stimulate the second pump component. Other reasons, however, may be considered such as dilution of the caffeine by the myoplasm, and dilution of the specific activity of the internal radiosodium as the result of the release of sequestered Na[11].

[11] Acknowledgment. This work was supported in part by grants from the Medical School Research Commitee, the Graduate School Research Committee, the Wisconsin Heart Association, the Office of Naval Research, and the National Science Foundation. The technical help of Mr. GEOFFREY CHAMBERS is gratefully acknowledged.

Zusammenfassung. Nachweis, dass Coffein zunächst den Auswärtstrom von Na-Ionen aus Einzelfasern der Bernakelmuskeln verringert, ihn hernach aber zu steigern vermag.

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17 January 1972.

Synaptic Frequency Demodulation

It is widely accepted that information is transmitted through nervous channels employing as a code the frequency of nerve impulses[1–3]. This code is demodulated at synapses into a quasi-analogue change of the post-synaptic membrane potential. This frequency-to-analogue conversion depends upon a great number of factors. Among these one can count pre-synaptic factors including presynaptic factilitation, potentiation and depression and also the statistical nature of the quantal release of transmitter. Post-synaptic factors include the duration of transmitter action, the membrane time constant, the membrane cable characteristics, and the non-linear summation of transmitter activity. Although a great deal is known about these various factors, it is not yet clear how all of them act in concert to produce synaptic frequency demodulation.

The neuromuscular junction is a well-known preparation where all the release parameters have been extensively investigated[4]. We here report preliminary results describing the dynamic characteristics of this synapse. We are currently engaged in defining the role of the various release parameters in determining synaptic frequency demodulation.

In the present study we employed the frog's (*Rana ridibunda*) sciatic nerve-sartorius preparation in vitro. Synaptic potentials were recorded intracellularly with conventional glass micropipettes, filled with 3 *M* KCl, which were connected through a cathode follower (Bioelectric Instruments) to the DC amplifier of a Tektronix 502A oscilloscope, and finally recorded on tape, using a Hewlett Packard FM tape recorder. The preparations were equilibrated in a medium containing a reduced calcium and increased magnesium ion concentration. In this medium the mean quantal content of the end plate potentials was low[5–7]. This served to prevent muscle contractions and also made the neuromuscular synapse resemble central synapses where synaptic potentials appear to have low quantal content[8,9]. For stimulation, a Wavetek waveform generator was connected to a frequency-modulation system which was employed to deliver supra-maximal stimuli to the sciatic nerve.

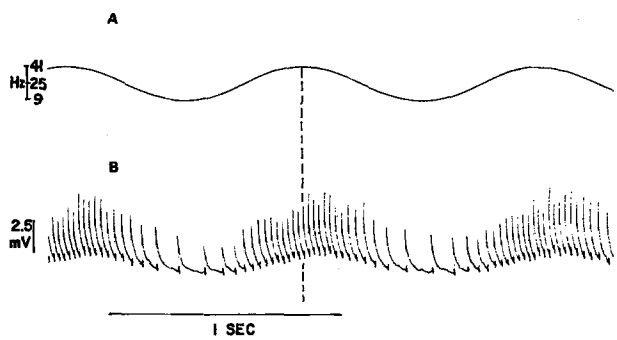

Fig. 1. Synaptic frequency to analogue conversion. The frequency of stimulation is shown as a continuous line, fluctuating between 41 and 9 isec. B) The post-synaptic membrane potential changes. Each impulse is a separate end-plate potential. Resting potential: 81 mV.

