

ASSOCIATIVE INDUCTION OF SHORT-TERM POTENTIATION IN
RAT HIPPOCAMPUS

By

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ABSTRACT

Tetanic stimulation of excitatory afferent pathways in the hippocampus can lead to long-term potentiation (LTP) of the postsynaptic response. Simultaneous tetanization of two separate but convergent afferent pathways can result in associative LTP, which exhibits a magnitude that is greater than the sum of the individual potentiations. Short-term potentiation (STP) of synaptic transmission also occurs in the tetanized pathway, and is a well known presynaptic phenomenon at other neuronal junctions. Studies were conducted to examine if STP in the hippocampus could be induced associatively and to determine the nature of pre- and postsynaptic interactions leading to this associative potentiation.

Experiments were conducted on transversely sectioned rat hippocampal slices maintained in-vitro. Population responses were recorded from the CA_{1b} pyramidal cells and were evoked using stimulating electrodes placed in stratum radiatum, stratum oriens and the alveus. Population excitatory postsynaptic potentials (EPSP) were recorded with extracellular recording electrodes placed at the apical dendrites of the CA₁ cells. Individual cellular EPSPs were recorded from the CA₁ cell somata. To assess the influence of tetanic conditioning on an untetanized afferent pathway, stimulating electrodes were placed in different pathways to deliver tetanic conditioning stimulation to the CA₁ cells. Another stimulating electrode was placed in a separate test pathway, which also converged on the same population of CA₁ cells. The test pathway either remained unstimulated or was stimulated once in conjunction with each tetanic conditioning train of stimulation.

Conditioning tetani delivered through strata oriens or radiatum induced associative STP of the test response when paired with a single test stimulation, but caused depression of the response when unpaired. Antidromic tetanization of CA₁ cells at the alveus or a depolarization of these cells by intracellular current injections produced the same pattern of potentiation with the paired/unpaired paradigm. By delivering a number of these paired test-plus-conditioning trains in rapid succession, the magnitude of the STP increased in a graded manner, and the size and time course resembled those of STP found at other junctions. At the maximum of ten pairings used in these studies, the evoked associative STP was succeeded by LTP.

Presynaptic excitability changes were assessed by monitoring the amount of current needed to fire an antidromic action potential from the Schaffer collateral terminals. These are the afferent terminals that form en passant synapses with the apical dendrites of the CA₁ cells. Pairing a single stimulation of these terminals with a conditioning tetanus of other afferents resulted in STP of the test EPSP, as well as a parallel decrease in the test afferent terminal excitability. These changes are in accord with a presynaptic mechanism of STP found in the spinal cord and neuromuscular junction.

The temporal overlap between the single test afferent volley and the conditioning tetanus was found to be a determinant both of the magnitude and the probability of STP induction. The single test volley could precede the conditioning tetanus by up to 50 msec or follow the tetanus by up to 80 msec and still induce a degree of STP. However, the greatest amount of STP was produced by simultaneous test and conditioning stimulations. These

lenient temporal limits suggest an altered excitability state due to pre- and postsynaptic interactions.

Taken together, the evidence indicates a postsynaptic initiating site for associative STP and LTP in the hippocampus. The initial postsynaptic depolarization appeared to interact with an afferent volley to alter presynaptic terminal excitability. It is proposed that a subliminal presynaptic release process follows an action potential in the terminal. This subliminal process may be facilitated by associative interactions between the postsynaptic depolarization and an action potential in the presynaptic terminal through an altered presynaptic terminal excitability. The associative interactions could lead to enhanced transmitter release by subsequent afferent volleys. The nature of this subliminal process is unknown, but several hypotheses were discussed. It was concluded that associative potentiation has a presynaptic locus of maintenance, and that STP and LTP in the hippocampus may be simply different multiples of the same unit potentiation event. However, the results do not rule out a possible additional postsynaptic locus for the maintenance of STP and LTP.

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1 INTRODUCTION

The mammalian hippocampus is under intensive study as the possible cortical structure subserving learning and memory (Goddard, 1980; McNaughton, 1983). The most promising phenomenon observed is long-term potentiation (LTP) (Bliss and Gardner-Medwin, 1973; Bliss and Lømo, 1973). Long-term potentiation is induced by the tetanic stimulation of an afferent pathway, leading to an enhanced post-tetanic response of the postsynaptic cell to afferent stimulation (Bliss and Gardner-Medwin, 1971). Since the initiating event is a short tetanus of several hundred milliseconds, and the ensuing potentiation can last for hours to days, (Bliss and Gardner-Medwin, 1973), LTP appears to share certain key properties with learning and memory and could be the physiological substrate for both.

A number of hypotheses have been advanced to explain the mechanism behind LTP (Baudry and Lynch, 1980; Collingridge, 1985; Malenka et al., 1986; Skrede and Mølne-Sørensen, 1981; Van Harreveld and Fifkova, 1975; Wigström and Gustafsson, 1985b), but its primary locus has yet to be determined. The associative nature of LTP was described by McNaughton et al. (1978), who showed that LTP can be produced in vitro by the simultaneous activation of separate converging afferent pathways; the potentiation thus produced was greater than the sum of that produced separately by each afferent pathway. Tetanic stimulation of an afferent pathway to field CA₁ produces LTP in this area and increases the excitability of other non-tetanized afferents here (Goh and Sastry, 1985). The mechanism of these interactions is unclear. It appears that a transmitter substance, potassium

released during the tetanus, or direct participation of the CA₁ neuron, is needed. This presynaptic interaction with the postsynaptic elements may play a role in the associative induction of LTP.

Short-term potentiation (STP) is a post-tetanic potentiation of the postsynaptic response lasting up to a few minutes (Magleby and Zengel, 1975a, 1975b). It has been observed at all excitable junctions examined, including those in the hippocampus (Feng, 1941b; Lloyd, 1949; Gloor et al., 1964). At peripheral nerve junctions, STP has been shown to be a presynaptic event mediated by a post-tetanic increase in evoked transmitter release (del Castillo and Katz, 1954d). The same mechanism is thought to mediate STP in the hippocampus (McNaughton, 1982). Because STP commonly precedes or accompanies LTP (McNaughton, 1982; Barrionuevo and Brown, 1983) and it was shown that presynaptic terminals interact with each other (Goh and Sastry, 1985), it is possible that STP could also be induced by associative interactions. The participation of the postsynaptic cell for the induction of a reportedly presynaptic phenomenon, namely STP, is certainly worthy of investigation.

Experiments were conducted in rat hippocampal slices in vitro. Studies were designed to examine whether STP of CA₁ neuronal responses could be induced through associative interactions between afferent inputs. If STP could be thus induced, then the temporal relationship between the tetanic conditioning and afferent test stimulations would be determined. Evidence in the literature indicates that tetanic stimulation of the conditioning input causes an extracellular negative wave near the synaptic terminations of a separate afferent test pathway (Wigström and Gustafsson, 1985b). This

wave may be interpreted as a dendritic depolarization, which could be involved in the associative induction of LTP. Therefore, direct depolarization of the postsynaptic cell could result in a condition that favours induction of associative LTP. This possibility was examined with depolarizing currents injected into the postsynaptic cell to mimic the effects of the conditioning tetanus.

Short-term potentiation at the neuromuscular junction and spinal cord is known to be accompanied by hyperpolarization and decreased excitability of the presynaptic terminal (Eccles and Krnjević, 1959a, 1959b; Hubbard and Willis, 1962). Intracellular recordings of hippocampal presynaptic terminals would demonstrate directly that presynaptic changes occur during the associative induction of STP. However, since the impalement of the fine boutons is not yet possible, the excitability of presynaptic terminals was indirectly assessed using a method that Wall employed in 1958 to measure primary afferent terminal changes in the spinal cord. In Wall's (1958) terminology, an increase in presynaptic excitability indicates a depolarization of the terminal membrane, and a decrease indicates a hyperpolarization.

2 REVIEW OF THE LITERATURE

The nervous system's capacity for facilitation of transmitter release is of great interest to neurobiologists. In general, activation of a neuronal input will affect in some way the postsynaptic response to a subsequent stimulation of that input. Depending on the nature of the stimuli and the excitable tissue involved, the enhanced synaptic response may range from a fleeting to a profound potentiation. The synaptic enhancements due to high frequency repetitive stimulation (tetanus or tetanic stimulation) are especially interesting. The term "post-tetanic potentiation" (PTP) was first coined to describe all synaptic enhancements due to a tetanus (Feng, 1941b; Grumbach and Wilber, 1940). Eccles (1953) defined PTP as an increased post-synaptic discharge elicited homosynaptically and due to increased presynaptic action. With the discovery of long-term potentiation (LTP) (Bliss and Gardner-Medwin, 1971), which has a time course of tens of minutes to days (Bliss and Gardner-Medwin, 1973; Bliss and Lømo, 1973), PTP has come to mean a sub-component of a post-tetanic short-term potentiation (STP) that lasts for no more than several minutes (Magleby and Zengel, 1982).

2.1 Short-term potentiation

Short-term potentiation has been found in all excitable systems studied to date and is considered a general phenomenon of synaptic plasticity (Eccles and Krnjevic, 1959a, 1959b; Feng, 1941a, 1941b; Hubbard and Schmidt, 1963; Lloyd, 1949; Martin and Pilar, 1964; Walters and Byrne, 1984; Zengel et al., 1980). There is evidence in the current literature to support three sub-components to post-tetanic STP: i) facilitation ii) augmentation iii)

post-tetanic potentiation. Their most salient differences lie in their respective time courses and time constants of decay (Magleby and Zengel, 1982).

Early work on facilitation and decurarization used nerve-muscle preparations from amphibians and mammals (Eccles et al., 1941; Feng, 1937, 1941a, 1941b). Spinal cord preparations were also favoured and many crucial findings about STP were made here (Gasser and Grundfest, 1936; Lloyd, 1949; Wall and Johnson, 1958). In the isolated heart, a phenomenon that closely resembles PTP was studied (Hadju and Szent-Gyorgyi, 1952). Evidence from the visual (Geldard, 1931; Granit, 1955; Hughes et al., 1956), auditory (Hughes, 1954; Hughes and Rosenblith, 1957) and olfactory (MacLean et al., 1957) pathways showed that a form of PTP is operational in these systems. Paired-pulse facilitation was first described at the neuromuscular junction (NMJ) of the frog (Eccles et al., 1941; Feng, 1941a, 1941b; Schaefer and Haass, 1939); a variant of paired-pulse facilitation is frequency facilitation, which some workers assert is wholly different (Creager et al., 1980). A relatively newly recognized form of STP is augmentation (Magleby and Zengel, 1976a), which has been neither fully elucidated nor accepted by workers in this field.

Another new form of post-tetanic potentiation is LTP, which was first observed in the rat hippocampus in 1971 (Bliss and Gardner-Medwin, 1971). It has also been found at the crustacean NMJ (Sherman and Atwood, 1971), marine mollusc sensory neuron (Castellucci and Kandel, 1976; Castellucci et al., 1970) and mammalian sympathetic ganglia (Briggs et al., 1983, 1985), but has not yet been demonstrated at the mammalian NMJ. Regarding the distinguishing characteristics, there is no confusion of PTP and LTP because

the latter has a decidedly long time course of 30 minutes to many weeks (Bliss and Gardner-Medwin, 1973; Bliss and Lømo, 1973).

2.1.1 Facilitation

Facilitation is a two component enhancement of transmitter release (Mallart and Martin, 1967). It is present immediately upon the onset of a tetanus and is evident for up to 600 msec into a tetanus (Magleby, 1973a, 1973b; Mallart and Martin, 1967). Indeed, tetanic stimulation is not essential for facilitation; a single antecedent impulse can facilitate the response to a stimulus, provided the intervening interval is not more than 50-100 msec. (Charlton and Bittner, 1978b; Creager et al., 1980; Larrabee and Bronk, 1947). This is called twin-pulse or paired-pulse facilitation and appears to be the elementary event behind frequency facilitation (Magleby and Zengel, 1975b; Mallart and Martin, 1967). It was determined that each impulse of a tetanic train of stimulation adds a linear component to the base rate of transmitter release and to the facilitated response; the magnitude of the aggregate potentiation ranged from 50% for frequency facilitation to about 100% for paired-pulse facilitation (Magleby, 1973a, 1973b; Magleby and Zengel, 1975a; Mallart and Martin, 1967). Assuming that the magnitude and time course of each impulse were the same, Mallart and Martin (1967) were able to describe facilitation in the frog NMJ with two decay constants of 35 msec and 250 msec. This two component characteristic of facilitation has been found in rabbit sympathetic ganglia (Zengel et al., 1980) and rat hippocampus (Creager et al., 1980).

Katz and Miledi (1968) showed the calcium (Ca^{++}) dependence of both paired-pulse facilitation and frequency facilitation and used these pheno-

mena to support their residual Ca^{++} theory of facilitated transmitter release. Although both facilitation and PTP have been attributed to residual Ca^{++} , it is not firmly established that both share a common active pool of Ca^{++} (Landau et al., 1973). For example, in the squid giant axon, facilitation may depend on the presence of a Ca^{++} current rather than residual Ca^{++} (Charlton and Bittner, 1978a). Furthermore, facilitation -- especially paired-pulse -- can be evoked independently of PTP. The degree of facilitation and its time course remained the same even if facilitation was induced during the maximal phase of PTP (Creager et al., 1980; Magleby, 1973a).

2.1.2 Augmentation

Augmentation is differentiated from facilitation and PTP on the basis of its time course and decay constant (Magleby and Zengel, 1976b). Most of the work on augmentation has been done by Magleby and Zengel on the frog NMJ blocked with high extracellular magnesium (Mg^{++}). In 1975(a), they proposed this intermediate phase of synaptic enhancement on the strength of a decay constant of 7 seconds: longer than facilitation and shorter than PTP. Increasing the number of impulses in the tetanus added linearly to the size of the augmentation without changing the time constant to any significant extent (Magleby and Zengel, 1976a). A multiplicative effect of augmentation on facilitation and potentiation was proposed, as was a common increase in quantal content, m , to account for the enhancement (Magleby and Zengel, 1982; Zengel and Magleby, 1982). Magleby and Zengel have suggested in their various studies that augmentation was present in the results of Larrabee and Bronk (1947), Liley (1956) and Landau et al. (1973). Notwith-

standing the common dependence on Ca^{++} , different divalent cations have differential effects on the stimulus-induced increases in transmitter release (Zengel and Magleby, 1977, 1980). Corroborating evidence for the existence of augmentation is still limited and, therefore, the concept is not yet generally accepted.

2.1.3 Post-tetanic potentiation

Post-tetanic potentiation can range from 3 minutes and up to 500% of control in the frog NMJ (Magleby and Zengel, 1975a) to 10 minutes and over 700% of control in the avian ciliary ganglion (Mallart and Martin, 1967). There had been early hopes that PTP would prove to be the elusive link between physiology and psychology as the substrate for such dynamic processes as learning and memory (Hughes, 1958). Since then, the focus for such a substrate has shifted to LTP (McNaughton et al., 1978; Levy and Steward, 1979). Much of the work on STP has actually been directed at PTP; therefore, the following sections on the various aspects of STP by necessity deal largely with PTP.

2.2 Locus of short-term potentiation

In 1858, Schiff reported on the post-tetanic potentiation of twitch tension in the frog gastrocnemius-sciatic preparation. Boehm (1894) observed a temporary decurarizing effect of tetanic stimulation in the heavily curarized sciatic-gastrocnemius preparation. In 1912, Forbes reported the facilitating effect of a tetanus to one nerve on the post-tetanic response of another adjacent nerve. Feng et al. (1938) described the post-tetanic repetitive discharge of the frog neuromuscular junction; however, the facilitating effect co-occurs with a prevailing inhibition (depression). Other

workers found that a few tetani of short duration induced a neuromuscular block, while additional tetani could remove this blockade (Brown and von Euler, 1938). There was much debate on the locus of this PTP; some workers maintained that it was a phenomenon of the muscle contractile elements (Walker, 1947), since direct stimulation of curarized muscle appeared to produce PTP. However, other workers found that direct stimulation did not produce PTP (Guttman et al., 1937), and concluded that PTP was an end-plate event at the NMJ.

Meanwhile, in the isolated feline dorsal root, Gasser and Grundfest (1936) made detailed observations of post-tetanic changes which correspond to the same changes seen in frog nerve (Gasser and Graham, 1932). They described two phases of post-tetanic hyperpolarization, a short first phase of several msec duration followed by a depression, and a prolonged second phase of hyperpolarization whose amplitude and duration were dependent upon the preceding tetanus: "After a maximal tetanus of 30 sec, the potential is + 0.6 to 0.7 mV, and the duration more than four minutes." (Gasser and Grundfest, 1936). Moreover, the associated post-tetanic spike potential (presynaptic spike) was also greatly enhanced. Larrabee and Bronk (1938) reported what they called "prolonged facilitation" in the cat stellate ganglion. Potentiation was observed with preganglionic tetanus, but not with antidromic postganglionic tetanus, leading the authors to conclude that "prolonged facilitation" must occur preganglionically.

