm x}$—the special averages obtained by alternate addition and subtraction followed by division by N. The N is assumed to be even, and these special averages are called (±) references.

$\mu[\ ]$—the mean of the random variable in brackets.

$\sigma[\ ]$—the standard deviation of the random variable.

$\rho[\ ]$—the autocorrelation ratio for the random variable.

$\delta(\ )$—the deviation of the random function about its mean function (also a random function).

The functions μ, σ, and ρ are all deterministic functions of time; μ and σ, of one time point; and ρ, of two time points. The deviation from the mean, $\delta(\ )$, is frequently referred to here as the variable component of the random function. Mean as used here is called population mean by statisticians and mathematical expectation by mathematicians.

The following relations governing averages of random functions under independent sampling are readily derived from the mathematical definition of μ, σ, and ρ:

$$\mu[\bar{v}] = \mu[v]; \mu[\delta(\bar{v})] = \mu[\overline{\pm v}] = 0 \tag{1}$$

$$\sigma[\bar{v}] = \sigma[\delta(\bar{v})] = \sigma[\overline{\pm v}] = N^{-\frac{1}{2}}\sigma[v] \tag{2}$$

$$\rho[\bar{v}] = \rho[\delta(\bar{v})] = \rho[\pm v] = \rho[v] \tag{3}$$

$\delta(\bar{v})$ and $\overline{\pm v}$ both tend to be derived from the same Gaussian random process. (4)

Equation 1 shows that the mean of the usual average, $\bar{v}$, is the same as that of the original function v. Equation 2 shows that the variability of $\bar{v}$ as well as that of $\overline{\pm v}$ has been reduced by a factor $N^{-1/2}$ as compared to the original random function, as measured by the standard deviation. This is the principal reason for averaging, and we call it the $N^{-1/2}$ principle.

Equation 4 is derived by application of the central limit theorem and is the fundamental mathematical relation underlying the use of the ($\pm$) reference. As one-dimensional random variables of time, $\delta\ (\bar{v})$ and $\overline{\pm v}$ both tend to the same normal distribution as N is increased. They do so because both have the same mean ($=0$) and the same standard deviation ($=\sigma\,[v]/\sqrt{N}$). As random functions of time, $\delta\ (\bar{v})$ and $\overline{\pm v}$ both tend to be derived from the same Gaussian random process as N increases. This is so because a Gaussian process is completely defined by μ, σ, and ρ, and, as Eqs. 1 to 3 show, these functions are the same for both $\delta\ (\bar{v})$ and $\overline{\pm v}$. Thus the variable component of the regular average, namely $\delta\ (\bar{v})$, and the ($\pm$) reference, $\overline{\pm v}$, tend to be samples, albeit independent ones, of the same Gaussian process.

The ($\pm$) reference is most useful when the assumed decomposition $v = u + x$ is valid and $\sigma\ (u) << \sigma\ (x)$. For then $\overline{\pm v} \simeq \overline{\pm x}$ and $\overline{\pm x}$ is approximately a stationary Gaussian random function with a constant mean (which may be assumed to be zero) and a constant standard deviation. Where the background activity x is rhythmic within a narrow frequency band, then $\overline{\pm x}$ ($\simeq \overline{\pm x}$) will have a high frequency component at approximately the mean of the frequency band whose amplitude will oscillate at a low frequency approximately equal to the width of the frequency band.

The average of squares, $\overline{v^2}$, is governed by the following relations:

$$\overline{v^2} = \mu[v^2] + \delta(\overline{v^2});\ \mu[v^2] = \mu^2[\bar{v}] + \sigma^2[u] + 2r\sigma[u]\sigma[x] + \sigma^2[x] \quad (5)$$

The $\overline{v^2}$ tends to become Gaussian with increasing N, and the $N^{-\frac{1}{2}}$ principle applies to ($\overline{v^2}$) in accordance with Eq. 2. The correlation ratio r between u and x has been introduced to allow for approximate representation of the interaction between the background activity, x, and the evoked activity, u. One has

$$\delta(\overline{v^2}) \simeq 2\mu[v]\bar{x} + \delta(\overline{x^2});\ \text{if } \sigma[u] << \sigma[x] \quad (6)$$

For narrow-band rhythmic processes, the band width is approximately doubled for $\delta\ (\overline{x^2})$ as compared to x or $\bar{x}$, and frequency content appears about zero frequency and double the frequency for x or $\bar{x}$. The standard deviation of $\delta\ (\overline{x^2})$ is of the order $\sigma^2\ [x]/\sqrt{N}$.

In averaging studies, although one can expect the variable components (noise) to be reduced by a factor $N^{-1/2}$ as compared to the mean (signal), it may not be possible to distinguish which features of the average are contributed by the signal and which by the noise. To determine this, one usually resorts to replication or systematic increase in N, or both. This may be both expensive and experimentally difficult. If this is not done, the results may be questioned.

