rats because we have observed a large number of both well-differentiated cells and mitoses at this age (unpublished data). White Holtzman rats were given [^{3}H]thymidine (50 μc per gram of body weight) by subcutaneous injection; uninjected rats of this same age served as controls. Four hours after injection, the hearts were fixed in glutaraldehyde-paraformaldehyde (*13*) and processed for electron microscope autoradiography (*14*).

Examination of autoradiographs revealed many myocardial nuclei that had incorporated [^{3}H]thymidine. Specific labeling of DNA was apparent from the very high counts (40 to 100) over the nuclei as compared to the very low background (one grain or less). Both labeled (Fig. 1) and unlabeled cells showed the same degree of differentiation. Only those cells in mitosis showed a lack of organized myofibril structure (Fig. 2).

Loss of myofibril structure may be necessary for mitosis in rat cardiocytes, as suggested by Rumyantsev (*10*). His electron microscopic studies of mitosis in 5- and 7-day-old rats indicate that during prometaphase, myofibrils near the nucleus partially disintegrate (*10*). The Z bands linking myofibril subunits disappear and bundles of thick and thin filaments remain during the various stages of mitosis (*10*). Our observations on the various stages of mitosis in 2-day neonatal rats confirm and extend these findings.

The presence of well-aligned myofibrils in labeled cells (Fig. 1) suggests that the mitotic cell in Fig. 2 has had a temporary loss of myofibril structure and was once as well differentiated as the cell synthesizing DNA (Fig. 1). Support for this interpretation comes from Kasten's (*15*) observation of beating ventricular cells of newborn rat in cultures. Some of these cells undergo mitosis but do not beat during the latter stages of cell division.

We show at the ultrastructural level in vivo synthesis of DNA in highly differentiated mammalian heart cells. The highly organized myofilament structure at the time of DNA synthesis confirms the suggestion of Rumyantsev and Snigirevskaya (*16*) that in rat cardiocytes the presence of well-aligned myofibrils does not act as a mitotic inhibitor or an inhibitor of DNA synthesis.

MARGARET A. GOLDSTEIN
WILLIAM C. CLAYCOMB
ARNOLD SCHWARTZ
Department of Cell Biophysics, Baylor College of Medicine, Houston, Texas

18 JANUARY 1974

References and Notes

1. F. E. Stockdale and H. Holtzer, *Exp. Cell Res.* **24**, 508 (1961).
2. K. Okazaki and H. Holtzer, *J. Histochem. Cytochem.* **13**, 726 (1965).
3. R. J. Przybylski and J. M. Blumberg, *Lab. Invest.* **15**, 836 (1966).
4. R. J. Przybylski and J. S. Chlebowski, *J. Morphol.* **137**, 414 (1972).
5. R. B. Weinstein and E. D. Hay, *J. Cell Biol.* **47**, 310 (1970).
6. F. J. Manasek, *ibid.* **37**, 191 (1968).
7. D. A. Hay and F. N. Low, *Am. J. Anat.* **134**, 175 (1972).
8. I. S. Polinger, *Exp. Cell Res.* **76**, 253 (1973).
9. K. Chacko, *Am. J. Anat.* **135**, 305 (1972).
10. P. P. Rumyantsev, *Z. Zellforsch. Mikrosk. Anat.* **129**, 471 (1972).
11. R. Zak, *Am. J. Cardiol.* **31**, 211 (1973).
12. W. C. Claycomb, *Biochem. Biophys. Res. Commun.* **54**, 715 (1973).
13. M. J. Karnovsky, *J. Cell Biol.* **27**, 137 (1965).
14. L. G. Caro and R. P. Van Tubergen, *ibid.* **15**, 173 (1962).
15. F. H. Kasten, *In Vitro* **8**, 128 (1972).
16. P. P. Rumyantsev and E. S. Snigirevskaya, *Acta Morphol. Acad. Sci. Hung.* **16**, 271 (1968).
17. Supported by NIH grants HL 05435, HL 07906, HL 05925; NIH contract 71-2493; and the American Heart Association, Texas affiliate, Houston chapter. We thank D. Murphy for technical assistance.

