

- difference when pattern alone was considered).
11. The median correlation of .29 between the IQ's of same-aged parents and their children is somewhat lower than the figure of .56 reported for the same kind of comparison by C. Burt [*Brit. J. Psychol.* 57, 137 (1966)] and E. W. Reed and S. C. Reed [*Mental Retardation: A Family Study* (Saunders, Philadelphia, 1965)]. The age of the subjects at testing is not clear in Burt's report but testing occurred during their early school years (as was true for Reed and Reed), an age when the coefficients presented in this report are highest. In the data presented here the low parent-child correlations relative to those for siblings may derive from the fact that more non-Stanford-Binet assessments were present in the parent-child than in the sibling protocols.
 12. G. A. Ferguson, *Nonparametric Trend Analysis* (McGill Univ. Press, Montreal, 1965).
 13. This research was made possible by more than 40 years of effort by the staff of the Fels Research Institute, L. W. Sontag, Director; by the continuous support of the Fels Fund of Philadelphia; and by PHS grants FR-05537, HD-00868, and FR-00222 to the Fels Research Institute and HD-04160 to R.B.M. I thank R. Q. Bell, V. Crandall, D. Eichorn, N. Hurlburt, A. R. Jensen, A. Kagan, J. I. Lacey, R. M. Liebert, J. C. Loehlin, N. Robinson, H. B. Robinson, and L. W. Sontag for their helpful comments on earlier drafts of this report and L. Christensen, K. Pryor, J. Peterson, and P. Savoy for their preparation of the data and manuscript.

8 July 1970; 25 August 1970

Neuromuscular Synapse: Stochastic Properties of Spontaneous Release of Transmitter

Abstract. *The spontaneous quantal release of transmitter from the motor nerve endings is a random process which follows the Poisson theorem; the liberation of each quantum is independent of the release of previous quanta. Increase in the extracellular calcium concentration produces a statistical interdependence in the release of the spontaneously appearing packages.*

Transmitter is liberated at the neuromuscular junction and at neuronal synapses as multimolecular packages or quanta (1). There are two main factors that determine the rate of appearance of these quanta: (i) the membrane potential of the presynaptic terminal (2) and (ii) the calcium ion concentration in the external medium (3). When an action potential reaches the motor nerve endings, several hundred quanta are released within a millisecond giving rise to an end plate potential (EPP), and thus transfer of the electrical information across the neuromuscular synapse is obtained. At rest, the rate of liberation is approximately one quantum per second. Our report deals with some of the statistical features of these spontaneously occurring quanta and with the effect of calcium ions on them (4).

The experiments were performed in vitro on the sartorius neuromuscular preparation of the frog *Rana ridibunda*. Conventional methods of intracellular recording were employed (5). The spontaneous activity (miniature end plate potential-MEPP) was first recorded in normal ionic environment (standard Ringer solution composition: 116 mM NaCl, 2.0 mM KCl, and 1.8 mM CaCl_2). The results (Table 1) show that the discharge is random in nature and fits the Poisson theorem (6). Thereafter, while the microelectrode was still lodged in the same fiber, the calcium concentration of the medium was elevated to 15 mmole/liter [by isotonic substitution for NaCl; the

resulting decrease in sodium concentration has a very little effect on transmitter release (7)], and a second series of records was taken. Under this condition the discharge of the MEPP's no longer fits the Poisson theorem (Table 1). Taking the chi-square test (8) as a measure of the agreement between the experimental results and the values predicted by the Poisson theorem, one can see that there is a profound change in the pattern of MEPP's brought about by elevating the extracellular concentration of calcium; the χ^2 for 1.8

Table 1. Fit of MEPP appearance to Poisson distribution at normal and high calcium. The expected values were derived from the equation $N_x = N_T e^{-\alpha} \cdot \alpha^x / x!$ where N_T is the total number of samples, N_x is the number of samples containing x number of MEPP's, and α is the mean number of MEPP's in a sample. For normal concentration of calcium, α equals 0.0255, and for high concentrations of calcium α equals 0.0956. Each sample is a 20-msec period. The total number of MEPP's for the normal calcium concentration is 1187; for the high calcium concentration it is 998.

MEPP in sample (x)	N_x	
	Observed	Expected
<i>Normal calcium concentration (1.8 mmole/liter)</i>		
0	45388	45393.02
1	1167	1157.07
2	10	14.77
3	0	0.12
<i>High calcium concentration (15.0 mmole/liter)</i>		
0	9601	9486.40
1	735	906.64
2	65	43.53
3	21	1.39
4	10	0.03
5	6	0.00064

mM Ca^{2+} is 1.6, showing a fair fit between the experimental and predicted values; the χ^2 for 15 mM Ca^{2+} is 56424, demonstrating the extreme poor fit to the Poisson distribution. Similar changes in the pattern of spontaneous release were obtained in ten additional experiments. The observed change in the pattern of release was reversible.