As can be seen in Figure 1, the frequency of stimulation of the sciatic nerve varied in a sinusoidal manner, each stimulus producing a distinct end-plate potential. In this particular experiment, the basic frequency of stimulation was 25 impulses/sec (isec) and was modulated in the range between 41 and 9 isec. The amplitude of the frequency modulation was thus 25 ± 16 isec (Figure 1A). It can be seen that both the base line of the post-synaptic potential and the peak amplitudes of the end-plate potentials varied in a sinusoidal manner, the membrane depolarization increasing with the rate of stimulation (Figure 1B). The pattern of frequency of stimulation of the nerve was thus translated into an analogue change in post-synaptic membrane potential. However, the post-synaptic changes show a small phase lag in respect to changes in the presynaptic rate of stimulation (see the dashed line denoting the peak frequency of stimulation).

It was next of interest to examine the amplitude response and the frequency response of the synapse. The results for amplitude response are shown in Figure 2. Here the amplitude of the frequency modulation was increased from 25 ± 0 isec (Figure 2A) through 25 ± 1.5 isec (Figure 2B), 25 ± 3 isec (Figure 2C), 25 ± 9 isec (Figure 2D) and 25 ± 16 isec (Figure 2E). It can be seen that,

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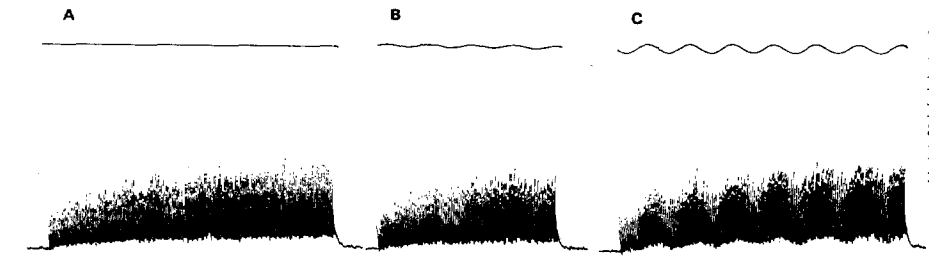

Fig. 2. Synaptic amplitude responses. The upper trace in each figure denotes the frequency of stimulation. The lower trace: post-synaptic membrane potential changes. Resting potential: 83 mV. A) Zero frequency modulation. B) 25 ± 1.5 isec. C) 25 ± 3 isec. D) 25 ± 9 isec. E) 25 ± 16 isec.