Woolsey and Larrabee (1940) tetanized the feline dorsal root and were able to show the same two phases of hyperpolarization, confirming that increases in the frequency and duration of the tetanus lead to increases in

both amplitude and duration of the second hyperpolarization. A concomitant facilitation of the evoked ipsilateral ventral root discharge lasted for the duration of the second prolonged hyperpolarization. This facilitated discharge could not be induced following antidromic tetani to the ventral root. In 1947, Larrabee and Bronk again examined this prolonged facilitation and were convinced that a preganglionic site was the locus of change. By recording from single ganglion cells as well as nerve trunks, it was found that the largest post-tetanic ganglionic response was the result of more ganglion cells responding (Larrabee and Bronk, 1947). Experiments with converging nerve trunks showed that tetanization of one preganglionic input actually results in decreased excitability of the postganglionic cell. This same decrease in excitability extended to responses to exogenous acetylcholine and lasted for about 1 minute. Other workers have since disputed the decreased excitability as a necessary result of a tetanus (Charlton and Bittner, 1978b; Martin and Pilar, 1964). In addition, Larrabee and Bronk (1947) described paired-pulse facilitation without naming it: a single preganglionic volley was sufficient to potentiate the response to a succeeding volley, much as Feng (1940) had shown in the NMJ of the toad.

Lloyd (1949) further advanced the causal relationship between the post-tetanic positive afterpotential (hyperpolarization) and PTP. He noted the parallels in the amplitude and time course between the post-tetanic afferent impulse, the period of hyperpolarization and the potentiated monosynaptic reflex. He proposed a presynaptic basis for PTP whereby a post-tetanic hyperpolarization results in a larger presynaptic spike that in turn leads to greater transmitter release. Eccles and Rall (1951) argued that a

tetanus of 30-300 volleys is followed by a brief post-tetanic potentiation of the synaptic potential, which occurs within 200 msec post-tetanus at a time when the presynaptic spike is actually smaller than the pre-tetanus control.

Wall and Johnson (1958) examined Lloyd's results and hypothesis more directly by testing the excitability changes in afferent fibers of a monosynaptic reflex loop (Wall, 1958). They found a marked decrease in the excitability of the afferent fiber terminal arborizations coincident with the magnitude and duration of PTP. Like Eccles and Rall (1951) and Lloyd (1949), Wall and Johnson found a discrepancy in the immediate post-tetanic period: there was a delay before the potentiated reflex maximized, whereas the decrease in afferent terminal excitability maximized almost immediately. Given that the presynaptic terminals were indeed hyperpolarized, they suggested that the intensity of this hyperpolarization immediately post-tetanus is sufficient to produce anodal block in some branches of the afferent fibers. Alternately, the hyperpolarization can desynchronize the afferent impulses (del Castillo and Katz, 1954d) such that the height of the presynaptic spike thus generated will be less than that of the control. Subsequent studies in spinal cord (Eccles and Krnjević, 1959a, 1959b), squid giant axon (Takeuchi and Takeuchi, 1962) and mammalian NMJ (Hubbard and Schmidt, 1963) supported these findings. Furthermore, Hubbard and Schmidt (1963) showed that small increases in presynaptic spike size are capable of inducing PTP by virtue of a logarithmic relationship between the spike height and end-plate potential (epp) amplitude. On the other hand, intracellular recordings at the nerve terminal of avian ciliary ganglia showed

neither changes in presynaptic membrane potential nor increased amplitude of the presynaptic spike during paired-pulse facilitation and PTP (Martin and Pilar, 1964). In spite of these conflicting results, the general consensus is that facilitation and PTP are presynaptic events.

2.3 Mechanism of short-term potentiation

The relationship between post-tetanic hyperpolarization and increased postsynaptic response rested upon the elucidation of synaptic transmission. In 1934, Dale and Feldberg first gave evidence that acetylcholine (ACh) was indeed the chemical responsible for synaptic transmission at mammalian NMJ. Continued efforts by various workers lent growing support to the idea of chemical transmission (Eccles, 1948; Eccles et al., 1942; Kuffler, 1948), but the debate between electrical and chemical transmission was not laid to rest until Eccles and MacFarlane's study (1949) of anticholinesterases and endplate potential. The overwhelming evidence supported chemical transmission mediated by ACh.

Del Castillo and Katz advanced their quantal theory of neuromuscular transmission in 1954. Drawing from Fatt and Katz' (1952a, 1952b; 1953) observations of spontaneous miniature end-plate potentials (mepp) at the frog NMJ, del Castillo and Katz observed in the isolated nerve-muscle preparation bathed in high magnesium (Mg^{++}) and/ or low calcium (Ca^{++}), that stimulating the nerve resulted in epps whose minimal size equals the mean amplitude of spontaneous mepps (del Castillo and Katz, 1954b). Most importantly, del Castillo and Katz noted that where the epps were not of the minimal size, their amplitudes could be closely predicted by whole number multiples of the mean mepp. Thus:

$$m \times \text{mepp} = \text{epp}$$

where mepp is the elementary unit of transmitter and quantal content (m) is the number of such units per epp. Quantal content is further defined as the product of the number of quanta available for release, n , and the probability of release, p ; therefore, the mean quantal content is:

$$m = n \times p$$

When the presynaptic terminal is hyperpolarized to or beyond a critical level, there is an increased frequency of spontaneous mepps in the form of bursts, but the quantal unit of these mepps is not increased (del Castillo and Katz, 1954d). During such a period of hyperpolarization, the amplitude of the epps increased, sometimes for a few seconds after the end of the hyperpolarization. In addition, evoked epps showed an increased amplitude that is attributable to increased quantal content. Earlier, Liley and North (1953) had concluded that PTP is a nerve terminal phenomenon that is mediated by an increased evoked release of ACh; increased end-plate sensitivity to the neurotransmitter appeared to have played very little part.

Another observation explained by the quantal mechanism is the post-tetanic depression that occurs after an intense tetanus (Eccles and Rall, 1951; Liley and North, 1953). Under conditions of normal release, when transmitter release is not impaired by agents such as high extracellular concentrations of Mg^{++} , a tetanus of 500 volleys is followed by a depression lasting several hundred msec (Eccles and Rall, 1951). This is succeeded by a period of PTP. However, in high Mg^{++} , which diminishes transmitter release, no depression is observed (del Castillo and Katz, 1954c; Feng, 1941b). Liley and North (1953) suggested a depletion of quanta during the tetanus to account for this depression. It appears that no significant

changes in p occurs during depression while there is an associated decrease in n (del Castillo and Katz, 1954c). Del Castillo and Katz (1954c) also showed that facilitation can be accounted for by an increased quantal content.

To further explain the quantal events during and post-tetanus, it is necessary to invoke the idea of transmitter mobilization. Elmqvist and Quastel's (1965) functional model of transmitter mobilization concurs with the evidence presented by others (Hubbard, 1963; Hubbard and Willis, 1962). In this model, the number of readily available quanta in the nerve terminal is relatively small. During a high intensity tetanus, this pool of transmitter is quickly depleted (Otsuka et al., 1962). A second pool of transmitter, presumably not in the form of packaged quanta, must then be mobilized into the readily available pool. Takeuchi (1958) estimates the time constant to restore n at the post-tetanic NMJ to be 4-5 seconds. This depletion and subsequent restoration of quanta at the terminal thus explains the observation that, although the amplitude and duration of PTP is proportional to the intensity and number of impulses in the tetanus, the latency to peak potentiation actually increases with the intensity of the tetanus (del Castillo and Katz, 1954c; Eccles and Rall, 1951; Feng, 1937; Feng et al., 1939; Gasser and Graham, 1932; Lloyd, 1952, 1959).

2.4 Ionic mechanism of short-term potentiation

Early work on neuromuscular preparations suggested that increased external potassium (K^+) had the same facilitatory and decurarizing effects as tetanic stimulation (Feldberg and Vartiainen, 1934; Wilson and Wright, 1936; Feng and Li, 1941). Other investigators proposed that an increased

extracellular K^+ was responsible for PTP (Rosenblueth and Morison, 1937; Grumbach and Wilber, 1940; Walker, 1948). Feng et al. (1939) stated that raised external K^+ cannot be involved since PTP in the presence of potassium chloride is less than that induced by tetanic stimulation alone. Furthermore, high K^+ leads to depression of the NMJ rather than potentiation (Feng and Li, 1941). Liley and North (1953) proposed another perspective: as it was known that tetanus causes increased release of both ACh and K^+ , perhaps the criterion for potentiation is an apparent decrease of intracellular K^+ , which can be mimicked by raising external K^+ .

The work on potassium proved inconclusive, and with the elucidation of the quantal mechanism of transmitter release, the focus of attention shifted to calcium. It was known that Ca^{++} and Mg^{++} have mutually 'antagonistic' effects at the NMJ: epps can be reduced and nerve transmission blocked by high extracellular Mg^{++} ; this nerve block could be relieved by raising extracellular Ca^{++} (del Castillo and Engbaek, 1954). In the absence of extracellular Ca^{++} , depolarization of the nerve terminal was in itself insufficient to cause transmitter release (Katz and Miledi, 1967). But if the depolarization was coupled with an iontophoretic pulse of Ca^{++} localized to the nerve terminals, then transmitter release was evoked (Katz and Miledi, 1967). Hence, transmitter release or multiplication of release requires Ca^{++} . Studies of Ca^{++} uptake in the squid giant axon using $^{45}Ca^{++}$ showed stimulus-dependent uptake (Hodgkin and Keynes, 1957). Aequorin loaded squid axons emit light upon depolarization, showing a Ca^{++} conductance whose time course is unaffected by tetrodotoxin and tetraethylammonium (Baker et al., 1971; Llinas et al., 1972).

Exactly how calcium effects transmitter release is unknown. It has been proposed that a Ca^{++} activated complex, CaX , somehow increases the probability of transmitter release at the terminal membrane (del Castillo and Katz, 1954a). Assuming that release is proportional to the fourth power of CaX (Dodge and Rahamimoff, 1967), Katz and Miledi (1968) advanced the residual calcium theory of facilitation at the frog NMJ. They proposed that the transient increase in Ca^{++} conductance during the depolarization leads to an influx of Ca^{++} that combines with X to form the active complex CaX . It is interesting to note that elevated levels of intracellular Ca^{++} persist beyond the duration of stimulus-evoked release (Miledi and Parker, 1981). If a subsequent stimulus invades the terminal within a certain interval after the first stimulus, then the residual Ca^{++} in the terminal will be augmented by another influx of Ca^{++} . The transmitter thus released will be proportional to the sum of the intracellular Ca^{++} (or CaX) raised to the fourth power.

Alternate mechanisms for PTP involving sodium (Na^+) currents or its accumulation in the presynaptic terminal are largely unsupported. Post-tetanic potentiation of the epp was inducible when all external Na^+ was replaced by isotonic calcium chloride, and when voltage sensitive Na^+ channels were blocked by tetrodotoxin (Weinreich, 1971). It is possible that Na^+ plays a supportive role, indirectly increasing intracellular calcium ions by competing at a common ion buffering system or for a limited energy source for extrusion (Birks and Cohen, 1968; Rahamimoff et al., 1980). Interestingly, Misler and Hurlbut (1983) were able to induce PTP at the frog NMJ in the absence of extracellular Ca^{++} . Intracellular recordings showed

that PTP of mepp frequency and epp size can be induced with repetitive stimulation in 0 mM Ca^{++} and 1-2 mM EGTA, provided that Ca^{++} is restored to the bathing medium immediately after the tetanus (Misler and Hurlbut, 1983). These authors also suggest a Na^+ dependent mechanism for PTP, but their speculation remains unsubstantiated.

2.5 Anatomy of the hippocampus

The hippocampus is part of the oldest cortical structure in the mammalian brain. During embryonic development, cells from the mantle layer at the rostral end of the neural tube proliferate. This cell mass migrates beyond the marginal layer, eventually surrounding the neural tube to become the cortical grey matter (Crelin, 1974). During proliferation and migration, the isocortex separates from the mantle layer to form the neocortex; the remaining allocortex, which is the more primitive, maintains its attachment to the mantle layer (Filiminoff, 1947). There is also a transitional cortex, called the periallocortex, that differs in cytoarchitecture from both neocortex and allocortex (Brodmann, 1909).

In mammals, the allocortex is found mostly around the brainstem in a cortical convolution that Broca (1878) called the limbic lobe. This true allocortex is subdivided into paleocortex and archicortex. The former comprises the olfactory bulb and associated structures; the latter comprises the subiculum, the hippocampus proper (Ammon's horn), the dentate gyrus (fascia dentata), precommissural hippocampus and supracommissural hippocampus (Schwerdtfeger, 1984). The obvious presence of olfactory pathways led to the name "rhinencephalon", for it was believed that olfaction was the limbic lobe's only function (Kolliker, 1896; Schaefer, 1898). Later work on

the limbic system (MacLean, 1952; Papez, 1937) dispelled the single function role for this old cortex.

The hippocampal formation consists of the periallocortical presubiculum, area retrosphenialis e, the parasubiculum and the entorhinal region (Chronister and White, 1975). Of the remaining archicortex, only Ammon's horn and the fascia dentata will be considered as the hippocampus. This will be further defined so that "hippocampus proper" will denote only Ammon's horn, excluding the subiculum.

The hippocampus is bilaterally symmetrical, shaped like commas or cashew nuts (Green, 1964; Teyler and DiScenna, 1984). They lie directly under the neocortex with their dorsal ends connected by the commissural fiber tract. The body of the hippocampus is pressed against the medial wall of the inferior horn of the lateral ventricle. The ventral tail of the hippocampus follows the lateral ventricle toward the corpus callosum (Figure 1).

Each hippocampus consists of two interdigitating archicortical parts, the cornu ammonis (CA) and the fascia dentata (FD). The ventricular surface of the hippocampus is covered by a white fiber layer, the alveus. These fibers are composed mainly of axons from cells of the CA fields and converge to form the fimbria on the medial surface of the hippocampus (Teyler and DiScenna, 1984). The cells of the hippocampus are in three basic layers: molecular, principle, and polymorph (Lorente de Nó, 1934). This is in contrast to the six (Brodmann, 1909) or seven (Rose, 1926) layers of the neocortex. It is interesting to note that Ramón y Cajal (1893) had actually described Ammon's horn as a seven layered cortical structure.

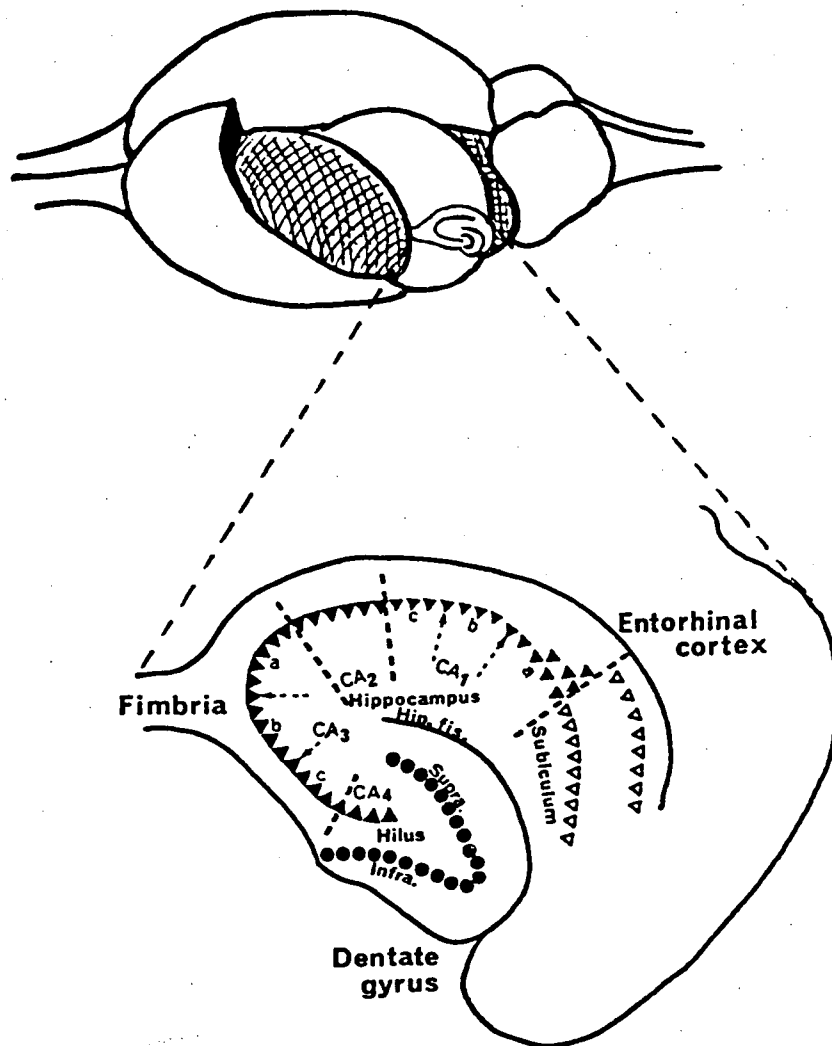


Figure 1

Anatomical location of the hippocampus in the rat brain. The top diagram shows the position of the hippocampus with respect to the rest of the brain and the bottom diagram illustrates the various subfields of a transverse section of the hippocampus.