13 August 1973; revised 26 September 1973

Miniature End Plate Potentials Recorded from Mammalian Myoneural Junctions in vivo

Abstract. *Fibers of the cat soleus muscle had a mean resting potential of 87 millivolts as measured with an intracellular microelectrode. Miniature end plate potentials had a mean amplitude of 0.95 millivolt, a mean frequency of 1.01 per second, a mean duration of 4.44 milliseconds, and a mean rate of rise of 0.99 volt per second. Two populations of response could be discerned on the basis of rate of rise. Neither α-chloralose nor nerve section had any significant effect on the response pattern. The in vivo preparation appears to provide a superior representation of the physiology of the myoneural junction.*

Most single cell data on synaptic function at the neuromuscular junction has been obtained with intracellular recordings from in vitro preparations (*1*). These studies have been important in developing our present concepts of the events at the neuromuscular junction, but a question about them persists. Are electrophysiological parameters measured in tissue excised and maintained in artificial salt solutions an accurate reflection of those in the living animal? Since the neuromuscular junction is extremely sensitive to hypoxia and to small changes in osmotic pressure (*2*), electrophysiological recordings made in vivo might be different from those made in vitro. The purposes of the present study were to adapt intracellular microelectrode recording techniques to an in vivo preparation of the cat soleus muscle; to establish the characteristics of the spontaneous postsynaptic response recorded under as nearly physiologic conditions as possible; and to compare these values with those recorded in vitro.

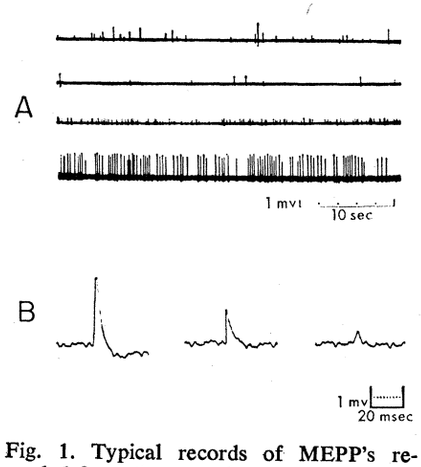

Fig. 1. Typical records of MEPP's recorded from the cat soleus muscle in vivo. The recordings in (A) show the range of amplitude and frequencies, and those in (B), with an expanded time base, show durations and rates of rise.

Cats were anesthetized either with α-chloralose or by C-1 spinal section under vinyl ether. The hindlimb was dissected, exposing the soleus muscle and isolating its nerve and blood supply by the method of Riker *et al.* (*3*). Steel pins were inserted through distal ends of the femur and tibia, and the leg was rigidly mounted in a Brown-Schuster myograph. Intracellular potentials were measured with glass capillary microelectrodes filled with 3*M* KCl (resistance, 8 to 15 megohms). Potentials were monitored on an oscilloscope and recorded on magnetic tape.

When a muscle cell was impaled at a point near the neuromuscular junction, a resting membrane potential of 80 to 90 mv was obtained. The mean, 87.0 ± 1.1 mv, is considerably higher than that reported for in vitro preparations such as the cat tenuissimus, 67 mv (*4*); rat soleus, 77.0 mv (*5*); and rat hemidiaphragm, 73 mv (*6*). The potential is stable; it can be recorded for hours with only 3- to 5-mv decreases.

Spontaneous miniature end plate po-

tentials (MEPP's) were also observed. Their frequency varied from cell to cell (Fig. 1A), but in each cell they occurred at random, that is, gave a good fit to a Poisson distribution (*8*). The upper record of Fig. 1A illustrates potentials at about 1 per second, the frequency observed most often. The distribution of average frequencies in a population of 20 cells is presented in Fig. 2A. The mean, 1.01 ± 0.29 per second is comparable to that observed in vitro (*4–6*). Chi-square analysis indicates that the distribution of mean frequencies is normal. Figure 1A also illustrates the variation in amplitude of the MEPP's; the smallest potentials are nearly 1.0 mv, many are 2 to 3 mv, and some (bottom trace) are 3 to 4 mv. Often, MEPP's of two amplitudes were recorded from a single penetration (upper two traces in Fig. 1A), but other penetrations produced only large or small potentials. A histogram of the amplitudes is given in Fig. 2B. The mean, 0.95 ± 0.01 mv, is substantially larger than that reported in vitro—for example, for cat tenuissimus, 0.45 mv (*4*); rat soleus, 0.5 mv (*5*); and rat hemidiaphragm, 0.6 mv (*6*). Chi-square analysis indicates that the distribution of amplitudes is not normal ($P > .05$). The poor fit is a consequence of the distinct secondary peak at 1.25 to 1.30 mv.

