Criteria for the appearance of post-tetanic potentiation of transmission at central synapses in *Helix aspersa*

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Summary. Long-lasting potentiation of inhibitory post-synaptic potentials occurs at 2 identifiable synapses in *Helix* brain. It appears only after tetanic stimulation and after a minimum of 20 impulses. Its amplitude and duration depend on the number of stimuli.

A long-lasting increase in the size of evoked potentials has been shown to take place in a number of preparations when repetitive afferent stimulation is applied at tetanic frequency. These include the vertebrate sympathetic ganglion[1], the neuromuscular junction[2,3] and the spinal cord[4]. Post-tetanic potentiation (PTP) is characterized by its long persistence[5], and recent evidence suggests that this is due to a long-term increase in stores of transmitter available for release at the synapse[6]. At a molluscan neuronal synapse a phase of heightened transmitter release has been described following a train of priming stimuli[7] which differs from short-term facilitation of potentials which is also observed in its duration and degree and its sensitivity to drugs[8]. In this paper we demonstrate potentiation of inhibitory post synaptic potentials in *Helix* brain following tetanic stimulation. This phenomenon is clearly separable in its criteria for appearance from non-tetanic facilitation.

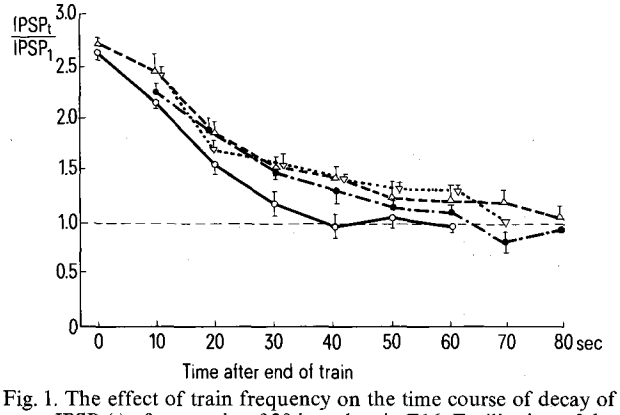

Fig. 1. The effect of train frequency on the time course of decay of a test IPSP (t) after a train of 20 impulses in E16. Facilitation of the exploratory response was plotted as the ratio of its amplitude to that of the initial amplitude at the start of the train (IPSP$_t$/IPSP$_1$) against the time after the end of the train of 20 responses. Different curves show different frequencies of stimulation. Circles 0.5 pulses/sec; triangles 2.0 pulses/sec; filled circles 8.0 pulses/sec; inverted triangles 20.0 pulses/sec.

Central ganglia from *Helix aspersa* were mounted on gel resin and perfused at room temperature with a physiological medium containing 80 mM NaCl, 4 mM KCl, 7 mM $CaCl_2$, 5 mM $MgCl_2$, 5 mM Tris-HCl buffer (pH 7.8–8.0) in Minimal Essential Medium Eagle supplemented with antibiotics. Intracellular recordings were made with glass microelectrodes filled with M potassium acetate (tip resistance 5–35 MΩ) and relayed via a cathode follower to a Wheatstone bridge circuit for measurement. Unitary postsynaptic potentials were generated in cells E16 and E17 on the dorsal surface of the visceral ganglion[9,10] by stimulation of the anal nerve (1 msec pulses applied from a Grass S44 stimulator) and their amplitude was measured from a permanent record on a Watanabe WTR 211 chart recorder. A regular repetition of the stimulus program was necessary to maintain a constant amplitude of unfacilitated potentials. Facilitation of all potentials during and after trains of priming stimuli has been measured as the ratio of amplitude at time t against the initial amplitude (IPSP$_t$/IPSP$_1$). Responses in cells E16 and E17 had an inhibitory component which in both cells underwent a very similar change in amplitude on repetitive stimulation at 1 pulse/sec, which increased in amplitude tending towards a maximum after a few responses. Thereafter, on discontinuation of stimulation, the response declined to its original value after approximately 60 sec. The unitary, monosynaptic nature of these inhibitory potentials and their behaviour on low-frequency stimulation have been fully described elsewhere[9].

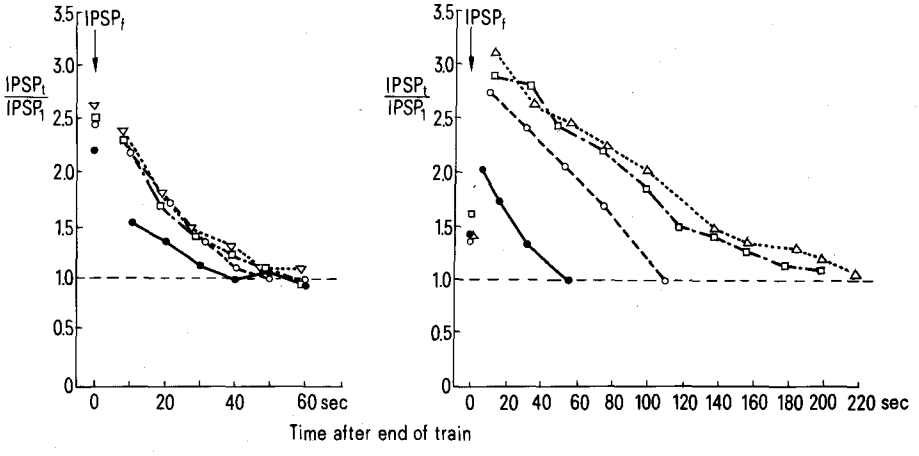

Fig. 2. The effect of train length upon the time course of decay of facilitation after stimulation at non-tetanic (left-hand graph) and tetanic (right-hand graph) frequencies. Graphs show results from a single experiment in cell E17. Filled circles 10 pulses; open circles 20 pulses, squares 40 pulses, inverted triangles 50 pulses, upright triangles 60 pulses. Facilitation plotted as ratio of size of response to exploratory test stimuli (t) evoked at intervals after the end of the train to the size of the response to the first stimulus in the train. Frequencies used: 1 pulse/sec for left-hand graph; 8 pulses/sec for right-hand graph.