Hip. fis. - hippocampal fissure

Infra - infrapyramidal blade of the dentate granule cell layer

Supra - suprapyramidal blade of the dentate granule cell layer

2.5.1 The dentate gyrus

The dentate gyrus, as its name suggests, is a V or U-shaped fold of cortex that caps the thin terminal edge of the hippocampus proper. The principle cells are the granule cells (Ramón y Cajal, 1893) found in a layer 5-10 cells deep, the stratum granulosum. These granule cells send dendrites that point towards the dentate hilus as well as dendrites that point to the ventricular surface. The granule cell axons course through the polymorph layer and converge about the hilus. In addition to polymorphic cells in this layer, there are inhibitory basket cells, which synapse with many granule cells through a supragranular axon plexus (Ramón y Cajal, 1893). Between the suprapyramidal and infrapyramidal blades of the FD is the hilus, a transition region of polymorphic cells and modified pyramidal cells (Lorente de Nó, 1934). These pyramidal cells are considered part of the tail of hippocampus proper and constitutes the field CA₄ (Lorente de Nó, 1934). Because the polymorphic layers of both CA and FD are confluent, the whole region demarcated by an imaginary line drawn between the ends of the stratum granulosum has been collectively labelled area dentata (Blackstad, 1956; Teyler and DiScenna, 1984). However, careful examination of the cytomorphology does not support this classification (Ramón y Cajal, 1893; Lorente de Nó, 1934).

2.5.2 The hippocampus proper

The thin edge of the cornu ammonis terminates in the hilus of the fascia dentata. The transitional pyramidal cells from the hilus gradually change to the pyramidal cells of area CA₄ (Lorente de Nó, 1934). Although the CA₄ field is difficult to detect (Blackstad, 1956; Chronister and

White, 1975), the cascade of pyramidal cells from CA₄ to form the stratum pyramidale of the hippocampus proper is easily discernible. These pyramidal cells are oriented with their apical dendrites pointed towards the center of the hippocampus at the blind end of the hippocampal fissure.

The stratification at the cornu ammonis is more involved than that in the dentate gyrus. A layer of axon fibers, the alveus, lies next to the surface at the lateral ventricle. Adjacent to the alveus is a fiber plexus of polymorphic cells, the stratum oriens. The next two layers are the stratum pyramidale and the stratum lucidum, the latter layer is rather poorly defined in the rodent hippocampus and is considered as one with the stratum pyramidale (Lorente de Nó, 1934). The same applies to the dense fiber plexus of the stratum radiatum and the stratum lacunosum, considered collectively as stratum radiatum (Lorente de Nó, 1934). Next to the stratum radiatum is the stratum moleculare (Lorente de Nó, 1934). The boundaries of strata radiatum and oriens mark the terminus of the hippocampus proper at the hilus. The boundary at the subicular end is very sharply defined by the abrupt termination of the stratum pyramidale in field CA_{1a} (Angevine, 1975; Blackstad, 1956; Ramón y Cajal, 1893; Lorente de Nó, 1934). Because of anatomical and physiological difference, the hippocampus proper is divided into several fields and subfields, each denoted by the letters CA and a numerical or alpha-numerical term (Lorente de Nó, 1934).

2.5.3 The fields of Ammon's horn

Figure 1 illustrates the different subfields of Ammon's horn and the dentate gyrus in a transverse section of the rat hippocampus.

2.5.3.1 Field CA₄: As mentioned above, this poorly demarcated zone consists of transitional pyramidal cells in a layer and a confluence of polymorphic cells. The actual presence and appearance of this field is extremely variable across species (Geneser-Jensen, 1972).

2.5.3.2 Field CA₃: This field is subdivided into CA_{3a}, CA_{3b}, and CA_{3c} (Lorente de Nó, 1934). The pyramidal cells of CA₃ (along with those of CA₂) are the giant pyramids of the hippocampus (Lorente de Nó, 1934). The apical dendrites of the CA₃ pyramid penetrate the stratum radiatum without lateral arborization and terminate with 2-3 vertical branches in stratum moleculare (Lorente de Nó, 1934). Thick spines at the proximal portion of these dendrites receive excitatory inputs from the mossy fibers of FD (Lorente de Nó, 1934). Subfield CA_{3c} pyramids have such synapses at both the apical and basal dendrites, whereas CA_{3a} and CA_{3b} pyramids have only apical synapses with mossy fibers (Lorente de Nó, 1934).

The axon of the CA₃ pyramid is a thick fiber that goes to the fimbria, where it gives off a number of collaterals (Ramón y Cajal, 1893; Lorente de Nó, 1934). Most of these collaterals are short, terminating in stratum oriens or interpyramidally. The most notable is a thick collateral discovered by Schaffer in 1892. This characteristic Schaffer collateral penetrates the stratum pyramidale to run in the stratum radiatum and terminates near the junction of CA_{1a} and CA_{1b} (Ramón y Cajal, 1893; Schaffer, 1892). The Schaffer collateral is present in nearly all CA_{3c} axons, about 50% of CA_{3b} and nearly absent in CA_{3a} axons (Lorente de Nó, 1934).

2.5.3.3 Field CA₂: This is a transition field of giant pyramidal cells between CA₃ and CA₁ (Lorente de Nó, 1934). These cells are smal-

ler than those of CA₃ and lack both the dendritic spines and the Schaffer collaterals. These pyramids are arranged in irregular rows which form a very thin stratum pyramidale. Toward subfield CA_{1c}, this thin layer thickens with the introduction of more pyramidal cells.

2.5.3.4 Field CA₁: The CA₁ field is also divided into subfields a, b, and c (Lorente de Nó, 1934). The border between the subfields are not well defined, but the respective pyramidal cells are morphologically different: amongst the CA_{1a} pyramids are subicular cells; CA_{1b} have the smallest pyramidal cells of all CA fields, and CA_{1c} pyramids are relatively large (Lorente de Nó, 1934). The apical dendrites of CA₁ pyramids lack synaptic spines at the proximal portion of the primary shaft (Ramón y Cajal, 1893). In the stratum radiatum, these dendrites arborize extensively into very fine branches, where numerous fine spines form synapses with the Schaffer collaterals (Lorente de Nó, 1934; Ramón y Cajal, 1893). Basal dendrites form short tufts of irregular branches in the stratum oriens (Ramón y Cajal, 1893).

The CA₁ primary axon is a thin fiber that essentially courses through the stratum oriens to the fimbria (Lorente de Nó, 1934). Axon collaterals may cross the stratum pyramidale to ramify in the stratum radiatum. Long recurrent collaterals arise from some CA₁ axons, traversing the stratum oriens to run in the alveus towards the fimbria and the subiculum (Lorente de Nó, 1934). At the border of CA₁ and the subiculum, the densely packed stratum pyramidale abruptly stops; the pyramidal cells disperse and the stratum oriens gradually thins to a single layer in the subiculum (Angevine, 1975; Chronister and White, 1975).

2.5.4 Neuronal pathways of the hippocampus

The orderly lamination of cellular structure in the hippocampus make it an ideal model for studying cortical organization. The various strata of the hippocampus maintain their relative orientation all along the longitudinal axis. In addition, the major intrahippocampal tri-synaptic system is organized in parallel planes that are roughly transverse to the longitudinal axis (Andersen et al., 1971). This lamellar organization has been clearly demonstrated with the in-vitro hippocampal slice (Skrede and Westgaard, 1971) (Figure 2). Although the tri-synaptic system within the hippocampus raises the possibility of fiber recruitment and a cascade effect of signal amplification (Teyler and DiScenna, 1984), there appears to be very little divergence from the lamellar organization throughout the entire hippocampus (Andersen et al., 1971).

2.5.5 Afferents to the hippocampus

2.5.5.1 Perforant Path: The perforant path (PP) carries the major excitatory input to the hippocampus (Lorente de Nó, 1934; Ramón y Cajal, 1893). These fibers originate from the ipsilateral medial and lateral entorhinal cortices (EC), crossing the hippocampal fissure to form synapses at the dendrites of the dentate granule cells, as well as with the dendrites of some CA₃ pyramids (Hjorth-Simonsen, 1973; Hjorth-Simonsen and Jeune, 1972). There is a topographical specificity to the synapses in that inputs from a given part of the EC project only to a limited part of the stratum granulosum, but with equal density along the entire length of the fascia dentata (Lømo, 1971). Another main excitatory input is the commissural fibers from the fimbria (Blackstad, 1956), which originate from the contralateral CA₃

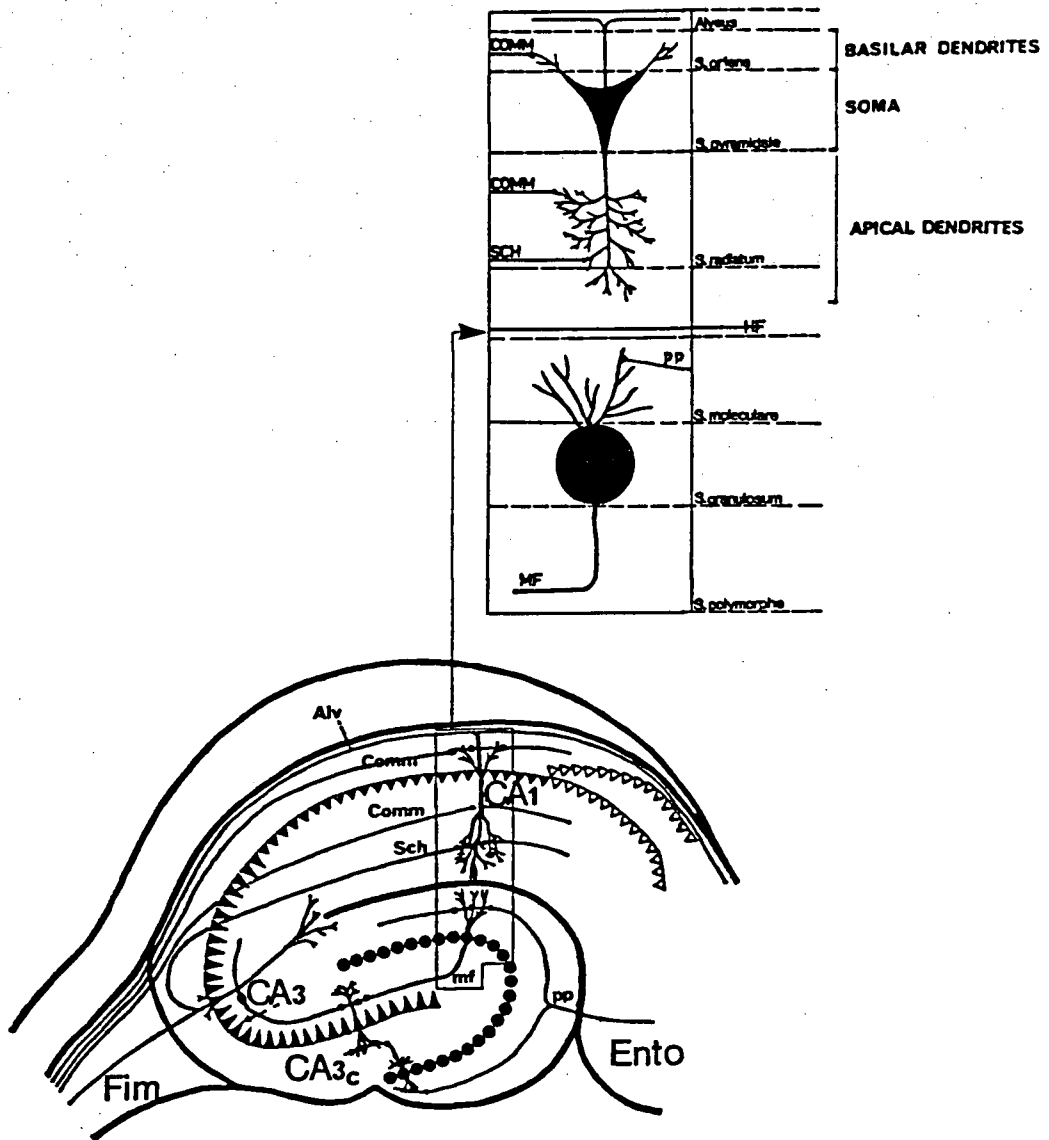


Figure 2

Anatomical diagram of a transversely sectioned rat hippocampal slice showing the various afferent, efferent and intrinsic pathways.

- Alv - alveus
- Comm - commissural input
- Ento - entorhinal cortex
- Fim - fimbria
- HF - hippocampal fissure
- mf - mossy fibers
- pp - perforant path
- Sch - Schaffer collaterals

and CA₄ pyramidal cells (Hjorth-Simonsen and Laurberg, 1977; Swanson et al., 1978).

2.5.5.2 Afferents to the CA₃ region: The axons of the dentate granule cells form the mossy fiber afferents to field CA₃. Their thin (0.2 μ m) fibers make en-passant synapses at the proximal primary shaft of CA₃ dendrites (Blackstad et al., 1970). Mossy fibers from the supra-pyramidal granule cells synapse with apical dendrites of the entire field CA₃, while the mossy fibers from the infrapyramidal granules synapse only with the basal dendrites of subfield CA_{3c} (Lorente de Nó, 1934; Swanson et al., 1978). The synaptic elements at the apical dendrites are noteworthy: mossy fiber boutons, 3-6 μ m in diameter and length, completely engulf the branched spines of the apical dendrite (Hamlyn, 1961). There is also evidence of mossy fiber synapses at the inhibitory basket cells of CA₃ (Frotscher, 1985), which is concrete evidence for feedforward inhibition (Douglas, 1978).

The commissural input to CA₃ originates in the homotopic region of the contralateral hippocampus (Andersen and Lømo, 1966). These fibers run through the fimbria and terminate on the basal dendrites of the CA₃ pyramids (Blackstad, 1956; Andersen and Lømo, 1966). There is some evidence of projections from the ipsilateral and contralateral field CA₄ (Schwerdtfeger and Sarvey, 1983).

2.5.5.3 Afferents to the CA₂ region: The distinction of field CA₂ is not embraced by all workers (Blackstad, 1956; Lorente de Nó, 1934; Teyler and DiScenna, 1984). However, from Lorente de Nó (1934), it is clear that mossy fibers do not make giant synapses with the apical dendritic spines, since the latter are absent from CA₂ pyramids. Local afferents

from CA₁ axon collaterals make synapses at the CA₂ basal dendrites (Lorente de Nó, 1934); commissural projections which have been shown to terminate at CA₁ in both strata radiatum and oriens, can be interpreted to have synapses at CA₂ by virtue of the ambiguous distinction between these two CA fields (Blackstad, 1956).

2.5.5.4 Afferents to the CA₁ region: The major input to CA₁ is from the ipsilateral CA₃ pyramids via the Schaffer collaterals in the stratum radiatum (Andersen, 1960; Andersen et al., 1971; Lorente de Nó, 1934). These Schaffer collaterals make numerous en passant synapses with the finely arborized apical dendrites (Ramón y Cajal, 1893). Stimulation of the ipsilateral CA₃ pyramids or the Schaffer collaterals elicits the greatest response from the CA₁ pyramids (Andersen, 1960; Andersen et al., 1971). Commissural projections come from the contralateral CA₄ and CA₃ pyramids, forming en passant synapses at both basal and apical dendrites (Andersen et al., 1980; Blackstad, 1956). The majority of these synapses are found at the apical dendrites in the stratum radiatum.

Lorente de Nó (1934) described collaterals of CA₁ that ascend from the alveus to terminate on the basal aspects of the CA₁-CA₂ pyramids. Consequently, activation of CA₁ pyramids generates an apparent synaptic potential in adjacent CA₁ pyramids (Andersen, 1975). Autoradiographic evidence neither supports nor rules out the existence of this short collateral (Swanson et al., 1978).

2.5.6 Efferents from the hippocampus

Aside from commissural output to the contralateral hippocampus, the only major efferent from the hippocampus is from CA₁ pyramids to the sub-

culum (Lorente de Nó, 1934; Swanson et al., 1978). There is anatomical evidence of a recurrent collateral system from some parts of CA₃ and all of CA₁ and CA₂ that terminates back in the entorhinal cortex (Hjorth-Simonsen, 1973; Lorente de Nó, 1934; Swanson et al., 1978). However, solid electrophysiological evidence for this recurrent network is lacking.

2.5.7 Interneurons

There are a number of interneuron types in the various strata of the hippocampus, the most prevalent being the basket cells (Lorente de Nó, 1934; Ramón y Cajal, 1893). These basket cells are distributed within the principle cell layers of both CA and FD, in close proximity to the principle cells (Andersen et al., 1964; Lorente de Nó, 1934). An axon of a basket cell arborizes extensively, making 200-500 synapses with primary cells within a dense plexus of fibers (Andersen et al., 1964; Struble et al., 1978). In the FD, the axon terminals synapse at the soma and dendrites of granule cells (Struble et al., 1978), while those at the CA pyramids terminate at the soma, proximal dendrite and initial segment of the axon (Blackstad and Flood, 1963; Kosaka, 1980; Seress and Ribak, 1983).