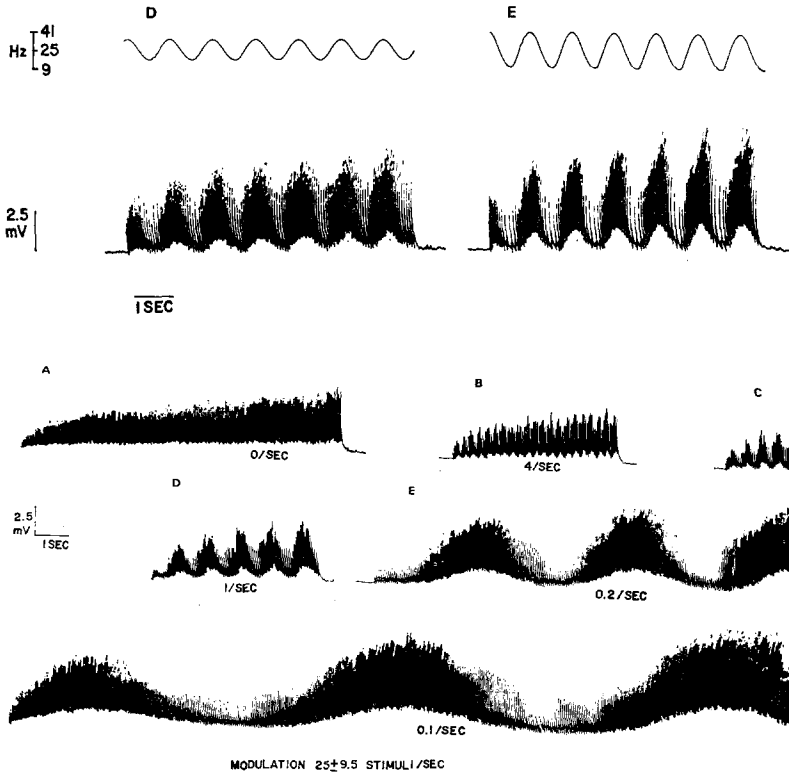

Fig. 3. Synaptic frequency response. Only post-synaptic membrane potential changes are shown. In this experiment, the amplitude of the frequency modulation was constant: 25 ± 9.5 isec. The following are the respective values for the frequency of modulation: A) 0 Hz. B) 4 Hz. C) 2 Hz. D) 1 Hz. E) 0.2 Hz. F) 0.1 Hz. Resting potential 80 mV.

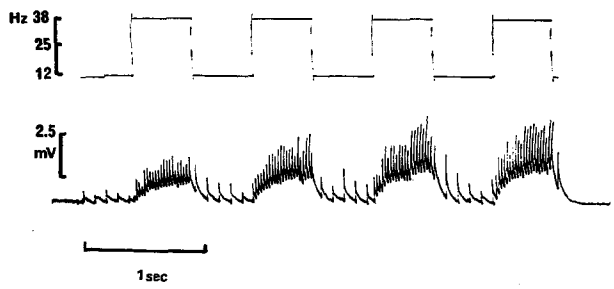

Fig. 4. The response to abrupt frequency changes. The upper trace designates frequency of stimulation. The lower trace: The post-synaptic membrane potential changes. Resting potential 80 mV.

qualitatively, post-synaptic potential changes grow proportionally with increasing presynaptic frequency variation. A quantitative analysis (LASS and LANDAU, unpublished) shows that for a considerable range, the post-synaptic potential changes grow linearly with increasing amplitude of frequency modulation. One must also take into account the gradual growth of the responses with time, probably due to the accumulated effect of presynaptic facilitation[10,11]. In order to minimize these prolonged effects, the preparation was always given a rest of 10–15 min between successive trains of stimuli.

Finally, the frequency response of this synapse was examined by employing sinusoidally varying rates of stimulation with a constant amplitude of frequency modulation (25 ± 9.5 isec) but with different sinusoidal cycle lengths. It can be seen in Figure 3 that the post-synaptic voltage changes are larger the greater the length of the sinusoidal cycle. Thus the largest post-synaptic potential variations were observed when a complete cycle of stimulation lasted 10 sec (Figure 3F). Smaller cycles lengths of 5 and 1 sec produced smaller post-synaptic changes (Figure 3D and E). The smallest membrane potential modulation was found with the shortest cycle length of 0.25 sec (frequency: 4/sec) (Figure 3B). The synapse thus acted like a low pass filter, transmitting best slow changes in presynaptic frequency. This can also be seen in Figure 4 where we demonstrate the post-synaptic changes which

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were found in response to a square change in presynaptic impulse frequency. The post-synaptic potential change is seen to grow and to decay slowly, in a manner reminiscent of the charging and of the discharging of a capacitor.

Our results confirm the low pass characteristics of synaptic transmission found by TERZUELO and BAYLY[12] from indirect evidence. Work is in progress to evaluate the role of various synaptic processes in determining synaptic amplitude and frequency response[13].

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Zusammenfassung. Die synaptische Übertragung von Reizmustern wurde an der neuromuskularen Synapse bei *Rana ridibunda* geprüft. Der Nerv wurde mit einer sinusoidal modulierten Frequenz gereizt und die postsynaptischen Potentiale intrazellulär abgeleitet. Daraus wird geschlossen, dass die Synapse Veränderungen in der Einsatzfrequenz überträgt, die einem «Low-pass»-Filter gleichkommt.