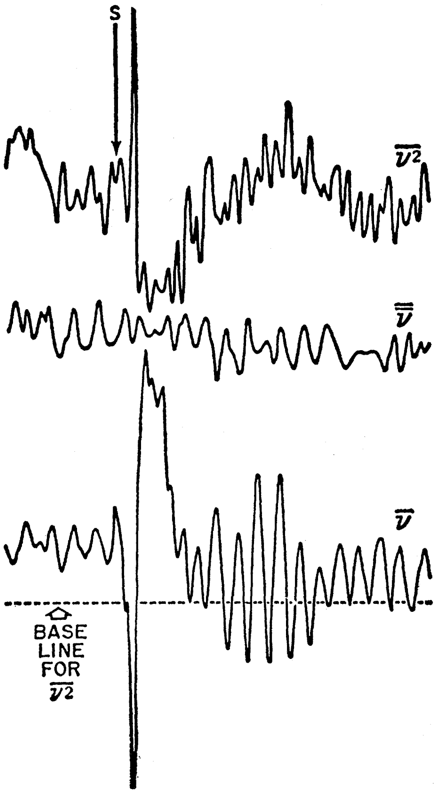

Fig. 1. Average, $\bar{\nu}$, ($\pm$) reference, $\overline{\pm\nu}$, and average of squares, $\overline{\nu^2}$; 800 photic stimuli; analysis time, 2 seconds; stimuli delivered 0.5 second after onset of analysis.

The ($\pm$) reference provides estimates of the noise levels (variable components) which are superimposed on the mean component. It is particularly advantageous to begin the analysis period some time prior to the delivery of the stimulus. Then both $\bar{v}$ and $\overline{\pm v}$ contain only variable components during the prestimulus interval, and accordingly one can detect more easily the appearance of a mean component in $\bar{v}$ following the delivery of a stimulus. The $\overline{v^2}$ not only provides estimates of the noise level (see Eqs. 5 and 6) but also is sensitive to interaction between background activity and stimulus, as in Fig. 1 where the mean component of $\overline{v^2}$ declines during the positive phase of $\bar{v}$. This decrease could be explained either by negative correlation between evoked activity and background activity (see next-to-last term of Eq. 5) or by partial blocking of the background rhythm during an interval of about two cycles, which would be expressed by a smaller value of $\sigma^2\ [x]$ during this period of time.

The later reappearance of magnified α rhythm in $\bar{v}$ suggests that the blocked α rhythm reemerges in phase, but that this coherence is gradually lost. The superimposed strong frequency variable component present in $\overline{v^2}$ has double the frequency of the α rhythm, and its amplitude is consistent with the formula $\sigma\ [\overline{x^2}] \simeq \sigma^2\ [x]/\sqrt{N}$ given after Eq. 6.

It should be noted that even though the independent sampling requirement is met (*3*), and μ, σ, and ρ are the same, $\delta\ (\bar{v})$ and $\overline{\pm v}$ tend to have the same statistical structure only as N is sufficiently large for the Gaussian approximation to be valid. The question of how large N should be for the statistics to be approximated by a Gaussian distribution must be studied either theoretically or experimentally for each case. For one-dimensional random variables the Gaussian distribution is approximated quickly, and $N \geqq 10$ is usually sufficient for the precision required in many biological experiments. Even for random functions, $N \geqq 10$ may be sufficient for the Gaussian approximation (*4*), although in average response studies N may have to be as large as 100 or even larger to bring out the principal features of the mean component.

In conclusion, a special kind of average, the ($\pm$) reference, is computed by alternate addition and subtraction of sample functions (for example, electrophysiological potentials). This measure, either alone or together with the average of squares, permits an estimation of the magnitude and statistical structure of the noise which is superimposed on a mean component of the average computed in the usual way.

It can be shown that the noise structure is related to the problem of estimating the precision and reliability of parameters derived from averaged traces (for example, latencies and amplitudes of peaks). However, this problem is a separate one and can be studied with conventional statistic tools, taking into account the special properties of averaged random functions (*5*).

HERBERT SCHIMMEL
Department of Neurology,
Albert Einstein College of Medicine,
Bronx, New York

References and Notes

1. N. W. Perry, *Science* **153**, 1022 (1966).
2. H. G. Vaughan, Jr., *Clin. Electroretinog. Suppl. to Vision Res. Proc. Symp. 3d, 1964* (1966), p. 203; I. Rapin and L. J. Graziani, *Neurology*, in press.

3. The sample may not be independent for biological reasons (for example, habituation) or as an experimental artifact (for example, regular consecutive sampling). See: J. S. Bendat, *IEEE Inst. Electron. Elect. Eng. Trans. Bio-Med. Electron.* **11**, 72 (1964); D. F. Ruchkin, *ibid.* **12**, 87 (1965).
4. Particularly pertinent are the results obtained in averaging the random sinusoidal process. See: W. R. Bennett, *Quart. Appl. Math.* **5**. (1948).
5. I. Rapin, A. Schimmel, L. M. Tourk, N. A. Krasnegor, C. Pollak, *Electroencephalog. Clin. Neurophysiol.* **21**, 335 (1966).
6. I thank Drs. Weitzman, Rapin, and Vaughan for discussion. Research supported by NIH grants NB-03356 and 5T1 MH-6418.