Electrophysiological evidence suggested that a recurrent inhibitory system was present in the CA field (Spencer and Kandel, 1961). Andersen et al. (1963, 1964) located the site of the inhibition at the soma of the pyramidal cells and proposed a circuit of feedback inhibition through which adjacent principle cells inhibit their neighbours via axon collaterals to the basket cells (Andersen et al., 1963, 1964; Kandel and Spencer, 1961). Immunoreactive staining of interneuron terminals for glutamic acid decarboxylase (GAD) showed that these interneurons elaborate γ -aminobutyric acid

(GABA) (Ribak et al., 1978; Seress and Ribak, 1983). The possibility of feedforward inhibition was suggested by the observation of very low threshold interneurons in the FD (Buzsaki and Eidelberg, 1981, 1982; Douglas et al., 1983). Labelling (Loy, 1978) and degeneration studies (Frotscher and Zimmer, 1983) support direct commissural innervation of basket cells from the contralateral homotropic primary cells. Electromicroscopy also show ipsilateral innervation of CA₃ basket cells by mossy fibers of the FD (Frotscher, 1985).

2.5.8 Longitudinal association pathway

Lorente de N6 (1934) first observed axon collaterals from CA pyramidal cells that run parallel to the long axis of the hippocampus. These collaterals arise from CA₃ pyramids which lack a Schaffer collateral (Lorente de N6, 1934), forming a dense plexus on the apical side of the pyramidal cells. The finely branched dendrites project along the axis to CA_{3a}, CA₂ and CA_{1c} dendrites, thereby linking the relatively isolated laminates of the tri-synaptic system into a powerful inter-lamellar pathway (Hjorth-Simonsen, 1973; Swanson et al., 1978). The physiological significance of this pathway has not been explored.

2.6 Short-term potentiation in the hippocampus

Gloor (1955) observed marked PTP in the feline hippocampus after stimulating the amygdala. Increased spike amplitude of the hippocampal response paralleled the decrease in latency to spike generation. In addition, a decrease in excitatory threshold and several phases of post-tetanic depression were described. Direct tetanic stimulation of afferents to the hippocampal pyramidal cell, presumably through commissural fibers via the fim-

bria, led to PTP of the population EPSP and population spike, and decreased latency (Campbell and Sutin, 1959). Tetanic stimulation of the perforant path resulted in PTP of pyramidal cell population EPSP and population spike (Gloor et al., 1964); these authors attributed the PTP to an increase in transmitter release, a pre-synaptic event. More recent studies of STP in the hippocampus and fascia dentata have revealed several components that are analogous to those found at the neuromuscular junction: facilitation (Creager et al., 1980), augmentation (McNaughton, 1982), and post-tetanic potentiation (Racine and Milgram, 1983). These authors agree that STP has a presynaptic locus and is due to elevated transmitter release (Abraham et al., 1985; McNaughton, 1982). However, there is no general agreement on the physiological significance of STP. Creager et al. (1980) suggested no significance in normal function, perhaps some role in epileptogenesis; others suggest a possible role for STP in short-term memory (Goddard, 1980; Racine and Milgram, 1983). The other point of concurrence is that STP and long-term potentiation are decidedly different processes; that is, STP does not lead, by graded increments or critical threshold, to LTP (McNaughton, 1982; Teyler et al., 1982).

2.7 Associative induction of potentiation: -afferent cooperativity

Long-term potentiation is now the most promising candidate as a substrate for learning and memory. Several characteristics of LTP are qualitatively similar to some salient features of learning and memory. For instance, LTP is input specific, showing potentiation only at the pathways that have been tetanized (homosynaptic potentiation) (Andersen et al., 1977; Lynch et al., 1977), and only a brief event (tetanus) is necessary for a

prolonged effect.

Recently, LTP in the dentate gyrus and the hippocampus have been induced via co-activated afferents (Barrionuevo and Brown, 1983; Lee, 1983; Levy and Steward, 1979; McNaughton et al., 1978). Long-term potentiation can be induced only if a large number of afferent fibers to the target cells are tetanized in synchrony, whereas STP can be produced by tetanic stimulation of only one (McNaughton, 1983). Rather than tetanic stimulation of one afferent pathway, associative induction allows two or more separate but convergent pathways to cooperate.

McNaughton et al., (1978) first showed cooperativity of co-active afferents in the fascia dentata. They tetanized the lateral and medial entorhinal pathways either separately or simultaneously to produce LTP. It should be noted that these separate but convergent pathways are independently capable of potentiation. When the two pathways were tetanized simultaneously, the resultant LTP was equal to and often greater than the sum of the independently produced LTP. Furthermore, these authors showed that spike discharge of the postsynaptic granule cell was in itself insufficient for the induction of LTP. Their conclusion that LTP is a cooperative phenomenon also rests on the finding that stimulus intensity, and not tetanus frequency or duration, is the determinant for LTP; high intensity tetanus can activate synchronously more fibers of differing thresholds to produce a larger LTP than a tetanus of lower intensity. This dependence of LTP magnitude on stimulus intensity has since been found by other workers (Lee, 1983; Wigström and Gustafsson, 1983a)

Another variation of associative induction of LTP involves the pairing of a weak and a strong tetanus (Barrionuevo and Brown, 1983). In this study, both a weak input and a strong input were each located in the afferent fibers of the stratum radiatum that converge on a population of CA₁ pyramidal cells. Stimulation of these non-overlapping pathways affected differentially the control response to a control stimulus at the weak input: tetanic stimulation of the strong input resulted in heterosynaptic depression (Lynch et al., 1977); tetanic stimulation of the weak input resulted in PTP. However, when both pathways were stimulated simultaneously, the weak control stimulus exhibited PTP and LTP. Kelso and Brown (1986) have shown that the two tetani will not induce associative LTP if separated by 200 msec. Levy and Steward (1983) have described the temporal requirements for associative LTP and long-term depression. These authors showed that LTP can be induced without temporal overlap, but the separation between weak and strong inputs cannot exceed 5-20 msec, otherwise, long-term depression masks any manifestations of LTP. Douglas (1978) had reported a 2-3 msec interval for a similar induction of LTP.

Thus, it appears that associative induction of potentiation has several criteria: 1. A critical tetanic stimulus intensity must be exceeded, the stimulus intensity being greater than the minimal needed to produce an EPSP (McNaughton et al., 1978); 2. The associatively potentiated pathway does not have to produce LTP by itself (Barrionuevo and Brown, 1983); 3. The pairing or association of the separate afferents must take place within a narrow temporal window (Douglas, 1978; Kelso and Brown, 1986; Levy and Steward, 1983); 4. Spike discharge of the postsynaptic cell is not neces-

sary for associative induction of LTP (Douglas, 1978; Lee, 1983; McNaughton et al., 1978; Wigström et al., 1983)

3 METHODS

3.1 Preparation of slices

Male Wistar rats (75–125 g) were placed on top of an ice pack inside a dessicator jar. The dessicator jar was closed and a mixture of anesthetic gas containing 2% halothane in 95% O₂ and 5% CO₂ (carbogen) was fed into the container. The ice pack lowered the body temperature to 31–32°C (measured rectally), presumably decreasing the body's metabolic demands. After 20–30 minutes, the rat was taken from the dessicator jar and the top of the skull exposed via an anteroposterior incision of the skin. The skull plates were carefully removed and the dura mater slit open to expose the brain. A copious amount (about 10 mL) of cold (4°C) normal perfusate was poured onto the brain to cool it as well as to clear the field of blood. The brain was separated from the spinal cord with a thin blade at the pontine level. The olfactory tracts were then severed, followed by severance of the optic nerves, and the whole brain was then removed from the cranial vault. Again about 5 mL of cold medium was poured over the brain before one or both hippocampi were dissected free.

The freed hippocampus was placed on the cutting platform with its long axis perpendicular to the blade of the McIlwain tissue chopper. The hippocampus was chopped into transverse slices of 500 µm thick and transferred as

a whole to a nylon net submerged in a petri dish of cold medium saturated with carbogen. The slices were then carefully separated with a spatula and about six slices were arranged on the nylon net; the remaining slices were discarded. A second nylon mesh was placed over the slices, effectively anchoring them in a sandwich arrangement. This prevented movement of the slices during the experiment. These sandwiched slices were taken from the petri dish and placed in the slice chamber, where they were perfused with normal medium at a rate of 3 ml/min. Elapsed time between the start of surgery and placement in the slice chamber did not exceed 3 minutes.

While in the slice chamber, the slices were submerged in carbogen saturated normal medium; about 0.5 mm of perfusate covered the top surface of the slices. In addition, a constant stream of humidified carbogen flowed over the top of the medium. A piece of parafilm was placed over the chamber opening for the duration of the one hour equilibration period to maintain an oxygen-saturated atmosphere. During equilibration, the bath was kept at room temperature (24°C). All experiments were done at a bath temperature of $32 \pm 0.2^\circ\text{C}$. Flow rate of the medium remained unchanged. To minimize variability due to different durations of ex-vivo perfusion, only one slice from each animal was used per experiment. Only those slices which exhibited a stable response to a test stimulus (60-100 μA , 0.2 msec duration, negative pulses) over a 30 minute period were used.

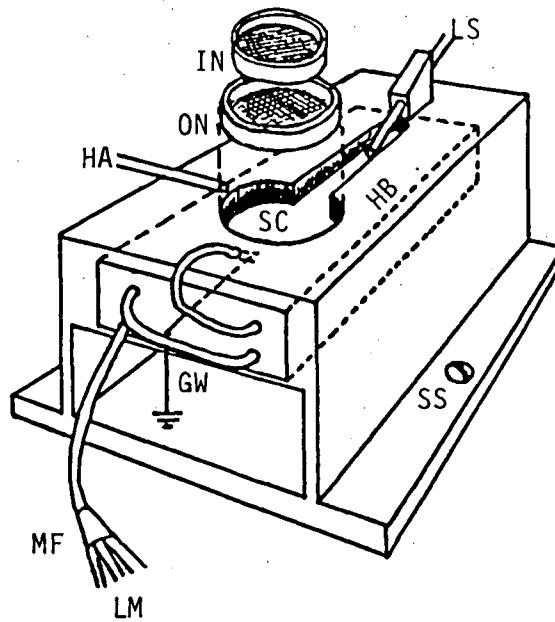
3.2 Slice-bath

The slice bath has been described in detail in a publication from this laboratory (Murali Mohan and Sastry, 1984). Figure 3 gives a diagrammatic illustration of the in-vitro slice bath. Basically, the bath has an alumi-

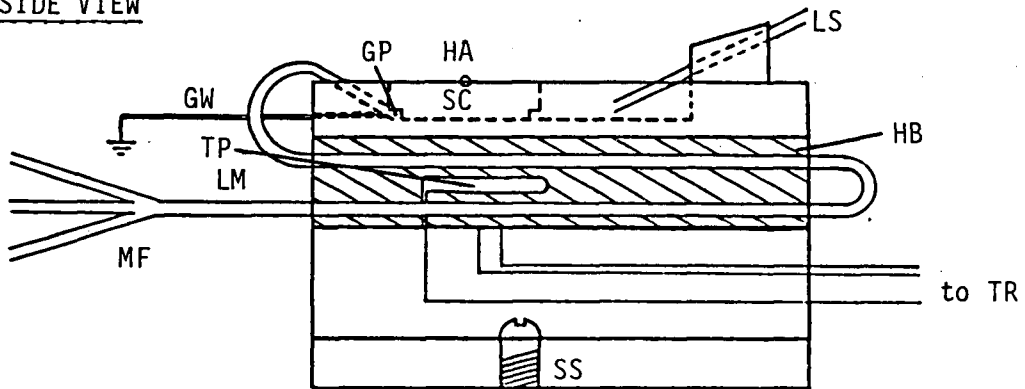
Figure 3

Diagrammatic illustration of the slice bath used for electrophysiological recordings from in vitro hippocampal slices.

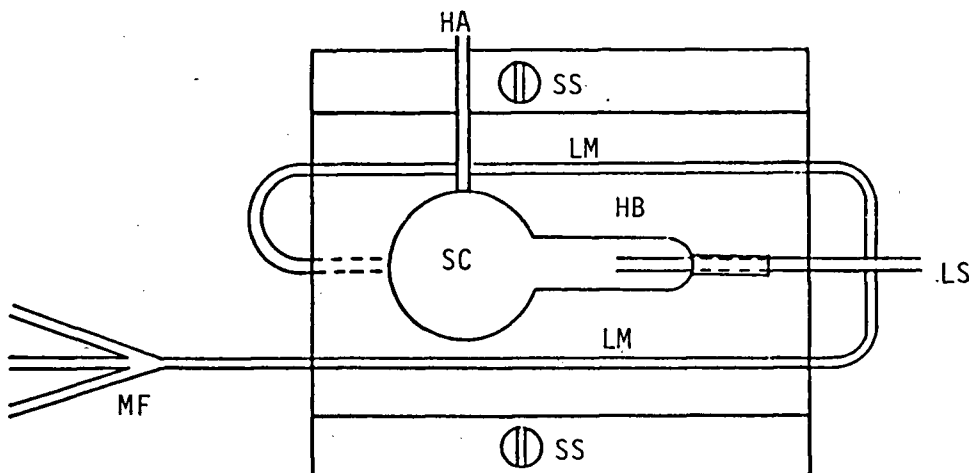
- GP - grounding pin
- GW - ground wire
- HA - humidified air
- HB - heater block
- IN - inner net
- LM - medium lines
- LS - suction line
- MF - manifold
- ON - outer net
- SC - slice chamber
- SS - securing screw
- TP - temperature probe
- TR - temperature regulator



SIDE VIEW



TOP VIEW



num heating block placed beneath the circular chamber where the hippocampal slices were perfused. A temperature sensing device attached to the block fed back to a regulator where the bath temperature was set and displayed. A length of polyethylene tubing was threaded through holes bored in the aluminum block. One end of this tubing was inserted through a hole drilled into the side of the chamber; this is the inlet. Perfusing medium fed into the other end of the tubing from a reservoir was heated via the aluminum block to approximately bath temperature before entering the chamber. Waste medium flowed beyond the circular chamber into a long trench where it was aspirated by gentle suction. The position of the suction tube determined the depth of the perfusate at the chamber. Humidified carbogen was blown over the slices through a tube fixed to the top of the bath and the whole bath was fixed to a stainless steel plate via two screws.

The switching of perfusates was facilitated by a manifold between the reservoir(s) and the bath inlet tubing. Changing the perfusing medium was accomplished simply by clamping shut all but the desired tubing to the manifold. The normal and picrotoxin media were contained in different reservoirs; each medium was independently saturated with carbogen at the reservoirs.

453 Perfusion media

The normal medium was of the following composition: NaCl, 120 mM; KCl, 3.1 mM; NaH_2PO_4 , 1.3 mM; NaHCO_3 , 26 mM; CaCl_2 , 2 mM; MgCl_2 , 2 mM; dextrose 10 mM. The pH of this medium was stable at 7.4 while aerated with carbogen. The picrotoxin medium contained the same components with the following changes: NaH_2PO_4 , 0 mM; CaCl_2 , 4 mM; MgCl_2 , 4 mM; picro

toxin 0.01 mM.

It has been reported that the GABA_A antagonist, picrotoxin, can free the in-vitro hippocampal slice of GABAergic inhibition, thereby facilitating the induction of LTP (Wigström and Gustafsson, 1983b, 1985a). However, picrotoxin also induces epileptiform activity in the hippocampus (Hablitz, 1984) which necessitated the increase of both divalent cations in the perfusing medium: calcium and magnesium were elevated to stabilize the cellular membrane. The higher CaCl₂ concentration led to solubility difficulties that were remedied by the omission of NaH₂PO₄. Notwithstanding the omission, the picrotoxin medium maintained its buffering capacity, showing a steady pH of 7.4 while aerated with carbogen.

3.4 Stimulation systems

In most experiments, the extracellular stimulation electrodes used were metallic (SNEX 100, Rhodes Electronics, resistance 1-2 MΩ). The concentric configuration of these bipolar electrodes minimized current spread beyond the stimulus site. Current pulses were generated by a 2 channel Grass Instruments S88 stimulator. Pulses from each channel were passed through a Grass Instruments PSIU6 constant current stimulus isolation unit before reaching their respective stimulating electrodes. All stimulation pulses were negative square waves.

For intracellular depolarization, a monopolar glass electrode filled with 1 M KCl and 1.6 M K citrate served the dual purpose of stimulating and recording (see following section). A 4 M NaCl-filled glass microelectrode or a fine-tip monopolar tungsten electrode was used to stimulate the Schaffer collateral terminal regions in excitability testing.