The stochastic features of the spontaneous release were further analyzed by the autocorrelation method. If the intervals between successive MEPP's are independent of one another, one would expect to obtain no correlation in a long series of intervals. However, if the appearance on one MEPP facilitates the appearance of the next MEPP or if a common cause increases the basis frequency of MEPP's occasionally, then a positive correlation among the intervals is expected. On the other hand, if the appearance of one MEPP inhibits the appearance of the next, a negative correlation is expected. In practice, the analysis of the experimental results was carried out as follows: first, the mean frequency of the MEPP's ($\bar{f}$) was calculated; then the frequency of the MEPP's in a given time interval after each event (f_i) was estimated by the autocorrelation method. For a process of independent release one would expect that the relative frequency, given by the ratio ($f_i/\bar{f}$) is unity for all time intervals. If a release of a quantum facilitates the appearance of the next quantum, the relative frequency will be greater than one, whereas if the release of a quantum inhibits the appearance of the next one, the relative frequency will be less than one. Figure 1A shows that under normal conditions (standard Ringer solution, 1.8 mM Ca^{2+}), the spontaneous release of transmitter is an independent process in the statistical sense; the appearance of a MEPP does not affect at all the probability of release of the successive quantum. At high external concentrations of calcium, the statistical behavior of the discharge is completely different; the relative frequency is significantly different from one (Fig. 1B). For example, in the 20-msec period after any given MEPP the probability of release is about five times larger than the basic probability. This enhanced probability of release decays slowly and reaches the basic value only after several seconds. The statistical interdependence between successive events explains the departure from a Poissonian process demonstrated in Table 1; if the probability of occurrence of a MEPP is

increased after any given event, then there are expected to be more multiple events in some samples than the predictions of the Poisson theorem.

The origin of the MEPP is not yet completely understood. The change in the statistical pattern of release brought about by changing the extracellular calcium concentration suggests that a main determinant of the probability is the state of the presynaptic membrane,

which can be modified by calcium ions.

Our experiments illustrate that under normal conditions a spontaneous synaptic event is independent of the preceding one. When the extracellular concentration of calcium is increased this independence no longer occurs. Thus, by changing the concentration of calcium one can transform the spontaneous release from one pattern of statistical behavior to another, from a Poissonian to a non-Poissonian random discharge.

S. ROTSHENKER
R. RAHAMIMOFF

Department of Physiology, Hebrew
University Hadassah Medical School,
Jerusalem, Israel

References and Notes

1. P. Fatt and B. Katz, *Nature* **166**, 597 (1950); *J. Physiol.* **117**, 109 (1952); J. Dudel and S. W. Kuffler, *ibid.* **155**, 514 (1961); B. Katz and R. Miledi, *ibid.* **168**, 389 (1963); A. R. Martin, *Physiol. Rev.* **46**, 51 (1966).

2. J. del Castillo and B. Katz, *J. Physiol.* **124**, 586 (1954); B. Katz, *Proc. Roy. Soc. Serv. Biol. Sci.* **155**, 455 (1962); J. R. Bloedel, P. W. Gage, R. Llinas, D. M. J. Quastel, *J. Physiol.* **188**, 52P (1967); B. Katz and R. Miledi, *ibid.* **192**, 407 (1967).
3. J. del Castillo and B. Katz, *J. Physiol.* **124**, 533 (1954); D. H. Jenkinson, *ibid.* **138**, 434 (1957); F. A. Dodge and R. Rahamimoff, *ibid.* **193**, 419 (1967); J. I. Hubbard, S. F. Jones, E. M. Landau, *ibid.* **196**, 75 (1968); B. Katz and R. Miledi, *ibid.* **207**, 789 (1970); R. Rahamimoff, in *Calcium and Cellular Function*, A. W. Cuthbert, Ed. (Macmillan, London, 1970), p. 131.
4. For effect of calcium concentration on the frequency of MEPP's in mammalian neuromuscular junction see: P. W. Gage and D. M. J. Quastel, *J. Physiol.* **185**, 95 (1966); J. I. Hubbard, S. F. Jones, E. M. Landau, *ibid.* **194**, 355 (1968).
5. W. L. Nastuk and A. L. Hodgkin, *J. Cell Comp. Physiol.* **35**, 39 (1950).
6. P. W. Gage and J. I. Hubbard, *Nature* **208**, 395 (1965).
7. R. I. Birks and M. W. Cohen, in *Muscle*, W. M. Paul, E. E. Daniel, C. M. Kay, G. Monckton, Eds. (Pergamon Press, Oxford, 1965), p. 403.
8. G. U. Yule and M. G. Kendall, *An Introduction to the Theory of Statistics* (Griffin, London, 1950), p. 701.
9. Supported in part by a Singer fund research grant.