Table 1. Effect of anesthetic procedures and sciatic nerve section on spontaneous miniature end plate potentials. Values are means ± standard error for ten cells; $P > .05$.

Treatment	Resting membrane potential (mv)	Frequency (per second)	Amplitude (mv)	Duration (msec)	Mean rate of rise (volt/sec)
Chloralose, nerve sectioned	87.0 ± 1.1	1.01 ± 0.29	0.95 ± 0.01	4.4 ± 0.05	0.99 ± 0.01
Spinal cord cut, nerve intact	85.2 ± 2.0	0.95 ± .18	1.01 ± .04	4.4 ± .1	.99 ± .01
Spinal cord cut, nerve sectioned	87.1 ± 1.3	.97 ± .20	1.00 ± .05	4.4 ± .1	.97 ± .03

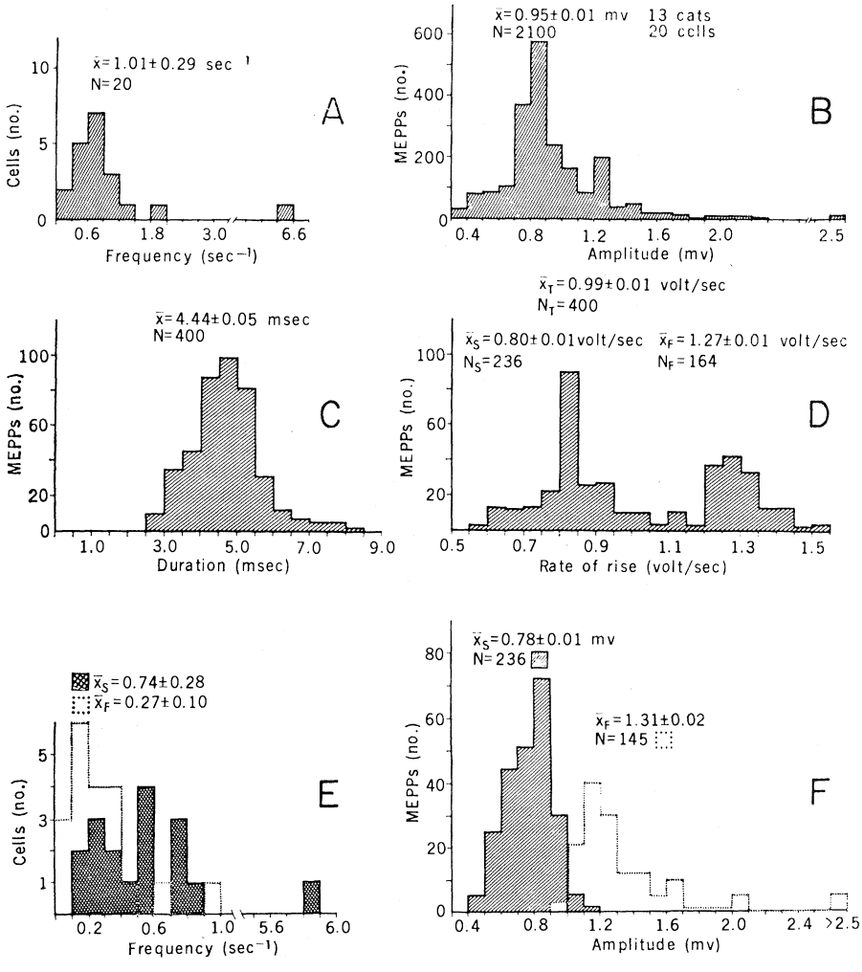

Fig. 2. Histograms of MEPP parameters recorded from the cat soleus muscle in vivo; values are means ± standard error. (A) The average frequency of MEPP's in a population of 20 cells is shown. (B) The amplitude distribution of MEPP's is shown for 20 cells. (C) The distribution of MEPP duration is shown for 10 cells. (D) The distribution of rate of rise of MEPP's is given for 10 cells; $\bar{X}_T$ is the overall mean rate of rise, $\bar{X}_S$ is the mean rate for data less than 1.0 volt/sec, and $\bar{X}_F$ is the mean rate for data greater than 1.0 volt/sec. (E) Distributions of average frequency are shown for two populations of MEPP's discriminated on the basis of rate of rise; $\bar{X}_F$ is the mean frequency for MEPP's that rise faster than 1.0 volt/sec, and $\bar{X}_S$ is the mean frequency for MEPP's with slower rates of rise. (F) Amplitude distributions of two populations of MEPP's discriminated as in (E).