3.5 Recording systems

Recording microelectrodes were pulled from fibre-filled capillary tubing (borosilicate glass, O.D. 1.5 mm, I.D. 1.0 mm, Frederick Haer and Co.) using a Narishige PE-2 microelectrode puller. Extracellular microelectrodes were filled with 4 M NaCl and had tips of approximately 1 μm . Typical resistance was 1-2 M Ω . Extracellular signals were amplified by either a World Precision Instruments DAM-5A differential preamplifier or a Medical Systems Neurolog AC-preamplifier and AC-DC amplifier. The amplified signals were then displayed on a Data Precision DATA 6000 waveform analyzer. Evoked responses were stored and averaged by the analyzer and hard copies of the averaged records (4-8 sweeps) were plotted on paper by a Hewlett-Packard 7470A graphics plotter.

Intracellular recording/current injection electrodes were also pulled from the same capillary tubing with the Narishige puller. Electrode tips were sub-micron in size and were filled with 1 M KCl and 1.6 M K citrate. Typical resistances were 40-50 M Ω . Signals were amplified with a World Precision Instruments (WPI) M-707 intracellular amplifier. Current pulses generated by the Grass Instruments S88 stimulator were directed to the current injection circuit built into the WPI M-707 amplifier. Current pulses and intracellular responses were monitored on a Tektronix type 5113 dual beam storage oscilloscope. Records were either captured on polaroid film or plotted using the Hewlett-Packard 7470A graphics plotter (in some experiments, the amplified responses were fed to the DATA 6000 unit where 4-8 sweeps were averaged and plotted).

3.6 Associative induction of STP

3.6.1 Conditioning by tetanic stimulation of fibers

These experiments were done in picrotoxin-containing medium. The effects of tetanic conditioning trains on the induction of STP were examined. A bipolar test stimulating electrode (S_2) was placed in the stratum radiatum of the CA_3 region to stimulate the Schaffer collaterals (Figures 4, 5). The stimulating current of the test input (S_2) was adjusted to produce a "weak" (200–600 μV) population EPSP as recorded in the apical dendritic area of CA_1 . The conditioning electrode was placed in one of three possible positions: in the stratum radiatum on the subicular side of the recording electrode (Figure 4); in the stratum oriens (Figure 4); and in the alveus (Figure 5) to stimulate the CA_1 pyramidal cells antidromically. This conditioning input was the "strong" input (S_1) which produced relatively large responses at the recording site: stratum radiatum EPSP was 1–3 mV; stratum oriens EPSP was 2–5 mV, with a superimposed population spike of 0.5–1 mV; and the alvear antidromic compound action potential was 3–7 mV.

The recording electrode was placed in the apical dendritic region of CA_1 where a test impulse from S_2 produced the maximal EPSP (Figures 4, 5). The stimulating electrodes for activating the S_1 and S_2 inputs were placed so that non-overlapping afferents would be stimulated. This criterion was tested by paired pulse experiments. The second test response to paired pulse stimulation (interstimulus interval of 50 msec) of S_2 was potentiated over control. If the "strong" input (S_1) was activated 50 msec before a single pulse of S_2 , then the test response showed either no change or a slight decrease in amplitude (heterosynaptic depression [Lynch et al.,

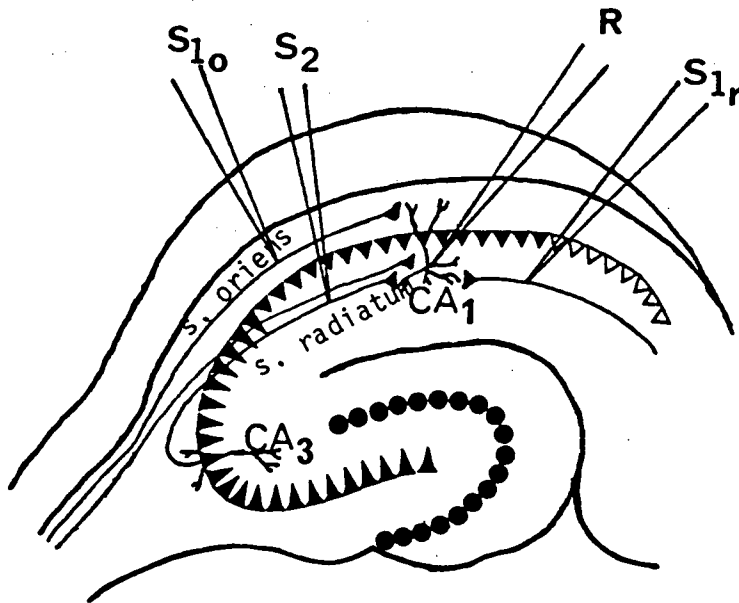


Figure 4

Experimental arrangement for associative induction of STP by conditioning of stratum radiatum and stratum oriens. Conditioning trains consisted of 10 pulses at 100 Hz (1-10 trains at 5 second intervals) and were either delivered alone or in conjunction with a single stimulus of the test (S₂) input at 1 msec following the initiation of each train. In some experiments, the temporal relationship between the conditioning train and the test EPSP for STP induction was determined. The test stimulation (S₂) was given at various intervals between -100 and +100 msec with respect to the onset of the conditioning train (S₁₀, stratum oriens).

R - extracellular recording electrode

S₁₀ - stimulating electrode for stratum oriens conditioning

S_{1r} - stimulating electrode for stratum radiatum conditioning

S₂ - stimulating electrode for evoking stratum radiatum
test population EPSP

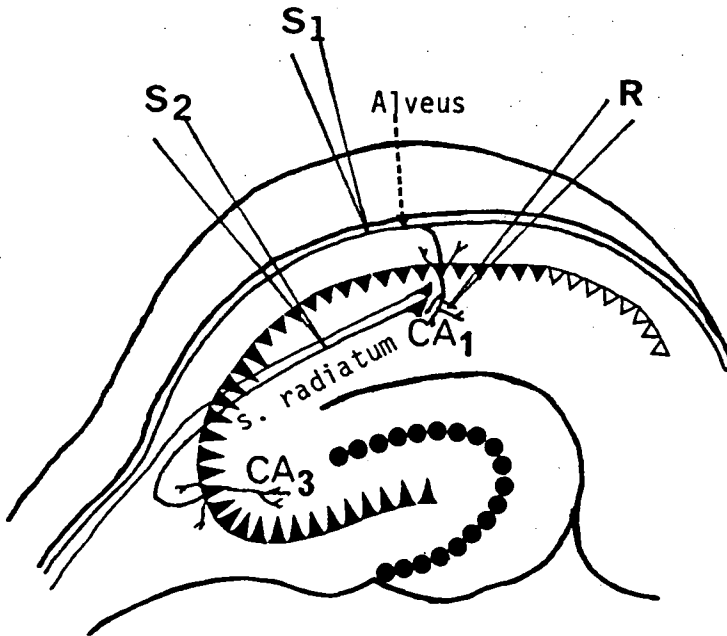


Figure 5

Experimental arrangement for associative induction of STP by alvear conditioning. As in the case of stratum radiatum and stratum oriens conditioning, alvear conditioning also consisted of trains of 10 pulses at 100 Hz (1-10 trains given every 5 seconds). These conditioning trains were either delivered alone or paired with a single stimulus of the stratum radiatum test input (S₂) at 1 msec following the onset of the train.

R - extracellular recording electrode

S₁ - stimulating electrode for alvear conditioning

S₂ - stimulating electrode for evoking test stratum radiatum population EPSP

1977])). This indicates no overlap between S_2 and S_1 ; overlapping afferents resulted in a potentiated S_2 response with the S_2 paired pulse experiments (see Figure 8A). Only non-overlapping inputs were used in the experiments. Since all experiments were done in 10 μ M picrotoxin, the possibility of an inhibition masking a potentiated S_2 response was minimized. The frequency of S_1 and S_2 stimulations was 0.1 Hz so that each stimulus alternated at 5 second intervals.

The conditioning tetanus was delivered through S_1 as trains of impulses. Each train consisted of 10 impulses at 100 Hz. One, five or ten trains were given; the frequency of multitrain conditioning was 0.2 Hz (one train every 5 seconds). Each train was either delivered alone (without the concomitant activation of the test input, S_2) or paired with a single stimulation of S_2 at 1 msec after the onset of the conditioning train. After tetanic conditioning, S_1 and S_2 were returned to the pre-tetanus frequency of 0.1 Hz.

3.6.2 Conditioning by intracellular depolarizing pulses

The role of postsynaptic cell depolarization in the associative induction of STP and LTP was examined using intracellular recording (see Figure 6 for experimental arrangement). Two separate test inputs were used here: one in stratum oriens and one in stratum radiatum. An intracellular recording electrode at the stratum pyramidale of the CA₁ area recorded single cell EPSPs. Stimulus intensity for each test input was adjusted to produce a "weak" EPSP of about 30% of maximum. Control stimulation frequency for each input was once every 15 seconds; they were alternated so that there was an interval of 7.5 seconds between each successive stimulation.

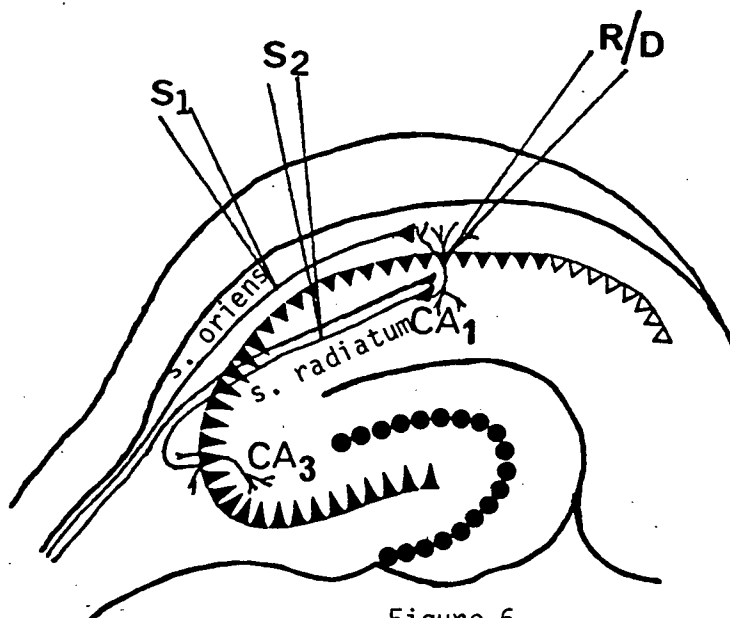
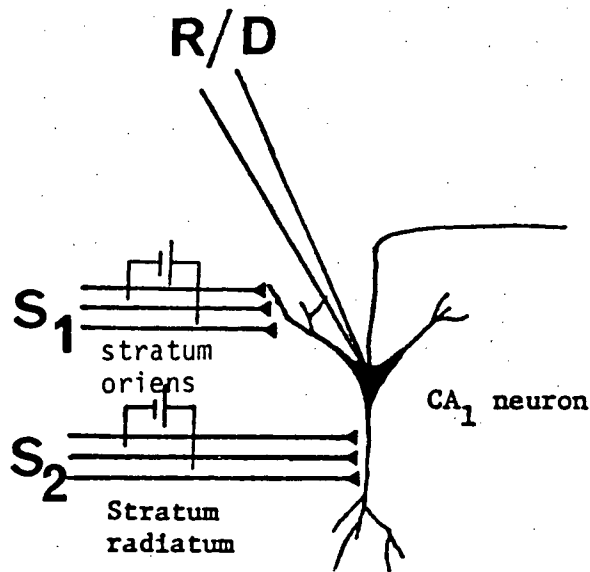


Figure 6

Experimental arrangement for associative induction of STP by intracellular injection of depolarizing current. Intracellular depolarizing pulses (3-10 nA, 75-200 msec duration) were either given alone or paired with a single stimulation of the stratum radiatum test EPSP (S₂) at 1 msec following onset of the pulse. The stratum oriens test EPSP (S₁) served as a control and was not paired with any depolarizing injections.

R/D - intracellular electrode for recording and injection of depolarizing current pulses

S₁ - stimulating electrode for evoking test stratum oriens EPSP

S₂ - stimulating electrode for evoking test stratum radiatum EPSP

Instead of a separate input for the conditioning tetanus trains, suprathreshold depolarizing pulses were injected into the impaled CA₁ cell through the intracellular electrode. These depolarizations (75–200 msec duration; 3–10 nA; one, five or ten depolarizing commands at 0.2 Hz) were used to mimic the effects of synaptically driven depolarizations induced by tetani of inputs. The depolarizations were given either alone or paired with one stimulation of the stratum radiatum test input at 1 msec after the onset of the depolarization. The stratum oriens input was never paired with any depolarizing current injections, thus serving as a secondary control in the experiment.

3.6.3 Temporal requirements governing the induction of STP

The temporal relationship between the conditioning tetanus and the test stimulus was examined by varying the interval between the onset of tetanic stimulation and the stimulation of the test input. The test input (S₂) was evoked by stimulation of stratum radiatum while the conditioning input (S₁) was evoked by stimulation of stratum oriens (Figure 4). Each input was stimulated at 0.1 Hz (alternating every 5 seconds) except during the conditioning tetanus, which was fixed at five trains at 0.2 Hz, each train consisting of 10 pulses at 100 Hz. The test stimulus (S₂) either preceded or succeeded the onset of each tetanic conditioning train by an interval of 0–100 msec.

3.7 Associative induction of Schaffer collateral terminal excitability changes

Changes in presynaptic terminal excitability were assessed using the method of Wall (1958). Figure 7 shows the experimental arrangement. A

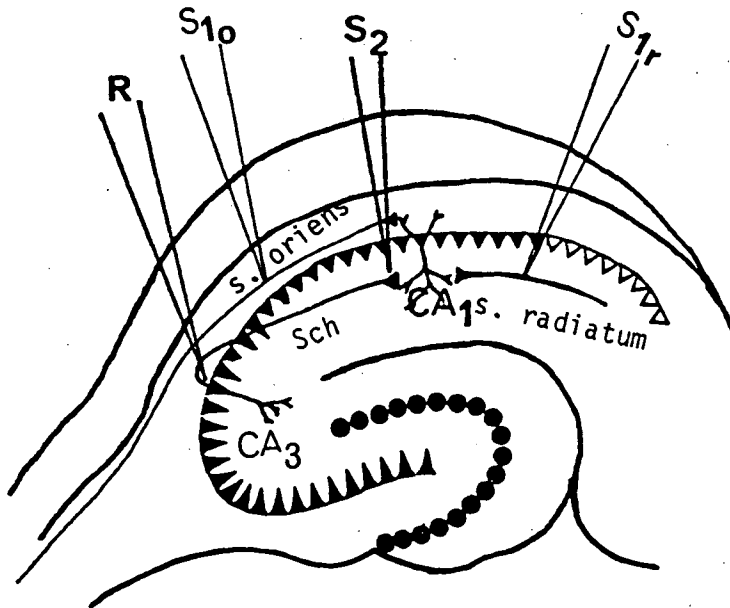


Figure 7

Experimental arrangement for excitability testing of Schaffer collateral terminal regions. The extracellular recording electrode (R) was placed in the CA₃ cell body layer to monitor all-or-none action potentials in single CA₃ neurons. The CA₃ neuron recorded from was activated by stimulation at the Schaffer collateral terminal regions (S₂) located at the apical dendritic region of CA₁. The excitability of the terminal regions (determined by the amount of current required to discharge the cell in 1-2 of 3 consecutive attempts) was monitored before and after conditioning. Conditioning trains were delivered either to stratum oriens (S_{1o}) or stratum radiatum (S_{1r}) and consisted of 1, 5 or 10 trains of 10 pulses at 100 Hz given every five seconds. The conditioning was either given alone or paired with a single suprathreshold stimulus of the test fibre at 1 msec following the onset of each train. It was confirmed that the conditioning trains did not activate the experimental cell.

monopolar glass microelectrode (S_2) or a fine-tip monopolar tungsten electrode was positioned in the stratum radiatum of field CA_1 , presumably in the area of Schaffer collateral synapses at the apical dendrites. Stimulation of the Schaffer collateral terminals through the S_2 electrode results in antidromic all-or-none action potentials (AP) in the CA_3 pyramidal soma. An extracellular recording electrode placed in the CA_3 stratum pyramidale recorded these action potentials. An electrode to activate a "strong" input (S_1) was positioned in stratum oriens or stratum radiatum in order to provide the conditioning tetanus. Stimulation of the "strong" input alone did not induce an antidromic action potential in the cell recorded from in field CA_3 . Tetanic stimulations of S_1 (10 pulses at 100 Hz; one, five or ten trains; one train every five seconds) were delivered alone or paired with a single stimulation of S_2 (a suprathreshold stimulus intensity was used) at 1 msec after the onset of the tetanus. Schaffer collateral terminal excitability was measured as the amount of current required to generate an action potential in the CA_3 neuron in 1 or 2 of 3 consecutive attempts by stimulation through the test (S_2) electrode. An increase in excitability would be reflected as a decrease in the amount of current required to discharge the cell and vice versa. A baseline level of current to produce the antidromic action potential by stimulation through the test (S_2) electrode was determined once every 2 to 3 minutes over 15 minutes before any conditioning stimulations were given. Three attempts were made for every stimulus intensity tested.