13 July 1970

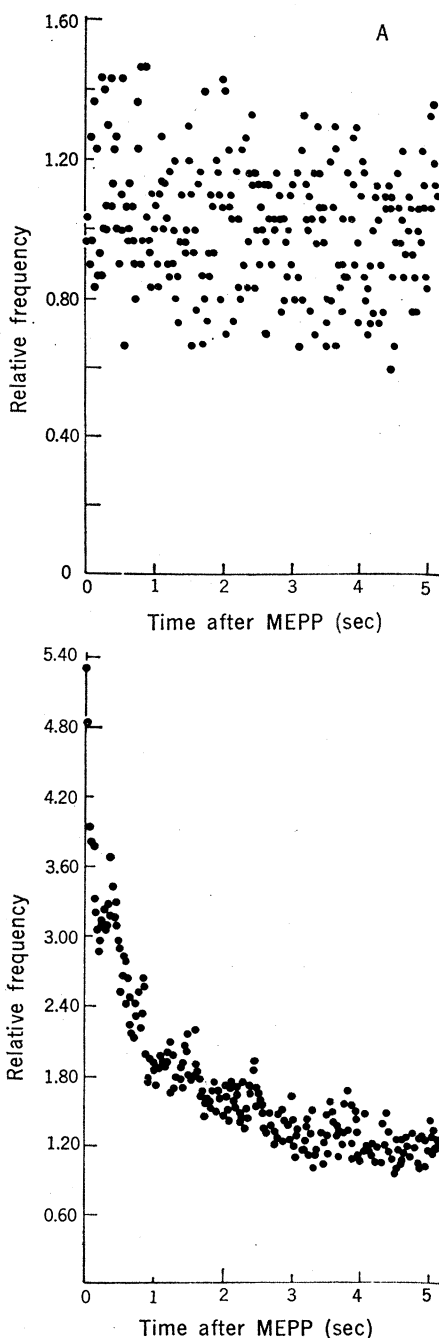


Fig. 1. Relative frequency of MEPP's following any given MEPP. The frequency of the MEPP's was estimated in successive 20-msec periods after each MEPP and divided by the mean frequency (see text). (A) Normal concentration of calcium (1.8 mmole/liter); (B) high concentration of calcium (15.0 mmole/liter).

Interference of HEPES with the Lowry Method

We, too, have experienced a similar difficulty in the determination of protein in the presence of HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid) (1). Our resolution of the problem was to measure the protein by a modified version of the microbiuret method (2), scaled down in volume such that the color developed from 0.4 ml of protein solution, when mixed with 0.2 ml of reagent, could be determined in silica microcells (0.5 ml). Any interference due to salt crystallization that might arise with buffers of high ionic strength is easily overcome by clarifying the mixed solutions in a bench centrifuge before measurement.

The method was unaffected by the presence of HEPES, is relatively nonspecific for the type of protein, and moreover produces a linear standard curve, with sensitivity not much less than with the Lowry procedure.

L. V. TURNER

K. L. MANCHESTER

Department of Biochemistry,
University College London,
Gower Street, London W.C.1, England

References

1. J. D. Gregory and S. W. Sajdera, *Science* **169**, 97 (1970).
2. R. F. Itzhaki and D. M. Gill, *Anal. Biochem.* **9**, 401 (1964).

15 September 1970

Prey Population: A Parsimonious Model for Evolution of Response to Predator Species Diversity

Ricklefs (1) proposes a model to account for "clutch size in birds" which seems hardly applicable to birds as a class. The model can be applied comfortably only to those birds which feed their nestlings on animal food, and more specifically to those which feed altricial young on motile prey. Pigeons (Columbidae) produce animal food from their own crops. Brood parasites do not feed their young at all. Young precocial birds feed themselves, many on vegetable matter. That apparent

cavil aside for the moment, it is worth while to consider the logical structure underlying the hypothesis, and the consequences logically to be expected from it.

One is immediately struck by the erection of an "adaptive system" which adjusts the strategies of foraging behavior among predatory birds to the productivity of their prey. As what seem to be his only examples of analogous "systems of predator-prey adaptation of diverse species" Ricklefs cites