Figure 1B shows MEPP's, all from a single cell, recorded on a different time base. The range of amplitudes (0.75 to 3.5 mv) is striking, as is the response duration (regardless of amplitude, each potential is complete in less than 7 msec). The distribution of MEPP durations is plotted in Fig. 2C. The mean is 4.44 ± 0.05 msec, short in comparison with in vitro values of 6 to 15 msec (*6, 8*). The rate of rise of MEPP's in vivo is high (Fig. 2D). The mean is 0.99 ± 0.01 volt/sec, but the distribution is badly skewed and has two distinct peaks. This suggests that MEPP's that rise faster than 1.0 volt/sec might be a separate population from those with lower rates of rise. Recalculation of data on this basis gave a mean value of 0.80 ± 0.01 volt/sec for slowly rising potentials and 1.27 ± 0.01 volt/sec for those rising rapidly. Even the lower rate of rise is higher than that for in vitro preparations (*4, 6*).

The bimodal distribution of rate of rise led to a revaluation of the distributions of frequency and amplitude. The frequency distribution is replotted (Fig. 2E) to discriminate on the basis of rate of rise. The mean frequency for MEPP's with a rate of rise less than 1.0 volt/sec is 0.74 ± 0.28 per second, and the mean for those with greater rates is 0.27 ± 0.10 per second (difference not significant, paired *t*-test). The amplitude distributions of slowly and rapidly rising potentials are given in Fig. 2F. The average amplitudes are 0.78 ± 0.01 mv for the former and 1.31 ± 0.02 mv for the later. Chi-square analysis indicates that each distribution is a good approximation to a normal curve ($P < .01$). This substantiates the notion gained from Fig.

1 that there are two populations of MEPP's—slow small and fast large. The large, rapidly rising potentials are probably generated near the recording site, and the smaller, slowly rising ones probably originate at some distance from it. This interpretation implies that if the electrode could be focally located with respect to each of the junctional sites, all MEPP amplitudes should be distributed about the 1.31-mv mean.

Because many parameters of MEPP's in vivo are strikingly different from those in vitro, we determined whether the anesthetic, α-chloralose, caused the differences. Data for animals anesthetized by cutting the spinal cord are given in Table 1, which also gives measurements obtained before and after the sciatic nerve was cut in cats with spinal section. No significant differences in resting membrane potential or MEPP frequency amplitude, duration, or rate of rise could be discerned (Duncan's new multiple range test). Thus, neither α-chloralose nor section of the sciatic nerve affects MEPP's recorded by our method. Thus, the activity we observed is probably an accurate representation of the physiological response.

Our results show that spontaneous MEPP's recorded in vivo are similar in frequency and randomness of occurrence to those observed in vitro. On the other hand, they have substantially larger amplitudes, higher rates of rise, and shorter durations, and the in vivo resting membrane potential is larger and more stable than that customarily observed in vitro. These differences are probably related to the intact circulation, which assures better oxygenation and osmotic milieu than those in vitro, but the magnitude of these differences is striking. Furthermore, since the resting membrane potential is 10 to 15 mv larger than that in vitro, the profile of the muscle action potential as well as that of the end plate potential may differ substantially from that observed in excised tissue. It may be necessary to reevaluate some of the conclusions related to transmitter release and quantal composition of the end plate potential which are based on data from in vitro records.

Our study demonstrated that the in vivo neuromuscular preparation offers a stable, highly sensitive system for examining the physiological events at the neuromuscular junction. The results raise some fundamental questions regarding the quantitative analysis of synaptic processes. Further use of in vivo recording should provide new insights into the nature of neuromuscular transmission. In addition, this preparation offers a unique opportunity for examining the pharmacological properties of the neuromuscular junction by administering drugs by routes identical to those used clinically.