4 RESULTS

4.1 Stratum-radiatum-conditioning

The effects of strong conditioning tetani on a weak population response were examined in these experiments. Tetanic stimulation of the "strong" conditioning input (S_1) without any concomitant activation of the "weak" test input (S_2) was insufficient in itself to induce any potentiation of the test input (S_2). All unpaired tetanic conditioning trains produced a degree of depression in the test response ($85 \pm 4\%$ SEM of control at 60 seconds post-10 unpaired tetanic trains of stratum radiatum, 7 of 8 expts., no change in 1 of 8) (Figure 8A). Potentiation of this population EPSP was induced if the conditioning tetanus was paired with one stimulation of the "weak" test input at 1 msec after the onset of each train. After one paired train, there was a brief, small potentiation of the test response lasting about two minutes. Increasing the number of paired trains to five markedly increased the size of the STP induced (Figure 8A). Repeated pairings of 1-5 trains led to repeated STP with no apparent changes in the duration of the potentiation; depression did not set in after repeated pairings. The STP, measured at 60 seconds post-10 paired trains, was $184 \pm 8\%$ SEM of control and lasted 2-3 minutes (6 of 8 expts., no change in 2 of 8). This STP was followed by LTP ($162 \pm 5\%$ SEM of control at 15 minutes post-10 paired trains, 6 of 8 expts., no change in 2 of 8). Note that depression due to unpaired trains did not increase proportionately with the STP.

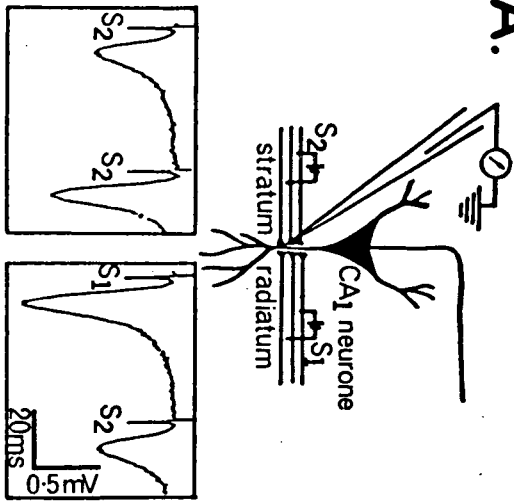
Figure 8

Associative induction of STP, LTP and the reduction in the Schaffer collateral terminal excitability. (A) The schematic diagram on the left illustrates the experimental arrangement. A bipolar test stimulating electrode (S_2) was positioned in the stratum radiatum and a bipolar conditioning stimulating electrode (S_1) was positioned in another area of the stratum radiatum. A recording microelectrode (containing 4 M NaCl) was positioned in the apical dendritic area of CA₁ neurones to monitor the test EPSP evoked at 0.2 Hz (stimulation strength was adjusted to obtain a response between 300-600 μ V). The conditioning stimulation strength was adjusted to evoke a population EPSP of 1-3 mV in size. If a twin stimulation of S_2 (50 ms interval) resulted in a facilitation of the second population EPSP (see inset, left) and if a stimulation of S_1 preceding S_2 stimulation by 50 ms resulted in no facilitation of the second population EPSP (see inset, right), then the S_1 and S_2 stimulations were presumed to activate separate input fibres. In all experiments, the effect of unpaired conditioning trains (UC; i.e., the test stimulation was off during the conditioning; each conditioning train contained 10 pulses at 100 Hz) and of paired conditioning trains (PC; i.e., the test stimulation was on 1 ms after the onset of each train) were examined on the test population EPSP. During the first 3 minutes after UC or PC, the response was monitored every 15 seconds and at all other times at 30 second intervals. The graph on the right shows results from one experiment. Note STP after 1 and 5 PCs, and LTP after 10 PCs.

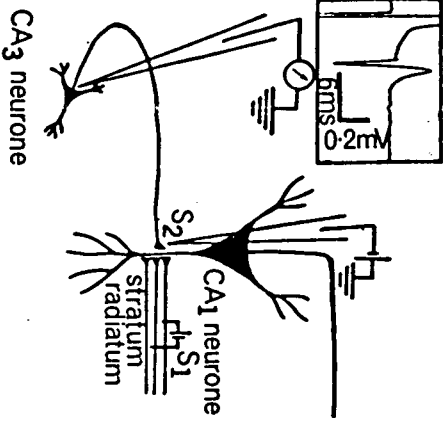
(B) Effects of the conditioning on the excitability of the terminal region of a Schaffer collateral. A monopolar test stimulating electrode (S_2) was positioned in the apical dendritic area of the CA₁ neurones to activate (0.2 ms negative pulses, 3-10 μ A, 0.2 Hz) the terminal regions of Schaffer collaterals so that antidromic all-or-none action potentials (see inset) could be recorded from the CA₃ cell bodies. A conditioning stimulation electrode (S_1) was positioned in the stratum radiatum and the unpaired (UC) and paired (PC) conditioning trains were applied as described in A. It was confirmed that the conditioning stimulation did not activate the test Schaffer collateral. During the PC, the stimulation strength to antidromically activate the test Schaffer collateral was increased to 2 times control to make sure that the fibre was activated during PC. A similar activation of the test fibre without the presence of the conditioning produced no changes in the excitability of the test fibre (results not shown). The amount of current required to produce an all-or-none action potential was taken as that which induced a spike in 1-2 of 3 consecutive attempts. In the graph to the right of the schematic diagram, recordings taken at 30 second intervals were plotted. Note that 1 and 5 PCs induced a 3 minute decrease while 10 PCs induced a prolonged decrease in the excitability of the test fibre terminal.

Results in (A) and (B) were from different experiments.

A.

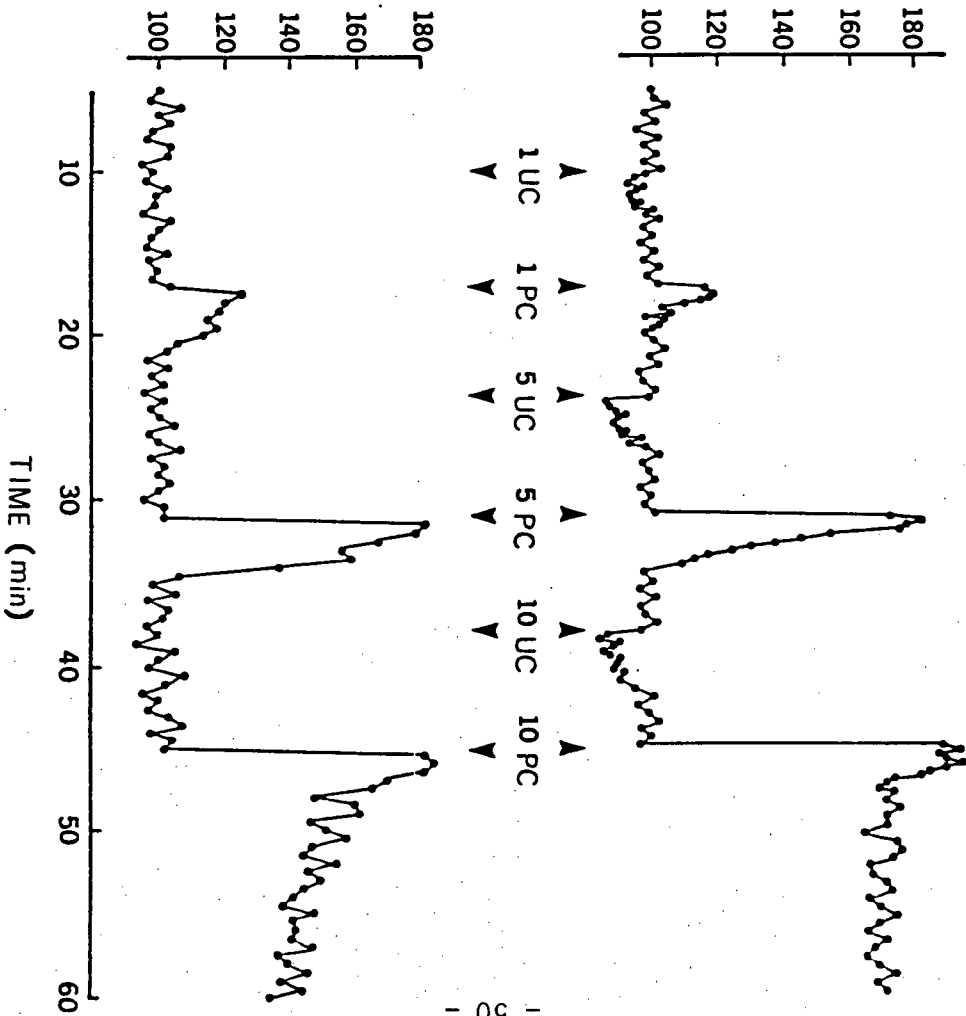


B.



AMOUNT OF CURRENT TO ACTIVATE
SCHAFER COLLATERAL TERMINAL
AS A % OF CONTROL

TEST POPULATION EPSP
AS A % OF CONTROL



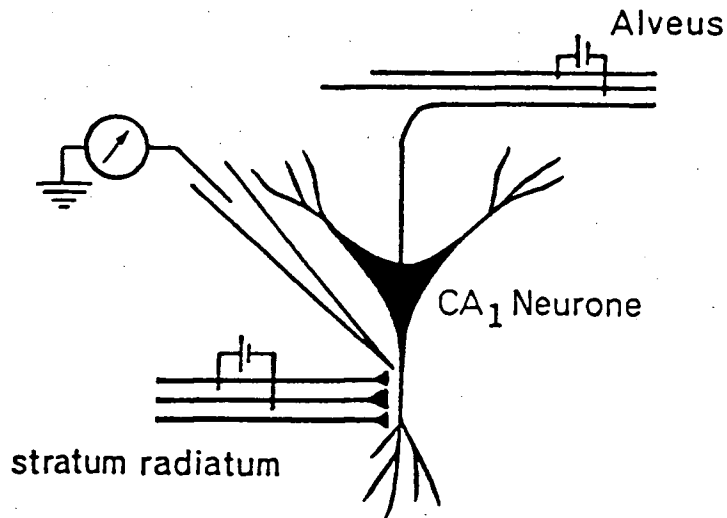
4.2 Stratum oriens conditioning

The effects of stratum oriens conditioning on the test stratum radiatum EPSP were quite similar to those seen above. Unpaired conditioning trains to the stratum oriens input (5 trains of 10 pulses at 100 Hz, one train every 5 seconds) led to depression of the test EPSP ($94 \pm 2\%$ SEM of control at 60 seconds post-5 unpaired trains, $n = 23$; see Figure 10). Pairing the conditioning tetanus with one stratum radiatum stimulation at 1 ms following the onset of each train led to STP (population EPSP as a % of control at 60 seconds post-5 paired trains: 121 ± 4 SEM, 5 of 5 expts.). Since the physical separation between the conditioning and test inputs did not appear to affect the associative induction of potentiation, it is possible to exclude presynaptic terminal interactions as necessary criteria for associative induction.

4.3 Alvear conditioning

The role of the postsynaptic cell in associative induction of STP and LTP is unclear. Using alvear stimulation to antidromically activate CA₁ cells, it was possible to depolarize the CA₁ cell through action potential discharge that did not involve transsynaptic responses. The CA₁ cell was stimulated with antidromic tetanic trains in the same paired and unpaired manner as the previous experiments. As with the above experiments, only the paired conditioning tetani induced potentiation; 10 paired conditioning trains produced both STP and LTP (Table 1). Unlike the synaptically driven tetani, however, unpaired antidromic tetani did not produce any significant depression of the test response ($98 \pm 3\%$ SEM of control at 60 seconds post-10 paired trains, 6 of 6 expts.).

Table 1. Post-Conditioning Potentiation Induced by Pairing Tetanic Trains of the Alveus with a Single Stimulation of the Test Input.



Time post-10 paired conditioning trains		
	60 s	15 min
Test population EPSP as a % of control	146 ± 7 SEM n = 6	133 ± 5 SEM n = 4

4.4 Intracellular current injections

Postsynaptic cell depolarization was further examined with intracellular current injections. All stratum oriens extracellular responses from the unpaired stimulations exhibited only depression after current injection (Table 2). In the case of the stratum radiatum responses, the EPSPs following pairing showed potentiation whereas the unpaired EPSPs were depressed (Table 2). This pattern of potentiation was similar to that of other experiments involving tetanic conditioning trains: increasing the number of paired depolarizing commands resulted in progressively larger STP. With ten paired conditioning commands, STP was superimposed on LTP (Figure 9, Table 2). This evidence clearly suggests the involvement of the postsynaptic cell in associative induction.

4.5 Schaffer collateral terminal excitability changes

Because STP has been shown to be a presynaptic phenomenon in other excitable junctions (Eccles and Krnjević 1959a, 1959b; Magleby and Zengel, 1975a, 1975b), it is pertinent to the argument to examine concomitant presynaptic changes during associatively-induced potentiation. Unpaired tetanic conditioning trains to stratum radiatum did not change the excitability of the Schaffer collateral terminals (amount of current to fire cell as a % of control: 98 ± 3 SEM at 60 seconds post-5 unpaired trains, 7 of 7 expts.; 99 ± 2 SEM at 60 seconds post-10 unpaired trains, 7 of 7 expts.). Similarly, unpaired tetani to stratum oriens also resulted in no alterations in terminal excitability (amount of current to discharge cell as a % of control: 99 ± 3 SEM at 60 seconds post-5 unpaired trains, 6 of 6 expts.). However, as can be seen in Figure 8, paired tetanic conditioning led to a

Table 2. Effects of intracellularly injected depolarizing current pulses on EPSPs evoked by stimulation of stratum radiatum and stratum oriens.

A. Stratum radiatum test EPSP

		Number of conditioning pulses					
		1		5		10	
		UP	P	UP	P	UP	P
1 min post-conditioning (EPSP as % of control)	Range	89	129-140	72-89	96-186	71-78	95-218
	Mean±SEM	-	135 ± 6	82 ± 3	134 ± 10	74 ± 1	145 ± 14
	n	1	2	5	9	4	9
15 min post-conditioning (EPSP as % of control)	Range	102	90-112	91-113	89-109	87-112	99-162
	Mean±SEM	-	98 ± 5	100 ± 6	103 ± 4	97 ± 5	122 ± 8
	n	1	2	5	9	4	9

B. Stratum oriens test EPSP

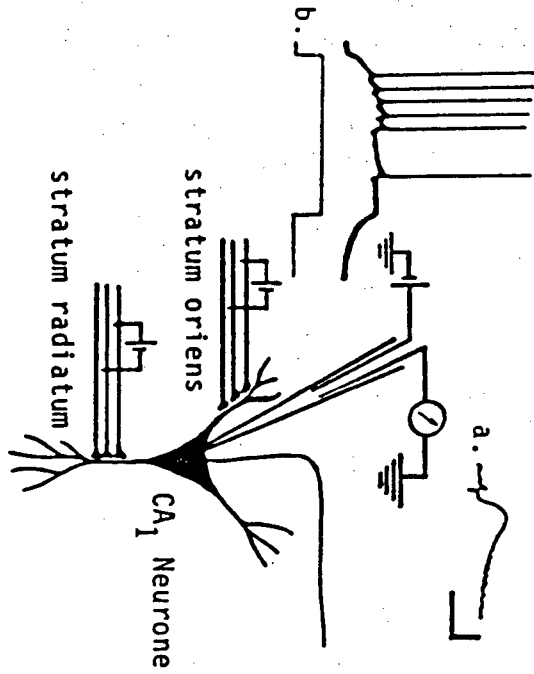
		Number of conditioning pulses		
		1	5	10
		UP	UP	UP
1 min post-conditioning (EPSP as % of control)	Range	86 - 87	71 - 88	71 - 86
	Mean±SEM	87 ± 1	82 ± 2	76 ± 2
	n	2	9	9
15 min post-conditioning (EPSP as % of control)	Range	-	91 - 110	88 - 111
	Mean±SEM	-	101 ± 4	98 ± 5
	n	-	9	9

P = each conditioning depolarizing command was paired with one stimulation of the test stratum radiatum EPSP at 1 msec following the onset of the conditioning pulse.

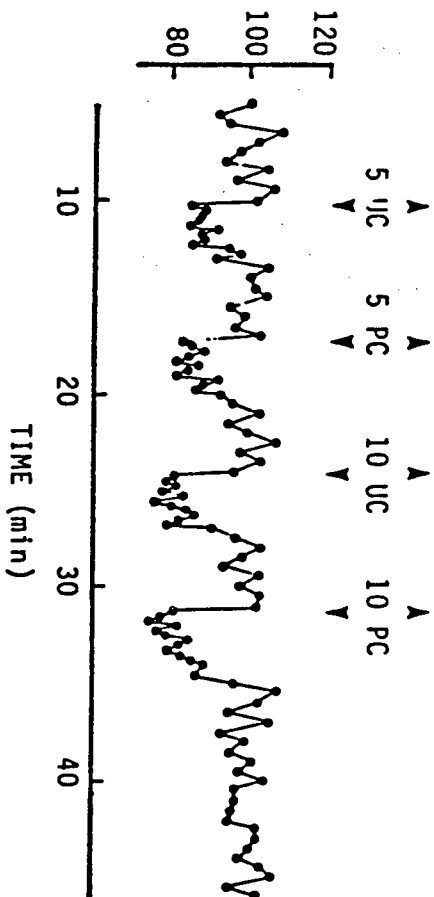
UP = conditioning depolarizing commands were given alone and not paired with test input stimulation.