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Electrocortical Correlates of Stimulus Response and Reinforcement

Abstract. *Three patterns of electrical response were identified in the occipital cortex of rhesus monkeys making a differential discrimination: an input pattern that identifies which stimulus has been displayed; a reinforcement pattern that indicates whether the outcome of the differential response was rewarded or in error; and an intention pattern that occurs prior to the response and predicts which response the monkey is about to make. Neither the reinforcement nor the intention pattern is present while the monkeys perform at chance; at this time, only the differences due to input can be distinguished. These results suggest that more than simple input transmission is occurring in the primary visual mechanism. The influence of the experience of the organism is apparently encoded in the averaged electrical potentials recorded from the striate cortex.*

To combine the techniques of electrophysiology with those of behavioral analysis of organisms subjected to cerebral ablations (*1*), we recorded potential changes that occur in the striate cortex of rhesus monkeys at various instants in a trial during which a visual discrimination is made. We placed a monkey in a restraining chair in front of, and within easy reach of, a 20- by 20-cm translucent panel split vertically down the center. Each half of the panel could be independently depressed; pressure closed a microswitch which sent a pulse to be recorded on magnetic tape (1.3 cm). The pulse also activated a circuit designed to deliver a food pellet into a cup placed under the panel whenever a correct response was made.

In front of the monkey, there was, attached to the chair, a small lever which, when pulled, activated a stimulus display. Thus there was reasonable assurance that the monkey would attend (make an observing response) to the display. Initially, during "shaping," the display covered the entire translucent panel until the animal pressed it; but the duration of exposure was gradually shortened until it lasted for only 0.01 msec. This short duration—in essence a flash—ensured that a transient response occurred in the visual pathways. A transient response was chosen because the techniques of analysis of neuroelectric phenomena are considerably more advanced at present for transients than for changes in steady state. Two stimulus patterns (vertical stripes and a circle) equated for area were generated in a relatively random sequence by slides in a modified Kodak Carousel projector facing the back of the panel. The order of the display of the two patterns was determined in advance, so that the report of the response would be collated by the reinforcing circuit with the pattern displayed. This collation determined whether the response made was correct or incorrect. The occurrence of reinforcement was also recorded on the magnetic tape.

Once "shaped," the monkeys were trained to press the right half of the panel whenever the circle was displayed and to press the left half of the panel whenever the vertical stripes were displayed. One monkey failed to learn the task (a difficult one because of the short duration of the display), and the other two monkeys reached a criterion of 85 percent correct in 200 consecutive trials after 1800 and 2800 trials. Two hundred trials were given daily 6 days a week.

The sequence of events that constitutes a trial is therefore as follows: (i) The monkey pulls a lever which initiates a pulse recorded on magnetic tape and (ii) turns on a stimulus display which lasts 0.01 msec. One of two patterns (vertical stripes or circle) is displayed; a pulse to indicate which display is flashed is reported to a reinforcing circuit and recorded on magnetic tape. (iii) After a variable period, the monkey depresses either the right or left half of the display panel. This pressure also initiates a pulse which is recorded on magnetic tape and reported to the reinforcing circuit. This circuit then delivers a food pellet whenever the vertical-stripe display is followed by a press of the left panel and whenever the circle display is followed by a press of the right side of the panel. Reinforcement is also recorded on the tape.

Recording of electrical activity from the brain was continuous over sample sessions of 200 trials and, of course, coincided with the recordings of the behavioral events. The sessions chosen were (i) at the beginning of training, after the monkey had been conditioned to press but while he was performing at chance, and (ii) after criterion performance was established. Recordings were made from 12 placements in the striate cortex. All were bipolar (depth of cortex to surface) from an insulated nichrome wire (300 μ in diameter). The electrical brain signals were adequately amplified before they were recorded on magnetic tape.

The tape-recorded results were processed on a small general-purpose digital computer (PDP-8). Brain activity was digitized by an A-to-D converter, and the results of conversion were stored on digital magnetic tape. We devised programs to average the digitized electrical activity forward in time from the onset of the stimulus display (the pulling of the lever) and from the response (the depression of either half of the display panel). Averages were also obtained by running the tape backward from the two time markers; these records indicated what was going on in the monkey's brain just prior to his turning on the display and making the differential response. Programs were also developed to equate records obtained from unequal numbers of trials, so that correct and incorrect performances could be compared at criterion. Finally, routines to smooth the curves were adapted for photographing the results.

For each of the samples recorded, compilations were made of the brain activity (i) after stimulus display, (ii) preceding differential response, and (iii) after differential response. These compilations were then broken down into three categories: circle as opposed to vertical stripes, right as opposed to left panel, and correct as opposed to incorrect outcomes (Fig. 1). Reliable differences (*2*) can be ascertained in the configuration of the brain record evoked by a stimulus display of 0.01 msec (*3*). In this instance, the circle generated a downward deflection; the two peaks of this deflection are more nearly equal than those generated by the vertical stripes. In the response to