THEODORE B. HOEKMAN
KENNETH L. DRETCHEN
FRANK G. STANDAERT

Department of Pharmacology, Georgetown University Schools of Medicine and *Dentistry, Washington, D.C. 20007*

References and Notes

1. W. F. Riker, Jr., and M. Okamoto, *Annu. Rev. Pharmacol.* **9**, 173 (1969).
2. J. I. Hubbard and Y. Loyning, *J. Physiol. (Lond.)* **185**, 205 (1966); J. I. Hubbard, S. F. Jones, E. M. Landau, *ibid.* **197**, 639 (1968).
3. W. F. Riker, Jr., J. Roberts, F. G. Standaert, H. Fujimori, *J. Pharmacol. Exp. Ther.* **121**, 286 (1957).
4. L. C. Blaber and D. C. Christ, *Int. J. Neuropharmacol.* **6**, 473 (1967).
5. J. J. McArdle and E. X. Albuquerque, *J. Gen. Physiol.* **61**, 1 (1973).
6. A. W. Liley, *J. Physiol. (Lond.)* **132**, 650 (1956).
7. P. W. Gage and J. I. Hubbard, *Nature* **208**, 395 (1965).
8. P. Fatt and B. Katz, *J. Physiol. (Lond.)* **117**, 109 (1952).
9. Supported by NIH grant NS-07651.

20 July 1973; revised 29 August 1973

Carcinogenicity of Methylchrysenes

Abstract. *Methylchrysenes are present in tobacco smoke and are suspected to contribute to the tumorigenicity of this inhalant. Chrysene and the six isomeric methylchrysenes were obtained in high purity (>99.9 percent); they were tested on mouse skin for tumor initiating activity and carcinogenicity. The 3- and 6-methylchrysenes are strong tumor initiators, whereas the other five chrysenes have moderate initiating activity. 5-Methylchrysene is a strong carcinogen; the other chrysenes are inactive or weak carcinogens.*

Tobacco smoke contains relatively high concentrations of alkylchrysenes in comparison to gasoline engine exhaust and other urban pollutants (*1*). Unsubstituted and alkylated chrysenes, benz[*a*]anthracenes, and benzo[*a*]pyrenes have been identified in neutral subfractions that amount to about 0.05

Table 1. Female, Swiss albino mice (Ha/ICR/Mil) were used. The tumor initiator dose for chrysene and the methylchrysenes was ten applications of 0.1 mg in 1 ml of acetone, and that for benzo[*a*]pyrene was ten applications of 0.0005 mg in 1 ml of acetone. The tumor promotor dose was 2.5 μg of TPA, given three times weekly. The acetone controls were negative.

Promotor application (weeks)	No. mice with tumors	Total tumors	Survivors	Promotor application (weeks)	No. mice with tumors	Total tumors	Survivors
	Chrysene				*4-Methylchrysene*		
1	0	0	20	1	0	0	20
10	0	0	19	10	0	0	20
12	1	1	18	12	2	2	20
14	3	6	18	14	2	2	20
16	6	9	18	16	3	3	20
18	8	15	18	18	7	9	20
20	11	19	18	20	7	9	20
	1-Methylchrysene				*5-Methylchrysene*		
1	0	0	20	1	0	0	20
10	0	0	20	10	3	3	19
12	0	0	20	12	6	9	19
14	2	2	19	14	12	31	19
16	2	2	19	16	13	49	19
18	5	5	19	18	17	92	18
20	6	6	19	20	17	96	18
	2-Methylchrysene				*6-Methylchrysene*		
1	0	0	20	1	0	0	20
10	0	0	19	10	0	0	19
12	0	0	19	12	1	1	19
14	2	2	19	14	1	1	19
16	6	9	19	16	5	8	19
18	8	13	19	18	7	11	19
20	8	13	19	20	7	11	19
	3-Methylchrysene				*Benzo*[a]*pyrene*		
1	0	0	20	1	0	0	20
10	0	0	20	10	0	0	20
12	1	1	20	12	1	1	20
14	3	5	20	14	4	5	20
16	6	11	20	16	6	9	20
18	11	21	20	18	6	10	20
20	14	26	20	20	6	10	20