Figure 9

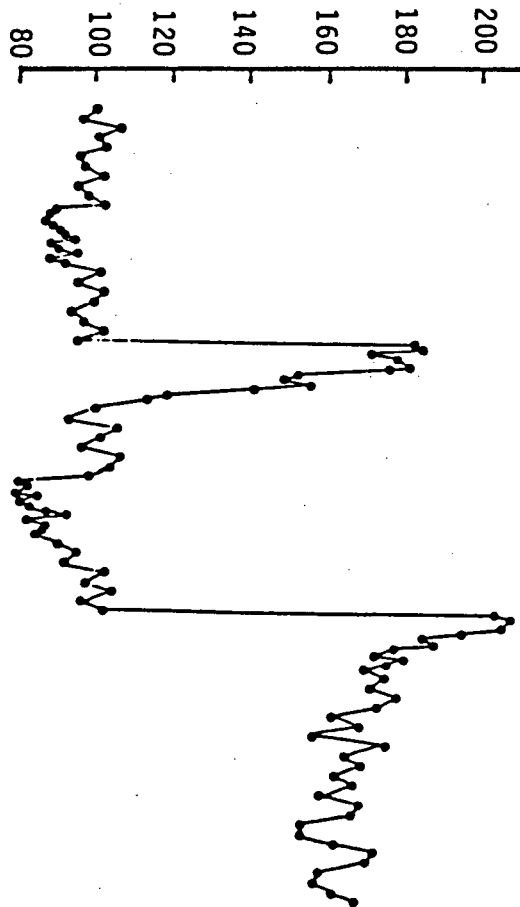
Induction of STP and LTP by paired conditioning depolarization of a CA₁ neuron. To evoke EPSPs in the CA₁ neuron, bipolar test stimulating electrodes were positioned in stratum radiatum and stratum oriens. A recording intracellular microelectrode in the CA₁ neuron was used to record the test EPSPs (a: the calibrations represent 10 msec and 5 mV) and to apply the conditioning depolarizing commands (3-10 nA, 75-200 msec, 1-10 commands at 0.2 Hz) (b: the square wave is 0.1 nA and 75 msec). The stimulation strengths were adjusted to evoke EPSPs at 30% of maximum size. The stimulation of stratum radiatum (every 15 seconds) and stratum oriens (every 15 seconds) was arranged in such a way that there was a 7.5 second delay between the two stimulations. During the unpaired conditioning depolarization (UC), the test EPSPs by stratum oriens and stratum radiatum were not evoked and during the paired conditioning depolarization (PC) the stratum radiatum-induced EPSP was evoked 1 msec after the onset of the depolarizing command while the stratum oriens was not stimulated. When more than one UC or PC was applied, they were given at 0.2 Hz. Stratum radiatum stimulation at 0.2 Hz without the presence of the conditioning depolarization of the CA₁ neuron did not result in a change in the size of the EPSP (results not shown). Note STP and LTP of the stratum radiatum-induced, but not of the stratum oriens-induced, EPSP following the PC. In the graphs, EPSPs were recorded at 30 second intervals. After UC and PC, however, recordings were taken at 15 second intervals for 3 minutes. The 'resting' membrane potential of the neuron at the beginning of the experiment was -65 mV and at the end of the experiment was -61 mV. This is a typical experiment; similar results were found in six cells.



Stratum oriens-induced EPSP as a % of control



Stratum radiatum-induced EPSP as a % of control



graded decrease in excitability that paralleled the post-tetanic potentiations in both time course and magnitude (amount of current to discharge cell as a % of control: 185 ± 6 SEM at 60 seconds, post-5 train paired conditioning by radiatum, 6 of 7 expts., no change in 1 of 7; 138 ± 6 SEM at 60 seconds post-5 train paired conditioning by oriens, 5 of 6 expts., no change in 1 of 6). Tetanic trains to conditioning inputs in either stratum oriens or stratum radiatum were able to induce the decrease in terminal excitability. Increasing the number of paired conditioning trains to ten led to a prolonged decreased in excitability that was associated with LTP of the test EPSP (amount of current to discharge cell as a % of control: 154 ± 5 SEM at 15 minutes post-10 train paired conditioning by radiatum, 5 of 6 expts., no change in 1 of 6) (Figure 8). A summary of all the results is shown in Table 3.

4.6 Temporal requirements for induction of STP

For these experiments, the number of paired conditioning trains was fixed at five because this paradigm was one that most reliably induced STP with no LTP. Two parameters were varied here: the order in which each test and conditioning inputs were paired and the interval between the two. In Figure 10, the x-axis is the interstimulus interval between the onset of the conditioning train and the test stimulus. Negative interstimulus intervals indicate that the test stimulus preceded the onset of the conditioning trains while positive intervals indicate the duration between the onset of the conditioning train and the succeeding test stimulus.

In the experiments where the test stimulus succeeded the onset of the conditioning tetanus, there was significant STP up to an interstimulus

Table 3. Effects of paired and unpaired conditioning trains on Schaffer collateral terminal excitability.

	60 s post-5 trains		15 min post-10 trains	
	unpaired	paired	unpaired	paired
stratum radiatum conditioning (threshold as a % of control)	98 ± 3 n = 7	185 ± 6 n = 6	99 ± 2 n = 7	154 ± 5 n = 5
stratum oriens conditioning (threshold as a % of control)	99 ± 3 n = 6	138 ± 6* n = 5		

*Results are expressed as mean ± SEM.

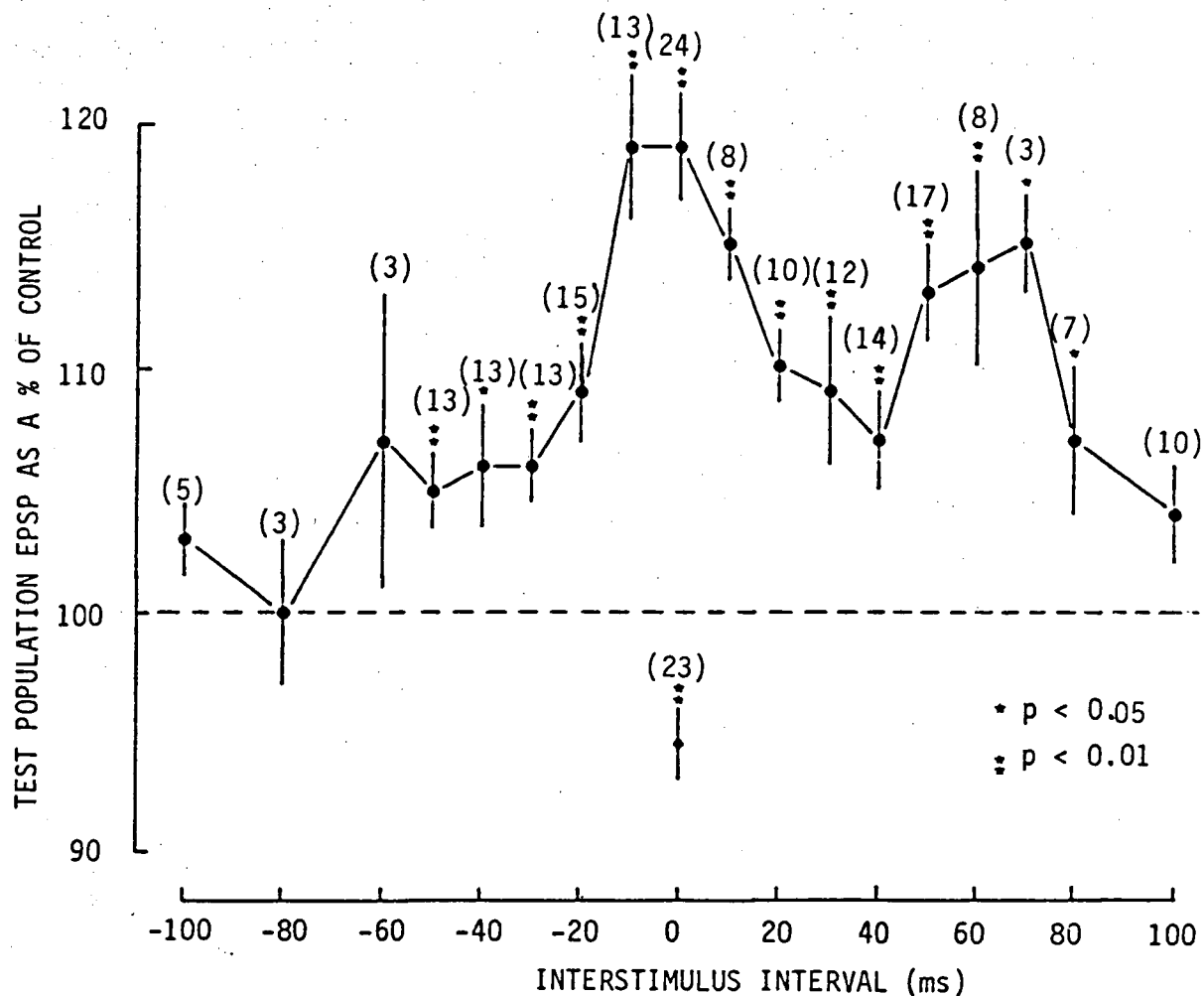


Figure 10

The limits of the temporal relationship between conditioning and test stimuli for the induction of associative potentiation. The test EPSP was evoked by stimulation of stratum radiatum and the conditioning was achieved through stimulation of stratum oriens (5 trains, 10 pulses in each train at 100 Hz, one train every 5 seconds). One test stimulus was paired with each of the five conditioning trains at every interstimulus interval examined. The conditioning-test interval was varied between -100 to +100 ms. A negative delay indicates that the test stimulus preceded the onset of the conditioning train and a positive delay indicates the time at which the test population EPSP was evoked following the onset of the conditioning tetanus. Each point on the graph (filled circles) represents mean \pm SEM of the test population EPSP magnitude measured at 1 minute post-5 paired trains. The one point represented by the filled diamond shows a significant depression of the test EPSP at 1 minute post-5 unpaired trains of stratum oriens (i.e., test EPSP was not evoked during conditioning). n is shown in parentheses above each point on the graph.

interval of 80 msec. The curve is bimodal with a dip in the potentiation at the 40-50 msec interval. Maximal potentiation was induced when both test and conditioning stimuli are activated simultaneously. Unpaired trains were given to the conditioning stratum oriens input to confirm non-overlap with the test input at stratum radiatum, as well as to show that tetanic conditioning trains alone did not induce STP.

The amount of STP induced by reversing the order of the stimuli appears to drop off markedly with respect to increasing the interstimulus interval. Significant potentiation was limited to an interstimulus interval of -50 msec. The significance of these results are examined in the following section.

5 DISCUSSION

The results show that STP and LTP in the hippocampal slice can be induced associatively without tetanic stimulation of the potentiated pathway. Studies in the current literature found associative induction of potentiation only in tetanized pathways (Barrionuevo and Brown, 1983; Lee, 1983; Levy and Steward, 1979; McNaughton et al., 1978). In the present experiments, the synaptic response to a single afferent volley is potentiated for a variable duration when it coincides with a conditioning tetanization given at a separate converging afferent pathway. This coincidental stimulation of the single afferent volley must occur within an asymmetrical temporal window around the conditioning tetanus.

No attempt was made to subdivide the STP induced by the present method into subcomponents such as augmentation and PTP, as was done with STP in other systems (Magleby and Zengel, 1975b, 1976a). Like the homosynaptic nature of tetanus-induced PTP (Eccles, 1953), only that afferent pathway paired with the conditioning stimulus is potentiated. This associatively induced STP increases in amplitude with the number of paired conditioning trains in a manner similar to PTP. However, the duration of potentiation does not seem to increase with the number of conditioning trains, a property that suggests augmentation. Since only 1, 5 and 10 paired conditioning trains were examined, it is possible that the graded nature of the STP was not fully evident, especially if the induction of LTP masked a prolonged duration of STP.

Previous studies of STP in spinal cord, neuromuscular junction and squid giant axon all concluded that STP was mediated post-tetanically via an increase in transmitter release (del Castillo and Katz, 1954c; Eccles and Krnjević, 1959a, 1959b; Lloyd, 1949; Magleby and Zengel, 1975b; Takeuchi and Takeuchi, 1962). In the hippocampus, where quantal analysis of transmitter release is still an equivocal procedure (Johnston and Brown, 1984), the locus of STP is also believed to be presynaptic (McNaughton, 1982; Racine and Milgram, 1983).

In the present experiments where the conditioning electrode was placed in the same stratum as the test electrode, there was the possibility of some undetected interaction of presynaptic terminals which might account for the post-conditioning potentiation. This communication may be through ephaptic interactions, as was suggested for interactions between somata and dendrites

(Richardson et al., 1984; Turner et al., 1984), electrotonic coupling (MacVicar and Dudek, 1981, 1982), the release of ions (Goh and Sastry, 1985; Weight and Erulkar, 1976) or neurotransmitter (Alger and Teyler, 1978; Goh and Sastry, 1985). However, the placement of the conditioning electrode in an anatomically separate stratum precludes the possibility that the post-conditioning effects are due entirely to presynaptic terminal or fiber interactions, especially since no axoaxonic synapses have been found in this area. Thus, it is interesting that the resultant associative STP and LTP from experiments using stratum oriens and stratum radiatum conditioning electrodes are remarkably similar. Without invoking hypothetical excitatory interneurons or inter-strata associational pathways, the most logical mediator of the conditioning effects is the postsynaptic CA₁ cell (McNaughton, 1982; McNaughton et al., 1978; Robinson and Racine, 1982). It is evident that the postsynaptic cell is capable of mediating heterosynaptic effects, since unpaired stratum oriens conditioning trains cause a heterosynaptic depression of the EPSP to stratum radiatum test stimulation (see Figure 10). Notwithstanding the postsynaptic nature of heterosynaptic depression (Dunwiddie and Lynch, 1978; Lynch et al., 1977; Sastry et al., 1984), the point is that activation of the CA₁ cell via the basilar dendrites will have an effect that can be observed at the apical dendrites.

The pivotal role of the postsynaptic cell in the present experiments is in partial agreement with the results of others who nonetheless found that postsynaptic cell spike discharge was not necessary for induction of potentiation (Douglas, 1978; Wigström et al., 1982). In the alveus conditioning experiments (see Table 1), it was a necessary condition of antidromic

dromic stimulation that the CA₁ cell does spike. Similarly, with the intracellular depolarization experiments, it was necessary to use supra-threshold currents in order to induce associative potentiation. Lee (1983) examined the ability of antidromic alvear tetanus to induce associative potentiation in the CA₁ cell and found that LTP is not enhanced when an orthodromic tetanus is paired with an antidromic train. This led him to conclude that postsynaptic cell discharge is not essential. A major difference between that study and the present one is that the former was done in normal perfusing medium whereas the latter included 10 μ m picrotoxin. In the absence of picrotoxin, the recurrent or feed-forward inhibitory neurons to the CA₁ cells would surely be activated, thereby shunting the depolarization of the soma and probably the dendrites. The probability of a sufficiently large depolarization reaching the subsynaptic dendritic site would be correspondingly low. Hence, Lee (1983) observed no enhancement with alvear conditioning. The need for cell spike discharge in the present experimental schemes is obvious if the initiating event for associative induction is at the subsynaptic zone in the dendrites. Orthodromic stimulation of afferents results in synaptic transmission that firstly depolarizes the subsynaptic dendrite. The electrotonic spread of this depolarization to adjacent dendritic sites could be independent of cell spike discharge. In the present studies, where single afferent volleys are paired with conditioning trains, the subsynaptic site must be invaded by a sufficiently large depolarization for the initiating event to occur. Unlike orthodromic conditioning tetani, the antidromic tetani do not depolarize the dendrites first; this is also true of the intracellular current injections. In addition, the

volleys do not depolarize the dendrites sufficiently for associative induction. Therefore, it is essential to activate the CA₁ cell above the threshold for an action potential, and to do so repeatedly with a prolonged tetanus to ensure that the decrementally propagated depolarization actually invades the dendrites.