Figure 1 shows a plot of the decay of IPSP amplitude following presentation of trains of 20 stimuli at different frequencies. The amplitude of test responses had an average period of decay of 60 sec to the baseline value, and the variation of the period was not considerable over the range 0.5–2.0 pulses/sec (40–70 sec). In figure 2, evidence is presented that the length of the train is important in governing the decay time of the response at tetanic frequency. The graph on the left shows the decay time course of the amplitude of responses after trains of different length presented at non-tetanic frequency. Points at time zero show the facilitated amplitude of the response at the end of the train. Full facilitation of 2.5 times the original response amplitude is partially developed after 10 pulses and more or less fully developed after 20–50 pulses. There is little variation in the timecourse of the decay process over this range of train lengths. The amplitude of potentials is back to normal after 40–60 sec, as shown by test responses evoked at 10 sec intervals after the train ends. There is no further increase in response amplitude during the period after the train. In the right-hand graph, the same range of train lengths was presented at tetanic frequency. Final responses in the train are much smaller due to tetanic fusion of the potentials. After 10 pulses, the amplitude and decay timecourse are those of simple facilitation. After 20 pulses, the timecourse is doubled and amplitude increased. After 40 and 60 pulses, a maximum potentiation and decay timecourse of 4 min are achieved. These are greater than those of facilitation and depend on tetanic stimulation for their appearance. In the 2 synapses described, both facilitation and PTP can be seen. In other cells, however, where little or no facilitation could be seen on sub-tetanic stimulation, potentiation of post-synaptic potentials could still be generated at higher frequency.

This long-lasting variety of plasticity of synaptic transmission resembles the post-tetanic potentiation observed widely in animal preparations[11]. In *Helix*, it does not appear after stimulation below the tetanic range, it also depends critically on the length of the priming train. On high-frequency stimulation of the synapse, the magnitude and duration of the potentiation developed as the train length was increased. This supports the view that PTP arises from a cumulative factor causing its increase with successive pulses[12].

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Extracellular fluid distribution in salt-hypertensive monkeys

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Summary. In salt-hypertensive monkeys, a decrease in plasma volume was found, inversely related to blood pressure level and not accompanied by a corresponding change in interstitial fluid volume.

Most cases of essential hypertension were found to be associated with a decrease in plasma volume (PV), inversely related to arterial blood pressure (BP) level and not accompanied by a concomitant decrease of interstitial fluid volume (IFV). This was evidenced by the decreased PV/IFV ratio, indicating that the distribution of extracellular fluid volume (ECFV) between the extra- and intravascular compartment is altered[1].

Such a situation was not observed in animal experiments, however. In rats with established 1-kidney renal hypertension, reverse changes were found, the PV being expanded and PV/IFV ratio increased[2]. Thus the only common feature with essential hypertension was an abnormality in ECFV partition.

The differences in PV and PV/IFV ratio may be due to different mechanisms involved in the pathogenesis of renal and essential hypertension, including species differences. We therefore measured the changes of PV and IFV in relation to BP levels in monkeys exposed to a chronically increased salt intake. This regime was found to cause hypertension in baboons[3], and a high salt intake was suggested to play a role in the pathogenesis of essential hypertension in man[4].

Material and methods. Measurements were carried out in monkeys (*Maccacus rhesus*) of both sexes aged 5–7 years, of 7 kg mean b.wt, exposed to a high-salt regime (about 1.5 g NaCl/kg b.wt/24 h[3]) for a period of 3 years. Animals of comparable age and weight, receiving about 0.06 g NaCl/kg b.wt/24 h, served as controls. All measurements were performed under pentobarbital anaesthesia (30 mg/kg).

PV was estimated by dilution method, using Evans blue (1% solution w/v, 0.8 g/kg b.wt) injected into the exposed femoral vein. Plasma dye concentration was measured after a 10-min equilibration period against standard samples prepared in appropriately diluted blood plasma of the same

Blood pressure and extracellular fluid compartments in control and experimental monkeys

		Controls (n = 7)	Experimental (n = 5)
BP	S	133.6 ± 4.18	158.0 ± 5.83*
	D	82.9 ± 1.84	106.0 ± 8.12*
	M	116.6 ± 3.13	140.0 ± 6.50*
PV		54.2 ± 2.54	42.7 ± 4.22**
Hct		37.1 ± 1.63	42.4 ± 1.60
ECFV		176.3 ± 5.69	146.4 ± 11.54**
IFV		122.1 ± 5.59	103.7 ± 9.89
PV/IFV		0.451 ± 0.0314	0.419 ± 0.0632

BP, blood pressure in mm Hg: S, systolic, D, diastolic, M, mean; PV, ECFV, IFV, volumes of blood plasma, extracellular and interstitial fluid respectively (ml/kg b.wt); Hct, haematocrit. Means ± SEM. n, number of values. *$p < 0.01$; **$p < 0.05$.