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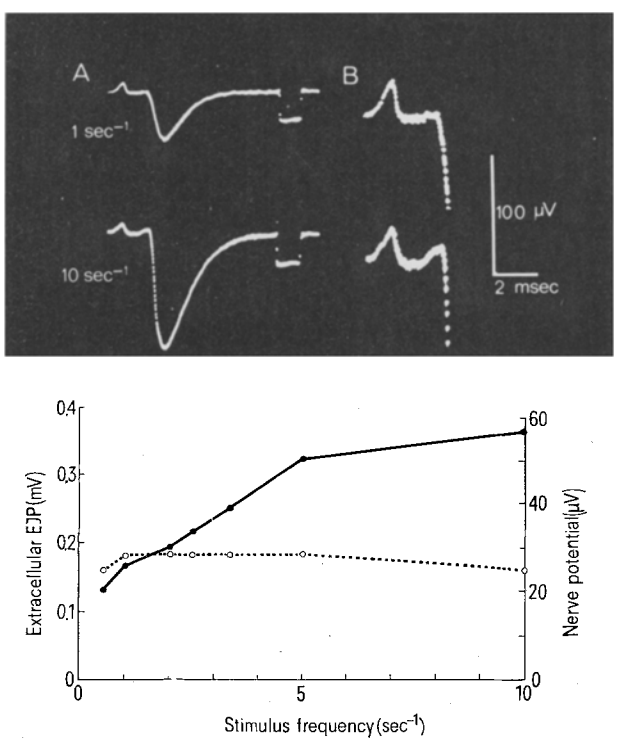
Crayfish Neuromuscular Junction: Facilitation with Constant Nerve Terminal Potential

At many excitatory, chemical synapses the average amplitude of the postsynaptic potential increases as the frequency of nerve stimulation is raised[1-5]. This increase has been shown to be a presynaptic phenomenon resulting from a greater number of transmitter quanta released per nerve impulse[6]. This process of enhanced transmitter release during repetitive stimulation, termed facilitation, has been explained in two ways.



A) Averaged extracellular junctional potentials (EJP) at synaptic region of a superficial crayfish muscle fiber. Average of 100 consecutive stimuli at 1 sec^{-1} (top) and 10 sec^{-1} (bottom). Calibration pulse equals 100 µV and 2 msec for both traces. B) High gain photographs of monophasic nerve terminal potential portion of A) Note: positive (upward) deflection following nerve terminal potential in lower (10 sec^{-1}) trace probably resulting from outward current from adjacent active postsynaptic spots. Temperature 9 °C. All recording A.C. Graph. Averaged extracellular EJP amplitude (filled circles, solid line) and nerve terminal potential amplitude (open circles, broken line) vs. stimulus frequency.

First, that repetitive stimulation leads to a progressive increase in 'active' calcium at transmitter release sites in the nerve terminal[7] and second, that the amplitude and/or duration of the presynaptic terminal depolarization leading to transmitter release increases with successive nerve action potentials[4,8]. It is known that an increase in external calcium and/or terminal depolarization results in increased transmitter output[9].

At both the chick ciliary ganglion[2] and frog neuromuscular junction[1] facilitation occurs without concomitant changes in nerve action potentials recorded from presynaptic nerve terminals. In contrast, at the squid giant synapse[10], mammalian neuromuscular junction[3], and the crayfish neuromuscular junction[4] an increase in presynaptic depolarization appears to accompany facilitation. In particular, at the crayfish neuromuscular junction it is reported that transmitter release varys linearly with nerve terminal depolarization which is in turn a linear function of stimulus frequency[4]. It is possible, however, that these extracellular records of presynaptic nerve terminal potentials are contaminated by postsynaptic currents arising from neighboring synaptic regions[11,12]. The present experiments re-examine the relation between the extracellularly recorded nerve terminal potential and the excitatory junctional potential (EJP), at the crayfish neuromuscular junction under conditions of reduced temperature. This procedure increases the synaptic delay[13] and allows temporally well differentiated recording of pre- and postsynaptic electrical events.

Materials and methods. The preparation, dissection and recording procedures were essentially the same as described by DUDEL and KUFFLER[14]. Signal averaging was

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