In agreement with the above line of reasoning are the results from intracellular current injection experiments. Indeed, there seems to be an exaggerated need for the postsynaptic cell to fire action potentials; the left inset in Figure 9 shows that the CA₁ cell actually fires repeatedly with a depolarizing current injection of 0.1 nA for 75 msec. Yet the current injections needed to induce associative STP and LTP were several orders of magnitude greater than the intensity needed to fire the cell. This apparent discrepancy can be easily explained if one examines the premise for associative potentiation, which requires that a number of afferent fibers interact more or less simultaneously. This means that a great number of afferent terminals -- and presumably subsynaptic dendritic sites -- must be activated. Since the number of activated synapses to any one CA₁ cell is finite, it may be necessary to depolarize the cell with several times the threshold current to ensure the depolarization of the maximum number of dendritic initiating sites. Alternately, the maximum number of initiating sites on the dendrites of one cell may be too small for associative induction, requiring the firing of the CA₁ cell so that additional cells could be recruited through ephaptic interactions (Richardson et al., 1984; Turner et al., 1984) or electrotonic coupling (MacVicar and Dudek, 1981, 1982). Whatever the mechanism, it is clear that the postsynaptic cell(s) initiates the induction

of associative potentiation but cannot be the only causal event.

To induce associative STP and LTP, the afferent pathway must be stimulated at least once in conjunction with the conditioning stimulus. Presumably, the postsynaptic depolarization sets up a synaptic environment that is conducive to potentiation. It can be argued that the crucial event for associative potentiation occurs at the presynaptic terminals; the input specificity of the associatively-induced potentiation supports this view. Since the site of the conditioning input is inconsequential to which afferent pathway is potentiated, the depolarization of the subsynaptic site can only be interpreted as a generalized change in the postsynaptic cell, namely the dendrites. Otherwise, subsequent stimulation of any afferent pathway would have produced a potentiated postsynaptic response. This is clearly not the case.

The excitability changes of the Schaffer collateral terminals indicate a critical role for the presynaptic fiber. Post-tetanic potentiation at the neuromuscular junction and spinal cord is accompanied by a parallel period of hyperpolarization at the presynaptic terminal (Gasser and Graham, 1932; Gasser and Grundfest, 1936; Larrabee and Bronk, 1938). Upon reaching the hyperpolarized terminal, a presynaptic action potential would be relatively larger in amplitude, thereby releasing more transmitter per action potential (Eccles and Krnjević, 1959a, 1959b; Hubbard and Schmidt, 1963; Lloyd, 1949). Another consequence of this hyperpolarization is a decrease in the excitability of the presynaptic terminal as defined by Wall (1958) (Wall and Johnson, 1958). This decreased excitability is also observed in the present study (see Figure 4B, Table 3).

Note that only the paired conditioning trains produced any changes in excitability of the Schaffer collateral terminals. Equally important is that unpaired conditioning trains did not alter excitability, thereby reaffirming the lack of lasting consequences due to any direct interactions between the test and conditioning inputs. The graded nature of the excitability decrease is readily seen. The parallel between Schaffer terminal excitability changes and the associatively-induced STP and LTP suggests a causal relationship. Assuming that the decreased excitability reflects a proportionate hyperpolarization of the terminals, one can infer that this hyperpolarization will lead to increased transmitter release per afferent volley. The results presented are far from conclusive, but they do suggest a plausible presynaptic mechanism for associatively-induced STP; similar decreases in afferent terminal excitability have been found to accompany LTP in the hippocampus (Sastry, 1982). A definitive quantal analysis of potentiated transmitter release in the hippocampus has yet to be done, but increased quantal content was found in the crayfish neuromuscular junction during LTP (Baxter et al., 1985). However, the relationship between presynaptic changes and hippocampal LTP is more tenuous.

What factor or process does the postsynaptic cell elaborate, and how does this translate into a presynaptic change. This elusive link between the pre- and postsynaptic elements may be one or more of several possible candidates. In the stratum pyramidale of field CA₁, extracellular potassium $[K^+]_o$ may reach a maximum of 12 mM during a tetanus (Benninger et al., 1980). Alger and Teyler (1978) found a good correlation between post-tetanic extracellular potassium $[K^+]_o$ and the amplitude of the poten-

tiated CA_1 population spike; no such correlation was found between $[K^+]_o$ and the population EPSP. However, the potentiation that they call STP is at least one order of magnitude greater in duration than the STP studied here and would be more properly called LTP. Their conclusion that elevated $[K^+]_o$ increases the excitability of the postsynaptic cell is in agreement with other mechanisms suggesting enhanced responsiveness of the postsynaptic cell after tetanic stimulation (Abraham et al., 1985; Fritz and Gardner-Medwin, 1976). The same study showed that $[K^+]_o$ at the dendrites is also elevated after a tetanus; such an ionic environment at the terminal region could increase terminal excitability by depolarization rather than decrease it. Indeed, Goh and Sastry (1985) found that untetanized afferent terminals adjacent to tetanized terminals actually exhibit increased excitability.

This theoretical impasse can be surmounted by examination of the ionic mechanism underlying post-tetanic hyperpolarization at peripheral neuronal junctions. Of the two phases of post-tetanic hyperpolarization (Gasser and Graham, 1932; Gasser and Grundfest, 1936), the second prolonged phase exhibits a duration of several minutes, which correlates well with that of associative STP. This second phase has been presumed to be the result of an activated electrogenic sodium pump (Na^+, K^+ -ATPase) (Nakajima and Takahashi, 1966; Rang and Ritchie, 1968a, 1968b). The activity of this ouabain-sensitive Na^+, K^+ -ATPase is directly dependent upon the intracellular sodium concentration $[Na^+]_i$ (Ritchie and Straub, 1957; Thomas, 1972), and to a lesser extent, on an elevated $[K^+]_o$ (Rang and Ritchie, 1968a, 1968b). It was found that both Na^+, K^+ -ATPase and post-tetanic

hyperpolarization exhibit the same sigmoidal dependence on $[K^+]_o$ (McDougal and Osborn, 1976). Tetanic stimulation of garfish olfactory nerve and rabbit vagus nerve increases Na^+, K^+ -ATPase activity as measured by inorganic phosphate efflux, which then returns to resting levels post-tetanically with an exponential rate constant of about 4 minutes (Ritchie and Straub, 1978). The rate of inorganic phosphate efflux also increases with increasing stimulus frequency (Ritchie and Straub, 1978).

In light of the above mechanism, associative induction of STP may involve the following series of events: a conditioning tetanus or intracellular current injection depolarizes the postsynaptic dendrites, which release a large amount of potassium. The transient increase in $[K^+]_o$ does not exert enough drive to activate the Na^+, K^+ -ATPase at the test presynaptic terminals; in fact, the excitability of the terminals may be increased. During this phase of increased excitability, the invasion of these terminals by a single afferent volley may induce an exaggerated Na^+, K^+ -ATPase response to the consequent Na^+ influx, thereby hyperpolarizing the terminals and preterminal axons. This hyperpolarization would be analogous to that induced by tetanic stimulation of the afferent fibers, leading to a facilitation of transmitter release to subsequent test stimulations of the afferents. The hyperpolarization is merely the priming event for some other process, such as an increase in the pool of available transmitter (Hubbard, 1963) or better propagation of a preterminal action potential to the terminal, that mediates the potentiation; hyperpolarization presumably elevates this process to a state of subliminal activation to await supra-activation by the afferent impulse. Increasing the number of paired conditioning

trains or current injections into CA_1 neurons could increase the $[K^+]_o$ so as to recruit more presynaptic terminals to the subliminal level of activation. The expression of the potentiated release would then follow mechanisms along the lines of the residual calcium theory (Katz and Miledi, 1968).

It must be borne in mind that the hyperpolarization is inferred from the decreased terminal excitability and, it is possible to have an apparent decrease in excitability without hyperpolarization. For example, a small depolarization, such as that induced by a slightly elevated $[K^+]_o$, can lead to inactivation of voltage dependent sodium channels at the terminal. To generate an antidromic action potential, a higher depolarizing current must be passed to activate those available sodium channels that are not right at the terminal. Furthermore, the excitability itself is inferred from higher current intensities needed to fire an antidromic action potential. The higher current may be needed to overcome an increase in resting conductance of any one of several ions.

Other possible mechanisms for the observed induction of STP may involve calcium-activated potassium channels at the presynaptic terminal (Mallart, 1984; Sastry, 1979), calcium-dependent chloride channels (Owen et al., 1984) or voltage-dependent calcium channels (MacVicar, 1984). In the periphery, Ca^{++} fluxes at the presynaptic terminal during normal and facilitated transmitter release has been well established (Hodgkin and Keynes, 1957; Katz and Miledi, 1967, 1968). Therefore, it is hardly surprising that Ca^{++} or Ca^{++} -mediated currents can be involved in associative STP. Some workers have suggested the activation of a Ca^{++} -mediated protein kinase C

in the presynaptic terminal to account for LTP (Malenka et al., 1986b). This kinase can be selectively activated by certain phorbol esters to produce LTP in the hippocampus; the LTP thus produced cannot be distinguished from tetanus induced LTP, and neither LTP can be induced when maximal potentiation has been achieved by either method (Malenka et al., 1986b).

Protein kinase C is found in presynaptic terminals (Girard et al., 1985) and may catalyze the phosphorylation of several proteins during LTP (Nelson and Routtenberg, 1985, Browning et al., 1979). In the hippocampal pyramidal cell, certain phorbol esters can block a Ca^{++} -mediated potassium current (Malenka et al., 1986a); similar channels have been found in presynaptic nerve terminals (Bartschat and Blaustein, 1985). The blockade of this outward K^+ conductance could delay membrane repolarization and lead to a prolonged Ca^{++} current, which would increase transmitter release. Similarly, the delayed repolarization may prolong sodium channel inactivation, resulting in an apparent decrease in terminal excitability. In addition, there is evidence that phorbol esters increase calcium currents by some action on protein kinase C (DeRiemer et al., 1985). However, phorbol esters do not induce post-tetanic potentiation independently of LTP (Malenka et al., 1986b).

Collingridge (1985) has proposed a postsynaptic inward current to account for LTP. This hypothesis involves a glutamate receptor subtype, the so-called N-methyl-D-aspartate (NMDA) receptor located on the postsynaptic dendrites (Baudry and Lynch, 1981). It is suggested that a voltage dependent magnesium (Mg^{++}) blockade of a NMDA-receptor coupled conductance is lifted upon depolarization of the postsynaptic cell (Mayer et al., 1984;

Nowak et al., 1984). Subsequent release of transmitter, presumably glutamate (Storm-Mathisen, 1977), from the presynaptic terminal then causes a greater late current through the NMDA receptor-coupled ion channel to further enhance the postsynaptic response (Harris et al., 1984; Wigström and Gustafsson, 1984, 1985b). This inward current is blocked by the NMDA antagonist 2-amino-5-phosphonovalerate (APV), which also blocks the induction of LTP (Collingridge et al., 1983; Harris et al., 1984; Wigström and Gustafsson, 1984). This mechanism thus accounts for the need to depolarize the postsynaptic cell for associative induction of LTP.

The presynaptic element could be activated by possible autoreceptors for glutamate (Collingridge et al., 1983; McBean and Roberts, 1981). If these autoreceptors are also NMDA receptors, then presynaptic depolarization by the afferent volley may be required to remove the Mg^{++} inhibition before current flow can occur. Assuming that Ca^{++} flows through these channels (Dingledine, 1983a, 1983b), the extra Ca^{++} in the terminal would act as the residual Ca^{++} in Katz and Miledi's (1968) theory for transmitter release. Again, the increased conductance may shunt the extracellular current injections, thereby necessitating higher current intensities to fire an antidromic action potential.

After the conclusion of the present studies, Wigström et al. (1986) published a paper examining the associative induction of LTP using intracellular depolarization in conjunction with single afferent volleys. These authors agree that the level of postsynaptic cell depolarization, rather than spike activity, is the determinant factor at the postsynaptic cell to induce LTP. However, they propose a postsynaptic locus for the induction of

LTP based on the voltage-sensitive NMDA receptor-activated current (Wigström et al., 1985). This current is apparent after a single high intensity stimulation or a brief tetanus (Wigström et al., 1985; Wigström and Gustafsson, 1984), and is blocked by the NMDA antagonist 2-amino-5-phosphonovalerate (APV). In their scheme of events, depolarization of the subsynaptic membrane containing NMDA receptors, in conjunction with transmitter released by afferent stimulation, is the associative event that results in potentiated postsynaptic responses.

This is a very attractive hypothesis, for these authors also showed that the conditioning tetanus and the single afferent stimulation can be separated by a period of 40 msec (Wigström and Gustafsson, 1985b). This temporal separation can be attributed to a residual current passing through the NMDA receptor channel and having a time course of about 50 msec for a single volley. (Wigström et al., 1985). These authors suggest that a temporal overlap of the NMDA current with the conditioning tetanus is essential for associative induction. Such a mechanism would explain the present observation that a test stimulus can precede the conditioning tetanus by 50 msec and still induce STP. Similarly, a conditioning tetanus would be expected to generate a current of longer duration; hence, the test stimulus can follow a conditioning tetanus by up to 80 msec and still produce STP. The greatest amount of potentiation is induced by simultaneous test and conditioning stimulations. Other workers have also found that the associative induction of LTP requires temporal overlap of test and conditioning stimulations (Kelso and Brown, 1986; Levy and Steward, 1983).

Wigström and Gustafsson's theory for the associative induction of LTP (1985b) does not account for the decreased terminal excitability in the present study. This decrease in terminal excitability is an important link between STP in the hippocampus and that in other systems. Although hyperpolarization of the Schaffer collateral terminals and accelerated Na^+, K^+ -ATPase activity was not demonstrated directly in the present study, their reported roles in PTP at other excitable junctions suggest equivalent roles in the hippocampus. With respect to the temporal limits of associative STP -- namely the 40 msec separation between a test stimulus and the succeeding conditioning tetanus, it is conceivable that each afferent volley induces an increment of subliminal activation at the presynaptic terminal, much as each afferent volley induces an inward current mediated by the NMDA channel. This increment of subliminal activation could be triggered by the transient increase in Na^+, K^+ -ATPase activity or intracellular Ca^{++} through voltage dependent channels, but only if a tetanic depolarization of the postsynaptic cell occurs within a short time. Since the exact nature of this subliminal activation is unknown, any number of possibilities could be advanced.

Notwithstanding this uncertainty, the results in this thesis strongly support a crucial presynaptic event for the associative induction of STP as well as LTP. Up until now, STP and LTP in the hippocampus have been considered totally separate phenomena. The proposed dendritic initiation site for the associative induction of STP is unusual because STP has already been shown to be a presynaptic event at other neuronal junctions. Furthermore, a single stimulation of afferents has not previously been shown to induce any

short-term synaptic efficacy changes. On the other hand, associatively induced STP in the hippocampus may be due to a postsynaptic mechanism involving NMDA receptor activation as suggested for LTP (Collingridge, 1985; Wigström and Gustafsson, 1985b). With respect to LTP, a great number of hypotheses regarding its mechanism focus on the postsynaptic cell for both induction and maintenance. Although there is evidence for increased transmitter release during LTP (Dolphin et al., 1982; Lynch et al., 1985; Skrede and Malthe-Sørensen, 1981), a plausible interaction between pre- and postsynaptic cells has only just been advanced (Wigström and Gustafsson, 1985b). Unfortunately, even this last hypothesis ignores the possible role of presynaptic causal events such as the lasting decrease in Schaffer collateral terminal excitability, which is a substantial link between LTP and presynaptic changes.

The present results also suggest a possible postsynaptic initiating site for LTP. This evidence indicates similar conditions underlying the two forms of potentiation and suggests a common mechanism of induction and maintenance. The fact that associative LTP can be induced in the same fashion as STP raises interesting possibilities regarding the locus of each potentiation; perhaps associative STP in the hippocampus (or central nervous system) is uniquely different from that in the periphery and tetanus-induced STP; there is also the possibility that some fundamental unit of potentiation is responsible for both associative STP and LTP, the difference between the two being only a matter of duration.

6 CONCLUSIONS

Ever since Bliss and Gardner-Medwin (1971) first reported LTP in the hippocampus, the working hypothesis for LTP has always been that LTP does not share a basic mechanism with STP (Bliss and Lømo, 1973; McNaughton, 1982; Abraham et al., 1985); the former is believed by some investigators to have a postsynaptic locus, the latter a presynaptic locus. In spite of the recent findings that associative induction of LTP depends on coincident pre- and postsynaptic activity, the study of synaptic potentiation in the hippocampus remains largely focussed on the postsynaptic cell. The present study shows that STP can also be induced by associative interactions between the pre- and postsynaptic cells. This STP demonstrates a magnitude and duration that parallel a presynaptic decrease in excitability, which has been shown to accompany STP in other synaptic junctions. A novel finding is that STP and LTP can be induced without tetanic stimulation of the afferent fibers. By increasing the number of interactions between the pre- and postsynaptic elements, greater STP can be induced. With ten pairs of such interactions, STP is followed by LTP. The site of action of the conditioning tetanus is narrowed to the postsynaptic cell; the conditioning effect itself is shown to be analogous to a generalized depolarization of the postsynaptic cell and can be mimicked by intracellular injections of depolarizing current. Temporal separation between the activation of the pre- and postsynaptic cells was examined, and possible mechanisms for the presynaptic changes were suggested. These findings suggest that STP induction is not confined to the presynaptic terminals and may share a common associative

induction with LTP. Conversely, LTP may have a very strong presynaptic component for induction and maintenance.

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