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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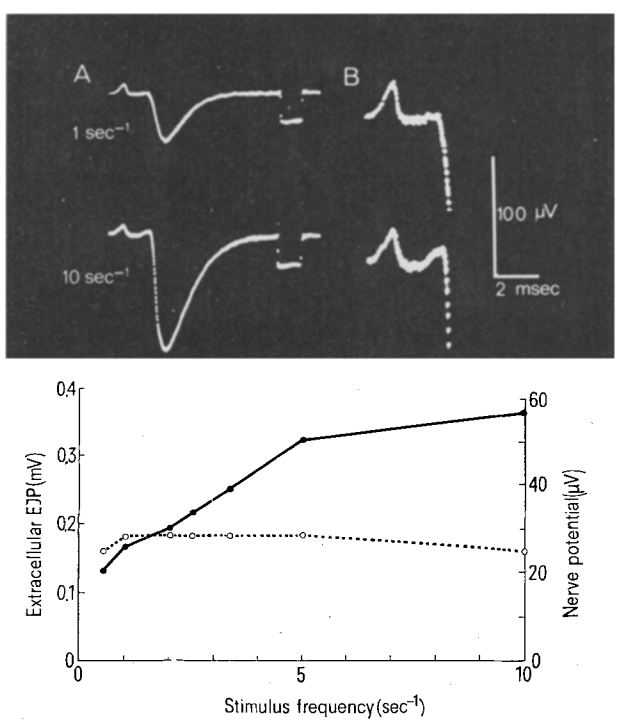
Crayfish Neuromuscular Junction: Facilitation with Constant Nerve Terminal Potential

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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accomplished with a Northern Scientific Digital Memory Oscilloscope (NS-550) with 1024 bit memory and 25 μsec bit^{-1} resolution. The temperature was controlled to within ± 0.5 °C by placing the experimental chamber on an aluminum plate containing 4 Peltier elements. A small thermistor, positioned approximately 5 mm from the recording site, continuously monitored bath temperature. The bathing solution contained NaCl, 195 m*M*; KCl, 5.5 m*M*; $CaCl_2$, 13.5 m*M*; $MgCl_2$, 3.0 m*M* and *Tris*-maleate buffer, 10 m*M*; pH 7.5.

Results. The Figure (A, B) shows the average response obtained with an extracellular microelectrode from an excitatory neuromuscular synapse at 9 °C and stimulus frequencies of 1 and 10 sec^{-1} in normal crayfish Ringer. The earliest response is a small, < 40 μV, monophasic positive (upward) potential which is all-or-none in individual records. This response results from the extracellular potential field produced by current flow across the nerve terminal membrane. Its shape indicates the site of recording to be in close proximity to the ultimate terminal of an excitatory axon branch[15–18].

Following the nerve terminal potential is the large, negative postsynaptic potential resulting from inward current through the postsynaptic membrane in response to transmitter action. However, comparison of the averaged high gain voltage traces (Figure B) reveals that at a stimulus frequency of 10 sec^{-1} (lower trace) the rising phase of a small positive potential slightly preceeds the EJP while at 1 sec^{-1} (upper trace) no significant voltage fluctuation occurs. The amplitude of this small positive potential is proportional to the negative synaptic potential recorded at various stimulus frequencies and probably results from outward synaptic current of neighboring junctions[12].

The Figure shows that with an increase in stimulus frequency from 1 to 10 sec^{-1} there is an ~ 2.5-fold increase in the extracellular EJP and essentially no change in the amplitude or duration (Figure, B) of the potential recorded from the presynaptic nerve terminal.

Discussion. The present experiments, performed at the excitatory neuromuscular junction of the crayfish *Orconectes virilis*, have utilized reduced temperature and signal averaging to differentiate the small presynaptic nerve potential from the large postsynaptic response resulting from released transmitter. It is concluded that at this synapse, facilitation in response to repetitive stimulation is not accompanied by any change in amplitude or duration of the extracellularly recorded nerve terminal potential. If such changes occur they are below the resolution of present recording techniques.

Zusammenfassung. Nachweis, dass die Verbindung in neuromuskulären Synapsen des Flusskrebses *Orconectes virilis* ohne Änderungen der Amplitude oder Dauer des extrazellulär registrierten Nervenendpotentials abläuft. Durch Abkühlen des Präparates auf 9 °C gelang eine zeitliche Trennung der prä- und postsynaptischen elektrischen Aktivitäten.

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Degeneration Secretion from the Parotid Gland of the Dog

During degeneration of efferent nerve fibres there is a stage when the nerves seem to be unable to retain in a normal way the transmitter still produced. At this stage the amount of transmitter leaking from the degenerating nerves exceeds the normal leakage from intact nerves and as a consequence the denervated effector organ is activated temporarily. This phenomenon was first described in the parotid of the cat and has later been observed in many other organs (EMMELIN[1]). Recently, secretion induced by different means was studied in parotid glands of dogs before and at various intervals after division of the postganglionic cholinergic nerves to the gland[2]. The secretory responses to eserine, locally administered, were found to be elevated above the preoperative values on the second postoperative day, indicating an increased leakage of acetylcholine at that time, but no degeneration secretion could be observed in the absence of eserine. Degeneration secretion has so far mainly been demonstrated in chloralose anaesthesia and it might be that the phenomenon did not appear in the experiments described above, due to the atropine-like effect of the barbiturate used as an anesthetic. Therefore, the present study was undertaken, where the parotid secretion was studied in dogs under chloralose anaesthesia after division of the postganglionic cholinergic nerves to the gland.

Methods. Five mongrel dogs were used. The auriculotemporal nerve and the secretory fibres on the internal maxillary artery[3] were divided bilaterally in 4 of the dogs, unilaterally in the 5th. In order to cause denervation supersensitivity, 1 parotid was preganglionically denervated by section of the tympanic nerve 7 days before the postganglionic denervation. Ether anaesthesia was used in these operations. When 42 to 50 h had elapsed after section of the postganglionic nerves, the animals were anaesthetized with chloralose (100 mg/kg i.v.) after induction with ether and a tracheal cannula was introduced. Supplementary doses of the anaesthetic were given when necessary. Both parotid ducts were cannulated with glass cannulae. The drops of saliva, falling from these cannulae, were recorded with manually operated electromagnetic pens on a smoked drum. Methacholine, 1 or 5 μg/kg; acetylcholine, 5 μg/kg; hexamethonium bromide, 20

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